

# **The Design of Animal Communication**

edited by Marc D. Hauser and Mark Konishi

A Bradford Book  
The MIT Press  
Cambridge, Massachusetts  
London, England

# 19 Phylogenetic Inference and the Evolution of Communication in Túngara Frogs

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Many biological phenomena engender issues that permeate various levels of analysis but which are often addressed in isolation. In contrast, Tinbergen's (1964) four questions describe a multidisciplinary approach to animal behavior in which issues of physiological mechanisms, ontogeny of behavioral acquisition, the adaptive significance of behavior, and its evolutionary history are addressed in an integrative manner. Studies of animal communication perhaps best exemplify how each of these questions impinge on the same general phenomenon.

Most studies of evolutionary history rely on interpreting the fossil record or deriving hypotheses about history from comparisons of extant taxa. The latter endeavor, which is the focus of phylogenetics, encompasses both studies of genealogical relationships and investigations of patterns by which characters have evolved. Ethological studies that have integrated phylogenetics and communication usually take one of two forms. One is the use of communication signals for taxonomic purposes. Animals themselves often rely on communication signals to identify conspecifics, and this is true for many taxonomists as well, especially when dealing with cryptic species. The other is the use of phylogenetics by ethologists to study the evolution of behavior, including how communication signals evolve. This approach is exemplified by Lorenz's (1941) studies of display evolution in ducks and Tinbergen's (1953) use of the comparative method to investigate the adaptive significance of social behavior.

The last decade has seen a resurgence in the use of explicit phylogenetic techniques to investigate behavioral evolution (see, e.g., Brooks and McLennan 1991; Harvey and Pagel 1991; Martins 1996). Many of these studies utilize hypotheses of phylogenetic relationships to derive the most parsimonious patterns by which behaviors have evolved (see, e.g., various chapters in Martins 1996), and to generate experimentally testable hypotheses about evolutionary processes (see examples discussed in Ryan 1996). The purpose of this chapter is to highlight some of these phylogenetic approaches to studying animal communication. To do so, we will introduce the acoustic communication system of the túngara frog, *Physalaemus pustulosus*. We will begin by reviewing studies that concentrate on the adaptive significance of this mate recognition system and the underlying mechanisms regulating it. The results of these studies suggest various hypotheses about the evolution of the communication system, which we then address using a variety of approaches combined with an underlying knowledge of the phylogenetic relationships of these frogs.

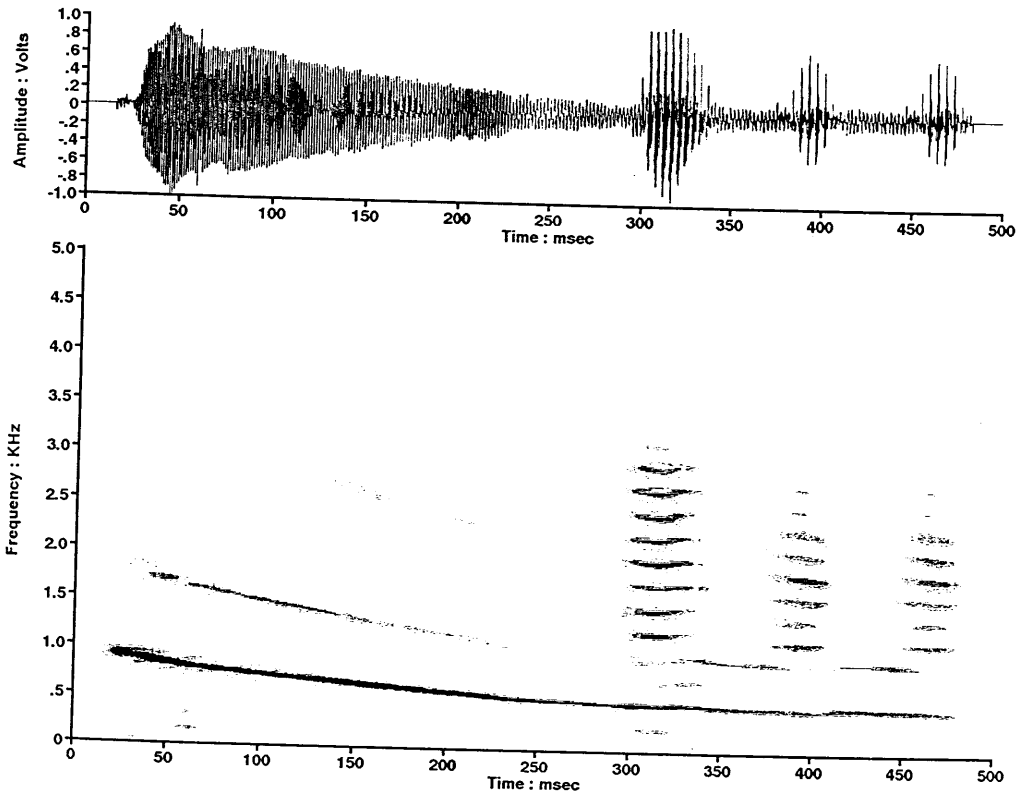
## Natural Call Variation and Intraspecific Call Preference in *Túngara* Frogs

Studies with our colleagues of the mechanisms and evolution of acoustic mate recognition in the *túngara* frog and its close relatives have addressed animal behavior, neurobiology, molecular phylogenetics, physiological energetics, and functional morphology. These studies have provided information as to how females utilize acoustic cues to assess potential mates, the neural mechanisms that guide such mating preferences, how these preferences bias male mating success in nature, how these preferences cause the evolution of attractive male traits, how costs of such traits increase predation risk, elevate metabolism, and are correlated with hormonal changes, and how preferences and traits have evolved during the history of these frogs as estimated by molecular phylogenies. These studies have made the *túngara* frog system a valuable one for addressing various issues in sexual communication. Here we will concentrate on how we use various phylogenetic approaches to understand the evolution of this communication system.

The *túngara* frog is a small (ca. 30mm snout-vent length [SVL]), common species found throughout much of the lowland tropics from central Mexico, south through Middle America, across the Darien gap into northern Colombia, and east through the lowlands of Venezuela into Trinidad and western Guyana (Ryan 1985; Ryan et al. 1996). This frog is abundant in central Panama near the facilities of the Smithsonian Tropical Research Institute, where most of these studies have been conducted.

The call of this species is unusually variable for a frog. There are two components to the call: a whine and a chuck (figure 19.1). The whine initiates the call, is always present, and can be produced alone or may be followed by one or several chucks. Thus the call can vary in the number of components from a whine only to a call with multiple chucks; we classify these as simple and complex calls, respectively. A typical whine is a frequency-modulated component whose fundamental has an onset of 900Hz and sweeps to a final frequency of 430Hz in 300ms. The whine has several harmonics with detectable energy in the second to fourth harmonic, but most of the whine's energy is in the fundamental. The whine's dominant frequency, the frequency with the greatest concentration of energy, is about 600Hz (Rand and Ryan 1981; Ryan 1985).

The chuck has a much different acoustic structure. When chucks are added to the call they are appended near the end of the whine, although an ongoing study suggests that the chuck's position relative to the whine might not be critical to female preferences. Up to six chucks can be added to a call although one, two, or sometimes three chucks are the more common occurrence. The typical chuck is 35ms in duration, has a fundamental frequency of 250Hz, and is characterized by 14 harmonics.



**Figure 19.1**

An advertisement call of the túngara frog, *Physalameus pustulosus*, consisting of a whine followed by three chucks.

thus having a frequency range from 250–3500Hz. More than 90% of the chuck's energy is within the higher half of the 14 harmonics, and the dominant frequency averages 2500Hz (Ryan 1985; Ryan et al. 1990).

Females are responsive to variation in call complexity. In nature, males call from stationary sites and females are free to move unimpeded throughout the chorus to choose a mate. As with most frogs, the call appears to be the primary cue used by females in mate location, assessment, and choice. Phonotaxis experiments in the laboratory offer a powerful tool to evaluate how the female's acoustic preferences might influence her choice of mates in nature. Phonotaxis experiments have shown that a whine is both necessary and sufficient to elicit female phonotaxis in controlled laboratory experiments. When females are given a choice between a whine only and

a whine with chucks, they prefer the latter. The further addition of chucks can enhance call attraction, but the relationship is not linear (Rand and Ryan 1981; Rand et al. 1992; Wilczynski et al. 1995).

Although males produce calls to attract mates, they do not always produce the most attractive calls—that is, calls with chucks. When calling in areas isolated from others, a male will tend to produce simple calls. In choruses, however, most males produce complex calls. Males vary call complexity (the number of chucks added) in response to vocal stimulation from other males, and this effect can be elicited by broadcasting calls to evoke vocal responses in the wild or in the laboratory. A male producing a call without chucks will add chucks in response to the call of another male—simple or complex. Playbacks of more complex calls can cause further escalation in a male's call complexity. Thus call complexity can be varied by the male, and it appears that all males can produce calls with a full range of chucks (Rand and Ryan 1981; Ryan 1985).

There is also substantial variation in the advertisement call among populations. In a survey of 30 populations along a 5000km transect encompassing most of the species' range, we found that there were significant differences among populations in all 12 call parameters of the whine and chuck that we measured (Ryan et al. 1996). We are currently determining if females are attendant to this variation among populations.

It had been a paradox as to why males do not enhance call attractiveness by maximizing the number of chucks. This suggested there might be a cost to adding chucks that would balance the benefit of increased attractiveness. Although calling is energetically expensive, there is no detectable increase in oxygen consumption or lactate concentration that results from increasing call complexity (Bucher et al. 1982; Ryan et al. 1983). There does appear to be a predation cost, however. The bat, *Trachops cirrhosus*, is common in many areas in which túngara frogs breed. This bat feeds on frogs and locates them by homing in on the frogs' calls; it does not produce echolocation signals when approaching the frog, and it has a series of neuro-anatomical features that enhance its low-frequency sensitivity (Barclay et al. 1981; Bruns et al. 1989; Tuttle and Ryan 1981). The frog-eating bat responds to call variation in a manner similar to the female túngara frog. Bats approach a speaker broadcasting only the whine, but when given a choice the bat also prefers calls with chucks (Ryan et al. 1982). Males are safer from bat predation in larger choruses due to a selfish herd effect; since females are relatively more common in larger choruses, safety from predation is complimented by increased per capita mating success (Ryan et al. 1981). Thus the countervailing forces of sexual selection and bat predation appear to have been responsible for the evolution of the variably complex call of the túngara frog.

What is it about the chuck that makes the call more attractive? Females not only prefer calls with chucks but tend to prefer calls with lower-frequency chucks. In nature, larger males are more likely to be chosen by females, and there is a significant and negative correlation between the fundamental frequency of the chuck, which ranged from 200–270Hz, and male body size, which ranged from 26–33mm. In phonotaxis experiments, females preferred synthetic calls with lower fundamental frequencies (Ryan 1980, 1983, 1985). In these earlier experiments, the energy distribution across harmonics in the synthetic chucks were not controlled to reflect the natural variation among chuck harmonics. Later studies, in which the energy distribution among harmonics reflected this in a typical chuck, tended to be consistent with this lower-frequency preference although the preference was not as strong as previously suggested ( $P = 0.10$  and  $P = 0.13$  in two experiments; Wilczynski et al. 1995). In these studies the dominant frequency is in the upper harmonics, and the fundamental and dominant frequency were correlated (see below).

Behavioral preferences can result from an interaction of properties of the stimulus with sensory biases. We attempted to uncover some of these biases that might lead to low-frequency preference in túngara frogs. Frogs have two inner ear organs sensitive to airborne sound. In túngara frogs, as in most frogs, the amphibian papilla (AP) is maximally sensitive to frequencies below 1200Hz, and the basilar papilla (BP) is maximally sensitive to frequencies above 1200Hz (Ryan et al. 1990). The dominant frequency of the whine is close to the frequencies to which the AP are most sensitive, about 600Hz. Although the chuck encompasses a wide frequency range (250–3500Hz), more than 90% of its energy is concentrated in the higher frequencies (>1500Hz) to which the BP is more sensitive. This suggested that most of the processing of the chuck initially occurs through the BP—at least at amplitudes near threshold (Ryan et al. 1990). Phonotaxis experiments support this interpretation. Females were presented with a whine versus a whine with either the low half (<1500Hz) or the high half (>1500Hz) of the chuck's harmonics with the natural energy distribution; thus the whine/low-chuck had less energy than the whine/high-chuck. Females preferred a whine/high-chuck to a whine, but did not discriminate between a whine and a whine/low-chuck (Ryan and Rand 1990; Wilczynski et al. 1995). Thus the BP seems to be important in processing the chuck when the relative amplitudes of the chuck harmonics are greatly skewed to the higher harmonics, as they are in natural chucks. This is not to suggest that if more acoustic energy were invested in the low-chuck this would not enhance its attractiveness. That issue is addressed below.

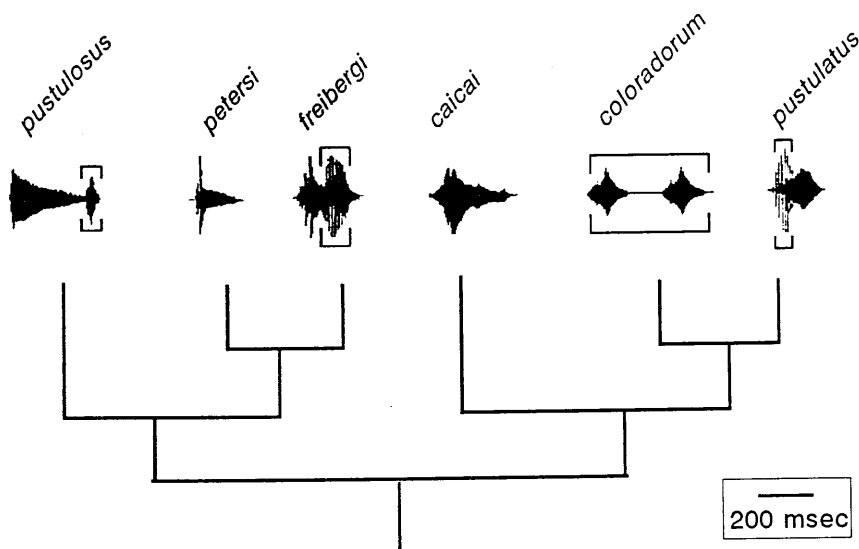
Although the tuning of the AP and BP tends to match the dominant frequencies of the whine and chuck, respectively, the BP is tuned, on average, slightly below

the dominant frequency of the average chuck in the population: 2100Hz for the BP versus 2500Hz for the chuck. This suggests that the female's behavioral preferences for chucks with a lower fundamental frequency, which also had a lower dominant frequency, might result from these calls effecting a better match to the BP's sensitivity. Computer models integrating an average tuning curve and digitized calls drawn at random from a population of túngara frogs support this contention. Chucks with lower dominant frequencies elicited greater excitation from the average tuning curve than did higher-frequency calls (Ryan et al. 1990). Phonotaxis experiments using single tones in place of the chuck, 2100Hz versus 3000Hz, offered qualified support for this hypothesis as well; more females preferred the lower-frequency tone, although the preference was not statistically significant ( $P = 0.10$ ; Wilczynski et al. 1995). Similar results were also achieved with another species of frog, cricket frogs (*Acris crepitans*): female BP sensitivity is below the average call's dominant frequency, and females were preferentially attracted to lower-frequency calls (Ryan et al. 1992).

### Acoustic Preferences and Sensory Exploitation

As discussed above, females prefer calls with chucks to calls without chucks, and there tends to be a weak preference for lower-frequency chucks over higher-frequency chucks. None of the other close relatives of the túngara frog, with the exception of a species in the jungles of Peru and Brazil that we tentatively identify as *P. freibergeri*, adds a suffix to the call (Cannatella et al. 1998; Ryan and Rand 1993a,c, 1995; figure 19.2). Morphological studies have identified the exaggeration of a fibrous mass associated with the vocal cords as being partly responsible for the production of the chuck (Drewry et al. 1982), and a comparative analysis shows that the evolutionary elaboration of the fibrous mass is associated with the addition of a call suffix (Ryan and Drewes 1990). The addition of more taxa and more characters to the phylogenetic analysis continues to support this concordance (Cannatella et al. 1998).

So how did it come about that male túngara frogs produce calls with chucks and that the females prefer them? For a communication system to evolve there must not only be a change in signal structure, but the receiver must be able to interpret these new signals appropriately. Thus it is often assumed that signals and receivers coevolve. This is an assumption about past history. No degree of experimentation demonstrating the congruence of a signal and receiver within a species has any bearing on how this congruence came to be. Instead, one could utilize information on phylogenetic relationships to deduce past patterns of signal evolution. This infor-



**Figure 19.2**

The phylogenetic relationships of taxa within the *Physalaemus pustulosus* species group. An oscillogram of the advertisement call of each species is shown. The brackets indicate derived call characters: *P. pustulosus*—chuck; *P. freibergi*—squawk; *P. coloradorum*—double calls; *P. pustulatus*—strongly amplitude-modulated prefix.

mation could then be used to design experiments that can test long-held assumptions about signal-receiver coevolution.

We utilized information on the phylogenetic relationships of these frogs to determine if, in fact, the female's preference for chucks evolved in concert with chucks (the coevolution hypothesis) or if females had a preexisting preference for chucks that was exploited by males (sensory exploitation hypothesis; Ryan 1990; Ryan et al. 1990; Ryan and Rand 1990; both hypotheses reviewed in Kirkpatrick and Ryan 1991; Andersson 1994; Ryan 1997). These hypotheses can be discriminated by reconstructing the phylogenetic history of the species group and inferring the historical sequence by which chucks and preferences for chucks evolved.

We utilized data on morphology, allozymes, and the sequences of the 12S ribosomal mitochondrial gene and the COI mitochondrial gene to estimate the phylogenetic relationships between the six species of the *Physalaemus pustulosus* group and three congeneric species that we used as outgroups (Cannatella et al. 1998; Ryan and Rand 1995; figures 19.2, 19.4). The six species in the species group comprise two smaller monophyletic groups. One group has a Middle American–Amazonian distribution and includes *P. pustulosus*, *P. petersi*, and *P. freibergi*. All of these species



are north or east of the Andes. The other group has three species that are all west of the Andes, *P. coloradorum*, *P. pustulosus*, and *P. caicai*. The three outgroup species are *P. enesefae*, *P. ehippifer*, and an undescribed species that we refer to as *P. "roraima"*. Allozyme variation, differences in DNA sequence, and the geology of the Andes all suggest that these two groups within the *P. pustulosus* species group separated about 20 million years ago, coincident with the rise of the Andes (Cannatella et al. 1998).

As mentioned previously, a call suffix is present only in the Middle American–Amazonian group, in *P. pustulosus* (a chuck) and *P. freibergi* (a squawk). Call suffixes appear to be absent in the western Andes group and in the roughly 35 species in the genus that we have examined (figure 19.2). Thus the call suffix seems to have evolved after the Middle American–Amazonian group diverged from the western Andes group. We asked if female *P. coloradorum*, a member of the western Andes group, would prefer calls with chucks if they were to evolve. Several chucks from a túngara frog call were digitally appended to the whine-like species-specific call of *P. coloradorum*. When presented with such a choice in a phonotaxis experiment, females preferred calls with chucks to the normal species call, which lacks chucks (Ryan and Rand 1993b,c). The fact that *P. pustulosus* and *P. coloradorum* both prefer calls with chucks suggests that this preference is shared through a common ancestor, as opposed to the hypothesis that *P. coloradorum* females happened to evolve the same preference for traits not existing in their own males. The former hypothesis is more parsimonious because it requires fewer evolutionary changes: one instead of two, assuming that the lack of a chuck is ancestral. This possibility is now being investigated with studies of *P. enesefae* (Z. Tarano, personal communication). It must be cautioned that parsimony by itself does not prove that this preference did not evolve independently in *P. pustulosus* and *P. coloradorum* (Ryan 1996). But in the absence of other data allowing historical reconstruction, such as a fossil record, parsimony is an acceptable criterion to interpret data on evolutionary pattern.

If our interpretation of the shared ancestry of the preference for chucks in *P. coloradorum* and *P. pustulosus* is correct, we can ask when the preference for chucks arose relative to the chucks themselves. The molecular data suggest that the most recent common ancestor of these two taxa existed before the Middle American–Amazonian and the western Andes groups diverged (figure 19.2). If so, then this suggests that the preference for chucks existed prior to the evolution of the chucks. So it seems that males evolved chucks to exploit a preexisting preference for chucks. Analogous results in a variety of other taxa suggest that sensory exploitation is not merely restricted to this one group of frogs (e.g., crabs, Christy 1995; Christy and Salmon 1991; fish, Basolo 1990a,b, 1995a,b; Endler 1992; lizards, Fleishman 1992).

Although female túngara frogs prefer calls with chucks to those without chucks, they also have preferences for call components existing in other species but absent in their own. Call prefixes and multiple call groups exist in the western Andes species group (in *P. pustulatus* and *P. coloradorum*, respectively) but appear to be absent in the rest of the genus (figure 19.2). Túngara frog females prefer their own conspecific call to which a prefix from *P. pustulatus* has been added to the normal, whine only call. They also prefer calls in multiple groups, which their males do not produce but which are produced by male *P. coloradorum*, to single calls. Finally, female túngara frogs prefer the squawk of *P. freibergi* added to their own simple call to the same call without the squawk (Ryan and Rand 1993a,b,c); merely increasing the whine's duration does not duplicate this effect (unpublished data). Thus call components that evolved in other species enhance the attractiveness of the túngara frog's simple whine call.

Interestingly, these various call additions not only increase the attractiveness of the call analogous to adding a chuck but are as attractive to female túngara frogs as chucks (figure 19.3). Thus it appears that any of these three call additions that evolved first, a chuck, a prefix, or a squawk, might have been an evolutionarily stable strategy (ESS); that is, once fixed in the population none of these stimuli would have been invaded by one of the others, assuming other forces such as predation act on these components similarly. Furthermore, the chuck might be an ESS relative to other more complex stimuli since females did not exhibit a preference between a whine-chuck and a prefix-whine-chuck (figure 19.3).

Preexisting preferences are not restricted to stimuli that have evolved in closely related heterospecifics. Artificial stimuli added to the call also affect preferences. As mentioned above, in the natural call-energy distribution, a high-half chuck but not a low-half chuck increases call attractiveness, suggesting a role for the BP in the peripheral processing of the chuck. If the amplitude of the low-half chuck is increased to that of a full chuck, however, the low-half chuck increases call attractiveness; thus there is potentially plasticity in which of the two peripheral end organs could be implicated in chuck preferences (Ryan and Rand 1990). Furthermore, the precise acoustic structure of the chuck does not influence preferences in these phonotaxis experiments; white noise and single tones added to a simple whine with the amplitude of the full chuck also elicit enhanced call attractiveness from females (Ryan and Rand 1990; Wilczynski et al. 1995). It appears that female call preferences favored the evolution of the complex call in *P. pustulosus*, but that they might have been permissive relative to the types of sounds that increased call complexity. Aspects of the male's calling morphology, on the other hand, might have been more important in determining the precise sounds used to make the call more complex.

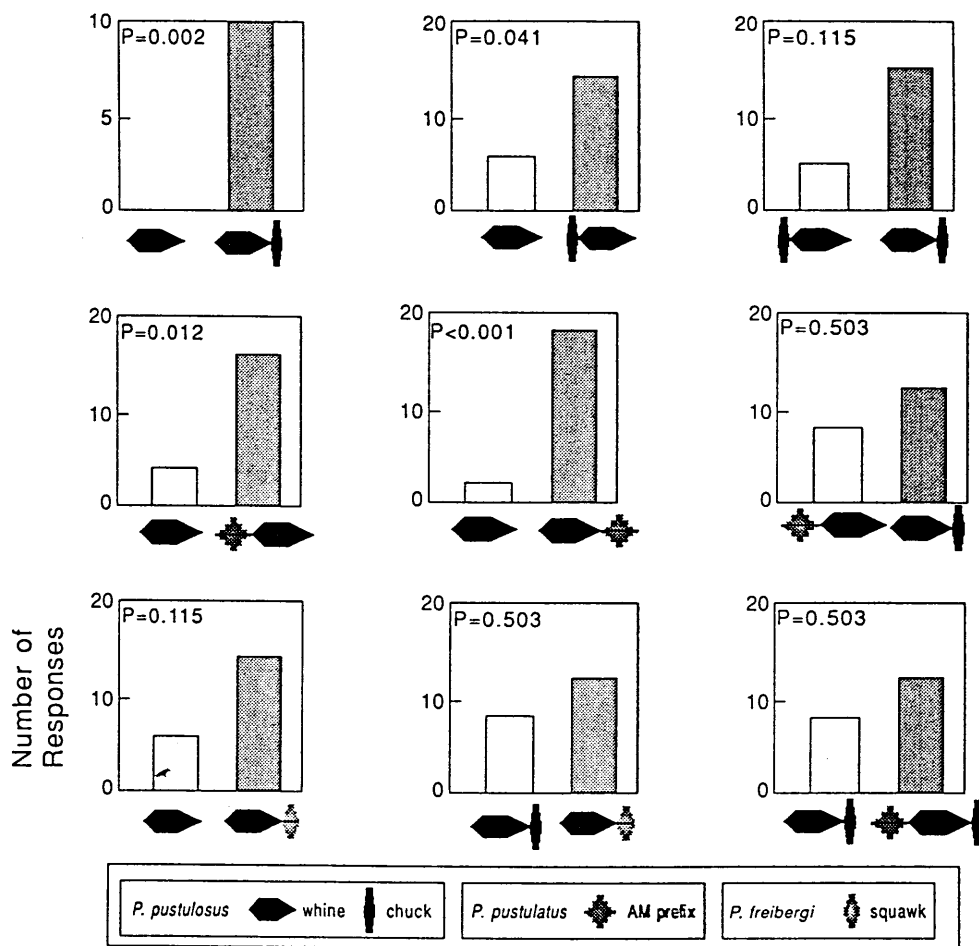


Figure 19.3

The results of phonotaxis experiments in which female túngara frogs were given a choice between pairs of stimuli that combined various call additions with the conspecific whine. In all experiments, except the whine versus whine-chuck with its sample size of 10, 20 females were tested. Exact binomial probabilities that test the null hypothesis of no preference are shown for each experiment.

It appears that there is a variety of stimuli that can enhance call attractiveness in túngara frogs, and that these stimuli are no less attractive than a natural chuck. Thus the female's acoustic preference space seems to be quite broad, encompassing a range of stimulus variation not exhibited by conspecific males, and even surpassing that exhibited by closely related heterospecifics. This observation has influenced how we now view the function and evolution of communication systems (see, e.g., Andersson 1994; Shaw 1995; Hauser 1996).

The concepts of sensory exploitation and preexisting biases are not nonadaptive (see, e.g., Dawkins and Guilford 1996; Ryan et al. 1998). If a preexisting bias had a direct, negative effect on female fitness, then selection should result in the evolution of the bias underlying the female preference (Kirkpatrick and Ryan 1991). Most hypotheses of adaptation, however, do not address the precise form of the phenotype that might result from selection. We suggest that knowledge of the evolutionary history of a lineage gives insights into those phenotypes that might plausibly evolve in response to selection. These studies provide an example.

Female túngara frogs gain some reproductive advantage by mating with larger males; more eggs are fertilized if the size difference between the typically larger female and the typically smaller male is minimized (Ryan 1985). To the extent that females are preferring lower-frequency chucks they are also preferring larger males (male size and chuck fundamental frequency are negatively correlated; Ryan 1980, 1985) and thus might be gaining a reproductive advantage derived from their call preference. If females had a different sensory bias and if males had a laryngeal morphology that biased them to other signal-phenotypes besides the chuck, then selection might still result in evolution and/or maintenance of female preference for sounds that signaled larger males. The resulting phenotype, however, could be quite different from the chuck; for example, males could add tones to the whine, or evolve visual displays that signaled body size. The inclusion of phylogenetic approaches that attempt to reconstruct evolutionary patterns of traits and preferences should be viewed as an addendum to and not an attack on hypotheses of adaptation.

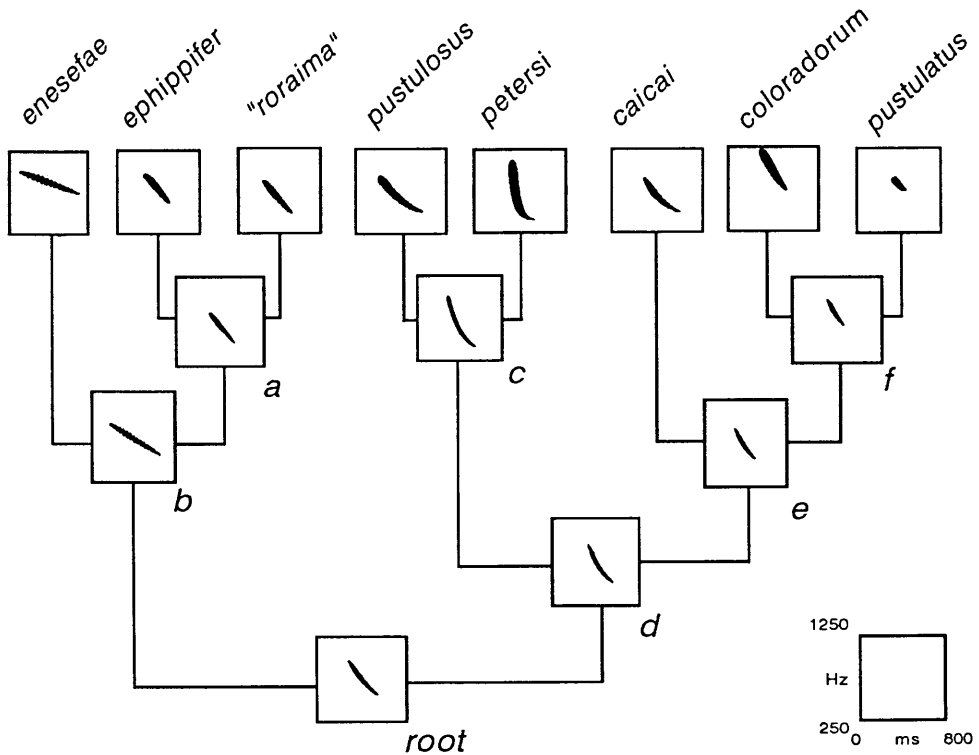
### **Conspecific versus Heterospecific Preferences**

By using phylogenetic information to design phonotaxis experiments, we have gained some insights into how sexual selection can influence the evolution of signal complexity. We have used this same approach to investigate the evolution of species-specific mate recognition in these frogs.

The túngara frog and all of the other species in the group produce whine-like frequency-modulated sweeps that appear to encode species-specificity and guide females toward conspecific males. Both *P. pustulosus* and *P. coloradorum* exhibit strong, near-unanimous preferences for the conspecific call over the calls of all the closely related species we tested. Females of both species, however, exhibit phonotaxis to some heterospecific calls when these calls are matched against white noise rather than the conspecific call (Ryan and Rand 1993b). These results suggest that like preferences for chucks, the female preferences for whines might be influenced by their historical legacy. Female call preferences are not deconstructed and reconstructed with every speciation event to result in a perfect match to the call of the newly evolved species. Instead, the precise forms that a newly evolved call and a call preference take are sensitive to their starting conditions or their ancestral states.

We utilized phylogenetic analyses to estimate the calls of ancestors of túngara frogs. We then asked if females discriminate between the calls of conspecifics and ancestors, and if calls of ancestors (when paired with white noise) are sufficient to elicit recognition from females (Ryan and Rand 1995). This analysis utilized differences in DNA sequences as an estimate of phylogenetic relationships and rates of divergence, quantitative measures of salient call parameters of all the extant species, mathematical algorithms that estimate ancestral characters by minimizing the amount of evolutionary change over the phylogenetic tree, and digital synthesis of the ancestral calls using the estimates for each call parameter (figure 19.4). We asked to what degree overall call similarity and phylogenetic relatedness might influence female phonotaxis. Overall call similarity is a phenetic measure of the acoustic similarity of the target call to the túngara frog call based on a principal component analysis of call variables. Phylogenetic distance is the degree of relatedness, based on purported changes in the DNA sequence between the túngara frog and the species or purported ancestor of the call being examined. These two variables were not highly correlated (Ryan and Rand 1995).

The phonotaxis studies using ancestral calls highlight the role of historical effects on the female's preferences for the conspecific whine. Although female túngara frogs discriminated between the calls of conspecifics and those of most ancestors, as they did between conspecific and heterospecific calls, they did not discriminate between the conspecific call and the call of their most recent ancestor (node c, figure 19.4) even though these two calls differ statistically in all call parameters (i.e., each of the ancestral's call parameters are outside of the mean plus 95% confidence limits of the analogous parameter for the túngara frog calls). These results suggest that, as with chuck and chuck-preference evolution, the evolution of the whine and whine-preference are not tightly correlated with one another. Furthermore, a multiple re-



**Figure 19.4**

The 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 (note that *P. freibergi* was not used in this analysis, cf. figure 19.2). "roraima" is an undescribed species. Sonograms illustrate the synthetic advertisement calls 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 (cf. FG, figure 19.5).

gression analysis of phylogenetic distance and overall call similarity regressed on phonotaxis preference showed that phylogenetic distance was a more important predictor of female preference than overall call similarity.

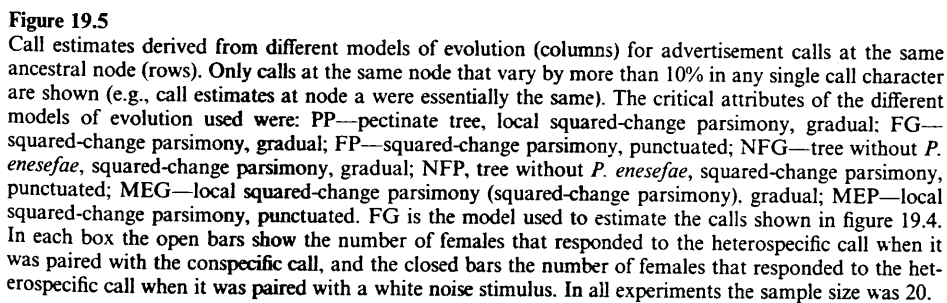
We also asked to what degree females would recognize a heterospecific call by presenting it in a phonotaxis experiment paired with white noise. A number of calls elicited statistically significant female phonotaxis. In contrast with the findings of the discrimination experiments, the overall call similarity between the conspecific call and the heterospecific call rather than phylogenetic distance better predicted female responses in the recognition experiments.

### How Robust Is the Phylogenetic Approach?

Behavioral interpretations based on phylogenetic hypotheses are only as strong as the phylogenetic foundation upon which they are based. One approach to estimating the robustness of such behavioral interpretations is to vary the assumptions underlying the phylogenetic hypothesis and ask to what extent changing such assumptions alters the behavioral interpretations.

In the previous study of female preferences for ancestral mating calls, we used one model of evolution to estimate calls at ancestral nodes. This model was based on the following characteristics: the most parsimonious tree topology (a bifurcating tree [two monophyletic groups] within the species group), the local squared-change parsimony algorithm, an assumption of gradual evolution, and inclusion of all three of the outgroups. We repeated the above procedure to determine how sensitive the results and conclusions of the previous study were to the particular model used to estimate the ancestral call characters (Ryan and Rand 1999). We asked: (1) if different models gave different call estimates for the same nodes; (2) if different call estimates at the same node were meaningful to females; and (3) if differences in female responses influenced conclusions from the previous study. We used seven different models that varied in at least one of the following parameters: tree topology (bifurcating versus pectinate tree), algorithms (local squared-change versus squared-change parsimony), tempo (gradual or punctuated evolution), and outgroups (two or three outgroup taxa used). In summary, we found that: (1) 51% of the 48 calls estimated at the various ancestral nodes differed by at least 10% in at least one of the eight call characters estimated at the same node; (2) 28% of the separate sets of female choice experiments revealed that different call estimates for the same node were perceived as meaningfully different by the females; and (3) these meaningful differences to females did not influence any of the major conclusions from the previous study (figure 19.5).

One conclusion of our previous study, for example, was based upon the female response to the ancestral call at a single node: the immediate ancestor of *P. pustulosus* and *P. petersi*, node c. In the previous study, females did not show a strong preference for the conspecific call versus the call estimated for the immediate ancestor (13 to conspecific versus 7 to ancestral call; two-tailed exact binomial probability  $P = 0.263$ ). In the other 13 tests in this study, there was always a significant preference for the conspecific call; in one case  $P = 0.045$  and in 12 cases  $P < 0.001$  ( $N = 20$  for all tests). The seven different models of evolution gave two different call estimates for node c. The pectinate tree gave one estimate, and the six models that varied in some assumptions but always used the most parsimonious tree topology





gave another estimate. The results of the G test, when not adjusted for experiment-wide error, suggest that the females responded to these two estimates differently in the discrimination tests. Using the call estimated from the pectinate tree, fewer females were attracted to the conspecific call. Only 6 females were attracted to the conspecific call, while 14 were attracted to the ancestral call ( $P = 0.115$ ), versus 13 to 7 in the previous experiment. Although the strength of female response differs between the two experiments, both fail to reject the hypothesis of statistically significant preference for the conspecific call. Although we would not argue strongly for a true lack of discrimination when  $P = 0.115$ , we point out that the trend in preference is actually in the opposite direction than would be predicted—more females were attracted to the call estimate for the immediate ancestor than to the conspecific call! This result offers an especially compelling argument for how the evolution of signals and receivers can proceed at different rates.

### How General Are Sensory Biases? A Neural Network Study

In the studies described above, the receiver (i.e., the female túngara frog) was an experimental constant and we measured its response to calls that varied over evolutionary time. We reached the conclusion that the receiver had biases toward other stimuli that might result from the historical legacy of this species. We took this approach one step further to explicitly address both how responses to heterospecific calls might be incidental consequences of conspecific recognition, and the degree to which historical effects influence these receiver biases. We did this by utilizing a technique from computational neurobiology: artificial neural networks.

Previously, Enquist and Arak (1993) showed that when a network was trained to a simple visual stimulus, such as a cross, there were a variety of hidden preferences for novel stimuli, including supernormal preferences. In another series of studies, Enquist and Arak (1994) and Johnstone (1994) showed that preference for signal symmetry can also emerge despite no specific selection on networks to recognize symmetric signals. The authors suggested that these results might be analogous to selection to recognize species-specific traits, and that they were in accord with studies of animal communication that showed hidden or preexisting preferences. There have been some criticisms of these studies, mostly based on the artificiality of the systems (Dawkins and Guilford 1995).

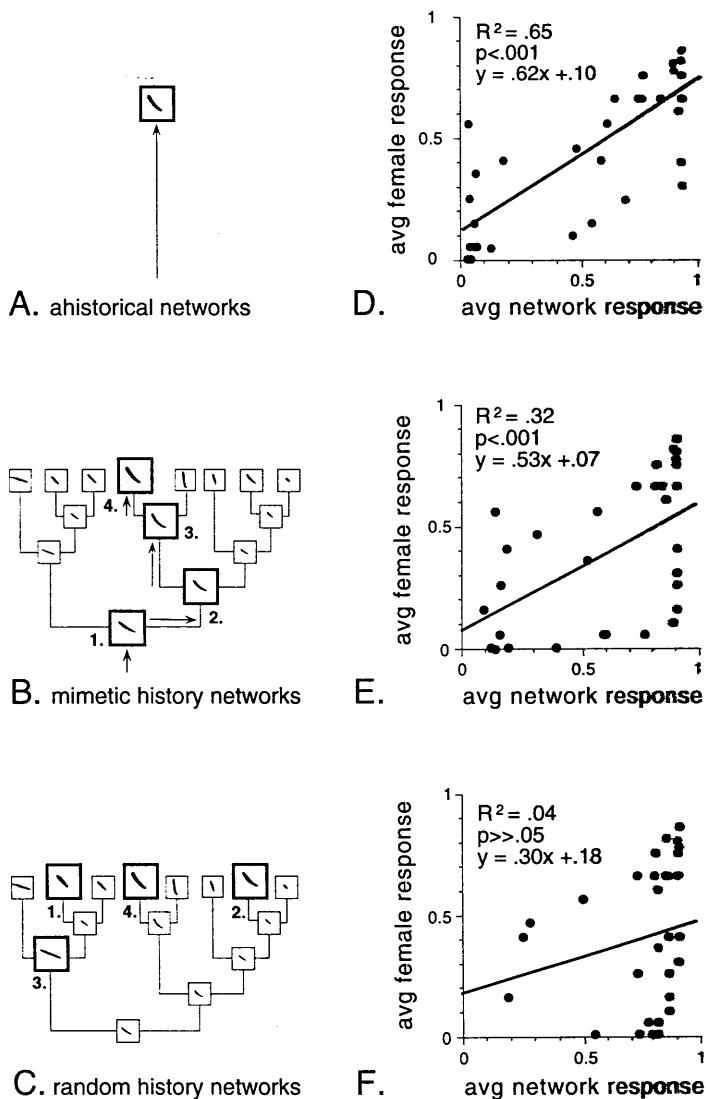
Phelps and Ryan (1998, in review) recently used neural networks to ask two questions: Would such artificial and relatively simple models predict the biases of female túngara frogs? And, would evolutionary history affect the models similarly to how it

affects female túngara frogs? We used a simple neural network that consisted of an input layer, a hidden layer, a recurrent layer, and an output (figure 19.6). As with other neural network models, the response of the network varied with the strength of the weights of the connections; these weightings were subject to selection, recombination, and mutation. We used synthetic calls that included all the heterospecific and ancestral calls used in Ryan and Rand (1995) as well as other stimuli with which females were tested. The other stimuli were from an ongoing study in which we tested female preferences to stimuli that were intermediate between the conspecific call and various heterospecific calls. These calls were digitized and presented to the networks in small time increments. The networks' performance was measured by comparing the strengths of response to the call and to noise; the greater the difference (favoring the call), the higher the value of call recognition.

Twenty populations of 100 networks per population were trained to recognize the túngara frog call. Most recognized the call at our arbitrary criterion after a thousand or so generations. The network that best recognized the call in each of the 20 populations was then tested with other stimuli (heterospecific, ancestral calls) with which female túngara frogs had already been tested. The results show that the response of the neural networks to these calls, with which they had no experience, predicts a significant and substantial amount of the variation in the biases of real túngara frogs ( $r^2 = 0.65$ ; Phelps and Ryan 1998).

These studies show that for a simple neural architecture, the task of learning a túngara frog call generates a variety of responses, some weak and some strong, for stimuli with which the network has had no previous experience. The emergence of hidden biases, as mentioned above, was also found in more artificial studies of neural network recognition of visual stimuli. The notable result from this study, however, is that these emergent biases of the artificial network explain a substantial proportion of the response biases of real females. The fact that these neural networks are far simpler systems than the frog's auditory system makes this result more rather than less interesting. In theoretical modeling in general, the simplicity of the model is a strength, increasing its generality and applicability. Although the frog's peripheral end organs and various auditory nuclei in the brain allow the latitude for various complex combinatorial analyses of signal structure, the neural net models suggest that these processes need not be overly complex. These models of course do not prove this assertion.

One limitation of the neural net model in the above simulations is that, unlike a real animal receiver, nets have no history of its ancestors having had to recognize different acoustic stimuli; that is, they are ahistorical. Our studies of preferences of real female túngara frogs for ancestral calls were designed to address the concern

**Figure 19.6**

The relationships between the responses of female túngara frogs and the responses of neural networks to a variety of heterospecific and synthetic stimuli. Networks were trained either only to the túngara frog call (A, D), to a series of calls along the evolutionary pathway from the most ancestral call to the túngara frog (B, E), or to a series of random histories that always ended with the túngara frog call (one example of which is shown in C, the responses of these networks compared to responses of females are in F).

that the history of the signal-receiver system could introduce hidden biases and pre-existing preferences into the receivers of extant species. We used the neural network studies to explore further the influence of history on female biases. We trained the neural networks to a series of calls representing our best estimate of the actual evolutionary history of the túngara frog call (the “mimetic” history) and compared how well these networks predicted female biases relative to a series of networks that were tested with random evolutionary histories.

In the mimetic-history simulations, networks were selected to respond to the root call (figure 19.6). When populations reached the response criterion, they were then trained on the call that was immediately descendent on the evolutionary pathway to the túngara frog—this is the node at the divergence of the two clades within the *Physalaemus pustulosus* species group. When those networks reached response criterion, they were trained to the call that was the immediate ancestor to *P. pustulosus* and *P. petersi*. Finally, these networks were trained to the túngara frog call. We also trained networks to random evolutionary histories (figure 19.6). As with the mimetic history, the networks were trained to a series of four calls, the last always being the túngara frog call. The previous three calls, however, were randomly chosen from the 15 heterospecific and ancestral calls in the call phylogeny. After the evolutionary simulations were completed, that is, were selected to respond to the túngara frog call, we measured their responses to the same set of heterospecific and ancestral stimuli that were used in the initial tests of the neural networks. We found that the networks with the mimetic evolutionary history better predicted the responses of female túngara frogs ( $r^2 = 0.45$  vs.  $r^2 = 0.11$ ; figure 19.6).

The neural networks that were trained only to the túngara frog call (ahistorical) and those that were trained to other calls prior to being trained to the túngara frog call (historical) were all eventually able to recognize the túngara frog call. The response biases in these networks, that is, how they responded to other calls, differed, however. A similar phenomenon might be expected in the evolution of receiver systems of real animals. For example, although most species show a preference for conspecific courtship signals over heterospecific ones, species might differ in the manner in which they respond to signals outside of the species’ range, and these response biases might be influenced by the receiver’s evolutionary history, that is, by the types of signals that the receivers of ancestors had evolved to decode. We make this interpretation because, as we discussed earlier, female call preferences are not deconstructed and reconstructed with every speciation event to result in a perfect match to the call of the newly evolved species. Instead, the precise forms that a newly evolved call and a call preference take are sensitive to their starting conditions or their ancestral states. This interpretation is supported by the results of the neural

network models that show that those neural networks with the history more closely resembling that of the túngara frog better predicts the responses biases of túngara frog females than those networks having the random history. We note that the neural network with the mimetic evolutionary history, however, did not predict female biases as well as did the ahistorical network in the initial study.

These studies show that neural network modeling can be used effectively to predict unintended response biases that occur in real animal communication systems. Furthermore, these models can be subject to evolution and the specific effects of history on these response biases can be quantified. With some obvious exceptions, such as information theory and game theory, animal communication has not benefited greatly from theoretical modeling. Artificial neural networks seem to offer the dual advantage of achieving generality by applying simple and artificial constructs to "hard" problems, as well as providing the advantage of hypothesis testing and assessing biological reality that comes from applying these models to specific communication signals and evolutionary histories.

## Summary

Our earlier studies of túngara frogs concentrated on the adaptive significance of the acoustic mate recognition system and the mechanisms that underlie receiver responses and signal generation. These studies provided substantial detail about the functions and mechanisms of communication in this system, but could not address questions about its evolutionary history. By deriving hypotheses of the phylogenetic relationships of the túngara frog and its close relatives, however, we were able to design experiments that explicitly tested hypotheses about evolutionary history. The phylogenetically based phonotaxis and neural network studies reveal that the evolutionary history of the communication system generates response biases to stimuli that are not part of the conspecific repertoire. In some cases, these response biases might be the starting point of sexual selection for new signals. We emphasize that these studies do not challenge the notion that female preferences and male signals are adaptive, but instead give insights into the phenotypes that are likely to evolve in response to selection. These insights could not have been gained without addressing the evolution of the communication system in an explicitly phylogenetic context. Since all communication systems have an evolutionary history, we believe a full understanding of any system can only be achieved when phylogenetic approaches are invoked.

Phylogenetic approaches by themselves, however, will offer little insight into the evolution of communication. Instead, we urge the development of more in-depth

model systems for a better understanding of animal communication. We feel that the success of the phylogenetic approach in our studies of túngara frogs depends, in part, upon our detailed understanding of the adaptive significance and sensory and morphological mechanisms underlying this communication system. Studies utilizing newly developed phylogenetic analyses in animal behavior will be lacking without otherwise detailed information about the behaviors in question. We will better understand the phenomenon of animal communication when we understand more systems that integrate Tinbergen's four questions of ontogeny, mechanisms, adaptive significance, and evolutionary history. Then we will have what we should be striving for—a general biology of animal communication (see also Hauser 1996).

### Acknowledgments

We appreciate logistical support from the Smithsonian Tropical Research Institute, financial support from the Smithsonian Institution's Scholarly Studies Program, the National Science Foundation (IBN 93-16185), and the University of Texas. We especially thank F. Bolonos, M. Briadorolli, M. Dantzker, L. Dries, J. Ellingson, M. Gridi Papp, A. S. Kapfer, N. Kime, D. Lombiedo, K. Mills, M. Monsivais, G. More, G. Rosenthal, S. Rodriguez, M. Sasa, Z. Tarana, and S. Yoon for assistance with the female choice tests.

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