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Individual Variation in Advertisement Calls of Territorial Male Green Frogs, *Rana clamitans*: Implications for Individual Discrimination

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Abstract

Individuals of many territorial species discriminate between familiar territorial neighbors and unfamiliar strangers based on individual differences in acoustic signals. Many anuran amphibians are territorial and use primarily acoustic communication during social interactions, but evidence for acoustically mediated individual discrimination is available only for one species. As a first step in research designed to investigate individual discrimination in a second species of territorial frog, we examined patterns of within-male and among-male variability in 198 advertisement calls of 20 male green frogs, *Rana clamitans*. The aim was to determine which acoustic properties could be used by males to recognize their territorial neighbors and to estimate limits of reliability afforded by these properties for identifying different neighbors. All of the call properties that we examined exhibited significant inter-individual variation. Discriminant function analyses assigned between 52% and 100% of calls to the correct individual, depending on sample size and the call properties included in the model. This suggests that there is sufficient among-male variability to statistically identify individuals by their advertisement calls. The call properties of fundamental frequency and dominant frequency contributed the most towards discrimination among individuals. Based on their natural history and behavior and the results reported here, we suggest that male green frogs likely discriminate between strangers and adjacently territorial neighbors based on individual variation in advertisement calls.

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Introduction

Animals demonstrate remarkable abilities to recognize and discriminate among other conspecifics at multiple levels of social organization (Colgan 1983). An important component of recognition systems concerns the properties by which animals can be identified as belonging to one or another class of conspecifics (Beer 1970; Beecher 1982, 1989, 1991). From a communication perspective, receivers can use signals to classify senders as members of one or another class as long as signals are somewhat distinctive between the classes of animals that pose the recognition problem (Beecher 1990, 1991). Signal distinctiveness results when classes differ in the presence or absence of certain signal attributes or when there is greater among-class variability than within-class variability in one or more properties of the signal (Beecher 1982, 1989; Falls 1982). Much previous research has focused on acoustically mediated recognition systems because of the ease with which acoustic signals can be recorded, analyzed, and broadcast in field and laboratory experiments. Several studies have demonstrated that acoustic communication signals identify particular individual conspecifics and that animals discriminate between conspecifics based on this signature information (e.g. Beecher et al. 1986; Rendall et al. 1996; Jouventin et al. 1999).

One kind of recognition system that has received considerable attention over the last four decades is territorial neighbor recognition (reviews in Ydenberg et al. 1988; Temeles 1994). Territorial animals often exhibit reduced levels of aggression towards familiar territorial neighbors compared to unfamiliar individuals ('strangers') based on familiarity with their neighbors' communication signals. By exhibiting lower levels of aggression toward established neighbors, residents can conserve time and energy by avoiding repeated aggressive interactions with individuals that pose little threat to territory ownership (Falls 1982; Peeke 1984). Acoustically mediated individual discrimination (*sensu* Halpin 1986) is prevalent among territorial songbirds (reviews in Falls 1982; Stoddard 1996) and is also known from one fish (Myrberg & Riggio 1985) and one anuran amphibian (Davis 1987). Like songbirds, male frogs and toads are highly vocal during their breeding seasons and often defend territories that contain female oviposition sites (Wells 1977b). Given the diversity of mating systems found among anurans (Wells 1977b), frogs represent an excellent opportunity to investigate the proximate and ultimate mechanisms of acoustically mediated individual discrimination. Nonetheless, anurans are an under-represented taxon in the individual discrimination literature.

Falls (1982) and others (e.g. Beer 1970) have suggested that determining the extent to which individuals can be accurately identified by their signals is a direct way to investigate the potential for individual discrimination, because the degree of signal distinctiveness determines the ultimate limit on recognition. Once the potential for some property or properties to identify individuals has been statistically quantified, then researchers can test the ability of receivers to behaviorally discriminate among individuals based on differences in these properties. As a first step in an investigation of individual discrimination in a territorial frog, we exam-

ined patterns of variability in the advertisement calls of territorial male green frogs (*Rana clamitans*, Anura, Ranidae) in order to quantify the potential for variation in these signals to identify individuals. We do not yet know whether male green frogs discriminate between neighbors and strangers. Thus, a second approach would also be to first establish that territorial male green frogs can discriminate between the calls of neighbors and strangers and then later quantify the variability in various acoustic properties that could mediate discrimination. However, in this case, a second round of behavioral testing would be needed to confirm that males actually discriminate between the calls of neighbors based on individual differences in these properties. The goal of our study is to focus future playback experiments on the acoustic properties that contribute most towards statistically discriminating among individual males.

Several aspects of their natural history and behavior suggest that the ability to discriminate among adjacently territorial neighbors is likely to be present in green frogs. First, territorial neighbor recognition is found predominantly in species in which territories function as multipurpose or breeding territories (Temeles 1994). Territories in green frogs contain important breeding resources. Female green frogs appear to choose mates based on the quality of the territory as an oviposition site (Wells 1977a). Secondly, male green frogs vigorously defend their territories using aggressive vocalizations, chases, splash displays, and physical attacks, and agonistic encounters can escalate to intense physical fighting (Schroeder 1968; Wells 1978). Repeated aggressive interactions with non-threatening neighbors are potentially very costly in terms of energy, lost mating opportunities and decreased vigilance that could increase the risk of predation. Thirdly, the potential exists for males to establish long-term relationships across common territorial borders with certain individuals that can last for weeks or months, even if the absolute spatial positions of these borders change during the breeding season (Martof 1953; Wells 1978). Finally, several investigators have suggested habituation to a new neighbor or its signals as a potential psychological mechanism underlying neighbor recognition (e.g. Brooks & Falls 1975a; Wiley & Wiley 1977; Peeke 1984). In a recent field playback study, Owen & Perrill (1998) demonstrated that the aggressive response of territorial male green frogs exhibits response decrements that are characteristic of short-term habituation and suggested that this learning process could permit males to learn about the calls of their nearby neighbors.

Methods

Study Organism

The green frog occurs throughout eastern North America and breeds between late Apr. and late Aug. in permanent bodies of water, such as lakes, ponds and streams. Male green frogs produce several distinct call types (see Wells 1978 and Bee & Perrill 1996 for descriptions of each type of call). The advertisement call (type I) is an explosive, short-duration signal that is often likened to the sound of a plucked loose banjo string. It is a broad-band signal with a roughly bimodal frequency spectrum consisting of a varying number of harmonics between 200 and

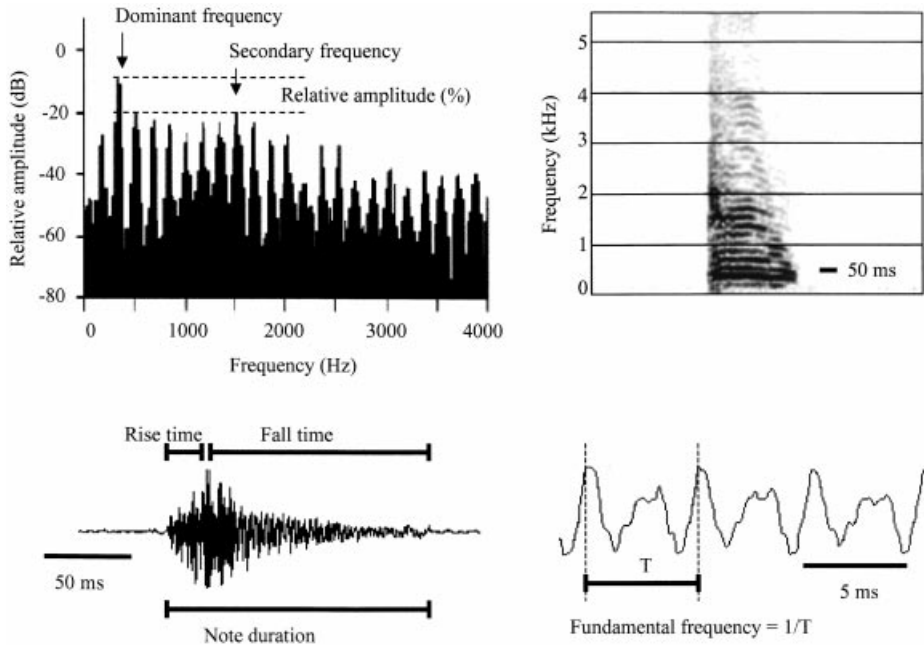


Fig. 1: Power spectrum (top left), spectrogram (top right), and oscillograms (bottom) of a type I advertisement call, with indications of the spectral and temporal properties measured in acoustic analyses. Fundamental frequency was calculated as the reciprocal of the average period (T) of five to six repetitions of the fine-temporal waveform

4000 Hz that are integer multiples of a fundamental frequency of approximately 200 Hz (Fig. 1). Type I advertisement calls are produced as a single-note call or a multiple-note call with up to six consecutive notes; they are the most common type of call heard in a green frog chorus. The number of notes per call typically increases with overall chorus activity during a night, and additional notes may function to increase detectability in a noisy environment (Wells 1978). These calls presumably function both to announce a male's position to other males and to attract females as prospective mates (Wells 1978).

Recording and Acoustic Analysis of Vocalizations

During June and July 1996 and 1998 we recorded between eight and 10 consecutive type I advertisement calls from 20 territorial male green frogs in three ponds located in the Baskett Wildlife Area ($n = 19$ males) and one pond at the Little Dixie Lake Conservation Area ($n = 1$ male) near Columbia, Missouri. The three ponds at the Baskett site were located within a 1-km radius, therefore, any influence of geographic variation on our analyses should be minimal. Recordings were

made with a Sennheiser MKH 815T or MKH 70 shotgun microphone equipped with a Windtech SG-3 windscreen and mounted on a tripod placed 1–2 m from a subject. Slight variation in the distance from subjects to the recording microphone between recording sessions is unlikely to accentuate inter-individual call differences (Boatright-Horowitz et al. 1999). During recording sessions, one to two observers sat quietly under ambient light conditions on the bank of the pond, approximately 3–6 m away from the subject, and recorded advertisement calls onto a Nagra IV-L recorder (tape speed = 9.5 cm/s) or an HHb PDR-1000 DAT recorder (sampling rate = 32 kHz). After each recording session, we captured the male, measured its body length (snout-to-vent length, SVL) to the nearest millimeter with a ruler (\pm SD = 77.4 ± 4.2 , $n = 17$ males) and recorded the water temperature at the recording site to the nearest 0.1°C ($25.6 \pm 3.3^\circ\text{C}$, $n = 20$). Recordings were made under ambient light conditions between 22:00 h and 05:00 h.

We analyzed power spectra and oscillograms of our recordings on a Kay DSP Sona-Graph Model 5500. The vast majority of type I calls consisted of single notes, and we limited the analysis of all multiple-note type I calls to the first note. We measured four spectral properties and three temporal properties of advertisement calls, including fundamental frequency, dominant frequency, secondary frequency, amplitude of the secondary frequency relative to that of the dominant frequency (hereafter 'relative amplitude'), note duration, rise time, and fall time (Fig. 1 and defined below). These call properties were chosen because they captured much of the among-male variation detected from visual inspection of spectrograms and oscillograms. Because male green frogs modify some of these call properties during agonistic interactions (Bee & Perrill 1996; Bee et al. 1999), we only recorded males that were not interacting with nearby frogs. Other call properties, such as note number and calling rate, were not analyzed because these properties likely vary depending on chorus density and overall chorus activity, both seasonally and within nights. Our recordings were of high enough quality that we had no difficulty distinguishing the signal from background noise. To insure a general level of consistency in these analyses, all advertisement calls were analyzed by one of us (C. E. Kozich) over a 6-wk period.

Dominant frequency is the harmonic of single greatest amplitude and corresponds to the second harmonic. Secondary frequency is defined as the harmonic of greatest relative amplitude in the higher frequency band of the bimodal spectrum. We determined the amplitude of the secondary frequency relative to that of the dominant frequency in dB. These values were also converted from the logarithmic dB scale to a linear scale and expressed as a percentage of the amplitude of the dominant frequency. This conversion was performed to satisfy requirements of mathematical operations (e.g. calculating means and variances). Our two measures of relative amplitude were highly correlated across individuals ($r = 0.98$, $p < 0.0001$, $n = 20$). Dominant frequency, secondary frequency and relative amplitude were determined from power spectra generated by averaging the frequency spectrum over a 100-ms section taken from the middle of the call (transform size = 1024 points, bandwidth = 14.5 Hz, 0–4 kHz setting).

We also determined the ratio of secondary frequency to dominant frequency (hereafter 'frequency ratio') as a configurational measure of the distribution of sound energy in the bimodal frequency spectrum. Dominant frequency and secondary frequency represent the peaks of two distinct frequency bands in the spectrum that may be analogous to formant frequencies (Simmons et al. 1992). Although the exact mechanism is unknown, these frequencies are likely emphasized as a result of differential filtering by resonating structures during sound production, such as the vocal cords, the vocal sac and the tympanic membranes (Purgue 1997). Such a measure is relevant because anurans have two separate hearing organs, the amphibian and basilar papillae, that are tuned to different frequency ranges (Fritzsch et al. 1988). In green frogs, the amphibian papilla is tuned to lower frequencies that are characteristic of the dominant frequency and the basilar papilla is tuned to higher frequencies that are characteristic of the secondary frequency (Sachs 1964).

The fundamental frequency is often a missing spectral component of green frog advertisement calls. We therefore measured fundamental frequency in the temporal domain by taking the reciprocal of the average period of 5–6 repetitions of the fine-temporal waveform (fundamental frequency = 1/waveform periodicity) from an oscillogram with an expanded time base (± 0.1 ms). The gross-temporal properties of note duration, rise time and fall time were also measured from oscillograms (± 0.8 ms). Note duration was the time between signal onset and offset. Rise time was defined as the time from signal onset to the time of maximum amplitude; fall time was defined as the time from maximum amplitude to signal offset. Signal onset and offset were identified as the beginning and ending, respectively, of the repeating fine-temporal waveform from an oscillogram with an expanded time base (± 0.1 ms).

Statistical Analysis of Vocalizations

Patterns of variability were assessed by analyzing a sample of 198 type I advertisement calls from 20 males (eight calls from one male and 10 calls from each of 19 males). For each call property, we determined a grand mean and standard deviation based on the mean for each male (see Table 2). We compared the variability among call properties by determining within-male and between-male coefficients of variation ($CV = [SD/\bar{x}] \times 100$). Within-male coefficients of variation (CV_w) were based on means and standard deviations calculated from the calls produced during a single recording session for each individual male. The between-male coefficients of variation (CV_b) were based on the grand mean and standard deviation. We calculated the ratio of between-male to within-male coefficients of variation (CV_b/CV_w) as a measure of relative between-male variability (e.g. Robison et al. 1993). If $CV_b/CV_w > 1.0$ for a given signal property, then the property is relatively more variable among individuals and could potentially function as a

recognition cue (Robisson et al. 1993; Jouventin et al. 1999). We used the model II analysis of variance (ANOVA) to determine whether call properties varied significantly among males (Sokal & Rohlf 1981; Falls 1982; Beecher 1989). Because green frogs are ectotherms and we recorded males that called exclusively while floating in the water, these analyses were performed on the residuals from the linear regressions of each call property on water temperature. We obtained similar results when we analyzed values that were not adjusted for differences in water temperature (Bee et al. unpubl. data).

We used discriminant function analysis (DFA) to statistically examine the individual distinctiveness of type I advertisement calls. Discriminant function analysis generates canonical discriminant functions that represent the linear combinations of the original variables that maximally separate groups (individual males in this case) in multidimensional signal space (Nelson & Marler 1990). The canonical coefficients, which represent the correlations between the original variables and the discriminant functions, describe how the original variables contribute towards discrimination between individuals. The discriminant functions generated in a DFA can be used to classify signals as belonging to particular individuals. Classification success serves as a measure of how well the linear combinations of variables in the discriminant functions distinguishes among individuals (Manly 1994). We classified each call using two different methods. First, we used a cross-validation procedure, in which each call was assigned to a particular individual based on the discriminant functions generated for the data set containing the $n-1$ remaining calls. Second, we assigned every other call from a male to a 'training' data set and a 'test' data set. The training set was used to generate the discriminant functions, which were then used to classify calls in the test set. Our classification procedures used prior probabilities that were proportional to the number of calls per male. The DFA was performed on the residuals from the regression of call properties on water temperature for the sample of 198 calls from 20 individuals. Similar results were obtained when we analyzed unadjusted call properties (Bee et al. unpubl. data).

In an earlier study of territoriality in green frogs, Martof (1953) noted a 'clustering effect' in which small groups of two to five frogs remained together when changes in territory residency occurred. Moreover, when these changes took place, males within clusters maintained the same relative spatial positions for periods up to about 2 mos. Hence, the sample size of 20 males for the DFA described above potentially exaggerates the magnitude of the recognition problem facing males by overestimating the number of individuals that a territorial resident might realistically be required to identify at any one time. Therefore, we also performed additional DFAs on the calls of smaller groups of males. We repeatedly re-sampled our sample of 20 males by randomly selecting 1000 groups of males at each of five sample sizes ($n = 2, 4, 5, 8, 16$). This analysis explored how estimates of correct classification derived using the cross-validation procedure changed as the number of individuals in the analyses was reduced from $n = 20$. The number of signals per individual in these analyses was either 10 calls (eight for one male) or five calls from each male.

Results

There were several significant correlations between call properties and between call properties and water temperature (Table 1). Both fundamental frequency and secondary frequency were significantly positively correlated with water temperature, and rise time was negatively correlated with water temperature. None of the measured call properties was correlated with body size. Dominant frequency and SVL were significantly negatively correlated in previous studies of green frogs ($r = -0.79$, Bee & Perrill 1996; $r = -0.83$, Bee et al. 1999). A likely explanation for this discrepancy is that the males sampled in our study represent a smaller absolute range of body sizes (70–86 mm) than in these previous studies (e.g. 56–97 mm, Bee et al. 1999).

Patterns of Call Variability

The calculated CV_b/CV_w ratios indicate that all nine of the acoustic properties examined in our study were relatively more variable among males than within males (Table 2). The model II ANOVAs confirm that all call properties exhibited significant among-male variability (Table 2) and thus are potentially useful as recognition cues.

Following Gerhardt (1991), we categorized fundamental frequency, dominant frequency and secondary frequency as static properties because they had mean within-male CVs of less than 5% (Table 2). We also consider frequency ratio to be a static property based on the range of within-male variability, although the mean within-male CV is slightly higher than 5%. Relative amplitude and rise time had mean within-male CVs greater than 20% and were categorized as dynamic properties. Within-male variation in note duration and fall time was intermediate between static and dynamic properties.

Static properties also had the lowest between-male CVs (Table 2). In all cases, the between-male CVs of static properties were less than 10%. Call properties categorized as intermediate based on within-male CVs also had intermediate between-male CVs (15–21%). Dynamic properties, on the other hand, had between-male CVs greater than 28%. Furthermore, the largest difference between the maximum and minimum values of a call property expressed as a percentage of the mean was 20.17% for static properties (dominant frequency) and 119.81% for dynamic properties (relative amplitude).

Individual Distinctiveness

Our initial discriminant function analysis of the call data included all nine variables. However, because there were strong correlations between some call properties (e.g. fundamental and dominant frequencies, measures of relative amplitude, note duration and fall time; Table 1), we also performed a number of separate DFAs in which we excluded either: fundamental frequency or dominant frequency; relative amplitude (in dB) or percentage relative amplitude; or note duration or fall time. Results from these analyses were quantitatively and qualitatively similar to the analysis that included all nine variables, using both the cross-

Table 1: Pearson product-moment correlations (r) between mean values of each call property (adjusted to 25°C)^a and between call properties and SVL and water temperature ($n = 20$ males)

	Fundamental frequency	Dominant frequency	Secondary frequency	Frequency ratio	Relative amplitude (%)	Note duration	Rise time	Fall time
Dominant frequency	0.94**							
Secondary frequency	0.20	0.16						
Frequency ratio	-0.71**	-0.74**	0.18					
Relative amplitude (%)	0.29	0.16	-0.01	-0.13				
Note duration	-0.22	-0.31	-0.41	0.38	-0.01			
Rise time	0.35	0.51*	-0.06	-0.13	-0.08	-0.29		
Fall time	-0.19	-0.33	-0.32	0.38	0.02	0.98**	-0.41	
SVL ^b	-0.16	-0.28	-0.12	0.28	0.41	0.27	-0.14	0.26
Water temperature ^a	0.53*	0.44	0.51*	0.11	0.05	0.04	-0.69**	-0.41

^aFor correlations with water temperature, values of call properties were not adjusted to 25°C.

^b $n = 17$; SVL was not measured for three males.

* $p < 0.05$, ** $p < 0.011$.

Table 2: Mean, SD, range, and within-male and between-male variability (CV) for nine acoustic properties of type I advertisement calls (adjusted to 25°C using linear regression) and the results of model II ANOVAs comparing among-male and within-male variability

Call Property	Grand Mean	Standard Deviation	Range	Type ^a	Mean within-male CV	Range of within-male CV	Between-male CV	Maximum between-male difference ^b	Ratio CV _b /CV _w	F ^c
Fundamental frequency (Hz)	196	9.1	182–218	Static	2.29%	1.02–4.10%	4.64%	18.18%	2.0	30.23*
Dominant frequency (Hz)	393	20.3	368–447	Static	2.04%	0.00–6.40%	5.16%	20.17%	2.5	39.38*
Secondary frequency (Hz)	1712	97.8	1526–1865	Static	4.59%	0.85–10.62%	5.71%	19.80%	1.2	8.75*
Frequency ratio	4.3	0.4	3.4–4.9	Static	5.11%	0.81–10.75%	8.60%	34.49%	1.7	13.04*
Relative amplitude (%)	46.8	16.7	19.1–75.2	Dynamic	28.50%	11.73–62.38%	35.73%	119.81%	1.3	16.00*
Relative amplitude (dB)	-6.6	+ 2.7/ - 3.8 ^d	- 14.4 to - 2.5							
Note duration (ms)	154	24.7	109–205	Intermediate	8.43%	2.53–20.38%	15.98%	62.13%	1.9	16.97
Rise time (ms)	19	5.4	12–33	Dynamic	20.09%	3.62–47.74%	28.56%	110.18%	1.4	16.39*
Fall time (ms)	130	27.3	79–187	Intermediate	11.30%	2.47–23.21%	20.96%	83.21%	1.9	20.26*

^aDetermination based on mean within-male CV after Gerhardt (1991).

^bMaximum difference between individual means expressed as a percentage of the grand mean.

^cdf = 19, 178.

^dAsymmetric standard deviations result when converting to the logarithmic decibel scale.

*p ≤ 0.05.

validation and split data set methods. Therefore, we focus primarily on the results of the DFA of all nine call properties from the sample of 198 calls ($n = 20$ males). This inclusive analysis generated seven significant discriminant functions, the first four of which had eigenvalues greater than 1.0 and accounted for 89.7% of the total variation among males (Table 3). All other analyses with fewer variables had three or four discriminant functions with eigenvalues greater than 1.0, which accounted for 79.6–91.4% of the among-male variation.

Inspection of the canonical coefficients in Table 3 revealed that the first canonical discriminant function, which accounted for 44.6% of the among-male variation, loaded most heavily on fundamental frequency and dominant frequency, and somewhat heavily on frequency ratio and note rise time. The second discriminant function accounted for 23.1% of the variation among males and loaded most heavily on the temporal properties of note duration, rise time and fall time. The third discriminant function loaded heavily on our measures of relative amplitude and accounted for 12.8% of the variation among males. In the other analyses, the first discriminant function accounted for 41.5–46.2% of the among-male variation and was consistently highly correlated with fundamental frequency ($0.80 < r < 0.92$), dominant frequency ($0.89 < r < 0.96$), or both.

In these analyses, mean values of classification success ranged between 52.4 and 60.3% (Table 4) and were significantly greater than the mean a priori probability of 5.0% (range 4.04–5.05%) correct classification expected by chance (paired t-test, all $p < 0.05$). Classification success improved markedly when we considered smaller groups of randomly selected males (Fig. 2). The values of classification success for the 1000 random groups of various sample sizes ($n = 2$ –16 males) ranged between 43.5 and 100%. There was an inverse linear relationship between percentage correct classification and the number of males included in the analysis (Fig. 2). In addition, estimates of correct classification were greater in analyses that included 10 calls per male than in those that included five calls per male.

When we analyzed groups of five randomly selected males, the mean correct classification was 81.9%. For groups of two randomly selected males, the mean percentage correct classification increased to 95.7%. For both analyses, the 95% confidence interval (81.3–82.5% and 94.4–97.0% for $n = 5$ and $n = 2$, respectively) indicated that significantly more calls were assigned to the correct individual than expected by chance alone (20% and 50%, respectively). A combination of the first two canonical discriminant functions allowed the unique identification of four out of 20 individuals when all males were considered together (Fig. 3). There was, however, considerable overlap among the remaining 16 males. Separation of males along the dimensions of the first two canonical discriminant functions improved considerably when smaller groups of males were considered (Fig. 3).

Discussion

All nine of the acoustic properties examined in this study exhibited significant inter-individual variation. Previous studies have also reported significant inter-individual variation in the acoustic properties of anuran communication signals,

Table 3: Canonical coefficients for the nine variables in the discriminant analysis using the cross-validation procedure (198 calls from 20 males). Calls adjusted for between-male differences in water temperature. Bold face indicates a correlation greater than 0.70

Variable	Canonical coefficient								
	1	2	3	4	5	6	7	8	9
Fundamental frequency	0.882	0.244	0.000	-0.151	0.013	-0.195	-0.230	-0.138	-0.169
Dominant frequency	0.955	-0.017	-0.058	-0.040	-0.022	0.086	-0.059	0.260	-0.060
Secondary frequency	-0.054	0.011	-0.246	-0.697	0.511	0.333	0.253	-0.094	-0.078
Frequency ratio	-0.584	-0.021	-0.155	-0.500	0.453	0.243	0.236	-0.252	-0.026
Relative amplitude (%)	0.177	0.381	0.809	-0.108	0.301	-0.027	0.208	0.132	-0.067
Relative amplitude (dB)	0.247	0.377	0.832	-0.146	0.111	0.092	0.246	0.022	0.042
Note duration	-0.064	0.727	-0.361	0.436	0.148	-0.112	0.284	-0.058	0.167
Rise time	0.577	-0.599	0.018	-0.172	0.121	-0.317	0.181	0.010	0.360
Fall time	-0.124	0.804	-0.355	0.325	0.196	-0.071	-0.020	-0.007	0.25127
Eigenvalue	6.775	3.503	1.948	1.403	0.763	0.356	0.267	0.105	0.081
Proportion of variation (%)	44.6	23.1	12.8	9.2	5.0	2.3	1.8	0.7	0.5
Cumulative proportion of variation	44.6	67.6	80.4	89.7	94.7	97.0	98.8	99.5	100.0
F	10.67**	8.00**	6.13**	4.78**	3.44**	2.47**	2.00**	1.37	1.31
df	171, 1408	144, 1272	119, 1131	96, 987	75, 838	56, 683	39, 522	24, 354	11, 178

*p < 0.01, ** p < 0.00011.

Table 4: Means and ranges of percentage correct classification from the discriminant function analyses using the cross-validation and split data methods (198 calls from 20 males)

Variables in analysis ^a	Cross-validation		Training/Test data sets	
	Mean	Range	Mean	Range
FF, DF, SF, Ratio, RA %RA, ND, RT, FT	55.4	20–100	60.3	0–100
FF, SF, Ratio, RA %RA, ND, RT, FT	58.4	20–100	60.3	0–100
DF, SF, Ratio, RA %RA, ND, RT, FT	52.4	10–100	57.3	20–100
FF, DF, SF, Ratio, %RA, ND, RT, FT	54.9	20–100	60.3	0–100
FF, DF, SF, Ratio, RA ND, RT, FT	57.9	20–100	60.3	20–100
FF, DF, SF, Ratio, RA ND, RT	54.9	10–100	58.0	0–100
FF, DF, SF, Ratio, RA RT, FT	54.9	10–100	55.0	0–100

^aFF, fundamental frequency; DF, dominant frequency; SF, secondary frequency; Ratio, frequency ratio; RA, relative amplitude (dB); %RA, relative amplitude (%); ND, note duration; RT, rise time; FT, fall time.

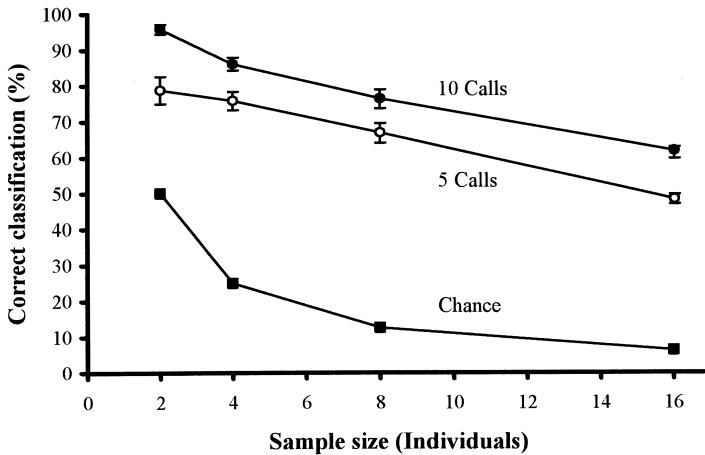


Fig. 2: Mean (\pm 95% confidence interval) percentage of calls assigned to the correct individuals for random samples of males of various sample sizes drawn from a larger sample of 20 males. Each point depicts the mean value for 1000 groups of randomly selected males with the specified sample size (i.e. number of individuals). Analyses were based on either 10 (closed circles) or five (open circles) calls per male. Closed squares depict the rate of correct classification expected from random assignment of calls to individuals

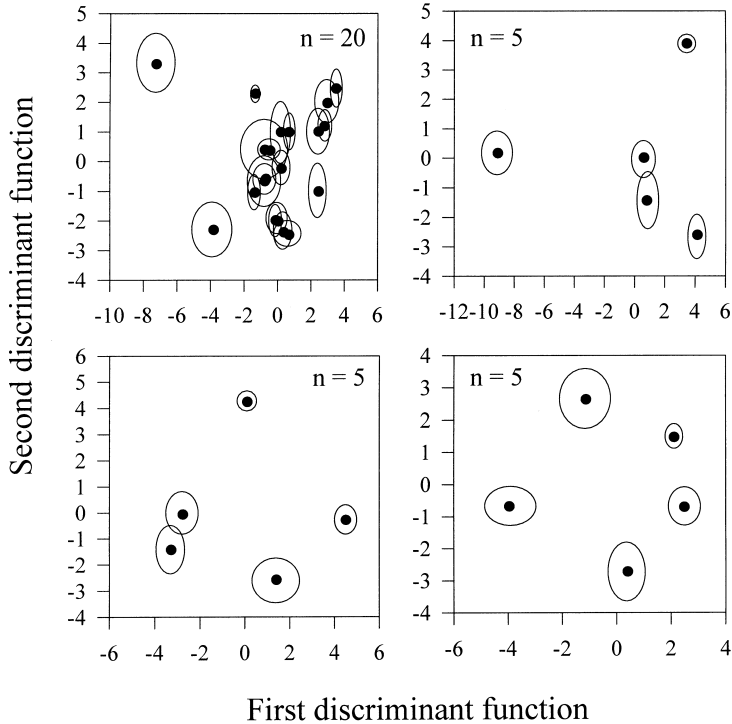


Fig. 3: Mean canonical coefficients (and 95% elliptical confidence intervals) of the first two canonical discriminant functions for all 20 males ($n = 198$ calls) and three groups of five males each ($n = 10$ calls per male)

most commonly within the context of sexual selection (e.g. Sullivan & Hinshaw 1990; Gerhardt 1991; Wagner & Sullivan 1995; Gerhardt et al. 1996; Howard & Young 1998). To our knowledge, only one study has previously examined individual differences in anuran acoustic signals specifically within a context of individual discrimination. Shy (1985) reported significant differences between individuals in nine properties of the advertisement calls of the treefrog *Hyla arborea savignyi*, and suggested that these differences could mediate individual discrimination. Moreover, Shy (1985) anticipated our study by suggesting that recognition between territorial males might take place in *Rana clamitans* based on individual differences in advertisement calls.

Patterns of Variability

The patterns of within-male and among-male variability reported here are similar to those reported for other anurans. For example, the properties of fundamental frequency, dominant frequency, secondary frequency and frequency ratio

were categorized as static properties because of their low within-male variability. Based on Gerhardt's (1991) criteria, spectral properties are also static in gray tree-frogs (*Hyla versicolor*), green tree-frogs (*H. cinerea*), spring peepers (*Pseudacris crucifer*), American toads (*Bufo americanus*) and bullfrogs (*Rana catesbeiana*) (Bee & Gerhardt unpubl. data; Gerhardt 1991; Howard & Young 1998). As Gerhardt (1991) pointed out, however, static and dynamic properties represent two ends of a continuum of variability. In support of this view, note duration (call duration) was an intermediately variable property in our study, but was classified as a static property in green tree-frogs and spring peepers, and as a dynamic property in gray tree-frogs and American toads (Gerhardt 1991; Howard & Young 1998).

Other studies of call variability in anurans have also measured repeatability (i.e. the intra-class correlation coefficient; Sokal & Rohlf 1981; Lessells & Boag 1987) of call properties across multiple nights of recording (e.g. Sullivan & Hinshaw 1990; Gerhardt 1991; Wagner & Sullivan 1995; Gerhardt et al. 1996; Howard & Young 1998). One limitation of our study is that subjects were recorded on one night only, and therefore, we did not calculate estimates of repeatability. However, similar acoustic analyses on the advertisement calls of male bullfrogs, *R. catesbeiana*, a close relative of green frogs (Hillis & Davis 1986), indicate that all of the call properties examined in that species exhibit significant variation among males when calls from two separate recordings made on different nights are analyzed (intraclass correlations: $0.43 < r < 0.93$; Bee & Gerhardt unpubl. data). More importantly, estimates of repeatability for fundamental frequency and dominant frequency were higher than estimates of these call properties in other species ($r > 0.90$; cf. Table 4 in Howard & Young 1998). It will be important to determine in the future whether the properties of green frog advertisement calls also exhibit significant among-male variability across multiple nights.

A general trend emerging from these studies is the direct relationship between within-male and among-male variability. In our study, and also in those of Gerhardt (1991) and Howard & Young (1998), properties categorized as static, based on within-male variability, also had the lowest between-male CVs, whereas dynamic properties varied more than static properties both within and between males. This result is apparent in the small range of variation in the ratios of CV_b/CV_w , which varied between 1.2 and 2.5, with several values near 2.0 (Table 2). As a result of this direct relationship, some static properties may fail to uniquely identify individuals, not because they are variable within males, but because of limited among-male variability (Gerhardt 1991). Alternatively, some dynamic properties could be useful for identifying individuals if the extent of among-male variability is sufficiently high (e.g. Sullivan & Hinshaw 1990; Gerhardt 1991; Gerhardt et al. 1996).

Individual Distinctiveness of Advertisement Calls

The results of a discriminant function analysis on the sample of 198 calls from 20 males indicated that, on average, 52–61% of the calls in our sample could be successfully assigned to the correct individual. When we analyzed smaller groups

of five males, mean classification success increased to 82%, and the calls from groups of two randomly selected males were correctly classified with even higher success (96%). These results suggest that type I advertisement calls of the male green frog are individually distinct, at least when smaller groups of individuals are considered. Furthermore, because we recorded the calls of males from a relatively small range of body sizes, and because spectral properties vary with SVL in green frogs (Bee & Perrill 1996; Bee et al. 1999), our estimates of among-male variability in these properties are conservative. Consequently, our estimates of classification success are probably also conservative.

As Beecher (1989) noted, estimates of percentage correct classification depend on the sample size in a discriminant function analysis. Our results suggest that estimates of correct classification increase as the number of individuals decreases and the number of signals per individual increases (Fig. 2). Other studies report considerably greater classification success than we report here (e.g. Smith et al. 1982; Robisson et al. 1993; Durbin 1998), but meaningful between-study comparisons are difficult given that these studies are based on variable numbers of individuals and signals per individual. In the context of individual discrimination, the scale of the potential recognition problem obviously must be considered when interpreting results. Martof's (1953) observation that male green frogs tended to form small, temporally stable clusters of 2–5 individuals suggests that an analysis with a large sample size (e.g. $n = 20$ males) may overestimate the complexity of the recognition problem facing territorial male green frogs. Even if clustering behavior is uncommon in green frog breeding aggregations, territorial residents only rarely share common boundaries with more than a few other males at any one time (pers. obs.). Hence, we believe that the discriminant function analyses on the smaller samples of 2–5 males are more biologically meaningful. To the extent that Martof's (1953) observations hold, spatial cues could also facilitate recognition, as they appear to in other territorial species (e.g. Falls & Brooks 1975; Wiley & Wiley 1977; Myrberg & Riggio 1985; Davis 1987; Godard 1991). Moreover, male green frogs would experience considerably more than five to 10 calls from a neighbor during the establishment of a mutually respected boundary, thus providing ample opportunity to encode into memory the individually distinct features of a neighbour's calls.

From an 'identification' perspective (Beecher 1991), all of the call properties examined here potentially function as cues that identify individuals. However, there are several reasons why we might expect males to discriminate among conspecifics based on a subset of signal properties that vary significantly among individuals. First, because different call properties exhibit different magnitudes of among-individual variation, receivers might be expected to more heavily weigh signal attributes that more reliably distinguish among individuals (Jouventin et al. 1999). Secondly, not all signal properties propagate equally well through the environment (Wiley & Richards 1978; Richards & Wiley 1980). In a noisy breeding chorus, males would be expected to weigh more heavily as recognition cues those properties that propagate through the environment with relatively less attenuation and degradation. Finally, the magnitude of among-male differences may be less than

the 'just noticeable difference' (JND) between two signals required for the nervous system to detect a difference. If this were the case for a given property, then discrimination based on that property would be impossible, regardless of the extent of reliable inter-individual variation. Alternatively, among-male differences may exceed the JND but fall below the 'just meaningful difference' (JMD) between two signals required to elicit a discriminative behavioral response (Nelson & Marler 1990).

We suggest that individual discrimination in green frogs could occur based on the individual distinctiveness of advertisement calls determined primarily by fine-temporal or spectral call properties. An inspection of the F-ratios and the CV_b/CV_w ratios in Table 3 and the canonical coefficients in Table 4 indicates that the correlated call properties of fundamental frequency (reciprocal of waveform periodicity) and dominant frequency are relatively more variable among individuals and contribute most heavily towards successfully assigning calls to particular individuals. Waveform periodicity and low-frequency spectral components, such as dominant frequency, propagate well through green frog breeding habitat (Boatright-Horowitz et al. 1999). In addition, the peripheral auditory system of green frogs probably extracts waveform periodicity or low-frequency spectral components of signals under a wide range of signal-to-noise conditions, as it does in the closely related bullfrogs (Schwartz & Simmons 1990; Simmons et al. 1992, 1993). More importantly, earlier work (Bee et al. 1999) has shown that male green frogs can discriminate between two stimuli that differ by 25 Hz in fundamental frequency (and corresponding differences in other correlated spectral properties), which is a magnitude of difference that falls within the range of among-male differences reported here (Table 2). Thus some among-male differences in spectral properties exceed both the JND and the JMD for male green frogs. Males appear not to discriminate between stimuli that differ only in note duration (Bee & Perrill unpubl. data), even though this signal property also varies significantly among males (Table 2).

In general, spectral properties are important for statistically discriminating between the acoustic signals of different individuals in a number of taxa (e.g. Weary et al. 1990; Hauser 1991; Scherrer & Wilkinson 1993; Lengagne et al. 1997; Durbin 1998). Experimental studies with songbirds suggest that territorial residents can use spectral song properties to recognize other individuals (Brooks & Falls 1975b; Nelson 1989). Although perception of individual differences may improve with some redundancy in the signal, we wish to emphasize that highly inter-correlated call properties, such as fundamental and dominant frequency (Table 1), obviously do not uniquely contribute to the total amount of information in the signal. Beecher (1989) has developed an analysis method based on information theory that measures the information content of a signal after eliminating inter-correlations among variables. We did not use this method because, as Beecher (1989) notes, the approach makes assumptions that are probably unrealistic when applied to determine the absolute amount of information in a signaling system, but is more useful for making comparisons among species (e.g. Beecher et al. 1986). Our results indicate that excluding each variable in a highly

correlated pair of variables did not significantly change the outcomes of the discriminant analyses.

Implications for a Recognition System

Do male green frogs discriminate between neighbors and strangers? Davis (1987) demonstrated that male bullfrogs, *R. catesbeiana*, discriminate between the advertisement calls of territorial neighbors and strangers. Green frogs are closely related to bullfrogs (Hillis & Davis 1986), and striking similarities exist between the mating systems of these two species (both are resource defense polygyny; Wells 1977a, 1978; Howard 1978). Given these aspects of their natural history and behavior, and the level of individual distinctiveness in advertisement calls reported here, we suggest that territorial male green frogs likely behaviorally discriminate between the calls of strangers and familiar neighbors in adjacent territories, and perhaps between different adjacent neighbors. Results from multivariate statistical analyses such as those reported here must be considered only as a starting point into the investigation of recognition systems (Beer 1970). Additional field studies of individual discrimination in green frogs would be worthwhile. Our present analysis should serve to focus experimental testing on the most statistically reliable features of the call and determine some a priori expectation about the magnitude of the minimum differences in these features that could, at best, set the upper limit on correct identification.

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