

In the Light
of
Evolution

Essays from the Laboratory and Field

Edited by

JONATHAN B. LOSOS

ROBERTS AND COMPANY
Greenwood Village, Colorado

Sexual Selection: A Tutorial from the Túngara Frog

MICHAEL J. RYAN

If we survey the animal kingdom we are struck by a spectacular diversity of forms: birds and bats, ants and aardvarks, lions and lamprey. Each has evolved suites of traits that allow it to make a living in a range of environments while dealing with a stunning set of ecological challenges. A closer look, a look within rather than among species, reveals another type of diversity, that between the sexes. In humans the differences can seem so profound that some have suggested in jest that we must be from different planets—“men are from Mars, women are from Venus.”

Darwin suggested that many of these differences between the sexes arise from sexual selection, which is variation in fitness that derives from variation in an individual's ability to acquire mates. Sexual selection can act on both sexes, but the results often seem to be more profound in males. In most species an increase in the number of matings has a greater effect on male mating success than on female mating success since males have more gametes than females. Also, as females invest more in reproduction than males, there are more males who are free to mate at any one time, thus promoting competition among males for access to females.

There are numerous strategies that males employ to gain matings, and they tend to fall into the general categories of competition and mate choice. Males can physically control females directly, they can control access to resources females require, or they can physically dominate and intimidate other males. In such cases selection has resulted in the evolution of males' weapons.

The mating strategy that has received the most attention, however, is mate choice. In numerous species the mating decision is made mostly by the female. Males advertise their wares to attract and seduce females, and females contrast and compare males and then decide on an appropriate partner. This aspect of sexual selection is also an exercise in a fundamental problem of animal communication: how does a sender utilize a signal to manipulate the behavior of the receiver to its own benefit, and how does the receiver respond to the signal to promote its selfish interests? To enhance their attractiveness males have evolved some of the most striking phenotypic traits of the animal kingdom, including dazzling visual displays of butterflies, coral reef fishes, and birds; the sonorous



FIGURE 1 A calling male túngara frog.

serenades of crickets, frogs, and birds; and the odiferous emissions that in some species can travel for miles and in our own has given rise to the multi-billion-dollar perfume industry.

In this essay I review some details of a long-term study of sexual selection and mate choice. The focus is acoustic communication and its behavioral ecology. A subtext however is integration. This investigation is grounded in the behavior of communication but meanders through various aspects of its neural mechanisms and past evolutionary history. It should become obvious that to obtain a deep understanding of sexual selection and communication it becomes necessary to address and integrate these various aspects of brain, behavior, and evolution.

THE TÚNGARA FROG AND ITS BREEDING NATURAL HISTORY

There are about 6,000 species of frogs and nearly all of them have a mating call. Typically the call is produced by the male. It is a very conspicuous vocalization, can travel long distances and, most importantly, it is specific to its species. The call usually functions in maintaining spacing among males and in attracting females for mating. Females rely on the call to identify males who are conspecifics and thus appropriate candidates for mating, and we know a good deal as to how the female's auditory system achieves this outcome. But there is also variation in calls among individuals, just as there is variation in the color of a fish's display or in the length of a peacock's tail. This variation might be a target of sexual selection if variation in these traits among conspecific males influences their ability to achieve mates.



FIGURE 2 A male (top) and female túngara frog in amplexus.

The target of this study is the túngara frog, *Physalaemus pustulosus*. This is a small frog (ca. 30 mm in body length) of the family Leptodactylidae, which is fairly common throughout much of its range. The frog is found from north of Veracruz, Mexico, and its range extends south to Panama, where it crosses the Darien Gap into South America. It also occurs in the Magdalena Valley of Colombia, throughout the llanos of Venezuela, and onto Guyana Shield and the island of Trinidad.

Túngara frogs breed in temporary pools of water in variety of habitats from deep in the forest to large flooded fields, and are also common in areas of human disturbance. Most of these studies have taken place in Panama at the facilities of the Smithsonian Tropical Research Institute and with the late Stan Rand, who was a staff scientist there. In Panama, the túngara frogs breed during the rainy season, which is from April to November. Males call from stationary calling sites and most of the calling occurs between 1900 to 0200 hours (FIGURE 1). Females are attracted to the calls and they are able to move among calling males without interference from them. At some point a female will initiate mating with a male by making physical contact with him, at which point the male clasps the female from the top, a condition known as amplexus (FIGURE 2). The newly formed pair usually leaves the water for a few hours and then returns to deposit its eggs. In this behavior túngara frogs are a bit unusual. As a female extrudes eggs from her cloaca, a few at a time, the males picks up the eggs with his hind feet and, while fertilizing them, beats the jelly matrix into a sparkling white foam (FIGURE 3). The eggs remain in the foam for a few days before they hatch into tadpoles and fall into the water below. They metamorphose from tadpoles into froglets in a few weeks and then in six months or so they are sexually mature and ready to breed themselves.



FIGURE 3 A male (top) and female túngara frog constructing a foam nest.

THE COMPLEX CALL

The male's primary sexual display is his call. And male túngara frogs call a lot, thousands of times in a typical night. None of this is unusual for a frog. What is unusual is the male's mating call. It has two components, a whine that can be followed by up to seven chucks. All calls have a whine. This part of the call starts higher in frequency and sweeps to a lower frequency in about 300 ms. It sounds as if it is being made by a video game. The second component is a chuck. It sounds like a very loud click. It is shorter than the whine, about 45 ms, and it has many frequency harmonics. Not all calls have chucks but males can add chucks to a whine seemingly whenever they please. We refer to the whine-only call as the simple call and whines followed by chucks, as complex calls.

When males are calling by themselves they tend to produce only a whine, whereas in choruses almost all of the males produce calls with chucks. Males increase the number of chucks in response to playbacks of conspecific calls. The more complex the call, the more likely the male will increase his call complexity. Males usually increase the number of chucks in a stepwise manner, going from 0–1–2–3 and then back down again, adding or subtracting one chuck at a time. Although they can add up to seven chucks, about 55% of the calls produced are simple calls, 40% have one chuck, and most of the rest have two chucks.

Why do males increase their call complexity? Since a primary function of male calling is to attract females, an obvious hypothesis is that females are more attracted to calls with chucks. One of the advantages of studying frogs is that they provide a robust and repeatable measure of female preferences. In most

frogs, females do not call except for a relatively inconspicuous release call they produce at times. The only reason a female approaches a male call, a behavior called phonotaxis, is to assess the caller as a potential mate. A phonotaxis experiment takes advantage of this natural behavior in a controlled laboratory experiment.

To test female preferences, we collect females at breeding sites in Panama who are in amplexus but have not yet produced a foam nest. We place a female under a cone in the center of a large acoustic chamber. She is in the dark and her movements are monitored remotely with an infrared video camera in the ceiling of the chamber. There are two speakers on opposite sides of the arena and they broadcast calls to the female at the male's natural calling rate, about one call per two seconds. The two calls are out of phase with one another, thus they do not overlap in time. The cone is lifted remotely and the female typically approaches and comes into contact with one of the speakers. The female must move 1.35 m to reach the speaker—scaled to human body size that would be about 80 m. Under such situations a female readily approaches a simple call, the whine only. This is true whether the whine is paired with noise being played from the other speaker or if the other speaker broadcasts calls of other species.

We used these tests to determine if complex calls are more attractive than simple calls. We presented females with a whine-only from one speaker and a whine-chuck from the other speaker. Females showed a preference for the complex call whether we used natural mating calls or calls that we synthesized on the computer to mimic the natural calls. We have a larger sample size using synthetic calls which shows the strength of the preference to be about 0.85 in favor of the complex call and 0.15 in favor of the simple call (3,135 versus 527); thus, females show a near six-fold preference for the complex call. Clearly males have evolved complex calls in response to sexual selection by female choice.

HOW TO MAKE A COMPLEX CALL

As we said above, males evolved a complex call because it makes them more attractive to females. But what evolved and how do these complex calls arise? Behavior is a motor output that involves a suite of morphological, neural, hormonal, and genetic components. In the case of a call, we might expect drastic changes in an acoustic signal to be accompanied by changes in the hardware that produces it; that is, the larynx. Also, if we can understand how the larynx changes to accommodate complex calls, we also would like to know when in evolutionary time this occurred. These questions require details of both anatomy and phylogenetics.

I will address phylogenetic relationships of the túngara frog and its close relatives in more detail below. Suffice it to say that of the other members of

the *Physalaemus pustulosus* species group, only the two species that are closely related to túngara frogs, *P. petersi* and *P. freibergi*, add secondary components to the end of the whine. Also, in both species the secondary components resemble chucks, although we sometimes refer to them as squawks to indicate the fact that they differ from the chucks in some of their acoustic aspects. Interestingly, some populations of *P. petersi* produce complex calls and others do not. This is in contrast to *P. pustulosus*, in which all males in all populations appear able to produce complex calls.

The anuran larynx consists of a pair of arytenoid cartilages that rest on the hyoid plate. Inside each cartilage there is a vocal fold. Much as in a mammalian larynx, air passes through the larynx and in doing so vibrates the vocal folds, and it is this vibration that produces changes in the ambient air pressure that we and the frogs perceive as sound. Túngara frogs have a relatively large larynx and the vocal folds of the túngara frog have elaborate extensions called fibrous masses. These masses are large and extend into the two bronchial processes towards the lung. It had been suggested that this mass is kept from vibrating during the whine, but that once the mass vibrates, a chuck results.

The variation in laryngeal morphology among frogs in the *Physalaemus pustulosus* species group matches the variation in the ability to produce complex calls. *P. coloradorum* does not produce complex calls and it has a small fibrous mass and a small larynx. Populations of *P. petersi* that do not produce complex calls have a larynx and a fibrous mass similar in size to that of *P. coloradorum*, while in the *P. petersi* that produce complex calls, the size of the larynx and the fibrous mass is similar to that of the túngara frog. Thus, there is a correlation between vocal output and vocal morphology, which leads us to think that for complex calls to evolve, males must evolve both a larger larynx and a larger fibrous mass.

Correlation does not demonstrate cause and effect. Experimental manipulations, however, have shown conclusively that the fibrous mass is implicated in chuck production. Recently, we recorded male túngara frogs calling in the field in Panama. We then anesthetized males and cut off the fibrous masses. Some males were anesthetized and the surgery replicated except for the final step—the masses were left intact. After the males recovered from the surgery, they then called. The sham operated males still produced chucks, but the males in the group in which the masses were removed failed to produce chucks. These males tried to make chucks. They increased call amplitude as they do at the end of the whine, but when they did so there were no chucks, only an increase in amplitude of the end of the whine. These manipulations demonstrate cause and effect for the role of the fibrous mass in chuck production.

Not only do males without fibrous masses lack chucks, but their feeble attempts at making their call more attractive—that is, their “pseudo-

chucks"—do not suffice in the ears of the females. When females were given a choice between the pre-treatment complex call of a male, and a post-treatment "pseudo-complex" call of the same male, females preferred the former. Whines with "pseudo-chucks" were not as attractive as whines with real chucks. No matter how hard a male lacking a fibrous mass tries to make his call more attractive, he just can't do it. Males need to have the hardware for complex calls, not just the motivation to make them.

TO CHUCK OR NOT TO CHUCK: IS THERE A TRADEOFF?

Males can make complex calls, females have a six-fold preference for complex calls, and the larynx evolved in response to this selection to allow the males to produce complex calls. Then why not produce complex calls all the time? All aspects of the phenotype have costs and benefits, and they usually evolve to the extent that costs and benefits are balanced. But what are the costs of calling, and is it more costly to make complex calls?

Any behavior involves expenditure of metabolic energy. When male túngara frogs call, there is a four-fold increase in the rate of oxygen consumption and thus substantial energy expenditure. Thus, calling is a very expensive venture. A male's rate of oxygen consumption, however, does not depend on the number of chucks he produces, only on the number of whines. Thus, calling costs, but complex calling does not cost more than simple calling, at least when we consider metabolic costs. But there are other costs to consider.

Any communication signal increases the conspicuousness of the sender to the receiver. If not, communication could not take place. But there are other receivers besides the intended ones. Eavesdroppers lurk everywhere. These unintended receivers exploit the signals intended for others as cues to locate the sender either to eat them or to parasitize them.

The bat, *Trachops cirrhosus*, is found throughout much of the neotropics and is relatively common in Panama at many of the sites where túngara frogs breed. This bat is unusual in that frogs form a substantial part of its diet. But it is their foraging behavior that makes the bats most interesting. These bats do not need to rely on echolocation to find frogs; instead they localize the frogs by homing in on the frog's mating call. These bats have a series of anatomical adaptations that enhance their sensitivity to the frequencies of frog calls, most all of which are below 5 kHz, while still maintaining their acute sensitivity to their echolocation calls, which range from 50–100 kHz.

Frog-eating bats eat substantial numbers of túngara frogs, more than 6/hour in one study on Barro Colorado Island in Panama (FIGURE 4). Do bats impose a cost of calling on males that can explain why males do not always produce



FIGURE 4 A frog-eating bat with a túngara frog. (Alex Baugh).

complex calls? To test this idea we placed two speakers in a large flight cage that contained a frog-eating bat. First, we broadcast the simple whine of the túngara frog from one of the speakers. The bat immediately flew from its perch towards the speaker. This confirmed that the bats can use the whine as a cue to find the frog. We then broadcast a simple call from one speaker and a complex call from the other. As with the female frogs, the bats were more likely to fly to the speaker with the complex call. These results suggest strongly that males vary the complexity of their calls to balance the costs of attracting predators with the benefit of attracting females.

Why do bats prefer complex calls? It does not indicate a better meal for the bats: males producing chucks are not larger absolutely or relative to their length. But the number of chucks a male produces is indicative of the density of frogs within a 1 m radius of the calling male. Thus, preferential attraction to complex calls also attracts the bats to areas of higher prey density. In addition, complex calls could be easier for the bats to localize.

In 1955 Peter Marler suggested that there was a strong congruence between structure and function of bird vocalizations. For example, in some instances birds want their calls to give away their location, as when they are mobbing predators, but not in other instances, as when they are giving alarm calls. Mob-

bing calls are usually short abrupt sounds that tend to be easier to localize, and alarm calls are longer and more tonal and prove more difficult to localize. The correlation between localizability and signal structure makes sense based on how mammals and birds are thought to localize sounds, by comparing differences in the sound between the two ears, but not necessarily how frogs do it. Interestingly, the whine resembles many avian alarm calls, while the chuck resembles mobbing calls. Thus, we predicted that chucks should make the call easier to localize.

Rachel Page conducted experiments with frog-eating bats to test this hypothesis. She placed small speakers out of sight of the bat and measured its accuracy in locating speakers that broadcast either simple calls or complex ones. She conducted these experiments in an outdoor flight cage in the forest on Barro Colorado Island, so the bats were in an acoustic environment similar to that in which they forage. In the simplest experiments, with frog calls broadcast continuously, no added background noise, and no obstacle to flight, the bats were able to locate both simple and complex calls with high accuracy. If the frog calls ceased when the bat left the perch, or background noise was broadcast during the experiment, or the bat had to navigate an obstacle course en route to the call, then the bats located the complex calls more accurately than the simple calls. If the task was made too difficult, by including two or more of the treatment variables, the bats either did not respond or their accuracy in locating both the simple and the complex calls was similarly poor. These results show that chucks, which make the call more attractive to female frogs, also make the call easier to locate by frog-eating bats. Furthermore, It seems that the whine and the chuck share structural similarities with the alarm calls and mobbing calls of birds: longer tonal signals (whines and alarm calls) are more difficult to locate, while the shorter more abrupt ones (chucks and mobbing calls) are easier to locate.

As if the túngara frogs did not have enough troubles with the bats tuning into their channel for sexual communication, it only gets worse. Panama is replete with mosquitoes, and it was their role as vectors of human disease that contributed to the French never being able to complete the Panama Canal. But mosquitoes have a lesser known cousin that is more of a threat to túngara frogs, the blood-sucking flies of the genus *Corethrella*. These flies are abundant and can be seen tormenting male túngara frogs, whose only defense is for the frogs to try to swat them from their heads as they call. Ximena Bernal showed that the flies walk around the back of the males until they reach their nostrils, where they take a blood meal (FIGURE 5). *Corethrella* is known to be attracted to frog calls, and the frog calls do not even need to be from local species. These flies, more than half-a-dozen different species, are attracted to calls of túngara frogs. Many more flies are caught at traps baited by a túngara frog chorus than



FIGURE 5 Blood-sucking flies on a túngara frog. (Kathrin Lampert)

a nearby silent bait. And, like the female frogs and frog-eating bats, the flies are more attracted to calls with chucks versus calls without chucks. We can add blood-sucking flies to frog-eating bats as exerting selection that keeps túngara frogs from always making complex calls.

THE CHUCK AS A TARGET OF SEXUAL SELECTION

Darwin got into trouble with some of his staunchest supporters, such as Alfred Wallace, when he formulated the theory of sexual selection. The problem was not that Darwin suggested that males evolved weapons to battle for access to females. The issue was that of female choice, and the notions that females made the mating decision in some species and that males evolved elaborate, often life-threatening, traits to entice them. Some have suggested that this notion of female control of the mating decision was anathema to Victorian views of the traditional role of women, and thus socially a difficult concept for some scientists of the time to accept.

We now know that in many species a male's sexual advertisement indicates his species membership. Females are usually under strong selection to mate with conspecific males since matings with heterospecifics can often go awry in several ways: eggs are not fertilized; development does not proceed properly; offspring have low survivorship; adults are infertile. If the only function of a mating call is to indicate species status, but there is no variation among conspe-

cific males in their attractiveness, then there is no sexual selection. Remember, sexual selection generates variation in the ability to acquire mates. An important criticism of sexual selection for 100 years was that there was no evidence that females attended to variation in traits among conspecific males. This was the main issue that I set out to remedy in the early studies of the túngara frogs.

The ideal demonstration of sexual selection by female choice involves several criteria. There must be (1) variation in male mating success; (2) part of that variation must result from female mate choice; (3) there must be a correlation between male mating success and a male trait; and (4) it must be demonstrated that female choice is influenced by variation in this trait.

In túngara frogs, the whine is necessary and sufficient to elicit mate attraction from females. If a female is presented with a chuck alone, she does not approach it. All other closely related species make a whine-like call, and the túngara frogs prefer their whine to the whines of other species. Thus, there is little question that preference for the conspecific whine results in females mating preferentially with conspecifics.

The only function of the chuck is to make males more attractive to females. There seems to be no doubt that chucks evolved in response to sexual selection by female choice. All males, however, appear to be able to produce chucks. Do any aspects of the chucks generate sexual selection, variation in mating success, among males? The answer appears to be yes.

I monitored male mating success in a túngara frog population for 152 nights. This consisted of measuring the size of every frog that arrived at the breeding site and marking it with a numbered tag and a unique series of toe clips. Each hour from 1900–0200 h I noted which males and females were at the pond, which males were calling, and which frogs were mating. During that time there were 617 males who came to the breeding site. On average males were at the breeding site for ca. 7 days over a 43-day time span. There was a total of 751 matings over the 4,456 frog-nights. The best predictor of a male's mating success was the number of nights he was present at the pond. Among the males at the pond, however, females are able to choose with whom to mate. A female often sits in front of one male while he calls and often moves among a number of males before she initiates mating by making physical contact with the male. Sometimes she will return to a male she has previously sampled, and in laboratory experiments she often will change her choice if the calls between two speakers are switched.

Larger males were more likely to mate. The call seemed like an obvious indicator of male body size. In many animals, the size of the larynx and the vocal cords increase with body size. These larger structures vibrate more slowly than smaller ones, and thus result in lower frequency sounds. In the túngara frog, there was a significant negative correlation between male size and the frequency

characteristics of the chuck. Larger males made lower-frequency chucks. This suggests, but does not prove, that female choice for larger males results from preferences for lower frequency chucks.

I constructed synthetic calls by adding sine waves of pure frequencies until a reasonable facsimile of the call was produced. In each of these synthetic calls the whine was identical, but chucks had either lower or higher frequencies. Females were presented with pairs of calls, and over a substantial range of variation in chuck frequencies they preferred the lower-frequency call. Thus, the preference for larger males seems to derive from the preference for lower-frequency calls.

Of course, there could be other, redundant information about male body size available to the female. Some of the frequency characteristics of the whine and the chuck are correlated; generally, both whines and chucks of larger males are lower in frequency than the calls of smaller males. Although the whine does not predict male body size as well as the chuck does, females also prefer lower- to higher-frequency whines. In addition, females could use visual cues. We know that the inflation of the male's vocal sac enhances the attraction of the call. We surmise, but do not yet know, that larger males have larger vocal sacs. It is possible that vocal sac size influences her choice as well.

In 1980, when these results of female mate choice based on variation in chuck frequency were published, this was the first study to experimentally manipulate variation in a trait among conspecific males and to show that this variation influenced female mate choice. Since then there have been hundreds of demonstrations of sexual selection by female choice. Darwin's second great theory, sexual selection, has been vindicated and is now a part of the canons of evolutionary biology after being in disrepute for more than 100 years.

WHY DO FEMALES CHOOSE?

Darwin did not offer much of an explanation as to why females prefer display traits of some males to others. He suggested that, like humans, other female animals might have an aesthetic sense. Interestingly, he did not give a "Darwinian" explanation for female choice, one grounded in females gaining adaptive advantages. In fact, Darwin was "out-Darwined" by Alfred Wallace, who did offer some possible explanations based more firmly in Darwinian theory, though perhaps more fanciful in its biological grounding.

As illustrated above, males pay a price for entering the sexual selection lottery. Female choice can also be costly. Searching for mates expends energy, and there is an energetic cost to the neural processing of calls. In addition, searching for mates exposes females to predators. We assume this danger increases with the female's visibility to predators, thus searching on darker nights should be safer than searching on nights with bright moon light. Túngara frog females

show about a six-fold preference for a whine-chuck to a whine when tested under dark conditions. When tested under conditions of total darkness versus dim light, female move more often, choose more often, and are more likely to choose a softer complex call over a louder simple call in dark versus dim light. All of these behavioral differences are consistent with the theory that females compromise the quality of mate if they choose when they are in greater danger from predators.

If females pay a cost to choose, there should be some offsetting advantage. There have been several competing hypotheses for the evolution of female mate choice: direct benefits, good genes, and Fisher's theory of runaway sexual selection. The latter two hypotheses are based on genetic aspects of mate choice and have been quite controversial, sometimes obscuring the fact that in many mating systems, females gain direct benefits from their mate choice. The túngara frog is one of these examples.

As far as we know, all male túngara frogs can add chucks. A male's propensity to add chucks is not indicative of his body size, nor of his relative physical condition. As males add chucks in response to calls of other males, the number of chucks produced by a male is indicative of the relative density of males. Thus, being attracted to complex calls brings females into areas where there are more potential mates from which to choose. But as mentioned above, the male's chuck is also an indication of his body size.

In most frogs, fertilization is external, taking place while the pair is in amplexus with the male hanging on to the female's back, and in most frogs females are larger than males. Thus, when a female prefers a larger male to a smaller one, as do females of many species of frogs, they prefer a male who is closer to them in size. In túngara frogs, and in some other species, more eggs are fertilized when the size differences between a female and her mate is smaller rather than larger. This is due to the juxtaposition of the female and male during external fertilization. If the male is too small, his sperm will be released on the female's back and fertilize fewer eggs, whereas when the cloacas are in juxtaposition, nearly all of the eggs are fertilized. Thus, female mate choice for larger males in túngara frogs is adaptive (below we will ask if it is an adaptation) in that it directly increases female reproductive success.

Different selection forces that favor the evolution of female mate choice could act simultaneously or sequentially. We have no direct test that female choice in túngara frogs favors males with higher genetic quality for survivorship. Frogs continue to grow after they reach sexual maturity, thus larger males should be older, and being older and larger is evidence that an individual has been able to survive. But male growth rates are very variable and body size might not be a very good predictor of age. In a book about honest signaling, Amotz Zahavi suggested that male túngara frogs that avoid bat predation are

demonstrating their good genes because they have lived longer. But this has never been tested directly. That is, we have no data to discriminate between the hypotheses that males that have not been eaten by bats have “good genes” rather than just “good luck.”

Another type of “good-genes advantage” is based on genetic complementarity rather than genetic superiority. By choosing a genetically less similar individual, as long as he is of the same species, a female can avoid some of the deleterious effects of inbreeding. A number of studies of mate choice based on olfactory cues in mammals have shown this to be true, and Bruce Waldman has shown that toads achieve the same end by relying on acoustic cues.

Túngara frogs do not exhibit mate choice based on genetic similarity, or at least not the way we tested it. We estimated the relatedness among male and female túngara frogs using molecular genetic markers. We showed that there was no relationship between the similarity of the calls of males and how related they were to one another. Also, female preferences for male calls were not biased by the relatedness of those males to her, and the male and female in a mated pair were no more or less related to each other than they were to other frogs that mated that night.

Why do female túngara frogs choose mates? As with most sexual animals, their choice delivers a conspecific rather than a heterospecific male for mating, and that is a good thing. Their preference for chucks guides the female to areas with more males from which to choose, and their choice of lower-frequency chucks results in them choosing larger males who are a “better fit” in amplexus and fertilize more eggs. There might be other advantages, some of them genetic, we have yet to uncover. But the main advantages seem to be direct effects on a female’s reproductive success rather than indirect effects on the genetic quality of her offspring.

We will next delve into the mechanisms underlying these preferences for complex calls and use those data to address the question of not just the current effect of female preferences but how they evolved.

HOW DOES THE BRAIN CHOOSE?

Niko Tinbergen suggested four major topics that can be addressed in animal behavior: development, mechanisms, adaptive significance, and evolutionary history. Ernst Mayr similarly pointed out that in evolutionary biology, one can address questions about proximate or ultimate causes. In the fields of behavioral ecology and evolution, questions about mechanisms are often ignored. But this is done with some peril. Ignoring mechanisms deprives us of knowing the complete biology of the organism. In addition, it does not allow us to understand how adaptations really work and why one set of adaptations rather

than another has evolved. With these goals in mind, we have endeavored to understand something about how the frog's brain processes acoustic signals and why it biases the response of females to some calls in favor of others.

The design of the frog's auditory system seems to be more directed toward detecting conspecific mating calls than to other sounds in the environment. Those other sounds can be important, but perhaps not as important as knowing the identity of who is calling.

Unlike mammals, who have one inner ear organ that is receptive to air-borne sound, frogs have two, the amphibian papilla (AP) and the basilar papilla (BP). There are many differences between these two end organs; the most important is that the AP is most sensitive to lower-frequency sounds, those below about 1,500 Hz, and the BP is sensitive to higher-frequency sounds, usually above 1,500 Hz. Robert Capranica suggested that these two end organs act as a pair of filters matched to the frequency characteristics of the conspecific mating call. If the call has two major peaks of energy in the spectral range they will tend to match the tuning of both the AP and the BP. If the call has only a single peak it will match the tuning of either the AP or the BP. In these cases the frogs still have both papillae and they are both tuned. Carl Gerhardt and Joshua Schwartz reviewed the literature on inner ear sensitivities and call frequency in frogs and showed that Capranica was correct.

Túngara frogs follow this pattern. Both the whine and the chuck have broad frequency ranges. The typical whine has most of its energy below 1,500 Hz with a dominant frequency of about 700 Hz, while a typical chuck has most of its energy about 1,500 Hz and a dominant frequency of about 2,500 Hz. These frequency peaks of the call coincide fairly well with the neurophysiological recordings of the tuning of the two end organs. With Walt Wilczynski and James Fox, we showed that on average the AP is most sensitive to 700 Hz and the BP to 2,100 Hz. These data support the matched filter hypothesis, and are further supported by phonotaxis experiments. We deconstructed synthetic versions of natural calls, piece by piece, until we were left with what were the sections of sound that were both necessary and sufficient to elicit phonotaxis response from females. The critical part of the whine was the part that contained the frequencies around 700 Hz and the critical part of the chuck was the upper harmonics from about 1,500-3,000 Hz. Thus, for the call to be perceived as a complex call, for the female to regard it as more attractive than a simple call, there must be sequential stimulation of these two frequency peaks. Thus, at a mechanistic level, one of the reasons females prefer a whine with chucks is that these calls sequentially stimulate the AP and BP more effectively than a whine alone.

We also addressed the preference for lower-frequency chucks at a mechanistic level. As noted above, the average tuning between the BP and the average dominant frequency of the chuck is not perfect; the average call is a bit higher

than the average tuning. Thus, lower-frequency calls, which are produced by larger males, will more closely match the tuning of the BP. We generated a computer model of the túngara frog's BP and showed that lower-frequency chucks stimulated it more than higher-frequency chucks. This might be one of the reasons why females are preferentially attracted to lower-frequency chucks.

CHUCKS AND PREFERENCES FOR CHUCKS: DID THEY COEVOLVE?

We now know that female túngara frogs prefer calls with chucks and lower-frequency chucks. This choice is adaptive as females gain a reproductive advantage by choosing lower-frequency chucks because it delivers larger males who fertilize more eggs. At the mechanistic level, the tuning of the BP seems to contribute to both preferences. Thus, one might conclude that túngara frogs evolved their BP tuning because of the adaptive benefits it provides in mate choice.

Earlier I mentioned a dichotomy in levels of biological analysis, that between proximate and ultimate questions. Within the latter there is also a dichotomy; it is that between current adaptive significance versus past evolutionary history. The question of whether a behavior is adaptive, whether it generates a fitness advantage relative to alternative behaviors, can be tested with experimental or correlational studies in the field or laboratory. Through these approaches we know female preference for low-frequency chucks is adaptive because it enhances her reproductive success. But whether it evolved to serve the function of choosing larger males, whether that preference in females arose as an adaptation, is a different question because it is historical in nature. If preferences for chucks and low-frequency chucks originally evolved as an adaptation, then we expect that the preferences and the calls evolved together. One alternative, referred to as sensory exploitation, is that there were preexisting biases in the female's sensory system to respond to lower-frequency sounds and when males evolved these calls they were immediately favored by female choice. How can we decide between these two alternatives?

These hypotheses can not be tested solely in the laboratory or in the field. Instead we need to recreate the historical pattern by which traits and preferences evolved. To do so, we first determined the phylogenetic relationships of the túngara frog and its closest relatives. Then for each species for which we have the information, we noted whether males produce chucks, whether females have the tuning of the BP that matches the chuck, and whether females showed a behavioral preference for the chuck.

When we conducted this analysis *P. petersi* and *P. freibergi* were considered a single species, there were three other species in the species group (more species have since been discovered), and we included three species in the genus but not

in the species groups for additional comparisons. Of all these species, only the túngara frog and *P. petersi* produce chucks. We thus conclude that the chuck evolved in the common ancestor of these two species.

As noted previously, the tuning of the BP contributes to the preference for chucks. The species that do not produce chucks usually have little energy in the frequency range that would stimulate the BP. We asked if the BP tuning that matches the chuck's frequencies evolved when the chuck did—that is, in the common ancestor of túngara frogs and *P. petersi*. The answer is no. The BP tuning in all of the frogs is very similar. Thus, we can reject the hypothesis that the chuck and the tuning of the BP which matches the chuck coevolved. Instead, the chucks evolved to match the preexisting tuning of the BP.

We can ask the same question about the preference for the chuck, not just the tuning that matches it. *P. coloradorum* occurs only in Ecuador on the western side of the Andes. They do not add chucks to their whines. We digitally excised three chucks from the call of a túngara frog and added it to a *P. coloradorum* call. Female *P. coloradorum* were given a choice between the typical whine-only call and the artificially created complex call. The female *P. coloradorum* preferred the complex call to the simple call. Although the preference for complex calls could have arisen independently in *P. coloradorum* and *P. pustulosus*, this seems less likely than the hypotheses that the preference is shared through a common ancestor. The latter hypothesis is favored by parsimony; that is, it requires us to posit fewer evolutionary changes and supports the hypothesis of sensory exploitation. Thus, we argued that it appears that male túngara frogs evolved traits to exploit preexisting preferences in females.

Recently, however, Santiago Ron conducted some similar studies with *Physalaemus* (= *Engystomops*) on the western side of the Andes. He tested *P. coloradorum* with stimuli a bit different from ours—he used one chuck, while we used three chucks, and details of the whine differed. Although he showed a numerical female preference for the complex call, the preference in his test was not significantly different from random nor, on the other hand, was it significantly different from our results. More decisively, he showed that two other species of *Physalaemus* did not even show a trend for a preference for complex calls. Although it is still clear that the chuck of *P. pustulosus* and *P. petersi* evolved to match the neural tuning of the BP, which is conserved across the *P. pustulosus* species group, these recent results suggest that the preexisting behavioral preference for the chuck is not distributed throughout all the close relatives of the túngara frog.

The studies of mate choice in túngara frogs show that females gain a reproductive advantage by preferring chucks and low-frequency chucks. The comparative studies suggest that the biases in the frog's auditory system existed prior to and thus promoted the evolution of chucks, while, on the other hand,

the preexisting behavioral preference for the chuck might exist in one species, *P. coloradorum*, it is not widely spread throughout the species group. These results do not change how we view the current function of complex calls and preferences, but it does influence our interpretation as to how they evolved.

CONCLUSION

These studies of túngara frogs have shown that variation in mating calls among conspecific males is salient to females. The females act on this variation, they prefer complex calls and lower-frequency complex calls with the result that they mate with males who fertilize more eggs. We have also shown that there is a conflict between sexual selection and natural selection on call variation. Although complex calls are favored by female choice, frog-eating bats and blood-sucking flies generate counter-selection on males that produce complex calls. These studies were generated by detailed field observations of the animal's natural history combined with laboratory experiments that tested hypotheses generated from correlational data collected in the field.

The information on the frog's auditory system has provided critical insights on how mate choice operates. It has told us how the basic characteristics of the tuning of the inner ear are matched to the frequency characteristics of the complex call. It also has suggested how the slight mismatch between BP tuning and chuck dominant frequency might explain the female preference for lower-frequency chucks, and where in the brain this information is synthesized and emerges into a decision.

The information on the phylogenetic relationships of the túngara frog to its relatives provides the platform for synthesizing the data on brain, behavior, and evolution. It suggests that the match between the chuck and the BP did not coevolve, but that the chuck evolved later to exploit the tuning characteristics of the inner ear. The same, it seems, might be true of the preference itself.

Biology has continued to become more integrative, and this is especially true of animal behavior. This integration is critical for we can not understand adaptations if we do not know how a phenotype is adaptive, and we can not fully appreciate how adaptations evolve if we do not cast our view toward evolutionary history itself. As I have shown here, Darwin was correct in his basic premise about sexual selection by female choice. Demonstrating this, however, required an understanding of brain, behavior and evolution and how they interact.

SUGGESTED READINGS

- Andersson, M. 1994. *Sexual Selection*. Princeton University Press: Princeton, NJ.
- Darwin, C. 1871. *The Descent of Man and Selection in Relation to Sex*. Murray: London.
- Gerhardt, H. C., and F. Huber. 2002. *Acoustic Communication in Insects and Anurans*. University of Chicago Press: Chicago.
- Kokko, H., R. Brooks, M. D. Jennions, and J. Morley. 2003. The evolution of mate choice and mating biases. *Proc. R. Soc. London B* 270:653–664.
- Ritchie, M. G. 2007. Sexual selection and speciation. *Ann. Rev. Ecol. Evol. Syst.* 38:79–102.
- Ryan, M. J. 1985. *The Túngara Frog: A Study in Sexual Selection and Communication*. University of Chicago Press: Chicago.
- Ryan, M. J. 1998. Receiver biases, sexual selection and the evolution of sex differences. *Science* 281:1999–2003.