

Feature Weighting in Signal Recognition and Discrimination by Túngara Frogs

Introduction

Communication between the sexes to identify appropriate mates is a critical component of the mating process. Since heterospecific matings often do not result in viable offspring, there should be strong selection on females to mate with conspecific males. Thus one would expect signalers to accurately convey species status and receivers to accurately decode this information (Dobzhansky 1940; Mayr 1942; Andersson 1994). Studies of communication systems involved in species recognition continue to make important contributions to our understanding of the process of speciation (Gerhardt 1988, 1994; Doherty and Howard 1996; Martens 1996; Veech et al. 1996). Furthermore receivers can generate selection among potential conspecific mates if there is a preference for some signals over others, and thus can generate variance in male mating success and influence signal evolution (Kirkpatrick and Ryan 1991; Andersson 1994). Studies of these interactions have enhanced our understanding of the process of sexual selection (Ryan 1991; Andersson 1994; Searcy and Yasakawa 1996). Species recognition and sexual selection, however, are related processes. Strong selection to avoid heterospecifics can incidentally cause females to avoid conspecifics that most closely re-

semble heterospecifics, thus generating sexual selection. Alternatively sexual selection for signal variants within the conspecific range can incidentally yield species recognition (Fisher 1930; West-Eberhard 1979; Ryan and Rand 1993b; Gerhardt 1994; Pfennig 1998).

Recognition and Discrimination

Studies of communication systems that result in conspecific matings are often couched in terms of species recognition and are concerned with what signals are recognized as indicating appropriate mates. Sexual selection studies, on the other hand, usually address the degree to which females discriminate among what are recognized as acceptable signal variants. Thus it is helpful to precisely define these terms and to conduct experiments that address each. We operationally define *recognize* as "to treat as valid" (*Compact Oxford English Dictionary*, 2nd edition, 1993). We determine if a stimulus is recognized by determining if it elicits an appropriate bioassay from the receiver. We define *discriminate* as "to make or constitute a difference in or between" (*Compact Oxford English Dictionary*). We test for discrimination by determining if the receiver responds to one rather than the other signal. This usage also has some parallels and some

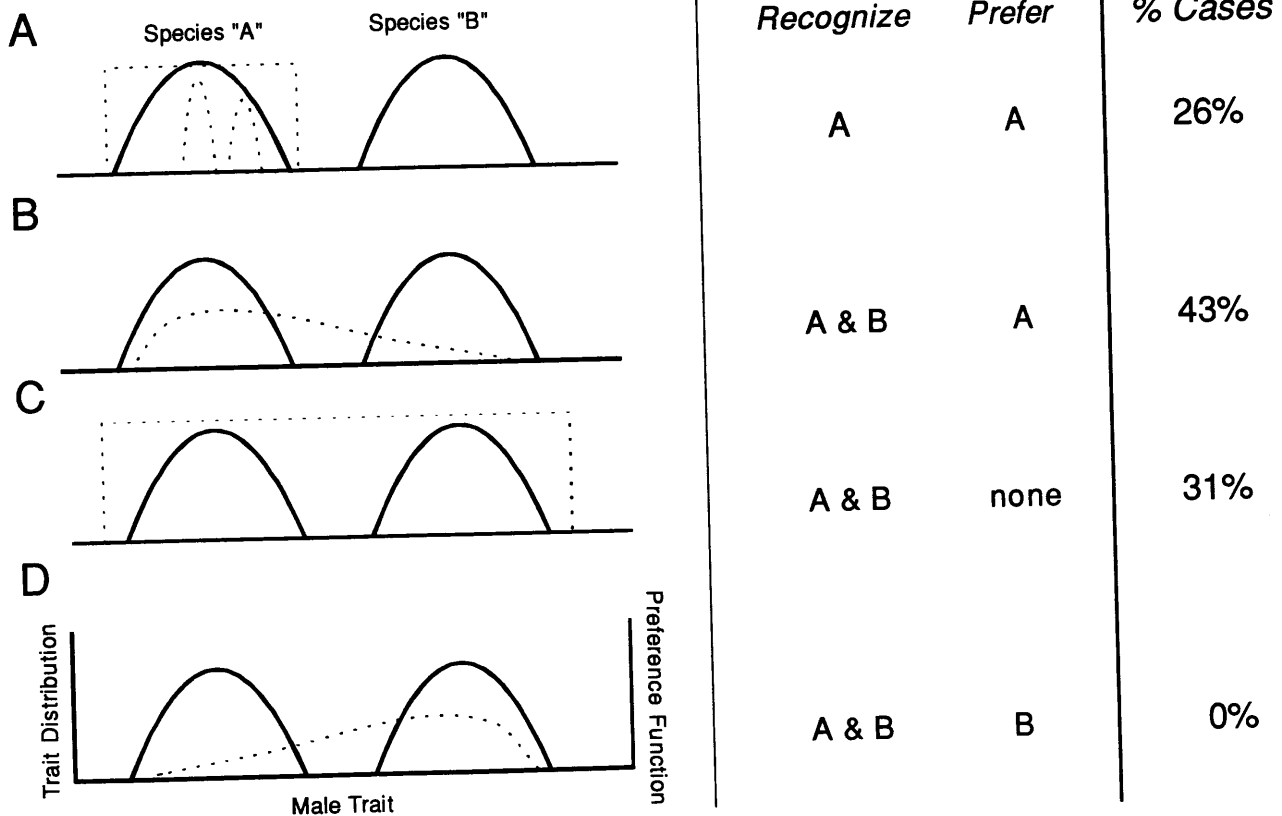


Figure 8.1. Possible patterns of signal recognition (test stimulus vs. white noise) and discrimination (test stimulus vs. conspecific call). We also show possible relationships between the trait distribution of male mating signals (solid lines) for two species, A and B, and the female preference function for species A (dashed line) that could result in each of the four patterns of recognition/discrimination. The right panels summarize the results of 36 phonotaxis experiments in which females were tested for recognition and discrimination with various heterospecific or ancestor calls.

differences with the terms *discrimination* and *identification* as used in the cognitive sciences (Harnad 1987).

As we use it, *recognition* is somewhat context-independent whereas *discrimination* is context-dependent. Recognition experiments do not allow us to determine if one signal would be preferred over another. Discrimination experiments tell us which signal of a pair is preferred, but do not tell us whether the unpreferred signal is recognized as appropriate but inferior or is not recognized at all. It is necessary to understand both of these factors to appreciate how receivers respond to signal variation.

We use these two classes of response to illustrate in Figure 8.1 how variation in preference functions and signal properties can result in different patterns of signal recognition and preference within and among species. The categories we show in Figure 8.1 are ranked (A–D) from a lesser to greater effect of the heterospecific signal on the female, and reflect what we think might be more or less likely out-

comes when a female is confronted with the mate recognition signal of a heterospecific.

The situation we assume would occur most commonly is that only the conspecific signal is recognized (Figure 8.1A). Three possible preference functions might result in this pattern of response: (1) all conspecific signals might be equally attractive, (2) there might be stabilizing selection on the mean signal, or (3) there might be directional selection favoring signals that depart from the mean. What might be considered the next most likely alternative is when females recognize both conspecific and heterospecific signals as appropriate but prefer the conspecific signal (Figure 8.1B). A less likely alternative is when females (mistakenly) recognize both a conspecific and heterospecific signal as indicating an appropriate mate and do not discriminate between these two signals (Figure 8.1C). The least likely scenario is when females recognize both the conspecific and heterospecific signal and actually prefer the latter (Figure 8.1D).

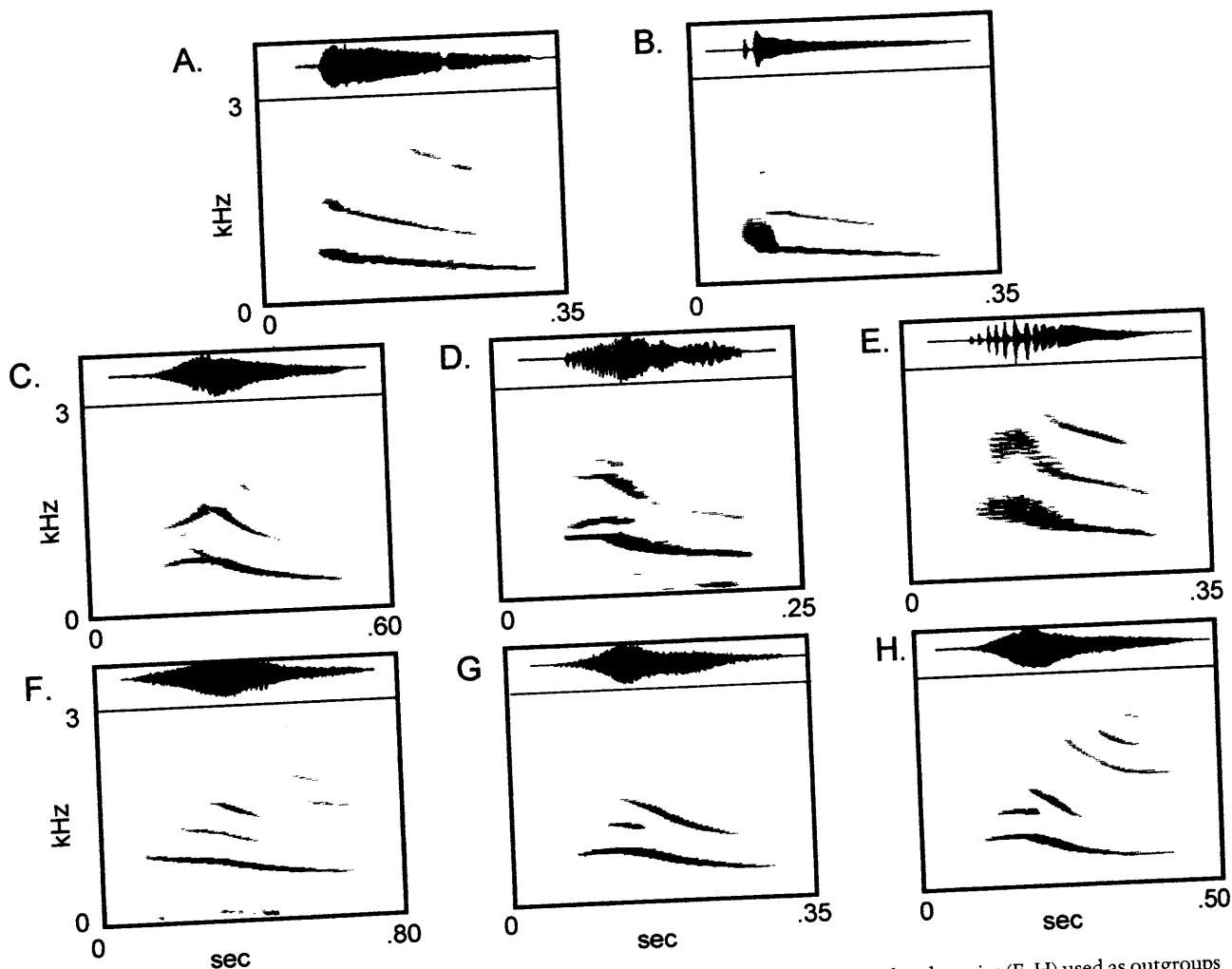


Figure 8.2. Calls of species of the *Physalaemus pustulosus* species group (A–E) and three closely related species (F–H) used as outgroups in the analysis (phylogenetic relationships are illustrated in Figure 8.3). Oscillograms (top) and sonograms (bottom): (A) *P. pustulosus*, (B) *P. petersi*, (C) *P. caicai*, (D) *P. coloradorum*, (E) *P. pustulatus*, (F) *P. enesefae*, (G) *P. ephippifer*, (H) *P. "roraima"* (an undescribed species). A species we tentatively identify as *P. freiberghi* (Cannatella et al. 1998) was not included in these studies.

This does not exhaust all possible interactions but we believe it presents the most likely ones.

Phylogenetic Comparisons

One of the goals of this study was to determine patterns of signal recognition and discrimination in female túngara frogs, *Physalaemus pustulosus*. We discerned these patterns over a wide range of calls of closely related heterospecifics and calls that we estimated at ancestral nodes of the phylogenetic tree of these species. For simplicity we refer to these calls as ancestral calls, but such shorthand should not suggest an arrogance of certainty about how ancestors of these frogs might have sounded. Testing female responses on this phylogenetic background is not a common approach but we feel it is a valid one because it might reveal how past history

influences responses of females (e.g., Phelps and Ryan 2000; Phelps, this volume).

Túngara frogs are almost never found in choruses with congeners (the one exception is the small area of overlap between *P. pustulosus* and *P. enesefae* in the llanos of Venezuela), only occasionally with another species in a closely related genus (*Pleurodema*), and sometimes with other members of their subfamily (Leptodactylinae: Cannatella and Duellman 1984; Cannatella et al. 1998). The calls of the other syntopically breeding species are rarely similar to the túngara frog call. Although these current ecological interactions might influence the evolution of the túngara frog's communication system, we have chosen to emphasize historical factors. Signals and receivers are not deconstructed and reconstructed with each species event. Instead they are jury-rigged innovations of ancestral conditions. Thus we feel a

phylogenetic approach to signal recognition can be a valuable addition to similar studies conducted in an ecological context, which might examine effects of habitat or acoustic competitors on signal evolution. An advantage of concentrating on either the phylogenetic or ecological approach is not to confound them.

In this study we determined how female túngara frogs recognize and discriminate calls of heterospecifics and ancestors, and how these patterns fit into the classification proposed in Figure 8.1.

Feature Weighting

Calls consist of a number of parameters or features. We cannot assume that variation in all of these features equally influences the females' responses (e.g., Tinbergen 1953). In a subsequent series of analyses, therefore, we asked how females weight these various features in making phonotactic decisions in the recognition and discrimination tests. We asked if their weighting schemes suggest they have been influenced by the need to discriminate closely related species, by phylogenetic divergence, and by the intraspecific variation in signal features.

The *Physalaemus pustulosus* Species Group

All members of the *Physalaemus pustulosus* species group and the three species we used as outgroups produce whine-like advertisement calls. These calls usually begin at around 1000 Hz and sweep to a frequency several hundred Hz lower in 200 to 800 ms (Figure 8.2, Table 8.1). Some calls are preceded by an amplitude-modulated segment of a few tens of milliseconds. The calls usually have several harmonics but the fundamental is the dominant. *P. pustulosus* and *P. freiberghi* are known to add suffixes facultatively to the whine (Ryan and Rand 1993a, 1993b, 1993c, 1999a).

The calls of frogs of the *Physalaemus pustulosus* species group and close relatives were recorded in the field between 1990 and 1993 using standard methods described in Ryan and Rand (1993a, 1993b, 1993c). The species group as defined by Cannatella and Duellman (1984) consists of two monophyletic groups: *P. pustulosus* and *P. petersi* are in one group, and *P. coloradorum* and *P. pustulatus* in the other. *P. pustulosus* occurs from north of Veracruz, Mexico, throughout much of Middle America and in northeastern South America in Venezuela and Guyana. *P. petersi* is distributed east of the Andes throughout much of the Amazon Basin. *P. coloradorum* and *P. pustulatus* are both found west of the Andes in Ecuador (Cannatella and Duellman 1984). Our more recent study (Cannatella et al. 1998) reaffirms the monophyly of the species group and each of the two clades

within the species group, and also suggests that there are two additional species. One is a taxon thought to have been *P. petersi* in Amazonian southern Peru that we tentatively refer to as *P. freiberghi*. A taxon thought to be *P. pustulatus* in northeastern Peru also proves to be worthy of separate species status, and we tentatively refer to this species as *P. caicai* (Cannatella et al. 1998; Figure 8.3). Further analysis of these species is necessary. Our preliminary data suggest another species in western Ecuador, and the entire *P. petersi*-*P. freiberghi* clade requires a thorough study of geographic variation in molecular and advertisement call characters similar to the one we have conducted with *P. pustulosus* (Ryan et al. 1996).

We used three congeners for outgroup analysis: *P. enesfae*, which is sympatric with *P. pustulosus* in Venezuela; *P. ephippifer*, which was recorded in Belem, Brazil; and an unidentified species from northern Brazil, similar to *P. ephippifer*, which we tentatively refer to as a *Roraima* species.

General Methods

Call Analysis and Synthesis

We measured eight call variables for one call each of 10 individuals from each of the species in the species group and the three outgroup species (Figure 8.4, Table 8.1). Túngara frog females do not attend to upper harmonics of the whine, thus the fundamental frequency, which is also the dominant, is both necessary and sufficient for species recognition (Wilczynski et al. 1995). We measured the following spectral variables of the fundamental frequency: the initial frequency, final frequency, and a measure of frequency-sweep shape, which is the time from the initial frequency to mid-frequency (Figure 8.4; see Ryan and Rand 1999b for details). The temporal variables were call duration, rise time, fall time, rise shape (time from call onset to midamplitude), and fall shape (time from midamplitude to call offset; Figure 8.4). Call duration was highly correlated with the sum of rise and fall times and thus was not used in the analysis.

We used these call variables of the species group and outgroup species, combined with our hypothesis of their phylogenetic relationships, branch lengths derived from DNA sequence changes, and a phylogenetic algorithm, either squared-change or local squared-change parsimony, to estimate each of the call variables at each ancestral node (Ryan and Rand 1995, 1999b). We then used these seven call variables to synthesize calls at the nodes; we refer to these as ancestral calls. We used the same variables to synthesize mean calls for all of the real species used.

We varied different parameters of the phylogenetic anal-

Table 8.1 Call parameters used in synthesis of phonotaxis stimuli under different assumptions of evolutionary history, and female responses to those stimuli in phonotaxis experiments

Species	Frequency (Hz)		Time (ms)			Shape			Female Response	
	Max	Final	Duration	Rise	Fall	Whine ^a	Fall ^b	Rise ^c	HvC ^d	HvN ^e
Species										
<i>Physalaemus</i>										
<i>pustulosus</i>	884	484	369.7	24.0	342.8	0.33	0.49	0.33	0	4
<i>petersi</i>	1220	384	246.1	13.7	230.3	0.11	0.79	0.84	1	11
<i>coloradorum</i>	1180	628	209.3	53.4	161.7	0.39	0.71	0.44	0	2
<i>pustulatus</i>	964	676	206.0	99.5	104.3	0.43	0.49	0.95	3	10
<i>caicai</i>	888	444	394.5	105.1	293.7	0.29	0.68	0.66	1	7
<i>ephippifer</i>	944	576	266.4	83.5	177.4	0.53	0.66	0.47	1	16
<i>roraima</i>	876	460	339.1	94.6	251.6	0.47	0.72	0.60	0	0
<i>enesefae</i>	976	692	745.7	301.5	445.7	0.52	0.54	0.55		
Assumptions^f										
Node a ^g										
TLSG	910	518	302.0	89.0	213.0	0.51	0.69	0.54	1	3
Node b										
TLSG	949	622	568.0	216.0	353.0	0.51	0.58	0.55	1	1
LSG	937	589	483.0	176.0	308.0	0.51	0.60	0.54	0	0
Node c										
TLSG	974	466	333.0	32.0	300.0	0.29	0.57	0.53	14	15
LSG	1050	434	309.0	19.0	287.0	0.25	0.61	0.53	7	17
Node d										
TLSG	1120	439	274.0	44.0	230.0	0.21	0.75	0.73	4	13
LSG	1015	507	297.0	59.0	238.0	0.30	0.63	0.69	5	9
LSP	1008	479	314.0	55.0	259.0	0.28	0.64	0.67	3	11
LSG*	1015	507	297.0	59.0	238.0	0.30	0.63	0.69	6	13
LSP*	1008	479	314.0	55.0	259.0	0.28	0.64	0.67	8	19
SG	998	526	339.0	85.0	254.0	0.36	0.62	0.62	10	16
SP	995	512	372.0	92.0	280.0	0.36	0.62	0.60	11	19
Node e										
TLSG	961	572	320.0	94.0	230.0	0.32	0.67	0.69	3	13
LSG	988	564	287.0	90.0	200.0	0.34	0.65	0.72	0	10
LSP	962	527	320.0	94.0	229.0	0.32	0.67	0.69	0	14
LSG*	988	564	287.0	90.0	200.0	0.34	0.65	0.72	4	9
LSP*	962	527	320	94	229	0.32	0.67	0.69	6	16
SG	992	547	311	88	225	0.35	0.64	0.68	5	16
SP	974	521	339	93	249	0.34	0.65	0.67	3	15
Node f										
TLSG	1072	652	208	76	133	0.41	0.62	0.78	2	5
LSG	1062	654	208	78	130	0.41	0.61	0.79	1	4
Root										
TLSG	961	545	451	125	326	0.43	0.57	0.54	2	8
LSG	995	528	345	89	257	0.37	0.63	0.62	1	12
LSP	977	554	448	140	308	0.44	0.60	0.57	6	12
LSG*	990	510	298	66	233	0.34	0.64	0.65	0	12
LSP*	955	506	308	73	235	0.40	0.66	0.59	3	12
SG	995	528	345	89	257	0.37	0.63	0.62	3	19
SP	977	554	448	140	308	0.44	0.60	0.57	8	18

^aWhine shape is the proportion of call duration when the frequency sweep reaches midfrequency.

^bFall shape is the proportion of the call duration when the call reaches half the amplitude from the peak amplitude to the end of the call.

^cRise shape is the proportion of the call duration when the call reaches half the amplitude from the initial to the peak amplitude.

^dHvC = number of female responses to the heterospecific call when presented together with a conspecific call (max. n = 20).

^eHvN = number of female responses to the heterospecific call when presented together with white noise (max. n = 20).

^fAssumptions: T = pectinate tree, L = local, S = squared-change parsimony, G = gradual, P = punctuated evolution.

^gRefers to nodes in Figure 8.3.

* Without *P. enesefae*.

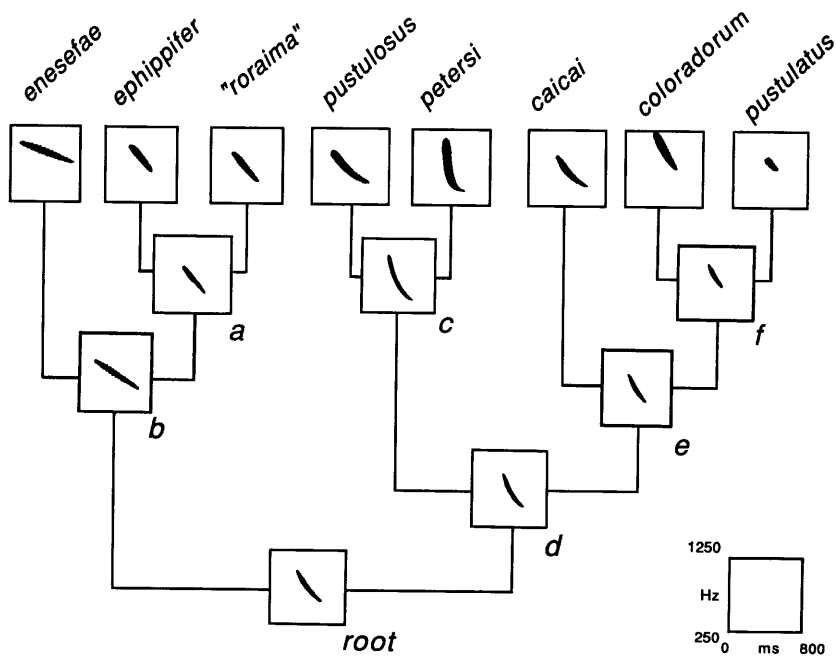


Figure 8.3. The phylogenetic tree illustrating the most parsimonious hypothesis for the relationships among members of the *Physalaemus pustulosus* species group and the three species we used as outgroups. "roraima" is an undescribed species. Sonograms illustrate the synthetic mating calls, which contain only the fundamental frequency of the whine (cf. Figure 8.2), for each taxon; the calls estimated for the ancestral nodes were derived from a local squared-change parsimony model assuming a gradual model of evolution (from Ryan and Rand 1995).

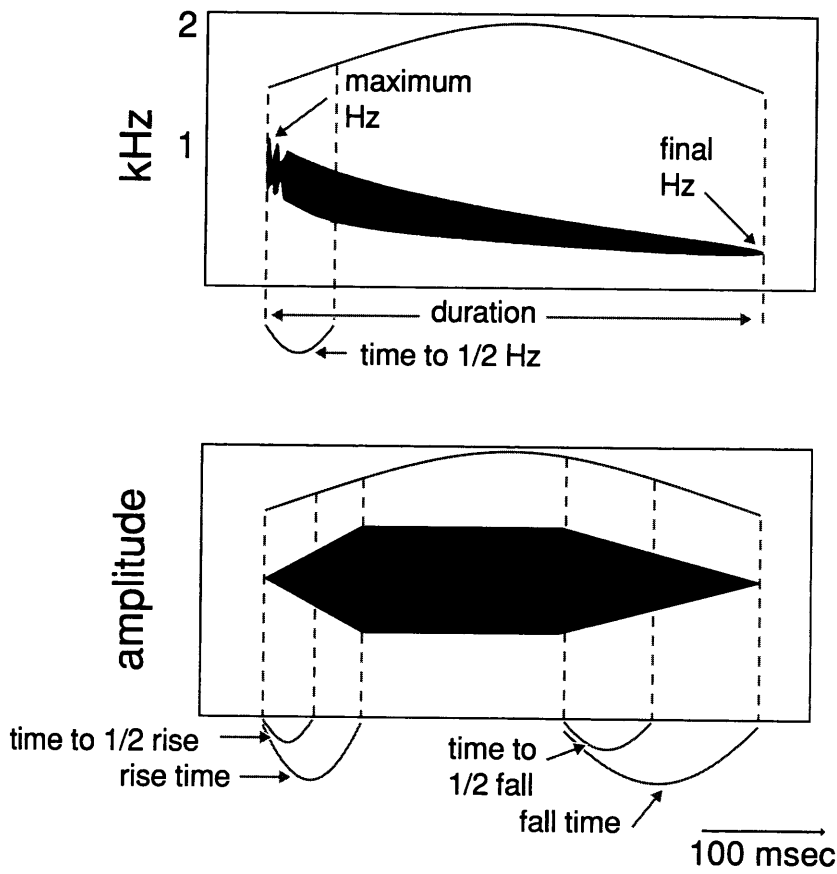


Figure 8.4. An illustration of the fundamental frequency of the túngara frog whine showing the various call parameters that were measured for analysis and synthesis; sonogram (top) and oscillogram (bottom). Measures of half frequency, half rise time, and half fall time were used to compute the shape of the frequency sweep, rise time, and fall time, respectively.

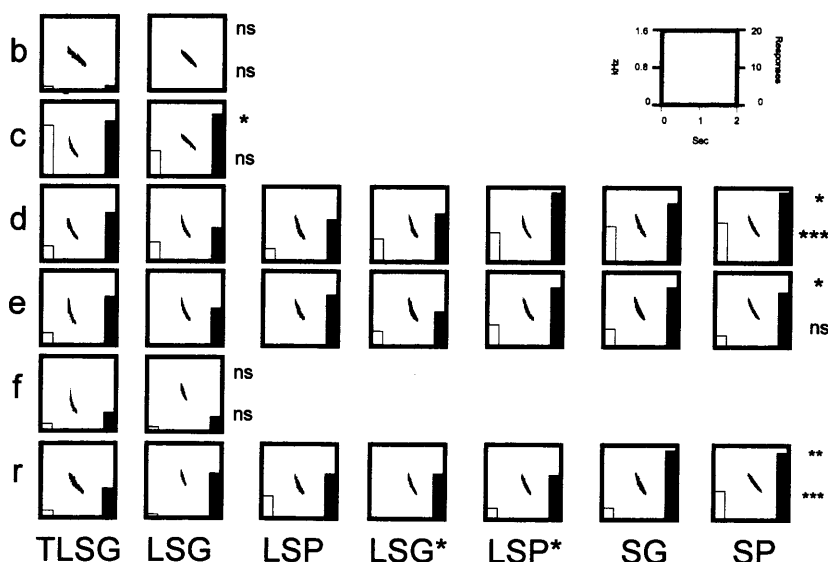


Figure 8.5. Sonograms of calls of the various nodes (rows; cf. Figure 8.3) that were estimated from different models of evolution (columns) that were considered different from one another and thus used in the phonotaxis experiments. (All estimates at node *a* were similar to one another given our criterion detailed in the text.) Open bars on the left of each box show the number of females responding to the call illustrated when it was presented with the túngara frog call. Closed bars on the right of each box show the number of females responding to that call when it was presented with a white noise stimulus. In each phonotaxis experiment, $n = 20$. Symbols at the end of each row indicate results of a *G* test, which tested the null hypothesis that the female responses to various calls at the same node did not differ for the discrimination tests (top symbol) and recognition tests (bottom symbol): ns = $p > 0.05$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Abbreviations are defined in Table 8.1 (see also Ryan and Rand 1999).

ysis to determine if they would yield different estimates of calls at the same ancestral nodes. The parameters were tree topology (the most parsimonious tree and the next most parsimonious tree); rate of evolution (gradual, branch lengths coded to DNA sequence divergence; punctuated, branch lengths equal); and outgroup species (*P. enesefae*, which had the most divergent call, included or excluded). We combined these phylogenetic parameters to make seven sets of estimates of ancestral calls; thus each node of the phylogeny had seven separate call estimates. We considered call estimates at the same node to differ if any of the seven call variables were more than 10% different. Most call estimates at a single node were slightly different (Figure 8.5).

Phonotaxis Experiments

We tested female túngara frogs from Gamboa, Panama, near the laboratory facilities of the Smithsonian Tropical Research Institute. The phonotaxis experiments have been explained in detail elsewhere (Ryan and Rand 1995, 1999b). We placed a female in the center of an acoustic chamber measuring 3 by 3 m. We broadcast the stimuli antiphonally from speakers in the center of walls opposite one another. A positive phonotactic response was noted if a female approached within 10 cm of one of the speakers. A “no re-

sponse” was noted if she remained motionless for 5 minutes at any time during the experiment, or if she did not approach a speaker within 15 minutes (see also Wilczynski et al. 1995; Ryan and Rand 1999a, 1999b).

In discrimination tests we presented a female with a túngara frog call synthesized from average values of the local Gamboa population versus one of the other synthetic heterospecific/ancestral calls. The null hypothesis of no preference was tested with an exact binomial probability. In recognition tests we presented a female with only one of the calls; the other speaker broadcast a white-noise control stimulus with the amplitude envelope of the túngara frog call. If a female did not respond in a recognition test, it could have been due to lack of attraction to the call being tested or a lack of motivation. To argue that a female’s failure to respond was not due to lack of motivation, these tests were preceded and followed by discrimination tests in which the conspecific call was always one of the alternatives. Only if females responded in these discrimination tests did we consider the no-response in the recognition test as valid. No-responses included both lack of response by otherwise motivated females and approaches to the white noise stimulus, although the latter was rare. We used a Fisher’s exact test to test the null hypothesis, which was the distribution of females approaching a silent speaker when it was paired

with a white-noise stimulus: 18-no approach, 2-approach. This null distribution empirically estimated the number of times a females encountered a speaker without reference to the stimulus it was broadcasting. The sample size in all tests was 20. Females were tested in more than one experiment. This does not violate any statistical assumptions of independence, which pertain to independence of data within and not among experiments.

Results

How Are Heterospecific Calls Classified?

The call variables for each species in the species group and outgroup, all the ancestral calls, which includes multiple estimates at the same node, and the results of the recognition and discrimination tests are shown in Table 8.1 (see also Ryan and Rand 1999b). We summarized these results relative to the scheme shown in Figure 8.1 to describe the pattern by which females classify calls of heterospecifics and ancestors. Our criterion for significant recognition and discrimination is a probability level of 0.05. We realize, however, that this probability level, although widely accepted as "proving" a significant difference, is arbitrary, and that there can be a great risk of a type II error by assuming that higher p values indicate that the null hypothesis should be accepted. Nevertheless we feel this is a useful approach for this classification scheme. A more detailed analysis of strength of response, without categorizing responses by p values, is considered below.

Only 26% of the 36 heterospecific/ancestral calls did not elicit significant recognition from females; the conspecific calls were always preferred to these calls in the discrimination experiments. This is what we had assumed would be the most likely pattern of recognition/discrimination (Figure 8.1A). However, females recognized the other 74% of heterospecific/ancestral call as indicating an appropriate mate (Figure 8.1B–D). Although many heterospecific/ancestral calls were recognized as appropriate, in 69% of the discrimination experiments the females preferred the conspecific call to the heterospecific/ancestral call (Figure 8.1A–B). In 31% of all experiments, however, females recognized the heterospecific/ancestral call and did not significantly discriminate between a conspecific and heterospecific/ancestral call (Figure 8.1C). Considering only the seven heterospecific calls and not those of purported ancestors, females always preferred the conspecific call to the heterospecific call; in three of seven cases the heterospecific call elicited significant recognition and in four of seven cases it did not (Table 8.1).

Feature Weighting and Call Preferences

The previous section details the responses of female túngara frogs to a variety of heterospecific and ancestral calls. Here we ask how females weighted various call features in deciding their phonotactic responses in those experiments. This analysis could suggest if the features weighted by females are those expected if female preferences evolved to discriminate closely related species, were subject to phylogenetic influences, or were guided by the amount of intraspecific variation in signal features.

Species Discrimination and Feature Weighting

The first question we addressed was if females weight more heavily those call features that best discriminate among species (conspecific, heterospecifics, and ancestors). But first a caveat. Túngara frogs are currently allopatric with all of the heterospecifics we tested, except for a small zone of sympatry with *P. enesefae*. The geographic relationships with any purported ancestors can not be determined. Thus túngara frogs have not previously been under selection to make the conspecific–heterospecific contrasts we test here. It would seem, therefore, that the conspecific preferences versus these heterospecific calls are incidental consequences of selection for self recognition (Passmore 1981; Paterson 1985) or for discriminating between conspecifics and other heterospecifics (Dobzhansky 1940; Coyne and Orr 1989). Nevertheless given the above studies showing to what degree females discriminated against and recognized heterospecific/ancestral calls, it is of interest to understand the call features that informed these phonotactic decisions.

We used a principal component analysis (PCA) to determine the importance of each call feature in statistically discriminating among species along the first three principal components (i.e., without reference to how females respond to signals). The standardized mean value of each heterospecific/ancestral call was assigned the scores for the first three components, which explained 44%, 21%, and 14% of the variation among calls, respectively (Table 8.2). Final frequency, initial frequency, and rise time loaded most heavily onto principal component 1; fall time, frequency-sweep shape, and fall shape onto principal component 2; and frequency-sweep shape onto principal component 3. Ancestral calls were assigned principal component scores based on the z scores of their call variables relative to those of the real species (Figure 8.6).

We determined to what degree the three PCA scores explained the female's responses in discrimination and recognition tests. The PCA scores were used as independent variables in a multiple regression analysis, whereas the number of female responses was the dependent variable. Principal

Table 8.2 Principal component loadings from the analysis of call variation among species of the *Physalaemus pustulosus* group and three closely related species used in the outgroup analysis

Call Variable	Principal Component		
	1	2	3
Maximum frequency	0.858	0.068	-0.401
Final frequency	0.935	-0.046	-0.183
Rise time	0.833	0.220	0.056
Fall time	0.171	0.875	0.226
Frequency-sweep shape	0.376	-0.529	0.689
Fall shape	-0.682	0.476	0.163
Rise shape	-0.716	-0.245	-0.393

component 1 did not enter into the multiple regression equation for either recognition or discrimination responses. Principal components 2 and 3 explained only 26% and 32% of the variation in discrimination and recognition, respectively. These results suggest that females are not weighting features in a manner that would best discriminate among the mean calls of species.

We conducted a similar analysis based on call variables of individuals. We used a discriminant function analysis (DFA) to ask how well individual call variables can discriminate between conspecific (túngara frog) calls and those of the other seven real species. We used the 10 calls from each species that were used to calculate the species means for the analyses above. Data were standardized to z scores before discriminant function analysis.

A jack-knifed classification matrix based on the discriminant function analysis using all call variables correctly classified all túngara frog calls as conspecific and all of the 70 other calls as heterospecific (we were not concerned with whether the heterospecifics were correctly classified to species). A forward, stepwise discriminant function analysis revealed that rise shape, rise time, and fall time, in that order, were the variables that best discriminated species. Rise shape by itself had a jack-knifed classification accuracy of 70%, adding rise time increased the accuracy to 80%, and adding fall time increased accuracy to 96%. Thus there is sufficient information in the seven call variables to discriminate perfectly between the conspecific call and the other calls of heterospecific/ancestors, and fairly accurate discrimination can result from using only a few of these variables. The question is whether females use these features in their phonotactic decisions.

We used a multiple regression analysis of the female choice results to determine what variables best describe female responses in phonotaxis tests. The seven standardized

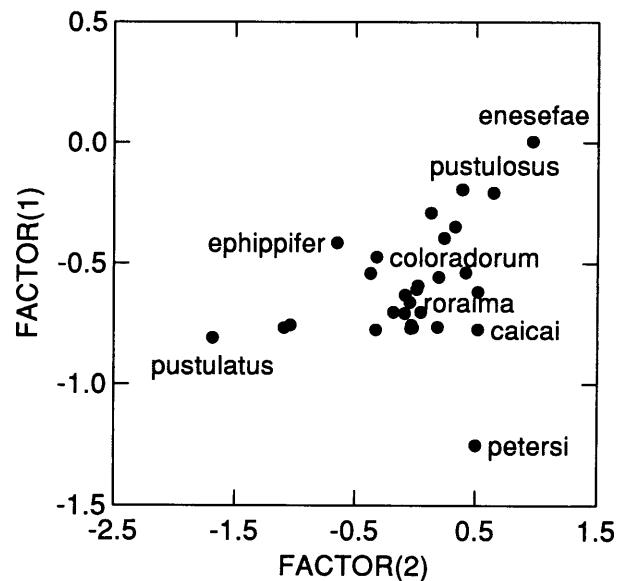


Figure 8.6. A plot of the first two axes of a principal components analysis of call variation among species of the *Physalaemus pustulosus* species group, the three closely related species used as outgroups, and the estimates of calls at ancestral nodes, including multiple estimates at the same node.

call variables were the independent variables and the number of female responses in either the recognition or discrimination tests was the dependent variable. We then asked how well those variables predicted conspecific identity.

Final frequency was the only variable that entered into the equation for recognition and explains 28% of the variation in these female responses ($r^2 = 0.28$). The shapes of the rise, fall, and frequency entered into the multiple regression equation for the discrimination responses, and together they explain 62% of the variation ($r^2 = 0.62$). How well do these call variables discriminate conspecific signals from the heterospecific/ancestral ones we used in this study? Only final frequency was identified as influencing female responses in the recognition experiments. A discriminant function analysis cannot be performed using only one variable. Thus we added a second variable to the analysis: fall time. This variable had the lowest partial correlation coefficient in the multiple regression analysis and thus should least influence the ability of final frequency to correctly classify conspecific versus heterospecific. These variables had a classification accuracy of 70%. The three variables that were weighted most heavily in the discrimination experiments, the shapes of rise, fall, and frequency, correctly classified 85% of the calls as conspecific or heterospecific.

These results show that in recognition tests females do not attend to variables that resulted in accurate discrimination between conspecific and heterospecific/ancestral calls

tested. In discrimination tests they weighted more heavily a suite of features that gave better species discrimination than in recognition tests (85% versus 70%), but were less accurate than the discrimination provided by the three best features for discriminating species (85% versus 96%).

Phylogenetic Divergence and Feature Weighting

Past history can influence how receivers respond to signal variation (Phelps, this volume and references therein). Thus we asked what call features are phylogenetically informative—those that best explain the amount of evolution between heterospecifics—and if these call features figure prominently in explaining female phonotactic responses. It is possible that the auditory system of these frogs are most sensitive to the phylogenetically informative signal features. Our measure of amount of evolution was derived from divergence of DNA sequences, which was the dependent variable in the stepwise multiple regression analyses (Ryan and Rand 1995, 1999b; Cannatella et al. 1998). We used only the heterospecific call variables and female responses and not the ancestors in the stepwise multiple regression analysis. Rise time, fall time, frequency-sweep shape, and fall shape entered into the equation, in that order, and predicted 98% of the variation in phylogenetic distance among species ($r^2 = 0.98$). Rise time and fall time, which were not weighted heavily in either the recognition or discrimination experiments, explained 66% of the variation ($r^2 = 0.66$) in a separate multiple regression analysis, as did frequency-sweep shape and fall shape ($r^2 = 0.66$), which are two of the three variables that were weighted heavily in the discrimination experiments.

The one variable that was weighted by the frogs in the recognition responses, final frequency, only explained 16% of the variation in phylogenetic distance ($r^2 = 0.16$), whereas the three variables that were heavily weighted in the discrimination experiments explained 80% of the variation in phylogenetic distance ($r^2 = 0.80$). These results suggest that females weight phylogenetically informative call features in discrimination experiments but not in recognition experiments.

Feature Invariance and Feature Weighting

A third factor that has been offered to explain feature weighting is the feature invariance hypothesis. This argument is that animals should weight most heavily those signal properties that vary little within the species regardless of how these signals compare with heterospecific signals (Nelson and Marler 1990; cf. Gerhardt 1991). In the sample of túngara frog calls used to estimate the mean, we find a pattern of variation among call variables that seems rather typical for frogs: spectral variables that are primarily deter-

mined by morphology have lower coefficients of variation than temporal properties that are usually under behavioral-physiological control (Ryan 1988; Cocroft and Ryan 1995). The coefficients of variation for the call variables analyzed are: initial frequency, 4.5%; final frequency, 11.3%; rise time, 67.1%; fall time, 20.1%; frequency-sweep shape, 21.3%; rise shape, 51.2%; fall shape, 46.2%. The variable that is weighted most heavily in the recognition responses has the second lowest coefficient of variation, whereas the three shape variables, which are weighted most heavily in discrimination responses, have three of the four highest coefficients of variation. Thus feature invariance predicts responses in recognition but not in discrimination experiments.

How Are Recognition and Discrimination Related?

It is usually assumed that the two types of experiments we use to assess phonotactic responses, recognition and discrimination, measure a similar phenomenon, but merely in different contexts. For example female choice tests usually use one but not both measures as assessments of female preferences. On the one extreme, recognition and discrimination might be two measures of the same phenomenon, as seems to be implicitly assumed, with the results of one perfectly predicting the results of the other. Alternatively these might be unrelated phenomena having only the bioassay in common but not being governed by any of the same rules or perceptual processes to evaluate the signals. We determined to what degree recognition and discrimination responses differ in feature weighting.

The responses of females to the same stimuli in the recognition and discrimination experiments were significantly correlated with one another ($r = 0.67$, $p < 0.001$). The amount of variation in female responses explained by this correlation is 45%, leaving 55% of the variation unexplained. The unexplained variance might be due to stochastic variation in two similar phenomena or to the fact that discrimination and recognition are two different phenomena. It is possible that there is a stronger relationship between the responses in the discrimination and recognition experiments, but that the relationship is not linear; transforming the data by logarithm, however, reduced the correlation slightly ($r = 0.64$).

Female responses in recognition and discrimination experiments were not influenced in the same way by overall similarity of the test call to the túngara frog call and phylogenetic distance of the test species/ancestor to túngara frogs. Differences among calls are not a good predictor of evolutionary relationships (Cannatella et al. 1998); the correlation between call similarity and DNA sequence divergence

explains only 28% of the variation in these data sets. An earlier study with a smaller data set (Ryan and Rand 1995) had shown that call similarity and phylogenetic distance might differently influence female responses in recognition and discrimination experiments—discrimination was more influenced by the phylogenetic distance between the call being compared with the túngara frog call, whereas recognition was more influenced by the similarity of the test call to the túngara frog call. In the expanded data set analyzed here, phylogenetic distance and similarity still differently influenced responses but in the opposite way. A forward stepwise multiple regression analysis showed that similarity alone explained 51% of the variation in discrimination responses, adding phylogeny explained a total of 67% of the variation. Alternatively phylogeny explained 40% of the variation in recognition responses, whereas adding similarity to the equation explained a total of 50% of the variation.

There were also differences in how responses in recognition and discrimination experiments scaled to independent variables, and these differences might be related to perceptual factors. For example response variation might be linear in some tasks but nonlinear (e.g., logarithmic) in others. It is also possible that females compare the test call with an internal representation of the conspecific call in recognition experiments, in which comparison between two simultaneous stimuli is not an option. Therefore we transformed both the female response data and calls as a means of uncovering such perceptual processes. The responses, the dependent variables, were log-transformed, and the standardized call variables, the independent variables, were coded by their absolute difference to the túngara frog call variables ($|\text{túngara call variable} - \text{test call variable}|$). If these transformations influenced the explanation of responses in discrimination and recognition experiments differently, it might indicate a difference in underlying perceptual processes.

These data manipulations only decreased the variance explained in discrimination responses. The unmanipulated data in the multiple regression explained 67% of the variation, as reported above. When the responses were log-transformed the variation explained fell to 35%, and was 45% when the call variables were also coded relative to the túngara frog call variables. Alternatively these data manipulations had a profound effect on the variance explained by the multiple regression in the analysis of the recognition responses. The unmanipulated data, reported above, explained 28% of the variation, and only final frequency contributed significantly to explaining variation in female responses in a stepwise multiple regression. Log-transforming the response data increased the variation explained to 63%, with frequency-sweep shape now contributing to the call variables significantly explaining

variation in preferences. Coding the call variables to the túngara frog call, in addition, further increased the variation explained to 77%, in which final frequency, frequency-sweep shape, and total duration became important predictors. Although these data transformations do not allow any insights into how the frogs are processing information in the discrimination and recognition experiments, it is yet another suggestion that the responses elicited in recognition and discrimination experiments might have been motivated by different weightings of factors.

Discussion

There are three general findings from the results of these phonotaxis experiments with túngara frogs. First, females often recognize other signals besides the conspecific signal as appropriate. Second, females do not weight signal features in a manner optimal for classifying conspecifics versus heterospecifics; in discrimination tests they weight heavily phylogenetically informative features and in recognition tests features with less intraspecific variation. Third, the two types of experiments we used to assess female phonotaxis preference, recognition and discrimination, are not highly correlated with one another and appear to involve different sets of feature weighting.

Classification of Conspecific versus Heterospecific

One of the general conclusions of animal communication is that females usually show a strong attraction for conspecific mate recognition signals relative to heterospecific signals, although exceptions to this trend are known (e.g., in katydids, Morris and Fullard 1983; swordtails, Ryan and Wagner 1987). Our results, however, show that a majority of non-conspecific (heterospecific and ancestor) calls are recognized as signaling an appropriate mate, and a substantial proportion of these calls are not significantly discriminated from the conspecific; this is unusual if not unprecedented. Considering only the seven heterospecific calls, females always preferred the conspecific call to those in discrimination tests, and in three of seven cases the heterospecific signals elicited significant phonotaxis. There are several possible explanations for these results.

It is possible that túngara frogs are unusual among species so far tested in possessing a quite permissive mate-recognition system. We doubt this is the case; nothing about the biology of these animals suggests that they are special, and other species of anurans, for example, will approach calls that are not conspecific even if they are sympatric (Oldham and Gerhardt 1975; Backwell and Jennions 1993; Gerhardt and Schwartz 1996).

We are sure that the experimental methodology we use, rather than the taxon we study, is responsible for revealing substantial evidence for attraction to heterospecific/ancestral signals. Most studies of mate recognition present pairs of stimuli simultaneously and the results are interpreted as females exhibiting a preference for one stimulus relative to the other or no preference (reviewed in Andersson 1994; Hauser 1996; Bradbury and Vehrencamp 1998). Although these studies reveal which stimulus is preferred, they tell us little about the unpreferred stimulus. Even in studies in which stimuli are presented separately, the strength of responses to two or several stimuli are usually compared to determine if one signal is preferred over another (Searcy and Marler 1981; Wagner 1998). Most studies have not compared the response in single-stimulus experiments with a null hypothesis of no response.

These methodological issues, however, cannot explain the lack of statistically significant discrimination between conspecific and some of the ancestral calls. This discrepancy with the bulk of previous studies on mate recognition is probably due to the types of signals we are testing in the phonotaxis experiments. Female túngara frogs always preferred a conspecific to a heterospecific call, but failed to discriminate in favor of the conspecific in 39% of the comparisons with 28 ancestral calls. The ancestral calls that were not significantly discriminated against were estimates of calls at the ancestral nodes closest to the túngara frogs: nodes c, d, e, and the root (Figures 8.3 and 8.5). Females discriminated against the call estimates at nodes a, b, and f. Thus it was the calls more similar to the túngara frog call that were not significantly discriminated against.

The ancestral calls were all significantly different from the túngara frog call. All of the values for their call variables were outside of the 95% confidence intervals of the analogous call variables for the túngara frog calls that were used to estimate the mean. It would be important, however, to know how the ancestral calls compare with a much larger sample of calls from the population of the túngara frog females tested. We tentatively conclude that these ancestral calls that were not significantly discriminated against are different from the conspecific call but, as seen in Figure 8.5, tend to be more similar to the túngara frog call than calls of the heterospecifics. We are probably testing signals more similar to the conspecific signal than other studies of species recognition.

Our study also differs from others in that our choice of test stimuli is guided by evolution rather than ecology. As noted above, we have not tested túngara frogs in response to calls of sympatric species. These calls are quite different from the túngara frog call, and we would expect near-perfect conspecific discrimination and little heterospecific recognition,

but this needs to be tested. Because calls of more closely related species will tend to be more similar than calls of more distantly related species, especially over large phylogenetic distances, our phylogenetic approach might be more likely to test responses to stimuli that are more similar to conspecific signals. Furthermore ancestral signals might not only be more similar to conspecific signals, but are more likely to have been the types of sounds that past receivers have had to decode. Thus our choice of using a phylogenetic approach to these studies (intentionally) biases us toward choosing signals that play to these response biases (Phelps and Ryan 1998, 2000; Phelps, this volume).

A more general conclusion from all of these experiments is that the female's perceptual map of what it considers an acceptable stimulus is not restricted to the conspecific signal. In fact some signals that are outside the conspecific range are not even discriminated from the conspecific call. This is one more piece of evidence against the notion that properties of the signal and receiver need be tightly matched. These results also suggest that interactions with other species are not necessary for effective conspecific versus heterospecific recognition. Female túngara frogs discriminated against all of the heterospecifics in favor of the conspecific call, and only some but not all of the heterospecifics elicited recognition from females. This is consistent with Paterson's (1985) notion that self recognition might suffice for species recognition, but might not be considered strong support for it, as we now address.

We suggest that the túngara frog females' positive responses to ancestral calls results from a combined process of generalization and relaxed selection. Females use signal processing strategies that are under strong selection to guide them to conspecific and only conspecific signals. The precision of the strategies required for such a task is context dependent. The strategy will, of course, depend on the properties of the conspecific signals but need not be restricted by the bounds on these properties. If the signal of interest has the lowest frequency to be encountered by a species, then an open-ended preference for low frequency will be as effective in identifying conspecifics as a preference for the precise frequency of the signal. But the degree of generalization, or over-generalization, that will be transparent to selection depends on the types of errors that could be made. Generalization might be favored by selection because it reduces costly neural computations (e.g., Bernays 1998), as long as it does not lead to errors in identification that reduce fitness, as would be true for responses to allopatric or ancestral species. Selection will add precision when necessary. Until necessary, however, generalization can be an important response bias that could influence future signal evolution.

Feature Weighting

One of the early findings of ethology was that animals are not equally sensitive to all stimuli in their environments. Instead they filter out much of the potential stimulation they encounter and are more influenced by some stimuli than others (Tinbergen 1953). Signals are usually a composite of multivariate traits, and we know that all features of the signals are not attended to equally. Nelson and Marler (1990) review discussions of the forces that might shape feature weighting and contrast two hypotheses, the sound environment hypothesis and the feature invariance hypothesis. The sound environment hypothesis states that features that best discriminate between sympatric and synchronic conspecifics and heterospecifics are those features that should be weighted most heavily. The feature invariance hypothesis, alternatively, states that the features that vary least within the species should be weighted most heavily. In Nelson and Marler's study these two hypotheses predict primacy for some of the same features, but the results tend to favor the sound environment hypothesis.

Our analysis parallels Nelson and Marler's except that we are contrasting species along a phylogenetic rather than an ecological axis; because most of the species we are testing are not sympatric with the túngara frog, we are not confounding these axes. Our hypothesis can be thought of as a phylogenetic influence hypothesis. The rationale for a study of feature weighting along a phylogenetic axis is not as immediately clear as for an investigation along an ecological axis. Our assumption for testing feature weighting in a phylogenetic context is that the evolutionary divergence of signal preferences within a group might match those features of the signal that most diverge. Thus females should weight those features that have diverged the most and thus best predict the dichotomous classification of conspecific/heterospecific when females are making the same choice. The same emphasis on phylogenetic influences on communication evolution makes another prediction: the call variables used by females should be those that are the best predictors of phylogenetic distance.

Our results show that females do not weight most heavily those features of the signal that would be ideal for classifying species as conspecific versus heterospecific. The principal component factor that best explains call variation among extant species does not explain a significant amount of variation in either recognition or discrimination experiments. The set of correlated variables that best separates the species in multivariate call space appears to be ignored by the females.

Our analysis of feature weighting of individual call variables leads us to the same conclusions. Using all of the call

variables results in 100% accurate classification, whereas using three variables can result in 96% accuracy. Túngara frog females only weight heavily three call variables in discrimination. However the three call variables they use are not the three that would result in optimal discrimination for that number of variables. Nevertheless, with the three variables the females use they would achieve 85% accuracy. The only variable heavily weighted in the recognition experiments gives only 28% accuracy; if the females were constrained for some reason to relying on only one variable, the most effective variable by itself would result in 70% accuracy.

Another view of phylogenetic influence on feature weighting, however, is less utilitarian. It does not assume that females have been selected to make the conspecific/heterospecific-ancestor discriminations we tested. Instead this view suggests that the features weighted most heavily by these kinds of frogs will be biased toward the acoustic features that are most likely to diverge. Four call variables explain almost all of the variation in phylogenetic distance, or evolutionary divergence, among the eight heterospecifics ($r^2 = 0.98$). Two of these call variables are not weighted heavily in female responses but two are. Each of the pairs of variables by themselves explains two-thirds of the variation in evolutionary divergence ($r^2 = 0.66$). Furthermore the three variables weighted most heavily in discrimination explained 85% of the variation in evolutionary divergence ($r^2 = 0.85$). The single call feature heavily weighted in recognition is not a good predictor of evolutionary divergence ($r^2 = 0.16$).

Our results suggest that there is a substantial phylogenetic influence on feature weighting in túngara frogs. This influence is not revealed in strategies that females use to discriminate against heterospecific/ancestral calls with which they have no current experience, in contrast to Nelson and Marler's (1990) result suggesting a significant influence of ecological signaling interactions on feature weighting strategies in some song birds. Instead it appears that there might be a very basic correlation between the properties of the signal and the receiver in that the receiver is more sensitive to signal features that are more likely to evolve. This is in contrast to the feature invariance hypothesis that suggests that feature weighting strategies are self referential and are biased to features that are less variable within the conspecific. We found no evidence for such an effect in this study when females were confronted with a discrimination task, but feature invariance appears to be relevant to recognition.

Recognition versus Discrimination

Studies of both species recognition and sexual selection by female choice have used laboratory experiments to ascertain female preferences based on male trait variation; this is

especially true for studies in the acoustic domain (reviewed in Ryan and Keddy-Hector 1992; Andersson 1994). In many studies of anurans as well as some in insects and birds, females are given a choice between a pair of sounds; a choice of one stimulus to exclusion of the other is interpreted as females having a preference within the confines of the laboratory setting and the female's current physiological condition (Wagner 1998). The strength of the discrimination between the two stimuli can be used as a measure of the strength of the preference. These experiments do not, however, tell us anything about the saliency of the unpreferred signal—is it merely not as attractive as the alternative; is it recognized as a signal that should be avoided; or is it not recognized as a signal and relegated by the auditory system to the general category of “noise”?

Other studies of mate preference use what we refer to as recognition experiments, and the strength of the response to single stimuli are compared among stimuli to ascertain female preference. In studies of *Drosophila*, flies are put together in pairs and mating speed is measured (Coyne and Orr 1989; Boake et al. 1997); in crickets the female's duration and acceleration in response to a single sound is measured (Wagner 1998), and in birds the number of courtship solicitation displays to a single acoustic stimulus is measured (Searcy and Yasakawa 1996; Stoddard 1996). If a stimulus elicits a strong response from a female we assume that the signal is recognized as being appropriate. Weaker responses, however, are usually not compared against a null hypothesis to test for recognition. The goal of these experiments is usually to compare responses across experiments to assess variation in the strength of preference relative to the stimuli being compared.

Both of these experimental approaches reveal important information about how females respond to stimulus variation, and the tacit assumption has been that these are different measures of the same phenomenon, female signal preference. Each might be more appropriate for different questions. Wagner (1998), for example, argued that a single-stimulus experiment with a continuous measure of the female's response is a better metric for measuring the strength of preference than a dichotomous choice among two stimuli. This comparison, however, suggests that these are measures of the same phenomenon. As far as we know, no one has investigated the possibility that recognition and discrimination are different phenomena, a caution also raised by Wagner (1998). If they are not, then serious consideration must be given to the appropriateness of the type of experiment for the hypothesis being tested, the validity of comparing results from single-stimulus and two-stimulus experiments, and, more importantly, what might be the differences between these phenomena at the perceptual level.

Our comparisons of recognition and discrimination experiments in túngara frogs suggest that these two responses might operate differently in some ways. All 36 of the heterospecific/ancestral calls we used were tested for both recognition versus white noise and discrimination versus the túngara frog call. The responses to the same stimuli were significantly and positively correlated: the more responses to the heterospecific/ancestral call versus noise, the more responses to the same call when it was paired with the túngara frog call. The variability in one response predicted by the other is 45%; the unexplained variance could be stochastic or could indicate different sets of feature weightings in the processes of recognition and discrimination. Also there is no overlap in the call variables that explains the variation in female response in the two types of experiments in a stepwise multiple regression. In the discrimination experiments the shapes of the frequency sweep and the rise and fall times explain about two-thirds of the variation in responses. In contrast in the recognition experiments final frequency is the only call variable that contributes significantly to explaining the variation in female responses, and at that it explains only about a quarter of the variation.

It is especially interesting how transformation of the data influences the degree to which call variables predict recognition versus discrimination responses. Our data transformations only decreased the variance explained by the discrimination experiments. The variation explained in the recognition experiments increased from one-quarter to three-quarters when the responses were transformed by logarithm and the call variables were coded by their relative differences to the túngara frog call. Concomitant with the increased variance explained is the recruitment of additional call variables in explaining the variance. But of the three that significantly explain recognition responses using the transformed and coded data, only one of those, the shape of the frequency sweep, is shared with the three call variables that best predict the discrimination responses.

The above results suggest that females might be attending to different cues in recognition and discrimination experiments, and their responses might be scaled to stimulus variation differently. Log-transforming only the responses in the recognition experiments substantially increases the predictability of the regression equation. This might be expected if recognition tended to be a stepwise function, and we might expect this to be more likely of recognition than discrimination. Coding the variables relative to the túngara frog call increases the predictability of the regression equation, and this might indicate the nature of an internal template to which females are comparing the call in the recognition experiments. The lack of such an effect in the discrimination experiments might be expected if the

túngara frog call is being presented to the female as one of the alternatives.

Some caveats need to be considered. It is possible that the lack of correspondence between recognition and discrimination results might derive from sampling error. Also many of our tentative conclusions are based on results of multiple regression analyses. Interpretation of such results should be made with caution. Ideally these results should be used to design experiments that test the importance of different variables in discrimination and recognition tests. If the phonotactic decisions being made in recognition and discrimination experiments are governed by different sets of feature weightings, however, we feel that we have revealed important context-dependent responses that have repercussions for methodological approaches to studies of acoustic preferences, and suggest a more complicated interaction of perceptual processes than previously appreciated.

Conclusions

The main conclusion of this study is that signals and receivers are not deconstructed and reconstructed with each speciation event. Instead they are jury-rigged innovations of ancestral conditions. Thus the kinds of recognition tasks that challenged the brains of ancestors will influence the manner in which brains of extant species solve analogous tasks. This is why responses of female túngara frogs to a variety of heterospecific and ancestral calls can be predicted by phylogenetic distance independent of any similarity of the test call to the conspecific call. Thus understanding the mechanisms underlying mate preferences requires a historical as well as a functional analysis.

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