



Sex differences in response to nonconspecific advertisement calls: receiver permissiveness in male and female túngara frogs

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In many species, males advertise to both male and female audiences. Given the asymmetry in fitness costs of recognition errors in response to mating signals for the sexes, usually higher for females than males, males are expected to be more permissive than females in their responses to signals. Few studies, however, have investigated such differences and there is no consensus on which sex is more permissive to signal variation. We examined the evoked vocal responses of male túngara frogs, *Physalaemus pustulosus*, to 14 species of heterospecific and ancestral male mating calls to evaluate the influence of call similarity and phylogenetic distance on their responses. We also compared male calling responses to female phonotactic responses to examine the propensity of response errors between the sexes. Recognition errors were higher for males than females, as predicted by the different costs associated with recognition errors for each sex. Males responded to the calls of most species with mating calls, and produced aggressive calls in response to two other heterospecific/ancestral calls. The responses of males were explained by phylogenetic distance but not by overall call similarity. Similarly, females were more likely to show phonotaxis to calls of species and ancestors that were more closely related. Therefore, evolutionary history has left a perceptual footprint on the brain of both sexes but the details seem to differ. We discuss proximate reasons underlying sexual differences in receiver permissiveness in túngara frogs and potential factors leading to their evolution.

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In animal communication systems, the same signal often targets multiple intended receivers. This double function is particularly common with mating signals, where in many species, males advertise to both male and female audiences (McGregor & Dabelsteen 1996; Searcy & Nowicki 2000; Gerhardt & Huber 2002). For example, male crickets, frogs and birds often use the same acoustic signal to attract females and to repel males. Males and females are not, however, expected to have the same threshold for responding to the same signal, especially when these signals are associated with reproduction (Trivers 1972). The sexes are likely to differ in their response criteria according to their consequences of erroneous responses. A female

basing a mate choice on the call of the wrong species, for example, could forfeit her reproductive investment for a substantial part of the breeding season, while a male calling in response to a heterospecific would bear less dire consequences, perhaps merely wasted time and effort.

In spite of the potential differences between the sexes in their propensity for recognition errors, few studies have investigated such differences (Searcy et al. 1981a, b; Searcy & Brenowitz 1988; Cynx & Nottebohm 1992; Dabelsteen & Pedersen 1993; Vicario et al. 2001; Nelson & Soha 2004). These studies, which have been limited to songbirds, show that females and males can respond to signals in different ways. Whether the female or the male is the more discriminatory sex, however, is not consistent in the few species tested. In red-winged blackbirds, *Agelaius phoeniceus*, imitations of conspecific calls by a mockingbird, *Mimus polyglottos*, did not elicit courtship solicitation displays in females (Searcy & Brenowitz 1988), while the vocal response of males was similar to both the imitation

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and normal song (Brenowitz 1982). Analogous results, in which females are more selective than males, were also found in response to conspecific red-winged blackbird songs modified by removing the final trill (Beletsky et al. 1980; Searcy & Brenowitz 1988), and unnatural temporal patterns of the conspecific song in swamp sparrows, *Melospiza georgiana* (Searcy et al. 1981a, b). In contrast, female blackbirds, *Turdus merula*, were less critical than males when responding to variation in song features such as the degree of frequency modulation of the song (Dabelsteen & Pedersen 1993). The same is true in white-crowned sparrows, *Zonotrichia albicollis*, and zebra finches, *Taeniopygia guttata*. Female white-crowned sparrows were less selective than males when responding to songs containing a phrase from a foreign dialect (Nelson & Soha 2004), and female zebra finches showed weaker discrimination than males in their responses to calls that advertised the sex of the signaller (Vicario et al. 2001).

In this study we investigated the responses of male túngara frogs, *Physalaemus pustulosus*, to nonconspecific signals and compared them to the results of studies on the responses of conspecific females to the same stimuli (Ryan & Rand 1995, 1999). We examined the responses of males to signals that varied in call similarity and phylogenetic distance, addressing the following issues: (1) male permissiveness in response to heterospecific signals, (2) the role of phylogenetic distance and call similarity in explaining these responses and (3) sexual differences in responses to nonconspecific calls.

Physalaemus pustulosus and Its Species Group

The túngara frog, *P. pustulosus*, is a small leptodactylid frog that is allopatric with other species in the genus *Physalaemus* throughout most of its range, with the exception of the llanos of Venezuela, where it is sympatric with *P. enesefae* (La Marca 1992). As in most species of frogs, male túngara frogs produce advertisement calls to attract females and at the same time deter rivals. Because the túngara frogs we study in Panama are allopatric with close relatives, evaluating recognition of advertisement calls of other members of the genus by female and male túngara frogs allows us to investigate the extent of signal generalization and permissiveness, excluding confounding factors associated with current selective pressures like character displacement (Ryan et al. 2003).

Túngara frogs mate in complex acoustic environments, and their recognition of conspecific calls has probably been shaped by the costs associated with failing to respond to relevant signals and with responding to erroneous ones. For túngara frogs, as for most receivers, the main challenge is to differentiate signals from background noise, which can consist both of signals from individuals of different species and environmental disturbances. Thus, the criterion for responding to a stimulus in túngara frogs is balanced by the benefits of correct responses (responding when a signal has occurred) and correct rejections (not responding to background stimulation) and the costs of false alarms (responding to background stimulation) and missed detections

(failing to respond when a signal occurred; Wiley 1994). As mentioned before, this trade-off is often different for each sex. Female túngara frogs responding to nonconspecific signals lose time and energy pursuing incorrect mates, and could forfeit their egg clutch if they do not find a conspecific male on time. This prediction is supported by evidence that females found at breeding sites often drop their eggs if left overnight without a male. On the other hand, males responding to incorrect signals would devote additional time and energy to calling, but would simultaneously increase their chances of attracting a mate. The trade-off further diverges between the sexes given the strongly male-biased operational sex ratio in this species (Ryan 1985). The costs of missed detections are lower for females than males, because females have multiple opportunities to mate, but mating opportunities for males are rare.

The *Physalaemus pustulosus* species group consists of two monophyletic groups (Cannatella & Duellman 1984; Cannatella et al. 1998). One clade is found east of the Andes in northern South America and includes *P. freibergi*, *P. petersi* and *P. pustulosus*. A second clade consists of species found west of the Andes in Ecuador and Peru, such as species B, *P. coloradorum* and *P. pustulatus* (Fig. 1). In this study, we used the advertisement calls of the species described in the *P. pustulosus* group by Cannatella et al. (1998) and included species B and three additional outgroups: *P. enesefae*, *P. ephippifer* and sp. A. Species A is an undescribed species from the state of Roraima in northern Brazil (Ryan & Rand 1995).

The advertisement calls of all species we used are frequency-modulated whines (Fig. 1). In these species, the fundamental frequency of the call is also the dominant frequency, and sweeps from about 1000 to 500 Hz. In some species, males facultatively produce complex calls, adding suffixes to the whines when they interact acoustically with other males. In *P. pustulosus*, males can add one to six short, multiharmonic components that are known as chucks (Ryan 1985). Chucks without whines, however, do not occur in nature. Complex calls are also known in some populations of *P. petersi*, the sister species of *P. pustulosus* (Boul & Ryan 2004). For the purposes of this study, however, we restricted the calls used as stimuli to only whines without including secondary components on the calls of any species.

METHODS

We collected male *P. pustulosus* between May and August 2003 at breeding sites near the research facilities of the Smithsonian Tropical Research Institute in Gamboa, Panama (9°07.0'N, 79°41.9'W). Calling males were found at choruses and brought to the laboratory to be tested. After testing them, we measured the snout–vent length of the frogs, and gave them a unique toe-clip number to prevent them from being retested and to contribute to the long-term data set on population demographics at this site. The frogs were marked following the Guidelines for the Use of Live Amphibians and Reptiles in Field Research compiled by the American Society of Ichthyologists and Herpetologists (ASIH), the Herpetologists' League (HL),

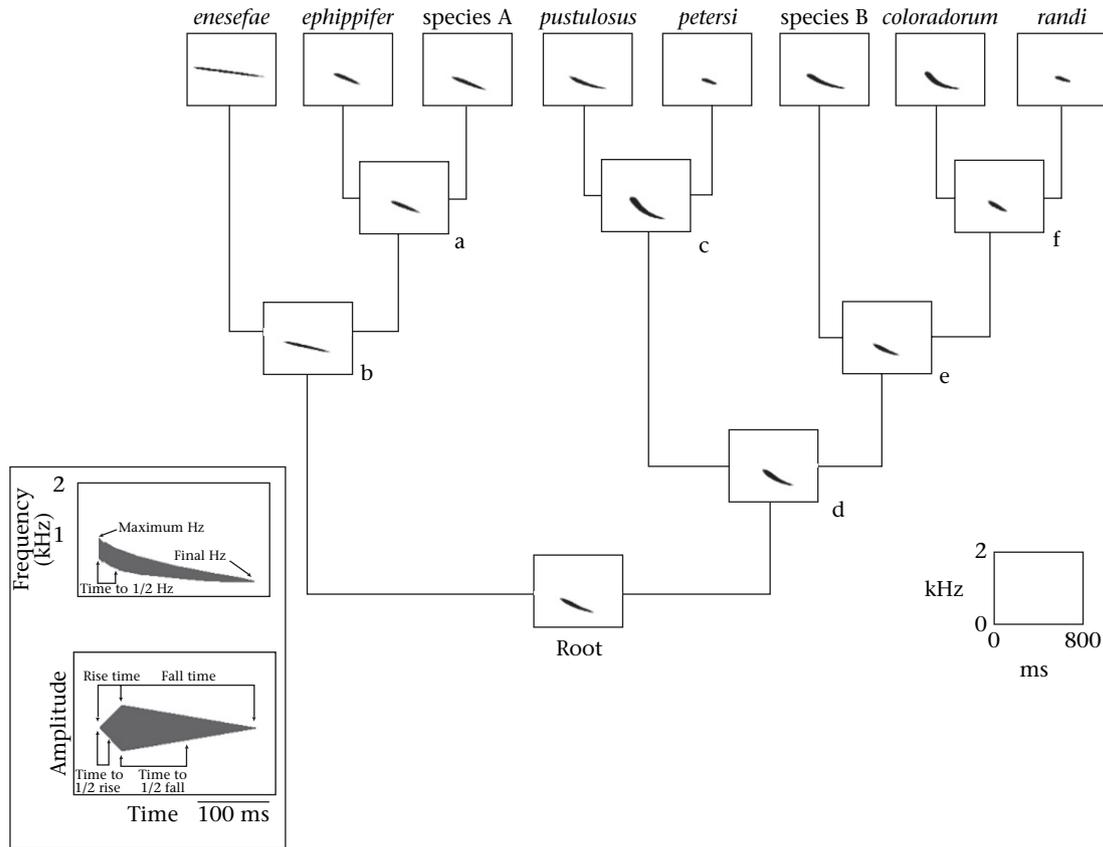


Figure 1. Phylogenetic relations of frogs in the *Physalaemus pustulosus* species group and three closely related species used as outgroups, *P. enesefae*, *P. ephippifer* and sp. A, illustrating their advertisement calls. Ryan & Rand (1999) refer to species B as *P. caicai*. In this study, we follow the designation by Cannatella et al. (1998) and Ron et al. (2006). Recent studies have confirmed that there are several new species in western Ecuador and have also designated the population studied by Ryan & Rand (1995, 1999), then known as *P. pustulatus*, as a new species, *P. randi* (Ron et al. 2004). Current studies, however, still support the monophyly of the species group and the presence of two clades (east and west of the Andes). Shown are sonograms of the synthetic advertisement calls of the extant species and estimations for the ancestral calls. The insert illustrates the call parameters measured to calculate species averages and synthesize test calls. Ancestral species are labelled in lowercase.

and the Society for the Study of Amphibians and Reptiles (SSAR), available at <http://www.asih.org/pubs/herpcoll.html>. Túngara frogs have been toe-clipped in this population for two decades without any detectable negative effect. We then returned the frogs to the site where they were captured. The frogs were frequently recaptured on following nights but were not used in the experiments again.

Males responding to the experimental stimuli called antiphonally with the heterospecific/ancestral calls as they do when interacting acoustically with other males in nature (Greenfield & Rand 2000). We use the evoked vocal responses as an indicator of call recognition, a common approach in anurans (e.g. Ryan & Rand 1998; Bee 2003). Here we evaluated the calling response to nonconspecific calls in male túngara frogs and assumed that if a signal elicited a response from a male, he mistakenly identified the stimulus as a conspecific call. Therefore, responses to nonconspecific calls represent recognition errors, specifically, false alarms.

Male-evoked Vocal Responses

We quantified vocal responses of males to 14 calls of species of the same genus and estimates of ancestral calls.

In the laboratory, we placed each male in an acoustically transparent plastic bag (Ryan & Rand 1998). Each bag had sufficient standing water for the males to call, and we placed the bags inside individual, acoustically isolated chambers (30.5 × 46 × 30.5 cm) following Bosch et al. (2000a, 2002). The chambers were lined with sound-absorbent material that attenuated sounds within the range of frequencies of the calls used in the experiment (500–1000 Hz). Each acoustic chamber contained a Radio Shack miniature microphone and a small, wide-frequency range speaker (Cambridge SoundWorks, Andover, Massachusetts, U.S.A., Ensemble IV). We placed males in the chambers the night that they were collected and we tested them the next night, because previous experience suggested that males were more likely to respond if tested on the second night. No male spent more than two consecutive nights in the laboratory. The chambers were in a well-ventilated room at typical calling temperatures, ca. 23–27°C. We maintained the males under a natural light–dark cycle and temperature regime. All males were tested between 1900 and 0200 hours.

We stimulated the males to call with a continuous tape of a high-density túngara chorus recorded by A.S.R. in Gamboa on 2 October 1990. Once a male began to call,

we initiated the playback experiments. Males were tested singly. Each test consisted of a set of five 60-s intervals: (1) control stimulus: white noise shaped with the amplitude envelope of the whine (0–10 kHz); (2) silence; (3) experimental stimulus: the heterospecific or ancestral call (see [Experimental Stimuli](#)); (4) silence; (5) control stimulus (see also [Ryan & Rand 1998](#); [Bosch et al. 2000a](#)). All calls and white noise were broadcast at a rate of one call every 2 s, a typical calling rate for túngara frogs ([Ryan 1985](#)). A total of 30 calls or whine-like noise bursts were presented in each 60-s interval. Only cases in which males called during both control stimuli were included in the analysis to eliminate cases of no response due to lack of motivation. After each test, the male was required to call again or respond to the chorus before starting a new test. Each male was tested with all test stimuli or until he no longer responded ([Ryan & Rand 1998](#)). We conducted 14 experiments, and in each one, we tested 10 males, for a total of 140 male trials. A total of 39 males contributed to the data set.

We presented the stimuli using a JVC XL-PG7 CD-player through a Realistic SA-10 amplifier at 90 dB SPL (re. 20 μ Pa) at 0.5 m measured by a GenRad sound pressure level meter (model 1982). All experiments were presented in random order and recorded with a Sony WM-D6 cassette recorder. We then digitized the tapes with CoolEdit 2000 (Syntrillium Software, Phoenix, Arizona, U.S.A.), at a sampling rate of 44.1 kHz and 16 bits/sample. Files were saved with coded names so that measurements would be taken blind. In each 60-s interval, we counted the total number of whines, total number of chucks and maximum number of chucks appended to a single whine.

Experimental Stimuli

We examined the evoked vocal response to synthetic advertisements calls of five species of frogs in the *P. pustulosus* species group, three congeners not in the species group and calls reconstructed at the ancestral nodes ([Ryan & Rand 1995](#); [Fig. 1](#)). Conspecific and heterospecific calls were recorded during previous studies ([Ryan & Rand 1993a, b, 1999](#)). Ancestral calls were estimated based on the calls of extant species ([Ryan & Rand 1995, 1999](#)). Variables for constructing the synthetic stimuli were based on mean values of a combination of the following eight spectral and temporal call parameters: maximum frequency, final frequency, duration, rise time, fall time, whine shape, rise shape and fall shape. Phylogenetic relations among extant species and seven ancestral nodes were based on the most parsimonious tree topology determined from an analysis of several morphological characters, 27 allozymes and 1200 base pairs of the 12S mitochondrial genome and its flanking regions, without including call characters in the analysis ([Cannatella et al. 1998](#)). Bootstrap estimates showed strong statistical support for all the nodes of the phylogeny (all $P < 0.05$). Estimates of the call characters for the ancestral nodes were calculated from local squared-change parsimony and used to synthesize such calls. [Ryan & Rand \(1999\)](#) showed that although other models of evolution generate different estimations of the ancestral calls, these differences are not perceived

by female *P. pustulosus*. For details on the estimation of the calls and the model of evolution, see [Ryan & Rand \(1999\)](#). We synthesized all stimuli using a program supplied by J. Schwartz (Pace University, Pleasantville, New York, U.S.A.; sample rate 20 kHz and 8 bit).

Comparison between Male and Female Responses

[Ryan & Rand \(1995, 1999\)](#) conducted phonotaxis experiments with female *P. pustulosus* to the same stimuli used in this study. We compare our results on male-evoked vocal response in this study to the responses of female túngara frogs from the same population investigated in that study. [Ryan & Rand \(1995\)](#) quantified the number of false alarms in phonotaxis by females to the test calls paired with a white-noise stimulus as the one used as a control in this study ([Experimental Stimuli](#)). In addition to those recognition experiments, [Ryan & Rand \(1995\)](#) also presented conspecific calls paired with heterospecific calls in discrimination experiments. For the purpose of comparing female and male permissiveness to heterospecific/ancestral calls, we used only the data on recognition experiments presented by [Ryan & Rand \(1995\)](#). In these experiments, a response was scored when the female approached a speaker within 10 cm. A 'no response' was noted if the female either remained motionless for 5 min after being released, stopped moving for 2 min at any time during the experiment or did not approach any speaker in 15 min. Tests to discern absence of response due to lack of motivation rather than lack of attraction to the call were also performed. For a complete description of the testing chamber and details of the protocol, see [Ryan & Rand \(1999\)](#).

Statistical Analysis

All statistical procedures were conducted using SYSTAT ([Wilkinson 1991](#)). To examine the recognition of heterospecific/ancestral calls in males, we contrasted their calling response to the experimental stimuli with their baseline calling behaviour (average silence periods preceding and following the experimental stimuli). We characterized the overall response of males using the first component of a principal component analysis (PCA) combining the total number of whines, total number of chucks and maximum number of chucks in a single call. The first component explained 83.43% of the variance, and all the variables highly contributed to the analysis (component loadings: number of whines = 0.954, number of chucks = 0.906, maximum chucks = 0.879; eigenvalue = 2.504). For each experimental stimulus, we performed a separate Wilcoxon matched-pairs signed-ranks test using the scores from the PCA for each male during the presentation of the experimental stimuli and baseline calling.

Individual frogs varied in their absolute calling responses; thus, to enable comparisons between individuals across stimuli, we examined the strength of calling in response to each stimuli by averaging the calling response of the two controls to calculate the ratio of responses as

follows: experiment/(average control + 1) (Bosch et al. 2000a, 2002). We then performed a PCA combining these ratios for the number of calls, number of chucks and maximum number of chucks per call. We used Pearson correlation analyses to investigate the extent to which call similarity and phylogenetic distance explained male calling response to nonconspecific calls. These analyses are particularly interesting because call similarity and phylogenetic distance are not significantly correlated in this group of species ($r_{12} = 0.47$, $P = 0.13$, 95% CI = -0.08, 0.8). Our metric of call similarity was based on a PCA of the standardized call variables. We computed the Euclidean distances between calls based on the first three axes of variation from the PCA, which explained 87% of the variation in calls among species and nodes. Phylogenetic distance between *P. pustulosus* and the other extant species and the ancestral nodes was calculated based on the most parsimonious estimated changes in DNA base sequence (Ryan & Rand 1995).

To contrast the responses of the sexes to nonconspecific calls, we converted the vocal response of males into a binary response equivalent to the one used for females (i.e. response, no response). If a male called more during the presentation of the heterospecific/ancestral call than during the silent intervals before and after it, his behaviour was scored as a response. A 'no response' was scored when a male called less to the experimental stimuli than during silence. We compared the number of males and females that responded to each heterospecific/ancestral call using two-tailed Fisher's exact tests.

RESULTS

Recognition errors in the form of false alarms were quite common. Most of the heterospecific calls that we tested evoked higher vocal responses from *P. pustulosus* males than spontaneous baseline levels (Fig. 2). In all but two cases (*P. enesefae* and ancestral node b), males had a significantly higher calling PCA score during the presentation of the heterospecific call than during the silent periods before and after it. Males generally responded with both simple and complex calls to the experimental stimulus. Simple calls were produced at the beginning of the stimulus period, but often males escalated calling by adding chucks. In most cases, males increased the complexity of their calls in response to the heterospecific call. Although males can add up to six or seven chucks to a whine, the maximum call complexity during the experiments was only three chucks per call, even though the total number of chucks summed over all calls varied considerably ($\bar{X} \pm SD = 15.09 \pm 10.85$, range 0–38 chucks).

Although males called less in response to *P. enesefae* and the ancestral node b, in those trials they produced 'mews', which are aggressive calls that function in maintaining fixed spatial distances among chorusing males of this species (Ryan 1985). The aggressive call is clearly different from the whine; it is longer and has a higher frequency and more amplitude modulation than the advertisement call (Fig. 3). When exposed to *P. enesefae* calls, 7 of 10 males produced mews, two did not call, and one produced

whines after about 30 s of the experimental stimulus presentation. During the calls of ancestor node b, three males produced mews, three did not call, and four males produced whines. None of the other calls tested evoked mews.

There was no correlation between the overall call similarity of the test calls to the conspecific calls and the vocal response elicited by experimental stimuli (Pearson correlation: $r_{12} = -0.341$, $P = 0.232$; 95% CI = -0.738, 0.231). In contrast, phylogenetic distance significantly predicted calling response ($r_{12} = -0.728$, $P = 0.003$; 95% CI = -0.907, -0.322). Multiple regression analysis was consistent with the results of the correlations. Phylogenetic distance best predicted calling response ($t = -3.123$, $N = 14$, $P = 0.010$), but call similarity did not significantly explain any of the variation ($t = -0.173$, $N = 14$, $P = 0.864$).

Comparison between Male and Female Responses

Males and females differed in their responses to most of the calls that we presented (Fig. 4). In 9 of 14 experimental stimuli, the proportion of individuals that recognized the nonconspecific call was significantly different between the sexes. In addition, in all of those cases, the proportion of males that responded to the heterospecific/ancestral call was higher than the proportion of females.

DISCUSSION

We quantified recognition errors, specifically false alarms, in response to nonconspecific calls in male túngara frogs. To establish whether the sexes differ in their responses as predicted by the differential cost of false alarms, we contrasted the responses of males and females to the same set of calls and found profound sexual differences.

Calling Responses of Male Túngara Frogs to Nonconspecific Advertisement Calls

Male *P. pustulosus* increased their calling response to most of the calls that we tested compared to their baseline calling during silence. In response to two calls (one heterospecific, one ancestral), the vocal response was not a mating call but an aggressive call. Given that males do not spontaneously produce aggressive calls, we consider the production of such calls an indication of call recognition, in this case a false alarm. Hence, we conclude that males recognized all the calls that we presented. They did not respond equally to all stimuli, however. Significant variation in male calling response was explained by phylogenetic distance but not by overall call similarity. The lack of a relation between calling response and call similarity probably results from males weighting call characteristics in a way not captured in our measure of call similarity. We used a principal component analysis that accurately quantified call parameters, which is an appropriate technique to statistically distinguish calls, but it need not reflect the degree to which male túngara frogs perceive those

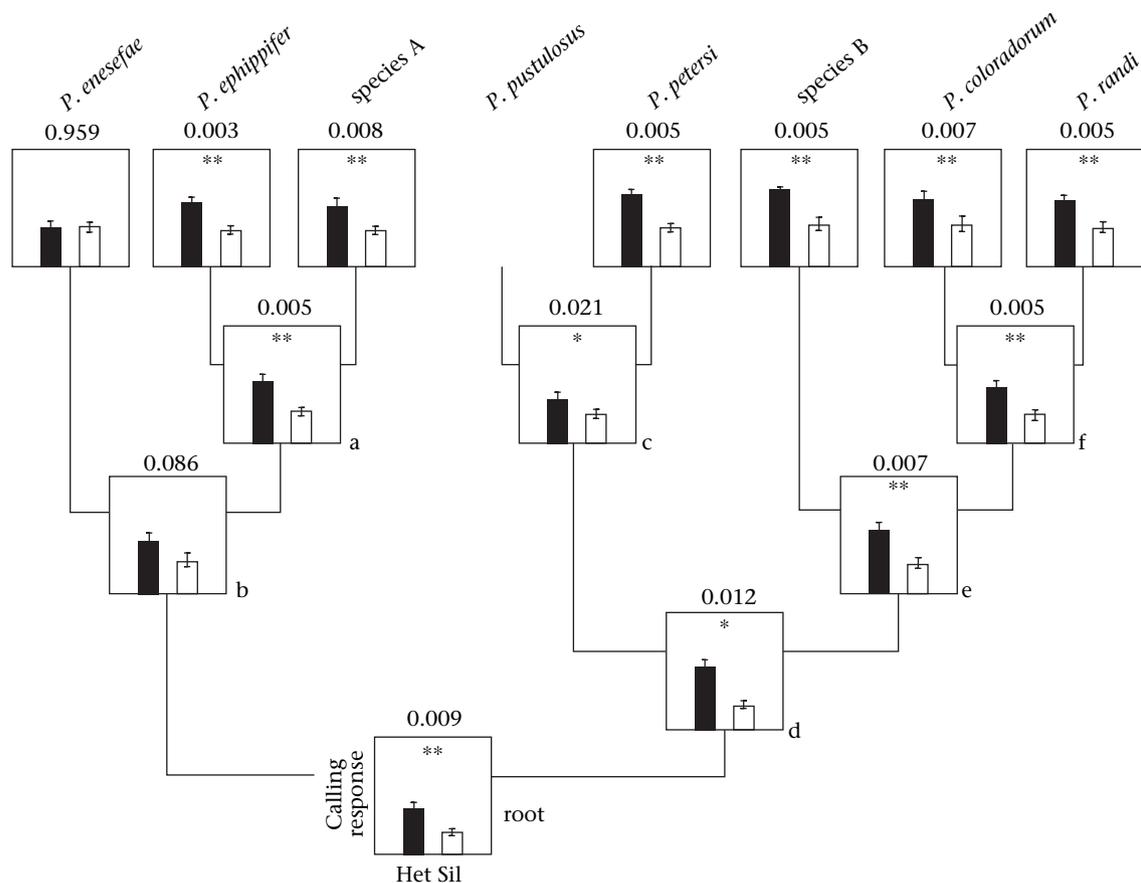


Figure 2. Responses of male túngara frogs, *P. pustulosus*, to 'heterospecific' calls (Het) and periods of silence (Sil; baseline). The calls were determined from species' means for the extant species and phylogenetic estimates for calls of the ancestral nodes. The responses of males (mean \pm SE) are based on the first component of a principal component analysis combining the number of whines, number of chucks and maximum chucks per call produced in response to the heterospecific/ancestral call (■) versus spontaneous calling (□). *P* values (Wilcoxon matched-pairs signed-ranks test) are shown above each graph. **P* < 0.05; ***P* < 0.001.

same calls as different. Ryan & Rand (2003), for example, showed substantial differences between statistical variation in a population of mating calls and the calls' perceptual variation to female túngara frogs.

Recognition of such a broad range of stimuli as the one found in this study is unexpected. Even though several studies have investigated the vocal response of males to variation in specific call parameters (e.g. Walkowiak & Brzoska 1982; Schwartz & Wells 1984a; Allan & Simmons 1994; Penna et al. 1997), few have evaluated their responses to heterospecific calls. In a pioneering study, Capranica (1965) determined the calling response of male bullfrogs, *Rana catesbeiana*, in response to the calls of 34 species, including nine species in the genus *Rana*, four of which are part of the *R. catesbeiana* species group (Hillis & Wilcox 2005). Male bullfrogs only called in response to conspecific calls. Capranica's results suggest high species specificity in the calling response of male bullfrogs. The difference between Capranica's (1965) results and ours may be related to the degree of similarity of the vocalizations used. The advertisement calls of all the *Physalaemus* that we studied consist of frequency-modulating whines similar to the one of *P. pustulosus*, while the call of *R. catesbeiana* has two distinctive frequency peaks that are absent in the calls of other frogs of the same genus.

The general permissiveness in the response of male túngara frogs in Panama to other *Physalaemus* calls might also result from their being allopatric with all of their congeners. In contrast, several closely related species of *R. catesbeiana* are sympatric with this species in the eastern U.S.A. One might expect sharpening of male call recognition in sympatry, just as one would expect reinforcement of female mating preferences. Evidence, however, suggests that the consequences of sympatry may be taxon specific. Although Australian leptodactylid males suppress their vocal activity when exposed to calls of sympatric species (Littlejohn & Martin 1969), the opposite is true for three species of Neotropical treefrogs (Schwartz & Wells 1983a, b, 1984a, b, 1985).

Male *P. pustulosus* gave aggressive calls in response to *P. enesefae* and the ancestral node b call. In the laboratory, males presented with this aggressive call in playback experiments produced aggressive calls in response, but often stopped calling immediately after the stimulus was broadcast. It thus appears that male túngara frogs perceived the calls of *P. enesefae* and node b as aggressive signals. This phenomenon may be the result of the longer duration of these test calls, which resembled the mew. Schwartz & Wells (1984a, 1985) observed that males of *H. ebraccata* and *H. microcephala* increased the number of aggressive

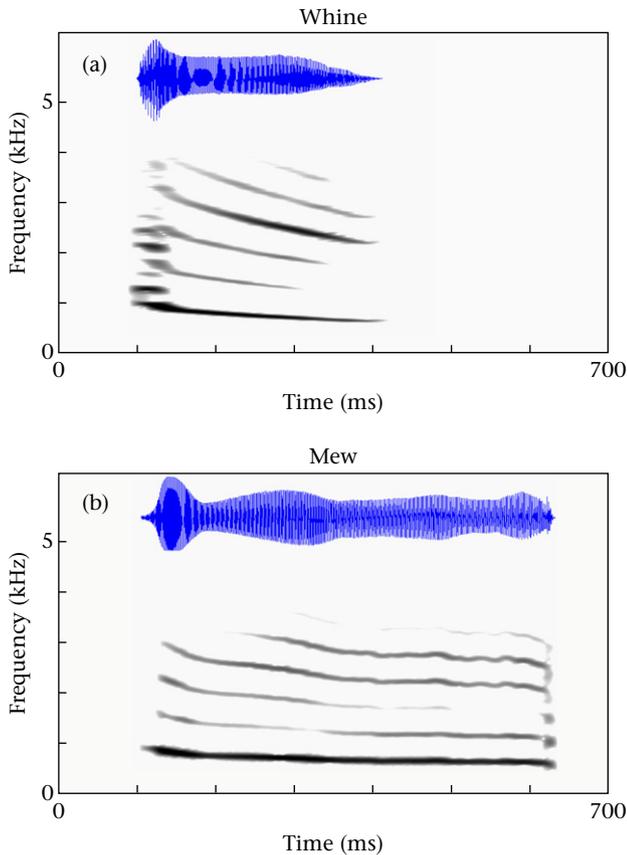


Figure 3. (a) Advertisement call or whine and (b) aggressive call or 'mew' of túngara frogs. Sonograms are shown at the bottom and oscillograms on the top. The relative intensity of the calls is not represented proportionally; the aggressive call is of much lower intensity than the whine.

calls in response to high-intensity playbacks of both conspecific and heterospecific calls. These species occur at the same breeding sites and call in close proximity, so they may have been favoured to respond to each other's aggressive calls. Testing the calling responses of male túngara frogs from the llanos of Venezuela where they co-occur with *P. enesefae* could provide valuable insights.

Sexual Differences in Receiver Permissiveness

Males made more recognition errors than females when responding to most of the nonconspecific calls. In spite of the differences between the sexes, all the heterospecific/ancestor calls recognized by females elicited only advertisement calls from males, but the calls of species that elicited aggressive calls in males were not recognized by females.

Contrary to the results found for male calling response in this study, Ryan & Rand (1995, 1999) found that female recognition of the test calls was explained by overall call similarity as well as by phylogenetic distance. Thus, calling males and females approaching a mate appear to weight signal variation differently. Our comparisons, however, reveal that although males and females differed in their

responses to the different calls, these responses showed an effect of evolutionary history. Females are more likely to show phonotaxis to calls of species and ancestors that are more closely related (Ryan & Rand 1995, 1999). Similarly, males call more in response to the calls of close relatives. Hence, there is an effect of evolutionary history on response to advertisement calls in both male and female túngara frogs. Therefore, just as history has left a perceptual footprint on the females' brain, the same is generally true for males, but the details seem to differ.

Differences in responses between the sexes could result from differences in the perception of signal variation, or in differences in how the same perceptual information influences decision making. In this mating system, as in many others, sexual signals elicit different tasks in the different sexes. Sexual differences in decision making could be either sex specific and independent of task, or task specific and independent of sex. For example, if females also called and males also showed phonotaxis, we might find that females are either more restrictive and less error prone to call variation whether hopping towards the call or calling to it, or more restrictive in the calls that they approach but less restrictive in their vocal responses. The analogous thought experiment could be conducted with males, and actual experiments could perhaps be conducted in duetting songbirds, in which both female vocal response and courtship solicitation displays could be measured in response to the same set of signal variation.

The same confound of task and sex also occurs in other systems, such as the studies of Searcy & Brenowitz (1988) in red-winged blackbirds, Dabelsteen & Pedersen (1993) in blackbirds and Searcy et al. (1981a, b) in swamp sparrows. At least two other studies, however, tested how males and females responded to signal variation using the same bioassay. Nelson & Soha (2004) measured calling responses of male and female white-crowned sparrows to songs containing a phrase from a foreign dialect and showed that males were more discriminating. Similarly, Vicario et al. (2001) evaluated the calling responses of both sexes of zebra finches to calls that indicated the sex of the signaller and found that females showed weaker sex discrimination than males. A relevant consideration, however, is that in animals where females and males characteristically perform different behaviours in response to mating signals, it is biologically meaningful to address their responses while performing their sex-typical task.

Regardless of where the sexual difference arises, there are three hypotheses that predict them. These hypotheses have been proposed to explain differences between males and females in responses to bird song and can be extended to understand the same phenomenon in other systems of acoustic animals such as frogs. First, Dabelsteen & Pedersen (1988) suggested that, if males and females differ in their habitats, they might experience different degrees of sound degradation and thus have evolved different responses to signals. Such a scenario is feasible in frogs (e.g. Witte et al. 2005). It seems unlikely, however, that male and female túngara frogs responding to mating calls at the same breeding site experience different acoustic environments. Second, Kroodsma (1999) proposed that, since male–male interactions lead to local song dialects and

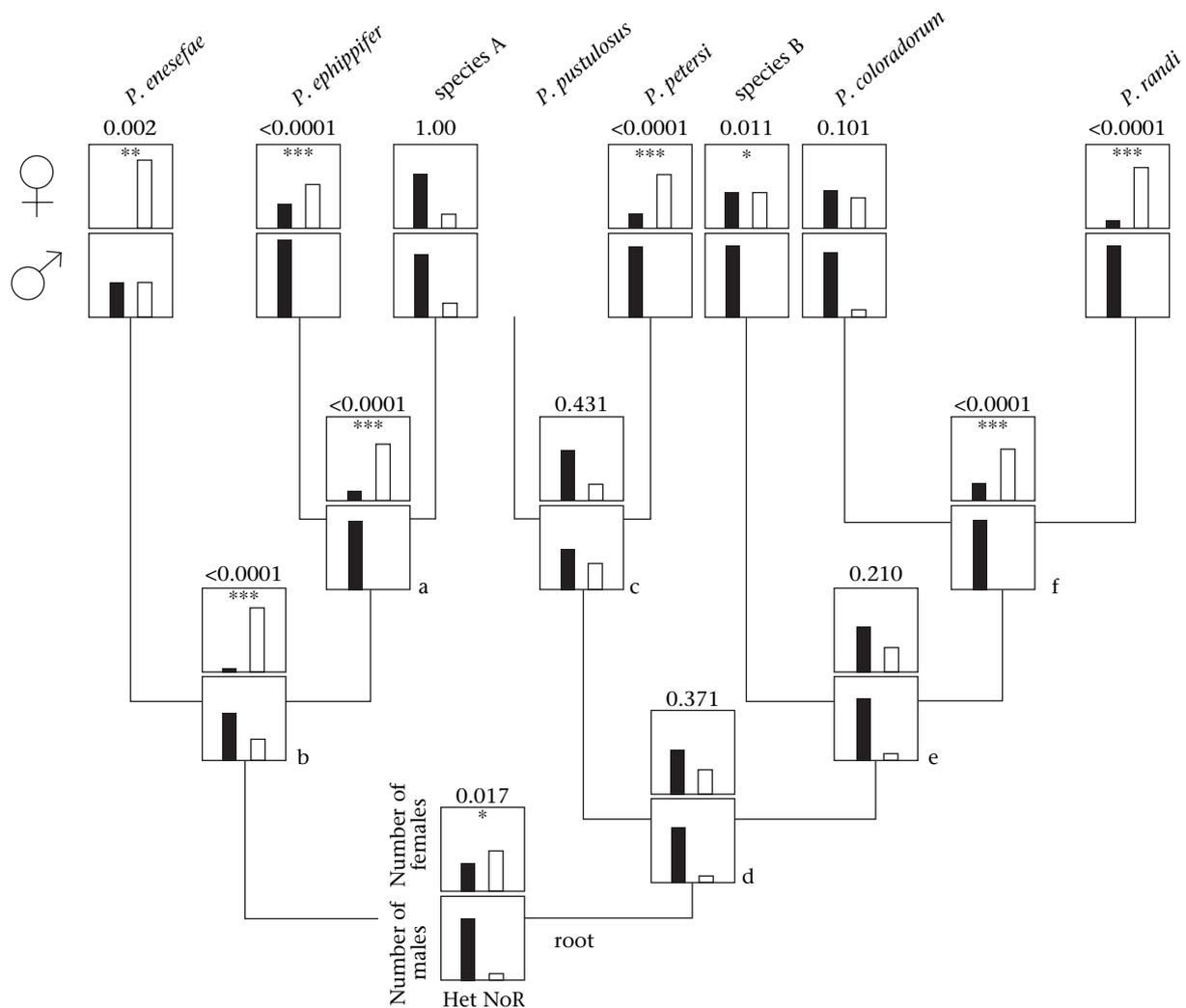


Figure 4. Responses of female (♀) and male (♂) *Physalaemus pustulosus* to the calls of species in the *P. pustulosus* species group, three closely related species, and calls estimated for the ancestral nodes. ■: responses to heterospecific/ancestral calls (Het); □: no response (NoR). Females' responses are from results of phonotaxis experiments by Ryan & Rand (1995). Males' evoked vocal responses are based on the first principal component of a principal component analysis combining the number and complexity of calls produced to score behaviour as response/no response. *P* values of Fisher's exact tests contrasting female and male responses are shown above each graph. **P* < 0.05; ***P* < 0.001; ****P* < 0.0001.

males would benefit from producing a common, widely distributed song to attract females over a wide area, males should therefore be highly responsive to local song variants, and females should attend to general, species-specific features of the call. Therefore, males are expected to be more selective than females. Our findings, however, are contrary to this prediction.

Third, the risk-of-investment hypothesis was initially proposed by Searcy & Brenowitz (1988) and later named by Dabelsteen & Pedersen (1993). As mentioned above, the consequences of false alarms and missed detections are more costly for females than for males, and thus females are predicted to be more coy. Another hypothesis, which to us seems an extension of this hypothesis, is the suggestion by Ratcliffe & Otter (1996) that females are under stronger selection to evaluate individual qualities of males. Female túngara frogs attend to spectral features of both the whine (Bosch et al. 2000b) and the chuck (Ryan 1980,

1985) of the mating call in a manner that results in females choosing larger males who then fertilize more eggs. Males also use spectral features of the conspecific whine; their calling behaviour escalates based on the frequency of their competitor's calls relative to their own call (Bosch et al. 2000a). Even though both sexes attend to call features that vary within the species, it is likely that males are not under such strong selection as females to ascertain species recognition. Relaxed selective pressures on male calling responses could generate broader recognition functions while still enjoying within-species selectivity.

Conclusion

We compared recognition errors, specifically false alarms, between the sexes by comparing sexual responses to mating calls of a variety of nonconspecific signals. Male

túngara frogs responded to an unexpectedly broad range of calls. Even though numerous studies have evaluated vocal behaviour of male frogs to variation in various temporal and spectral parameters, few have examined variation outside the range of parameters of the conspecific call. In accord with the predictions based on the consequences of false alarms and missed detections, males were much more permissive in their response to signal variation than females. Several targets of selection could be responsible for such differences. They could result from differences between the sexes in signal perception, or from how the same information results in different decisions for sex-specific tasks.

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