A male túngara frog, his vocal sac inflated, calls to females in a Panamanian pond.
Michael J. Ryan

The Panamanian Love Call

What awaits the calling male túngara frog—a sexual rendezvous or sudden death?

by Michael J. Ryan

Every spring, many parts of the United States explode into a cacophony of whistles, groans, burps, and shrieks. These sounds indicate that the local frog fauna has established residence in nearby ponds, and that males are advertising their readiness for their yearly sexual rendezvous. Thus the call of these animals is sexual in nature, and is produced, for the most part, by males to attract females and to ward off other males that might have stealing a mate in mind. Since the biological significance of this calling behavior is seemingly straightforward, there should be little difficulty in understanding its evolutionary history. On the contrary, my research into the communication behavior of a neotropical frog, the túngara frog, has shown that while the many compromises and contingencies that shape the evolution of this behavior are far from obvious, these factors can be documented, thereby leading to a better understanding of the evolution of behavior.

The animal kingdom is resplendent with various morphological, physiological, and behavioral traits that enhance an animal's ability to survive. The widespread occurrence of these traits, or adaptations, together with observations of the resemblance between parents and offspring, was what led Darwin to formulate his theory of natural selection. How then did Darwin explain the collection of elaborate and sometime bizarre traits associated with animal courtship, such as frog calls, that hardly enhance survival and may even promote an early death? Darwin believed the evolution of these traits occurred under the influence of sexual selection. He proposed that if a characteristic enhances an individual's (usually a male's) ability to gain access to a mate, even at some cost to survival, it should occur more frequently in the population through evolutionary time. An intriguing proposition since selection is normally thought of as a force that results in the evolution of traits that enhance survival, not traits that are maladaptive for survival.

One characteristic of frog calls, well documented during the past three decades, is the species specificity of the mating call: females prefer the calls of their own species to the calls of other species. Studies of the neurobiology of frogs and toads have also shown that the frog's auditory system is "tuned" so that it detects the conspecific mating call better than other types of sounds. The advantage of the female's discriminating behavior is obvious—mating with males from other species usually does not result in viable offspring. A female making this kind of mistake is wasting her reproductive investment, and thus we expect strong selection for female attraction to the conspecific call.

During a study of bullfrog mating behavior, however, I noticed that calls differed among males. I became intrigued by the possibility that this variation was not meaningless noise contained within the species-specific vocalization but, in fact, might influence the female's choice of a mate. It was this question that brought me to the Smithsonian Tropical Research Institute in 1978 to study the mating and communication behavior of the túngara frog on Barro Colorado Island, in the middle of the Panama Canal. This study encompassed almost three years of field research between 1978 and 1981.

Túngara frogs (*Physalaemus pustulosus*) are small, a little more than one inch in body length, and weigh less than one and one-half grams. They are fairly nondescript brown frogs that are common throughout much of Central America. In Panama they breed during the rainy season, which extends approximately from April to December. Among frogs, those with long breeding seasons are characterized by mating systems in which males typically remain stationary and vocally advertise their presence to females. Túngara frogs are no exception. These frogs breed in stagnant pools of water that range in size from the hoofprint of a tapir to a large flooded field. Much of my investigation was conducted in a small cement pool, constructed in the mid-1960s by A. Stanley Rand, a research scientist at the Smithsonian Tropical Research Institute, for his earlier studies of túngara frogs. The frogs have been breeding there naturally ever since.

Males arrive at the breeding site around dusk and immediately begin to call, some producing more than 7,000 calls in a single night. The males are almost evenly spaced around the perimeter of the pool, and any male intruding on a calling male's personal space is threatened by aggressive, mewlike calls. If the intruder is not discouraged, a wrestling bout often ensues. Although physical combat is not frequent, on several occasions it resulted in the death of one of the participants. As with many frogs, females come to the breeding site only on the nights they mate. When a female arrives she spends a fair amount of time, sometimes hours, swimming among the chorusing frogs and sitting stationary in front of calling males. One female sat in the corner of the breeding site in front of a calling male for approximately twenty minutes, swam away to position herself in front of another call-

Less than one and one-half grams in weight and a little more than one inch long, a túngara frog perches comfortably on the head of a giant toad, Bufo marinus.

Merlin D. Tuttle; Bat Conservation International

ing male about three feet away for the same length of time, and eventually returned to her original position where mating took place. Males do not attempt to mate with the female during these sojourns. Instead, mating is initiated when the female makes physical contact with a calling male, the male then clasping the female from the top.

An analysis of 751 matings by 617 measured and tagged individuals revealed that larger males were more likely to mate. The pair remain clasped, or in amplexus, until the calling subsides, usually about midnight. Often they leave the water and wait on the shore until this time. Sometime after midnight, the pair return to the water, settle on a site, and construct their foam nest; this site usually bears no relationship to the place from which the male was calling.

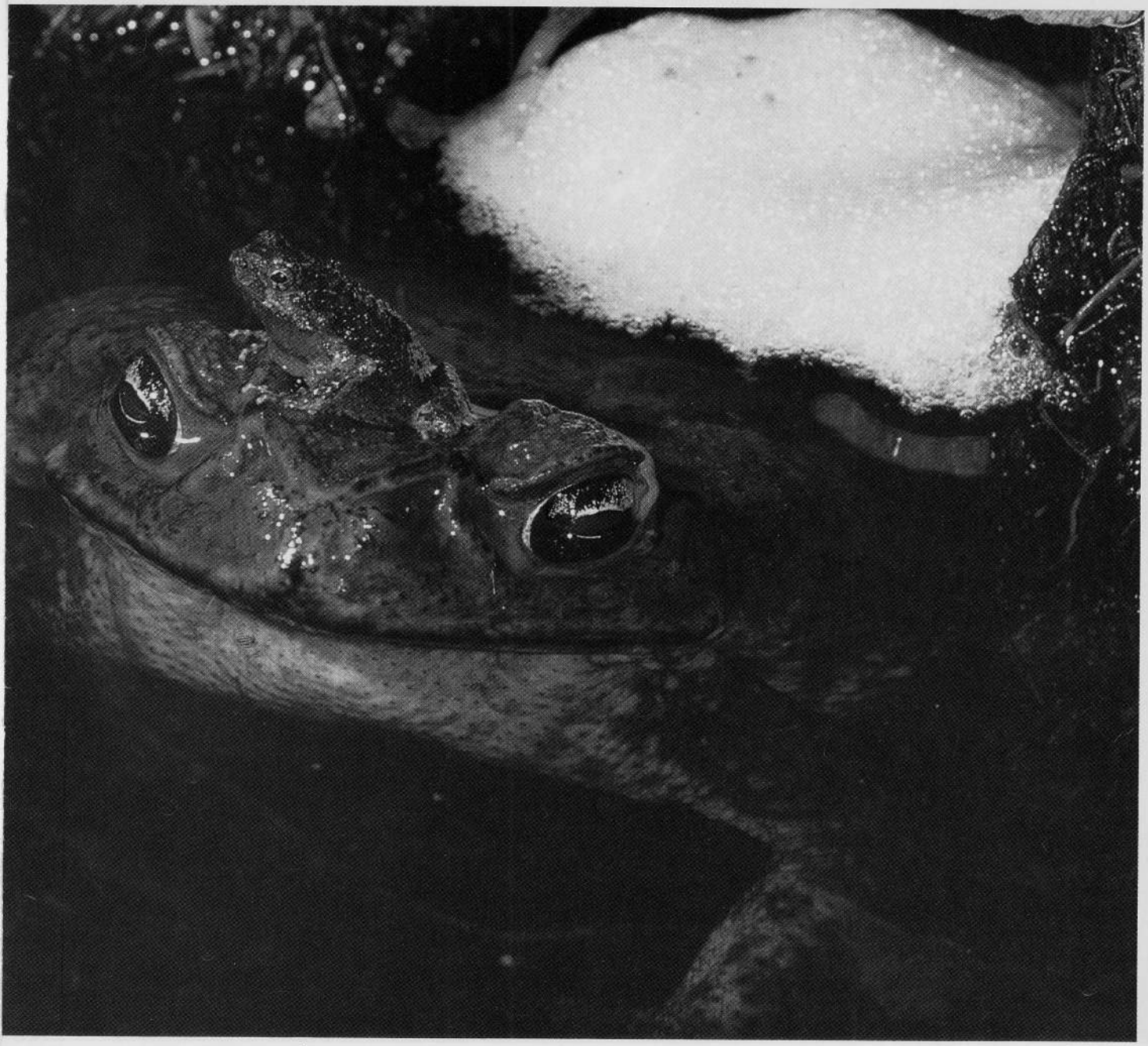
Nest construction is an arduous task. The female passes her eggs, a few at a time, from her cloaca. The male grasps them with his hind legs, moves them past his cloaca where fertilization takes place, and, in a quick, beating motion, fluffs the jelly matrix surrounding the eggs into a foam, much the way we beat egg white into a meringue. Nest construction takes about an hour, and the male is visibly tired toward the end. Both frogs then abandon their future offspring, which will hatch into tadpoles in a few days. If they are lucky enough to avoid the dragonfly nymphs and other voracious predators inhabiting the pools, the tadpoles will metamorphose into small frogs in about six weeks time.

Females appear to choose larger males as mates, and I wanted to know how they do this. Since males have little of value to offer the females besides themselves—they do not defend any resource of potential use, such as a nesting site—some aspect of the male's characteristics must be important. And since the males put so much time into calling, that seemed a likely place to start. The complex call of the túngara frog consists of two components: one whine and from zero to six chucks (much of this complexity was deciphered by Stanley Rand in the 1960s). The whine is almost half a second long and resembles the sounds emanating from a Star Wars video game. The chuck is of much shorter duration and sounds like an abbreviated burp. Males always produce a whine and females are attracted to a loudspeaker broadcasting only the whine. Chucks never occur by themselves in nature, but if dissected from a call and broadcast to frogs, they do not elicit a response from either sex. The whine, then, appears to be necessary and sufficient for species recognition. Is the chuck, perhaps, a meaningless frill? Not quite. When a male is part of a chorus he usually produces chucks, and larger choruses are characterized by more chucks. Males also add chucks to their calls as a female approaches. In one case, a male increased the number of chucks in his call from two to six in a matter of seconds—the largest number of chucks I recorded.

These observations suggested that the social milieu of the animal influences the complexity of its call. Rand demonstrated this by presenting a male with calls of varying complexity. As the broadcast call increased in number of chucks, so did the male's response. The reason males add chucks is also clear: when presented with two calls, one without chucks and one with chucks, females preferred the latter.

But these results presented a paradox. If males were at the breeding site to attract females, why didn't they always produce calls that females found most attractive? The benefit of such a behavior is evident—a greater chance of mating. Perhaps there is a cost to producing chucks. One obvious cost is energy. Although at the time of my study no one had ever measured the amount of energy used by a vertebrate in sexual display, many studies had assumed that the cost was significant. Together with Terri Bucher and George Bartholomew of the University of California, Los Angeles, I investigated the energetic costs associated with calling. We did this by placing a male in a small respirometer. To stimulate the frog to call, a speaker broadcasting calls was placed outside the respirometer, and a microphone recorded the number of calls produced. After about thirty minutes of calling, we measured the amount of oxygen that was depleted in the respirometer and found that calling was a very expensive venture for the male. Metabolic rate increased about 500 percent above what it was when the frog was at rest. However, no more energy was used to produce calls with chucks than calls without chucks. So, while there is a substantial cost to calling, there is no increase in cost for making those calls more attractive.

In the 1950s, Peter Marler, now at The Rockefeller University, suggested that some calls of distantly related bird species have evolved similar structures because they serve similar functions. Alarm calls warn others of the presence of a predator without revealing the warner's location. Mobbing calls elicit assistance from others by revealing the signaler's location.



Thus alarm calls should be ventriloquial, while mobbing calls should be easy to locate. These calls each share a similar structure. But the alarm call is also amazingly similar to that of the whine, while the mobbing call resembles the chuck. Rand suggested that just as predation had molded the structure of these calls in birds, it played a similar role in the call of the túngara frog. At the time he made this suggestion, however, there were no known predators of frogs that used the frog's call for locational cues.

That changed quickly when Merlin Tuttle of the Milwaukee Public Museum netted a bat with a frog in its mouth on Barro Colorado Island. Tuttle suggested that this bat (*Trachops cirrhosus*), which has become popularly known as the frog-eating bat, not only eats frogs regularly but locates them by homing in on their mating calls. Tuttle returned to Barro Colorado Island in 1980 to begin studies of bat–frog interactions. We then teamed up to investigate the possibility that the bats homed in on the call of the túngara frog and, especially, the possibility that they preferred calls with chucks.

Frog-eating bats eat túngara frogs more than they eat any other species of frog. At one pond, Tuttle, our assistant Cindy Taft, and I observed ninety-five instances of predation during fourteen and one-half hours of observation—6.6 frogs eaten per hour. We captured some of these bats, placed them in a large flight cage, and found that they were readily attracted to túngara frog calls that were broadcast from speakers. Echolocation signals and vision played no role—the bats were attracted to the calls alone—a behavior then thought unusual. Subsequent studies, however, especially ours of the African bat *Cardioderma cor,* demonstrated that a variety of bats use prey-generated cues while hunting. In the flight cage, we presented bats with a choice between calls without chucks and calls with chucks. Of the five bats tested, all were more likely to fly to the speaker producing the calls with chucks. We also tested this hypothesis in the field. Placing two small tape recorders in areas that we knew the bats frequented, and using a night-vision scope, we counted the number of passes the bats made over the recorders broadcasting each call type. Again, there was an overwhelming prefer-

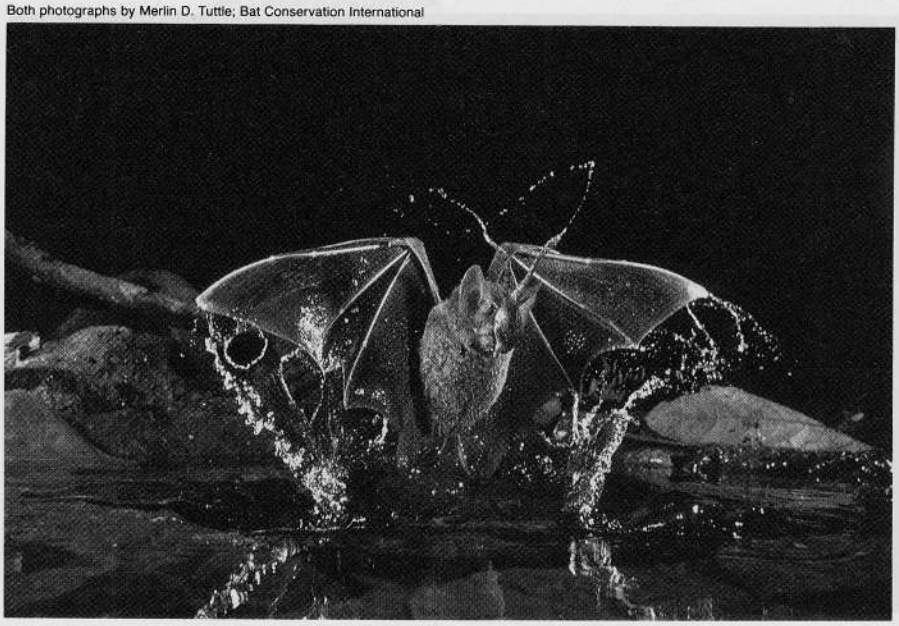
ence for calls with chucks. These results demonstrate the predicament faced by males trying to attract mates. They can increase their attractiveness to females at the cost of a higher predation risk or they can play it safe but take the chance of not being selected as a mate. The variable complexity of the mating call in these frogs seems to have evolved to allow males to strike a compromise between the benefits of attracting a mate and the costs of attracting a predator.

If the calling of a single male will attract a predator, what about the cacophony of an entire chorus, which can consist of more than 400 males vocalizing their sexual intent? Would more calling males attract more bats? And how does chorus size influence a male's ability to attract a mate? Is he less likely to succeed when he is one of many attempting to attract a female's attention? These questions led us to quantify the costs and benefits of chorusing behavior for this species of frog. We monitored predation rate and mating success at a single pond where chorus size fluctuated each night, and found that it was advantageous for male frogs interested in both mating and surviving to join larger choruses. The number of frogs eaten by bats did not increase with chorus size; therefore, the actual risk of predation for any individual frog was lower in larger choruses. The larger choruses appear to offer some predator protection, probably through safety in numbers. A male's mating success also does not suffer when he is a member of a larger aggregation of calling males. On average, males are more likely to mate when part of a larger chorus because proportionately more females are present at larger choruses. Why this is so is not clear.

But just what is it about a male, besides his complex calls, that makes him more attractive to females? Since larger males are more likely to be chosen by females, I asked what characteristics of the male would result in this type of mating preference? In his study of bullfrogs (*Natural History,* April 1979), Richard Howard also showed that females preferred to mate with large males and that these large males defended higher-quality territories, used as oviposition sites, than did smaller males. In his study, females could have been assessing territory quality or some

Using a frog's mating call as a locational cue, a frog-eating bat swoops down to capture a calling male, left, and flies off with the prey in its mouth, below.

Both photographs by Merlin D. Tuttle; Bat Conservation International



other aspect of the male's characteristics. One reason I decided to study túngara frogs is that males do not defend resources used by females, thus the female's choice should be influenced only by the male's characteristics.

Given the demonstrated costs in terms of energy and predation incurred by calling males, the mating call is of obvious importance in mate attraction. There was reason to believe that the call might contain information about male body size, and that female choice might be based on this information. The frequency, or pitch, of a vocalization is determined largely by the mass of the vocal cords. This is true for both frogs and humans. For example, it is the hormone-induced increase in vocal cord mass that causes the sometimes embarrassing voice change experienced by young men as they reach puberty. Biologists had long noted that in frogs there was a correlation, both among and within species, between the pitch of the mating call and the frog's body size. This is true simply because larger frogs have more massive vocal cords. When I examined the mating calls of 132 males, there was a significant correlation between body size and the pitch of the chuck. Together with the observation that females are more likely to choose larger males as mates, this suggested that females are more attracted to lower-frequency calls, and it is this preference that results in the mating success of larger males.

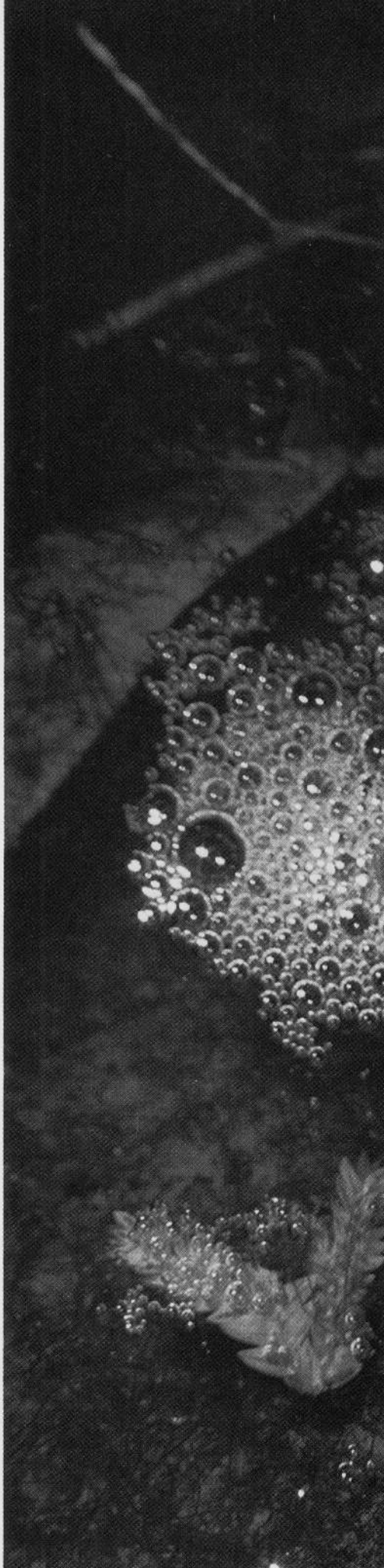
One of the advantages of working with frogs is the ability to test female choice through playback experiments. I used synthetic calls in order to control for variation in the whine. When I presented females with simultaneous calls containing a high-frequency chuck and a low-frequency chuck, females were attracted preferentially to the latter. I then used two calls that were closer in frequency and the females still preferred the lower-frequency call. Thus, experimental evidence supports the hypothesis that females mate with larger males because they are preferentially attracted to their calls.

These results demonstrate that there is selection, through the action of female mate choice, on the male's call. They do not demonstrate, however, that the calls have evolved in response to this selection. Evolution need not take place in response to selection. Although selection can be measured within one generation, evolution is a historical process. If generation times are long, as they are for almost all vertebrates, it is not possible to witness evolution in action. Instead, a retrospective analysis is necessary. If sexual selection has influenced the evolution of the call of túngara frogs, then these frogs should have a call frequency that is lower for their body size than that of closely related frogs, provided that sexual selection has not acted similarly in these other species. The latter assumption is probably valid. Only túngara frogs have the two-component call—thus only in this species can selection act on a component of the call that is used for intraspecific interactions without affecting the call component used for species recognition.

I compared the call of túngara frogs with the calls of twenty-nine species from the same subfamily (Leptodactylinae). First, the relationship between body size and frequency was determined for all species. This yielded a prediction of the fre-

Below: Prior to fertilization, the male clasps the female from above with his underside resting against her back. Right: Still clasping the female, the male uses his hind legs to beat the jelly matrix surrounding the eggs into a foamy nest.

Both photographs by Michael J. Ryan



quency of the call for any species given its body size. Species were then compared by determining the magnitude by which their frequency departed from that prediction. Túngara frogs have a frequency that is the lowest for its body size of all the species that were examined; in fact, it is 252 percent lower than that predicted by the body size–frequency relationship for the subfamily. If the analysis is restricted to only members of the genus *Physalaemus*, túngara frogs still have the lowest frequency call for their body size—314 percent lower than predicted. Clearly, these frogs have evolved very-low-frequency calls.

Although call frequency has decreased without a concomitant change in body size, vocal cord mass must have increased to achieve such a result. Together with George Drewry and W. Ronald Heyer of the Smithsonian Institution's National Museum of Natural History, Rand examined the vocal morphology of this species. Túngara frogs have achieved this low-frequency call by the addition of two large, pendulous masses that hang from the vocal cords, thus drastically enhancing the mass and lowering the frequency of vibration. Robert Drewes of the California Academy of Sciences and I have surveyed the vocal morphology of frogs in the species group to which túngara frogs belong. Although other members of the genus may possess additional masses associated with the vocal cords, we only find the addition of very large, pendulous masses in túngara frogs. Peacock tails and deer antlers are usually cited as examples of elaborate traits that have evolved under the influence of sexual selection. Although I am admittedly biased, these traits seem to pale in comparison with the spectacularly pendulous masses, resembling a pair of testes, that hang from the vocal cords of a male túngara frog.

The seemingly simple behavior of a male frog calling to attract a mate appears to be the result of a series of complex, interacting factors. The case of the túngara frogs demonstrates how the true evolutionary history of a behavior can only be glimpsed by a combination of approaches that consider mating behavior, energetics, predation, phylogeny, sensory physiology, and morphology. The various disciplines within biology are only artificial constructs in the pursuit of science and are not adhered to by real organisms evolving in the real world. The necessity of a broad, integrative approach is becoming clear not only in studies of behavioral evolution but within the whole field of evolutionary biology. □