How to Design Experiments in Animal Behaviour*

12. Male Frogs Sing for Sex but Why Don't They Do Their Best?

Raghavendra Gadagkar

Charles Darwin proposed a separate theory of sexual selection, as distinct from his theory of natural selection, to account for adaptations that confer success in finding a mate, which may sometimes be quite the opposite of what is best for survival. Darwin's proposal that females have a sense of beauty and choose mating partners that appear beautiful to them was met with much scepticism. But today we have a rather detailed understanding of what animals find beautiful and why. In this article, I will describe a few very simple experiments performed by Michael J. Ryan, in collaboration with A. Stanley Rand, herpetologist extraordinaire and Merlin D Tuttle of the Bat Conservation International fame, that laid the foundation for our current understanding of the meaning and evolution of beauty. Studying the túngara frog on Barro Colorado Island, a research station of the Smithsonian Tropical Research Institute in Panama, they showed that (1) male túngara frogs can produce both simple calls, consisting of just a whine, or complex calls in which one or more chucks are added to the whine, (2) female túngara frogs have a decided preference to mate with males giving complex calls, (3) males are nevertheless reluctant to add chucks to their calls and generally do so only when they hear other males calling, and (4) the local predatory fringe-lipped bat also has a decided preference to eat males giving complex calls. Male túngara frogs thus face a trade-off between sex and survival. These experiments not only answered the question of why males don't do their best when it comes to singing, but they also set the stage for many more sophisticated investigations

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*Vol.25, No.6, DOI: https://doi.org/10.1007/s12045-020-0998-7

that have led to an understanding of how and why natural selection has favoured this particular sexual aesthetic in the frogs and this particular culinary aesthetic in the bats.

Keywords
Túngara frogs, mating call, *Physalaemus pustulosus*, sexual selection, female choice.

The Cost of Sex

Nearly all multicellular organisms practice sexual reproduction—the union of male and female haploid gametes to form a diploid zygote in every generation. Very few multicellular species routinely use the ancient practice of asexual reproduction, although many have retained it for use under certain conditions. This is something of an evolutionary paradox because sexual reproduction is costly—it requires two parents to produce offspring. Unlike in most sexual species where about half the population consists of males, in an asexual population all individuals are identical (not differentiated into males and females, but traditionally referred to as females because they give birth to offspring) and reproduce all by themselves. This two-fold cost of sex is the subject of much research and many theories. That apart, there is also the problem in sexually reproducing species that males and females have to find each other in every single generation. Every time I see an obscure insect I can't help marvelling at the fact that at least some members of that species must have found sexual partners in every generation without fail, for the millions of years that the species has been around. Not surprisingly, sexually reproducing organisms go to a great deal of trouble to ensure the union of males and females.

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It is a curious fact of the biology of sexual reproduction that the burden of finding a sexual partner falls more heavily on males than on females. Because females generally produce relatively small numbers of costly eggs and devote relatively more time and effort in caring for their offspring, they are the limiting resource. Males on the other hand, generally produce large numbers of cheap sperm, spend relatively less time and effort caring for their offspring and can, therefore, mate with many more partners than is possible for a typical female. The mass of a nutrientfilled human egg, for instance, is about a million times the mass of a human sperm, which contains little more than a haploid set of chromosomes! In this asymmetric situation, success in finding a sexual partner is relatively more uniformly distributed among the females but is highly skewed among the males—while most females will find a sexual partner, some males will find many partners and many males will inevitably be left without any. Males, therefore, have to work much harder to find a mate—they either have to fight with other males in order to win in the male-male competition and/or they have to advertise themselves to the females and emerge successfully through the filter of female choice. Darwin realised that what it takes to succeed in the sexual market is not always the same that is required to succeed in the struggle for survival. He, therefore, proposed a separate theory of Sexual Selection, to account for adaptations that confer success in finding a mate, which may sometimes be quite the opposite of what is best for survival.

Perhaps not so surprisingly, male-male competition for females has been much better studied while female choice has been rather neglected, at least until recently. Darwin's proposal that females have a sense of beauty and choose mating partners that appear beautiful to them was met with much skepticism, including by Alfred Russel Wallace, the co-discoverer of the principle of natural selection. That females can indeed choose, and have a sense of beauty is the theme of this article, but of course, we will mainly use it as a context in which to continue to explore the practice of science and illustrate more ways of designing experiments and doing low-cost research. In particular, we will dwell on how male frogs advertise their beauty and what female frogs find beautiful.

Frogs may seem an unlikely subject to explore beauty. Our common perception of frogs is perhaps not one they would be proud of! There are over 7000 species of frogs, and a great deal is known about them—at least biologists have found them worthy of attention. In 2007 Professor Kentwood D Wells of the University of Connecticut in the USA published a 1400-page book entitled *The Ecology & Behaviour of Amphibians* [1], which has

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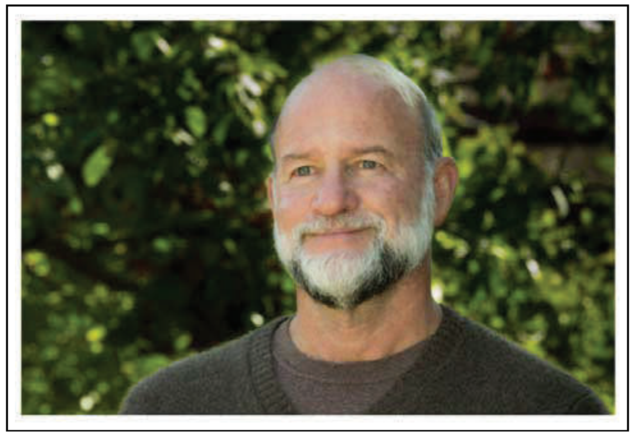

Figure 1. Michael J Ryan (1953–). Born to a truck driver in Bronx, New York as the oldest of eleven siblings, Michael J Ryan rose to become the Clark Hubbs Regents Professor in Zoology at the University of Texas, Austin, USA. Michael Ryan has spent a lifetime studying the túngara frog in an attempt to understand what is beauty and how and why some traits come to be regarded as beautiful while others not. His research, culminating in the theory of sensory exploitation, has shown that while males have exploited the sensory biases of females over evolutionary time, at the present time females are the biological puppeteers, making the males sing exactly what their brains desire—a paradigm shift in the study of sexual selection. The latter part of *A Taste for the Beautiful: The Evolution of Attraction* [3] was written after Mike suffered a serious accident which has left him in a wheelchair with a spinal cord injury.

been described as "the definitive one-volume work on the world's amphibians". Professor Wells begins his book with an amazing quote by Carl von Linnaeus, the father of taxonomy: "These foul and loathsome animals ... are abhorrent because of their cold body, pale colour, cartilaginous skeleton, filthy skin, fierce aspect, calculating eye, offensive smell, harsh voice, squalid habitation, and terrible venom; and so their creator has not exerted His powers to make many of them."

This reputation of amphibians apparently did not deter Professor Michael J. Ryan (*Figure* 1), one of the protagonists of this article, to choose a frog to explore beauty. Clearly, he made a wise choice as his 40-year research [2] on the Túngara Frog has led recently to the publication of his fascinating book, *A Taste for the Beautiful: The Evolution of Attraction* [3].

The Túngara Frog

Physalaemus pustulosus is a small, brownish frog, about 3 cm in length (length of frogs is usually measured as the distance between the tips of their snouts to the end of their vents, i.e. not measuring the legs) (*Figure* 2). Its pustular skin, which gave it the specific name *pustulosus*, and the bumps on its skin make it look more like an 'ugly' toad than a 'beautiful' frog. In fact, Ryan went to Panama to study the much more beautiful red-eyed tree

Figure 2. A calling male túngara frog, *Physalaemus pustulosus*. Notice the large vocal sacs. (Photo: Ryan Taylor).

frog *Agalychnis callidryas* but gave it up in favour of the fairly non-descript túngara frog that the Panamanians consider a toad, rather than a frog, but more about that later.

Frogs and toads belong to the order Anura, in the vertebrate class Amphibia. Anura is divided into some 55 families, one of which is Leptodactylidae with 57 genera and some 650 species, including *P. pustulosus*. The genus *Physalaemus* is distributed throughout most of middle and south America and few additional places. Michael Ryan studied *P. pustulosus* in Barro Colorado Island (BCI) in the Isthmus of Panama. BCI is an island of about 15 km^2 in the man-made Gatun Lake in the Panama canal. Preserved as a nature reserve and administered by the Smithsonian Institution, it is the main research station of the Smithsonian Tropical Research Institute (STRI), making it one of the best-studied tropical forests in the world, and not surprisingly, a mecca for tropical biologists. During my three-month stay on BCI in 1980-81, I had the pleasure of meeting, in addition to the large number of outstanding staff scientists (including Stanley Rand, another protagonist of this article), many famous visiting scientists including John Maynard Smith and Richard Dawkins and of course, Michael Ryan.

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On BCI and elsewhere, túngara frogs breed in temporary water bodies where the males advertise themselves to the females by calling thousands of times from dusk to midnight. Females arrive only when they are ready to mate, so ready that all their eggs will ooze out of their bodies and go to waste if they do not mate within the next few hours. In spite of being in a hurry to mate, females are choosy, and they have plenty of males to choose from. To quote from Michael Ryan's *A Taste for the Beautiful*, "A female gives her choice of a mate some serious thought. She will sit in front of one male for a time, often move on to others, and sometimes return to a male she has already sampled. She checks out the males by listening to what they have to say, that is, their whining and chucking. When a female decides to mate, she slowly moves to the male. He clasps her from the top. They are now mating, although the mechanics are a bit different from those to which we are accustomed." When the eggs are extruded by the female the male catches them in his hind feet, fertilizes them and makes a foam nest for them. Michael Ryan found the sex life of the túngara frog so fascinating that he watched them for "186 consecutive nights ...watching everything [they] did from sundown to sunup—more than one thousand of them, all individually marked so [he] could tell them apart ..." He had one simple question—how do the females choose their mates, what is their standard of beauty?

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How can we answer this question? As Michael Ryan says, a few well-designed behavioural experiments "can have the precision of a surgeon's scalpel". So, let us now study some of these well-designed experiments that he conducted.

What Does a Túngara Frog Call Sound Like?

One way to answer this question is to listen to the frogs themselves, and you can do so here [`https://www.youtube.com/watch?v=UoUL-jGgU1I`]. While it is easy to hear sounds, enjoy them, and even classify them, it is often difficult to describe them in words. Apparently, some people have attempted to do so by creating words whose pronunciations imitate the very sound that they are supposed to describe. This exercise even has a name—onoma- topoeia, and common examples are cuckoo, sizzle, splash, crack and bang. In his earlier monograph *The Túngara Frog* [4], Michael Ryan claims that the word túngara sounds remarkably like the call of the frog itself, if the first syllable is stressed and the second syllable is uttered very rapidly. I am not convinced, but of course, you should check it out for yourself. I am quite comfortable with his verbal description, "The ... call has two components, a "whine" and a 'chuck'; the call resembles the sounds produced by some "star wars" video games. The call always contains a whine and can contain from 0 to 6 chucks...". My dictionary defines a 'whine' as "a long, high-pitched complaining cry" which sounds about right to me, and the chuck, I would agree, is onomatopoeic.

But scientists need something much more precise and objective. Recall the words of Dr Louise Banks in the film *Arrival* (2016) (based on the short story entitled *Story of Your Life* by the inimitable American science fiction writer Ted Chiang; I cannot resist the temptation to say that Ted Chiang's short story is infinitely more profound than the movie): "Maybe we'll be able to hear the difference between alien phonemes, given enough practice, but it's possible our ears simply can't recognize the distinctions they consider meaningful. In that case, we'd need a sound spectrograph to know what an alien is saying." Even though we can hear the calls, in the interests of precision and objectivity, A. Stanley Rand, staff scientist at STRI and herpetologist extraordinaire (*Figure* 3) [5] and Michael Ryan began by recording the túngara frog calls and analysing them, just as Dr Louise Banks does for the sounds produced by the (alien) *heptapods*. In such analyses, the whines and chucks can be unambiguously distinguished and the numbers of chucks following each whine can be accurately determined. See a graphic representation of four kinds of calls, whine only and whine with 1, 2 or 3 chucks in *Figure* 4. Oscillograms plot the energy contained in the calls on the Y-axis and time on the X-axis, while the sonograms ('sound spectrographs' in the terminology of Dr Louise Banks) graph the frequency of the oscillations on the Y-axis and time on the X-axis. They then labelled the call with only a whine and zero chucks as a 'simple

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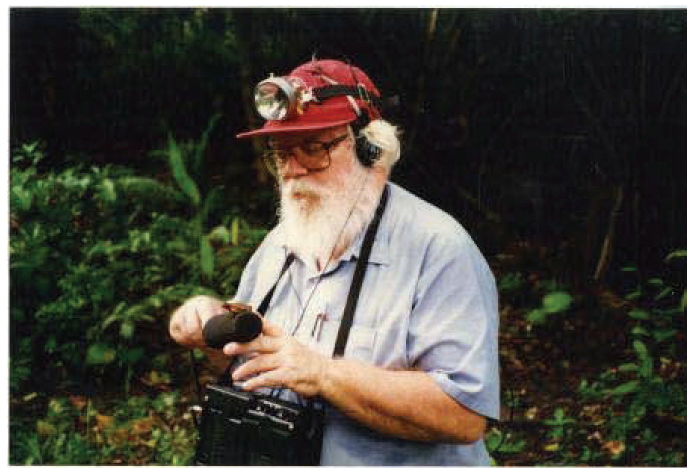

Figure 3. A. Stanley Rand—herpetologist extraordinaire (1932–2005). A world expert on lizards, iguanas, crocodiles and frogs, Stan, as he was affectionately known, spent most of his career at STRI in Panama. When Stan died in 2005, Michael Ryan wrote "[except] perhaps for BCI, Stan was STRI's most valuable resource ... When I informed by mass e-mail numerous colleagues of Stan's death, I received a plethora of responses in which the word "love" was used much more than one might associate with "macho" (and "macha") field biologists.".

call' and a call with whines followed by one or more chucks as a 'complex call'.

What Kind of Calls Does the Female Frog Like?

Well, we should ask her, shouldn't we? More generally, such 'asking' is called a bioassay. David John Finney, a famous British statistician and a student of the even more famous statistician Ronald A Fisher, and who was one-time President of the Royal Statistical Society, and who lived to be 101 (in 2018), defined bioassay as "the measurement of the potency of any stimulus, physical, chemical or biological, physiological or psychological, by means of the reactions which it produces in living matter". While bioassays are most commonly used to determine the concentration or potency of a substance by its effect on living cells or tissues, the most interesting bioassays, I think, are those that involve whole animals and the effect being sought is in terms of their behaviour. The first bioassay I ever witnessed was so impressive that the experience is etched in my memory.

I was visiting Delhi University in 1970 as an undergraduate Summer Research Fellow and one Professor Kailash Narain Saxena kindly and most enthusiastically showed me around his labora-

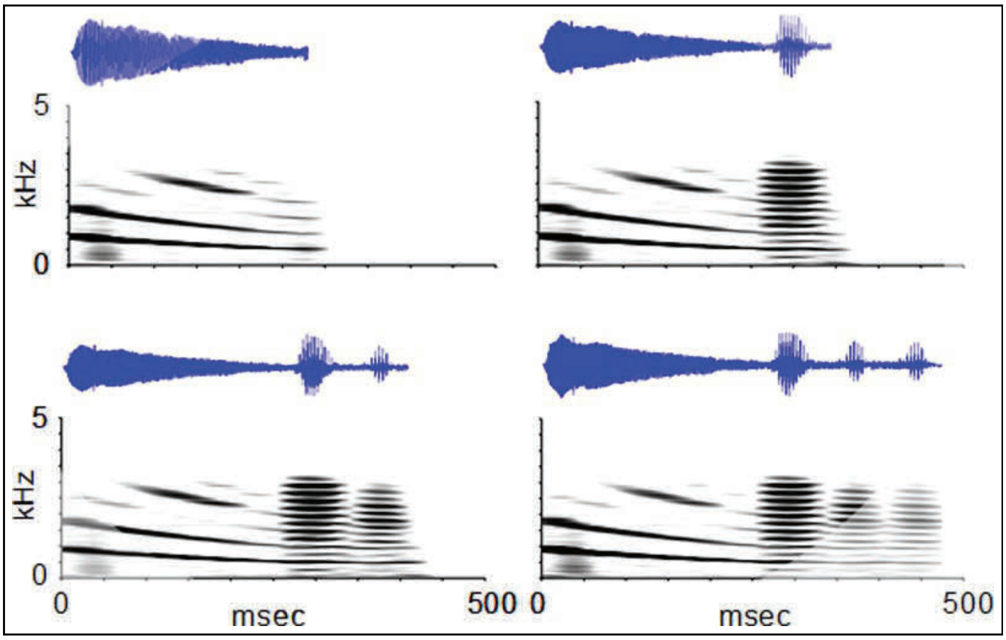

Figure 4. Oscillograms (in blue) and Sonograms of the calls of the male túngara frog, *Physalaemus pustulosus* of increasing complexity (top left: whine only, top right, bottom left and bottom right: whine with 1, 2 and 3 chucks, respectively). (Courtesy: Michael J Ryan).

tory. He was studying the sex pheromones of some agricultural pest insects in the hope of using the sex pheromones to trap the insects in the agricultural fields, before they did much damage. To identify and isolate the sex pheromones, he would make a crude extract of some glands of female insects (which produce the sex pheromone) and separate their constituents by means of a marvellous (now old) technique called paper chromatography. The crude extract would be applied to one end of a large sheet of filter paper and that end would be dipped into a tray containing an organic solvent. The paper would absorb the solvent, and the solvent would move across the paper carrying the molecules in the crude extract with it. The interesting thing is that different molecules in the extract move to different distances along with the solvent based on how soluble they were in that solvent. At the end of their journey, different molecules would make distinct spots on the filter paper. Generally, the molecules would have some colour, and the spots could thus be located. The next step would normally be to re-extract the compounds from all the different spots and test each of them to see which ones would be attractive to the male insects. This would be the usual bioassay. But what impressed me was how Professor Saxena used a clever short-cut to save himself a lot of time and money. He would simply release the male insects onto the filter paper, and they would promptly go and sit on the spot that they found most attractive—pheromone located. Talk of low-cost research.

Now, just as Professor Saxena got his male insects to tell him which of the many compounds that the female insects produced, they liked best, Professor Ryan had to get his female frogs to tell him which of the two kinds of calls his male frogs produced (simple or complex), that they liked best. And he did, with equal success. Stanley Rand and Michael Ryan recorded the calls of male frogs, both simple and complex, using a tape recorder. Soon, they also learned to artificially synthesise the calls so that they could playback either the naturally recorded calls or the synthesised calls, with the whine only or with the whine and the desired number of chucks, at will.

Next, they placed a female túngara frog in a small cage in the middle of an octagonal arena. Now they played back the male túngara frog calls, either natural or synthesized, from two speakers placed on either side of the female at 75 cm. One speaker played the simple call while the other played the complex call, alternately. At this point, they removed the cage enclosing the female so that she was free to hop towards whichever speaker (call) she liked, and they recorded her choice she thus revealed. After testing 15 females the result was clear—in 14 out of 15 cases the females clearly preferred the complex call (*Figure* 5). A simple bioassay yielded a clear result. Technically, the movement of the frog towards the sound she preferred is called 'phonotaxis'. As you can imagine, such movement of animals in response to external stimuli, phonotaxis (sound), phototaxis (light) or chemotaxis (chemical) can be conveniently used to perform bioassays with whole animals. Coming back to the frogs, it is clear that female frogs like complex calls, with not just whines but with added chucks [6].

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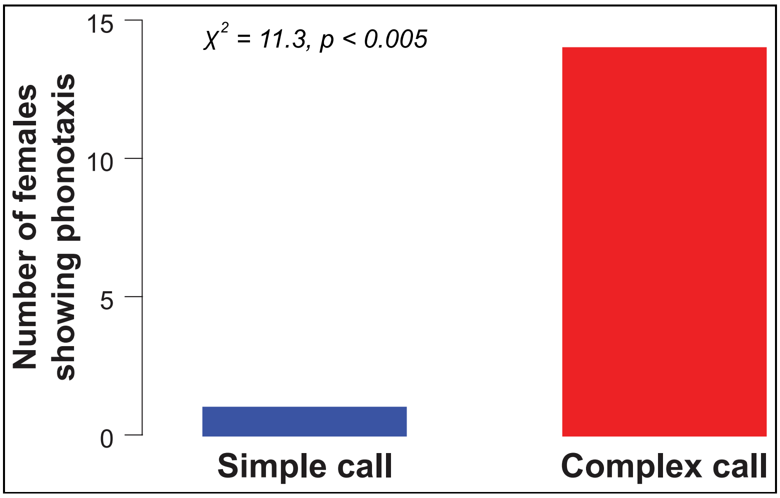

Figure 5. Female túngara frogs prefer males who give complex (whines followed by chucks) rather than simple (whines only) calls. Figure drawn by the author, based on data in A. S. Rand, and M. J. Ryan. The adaptive significance of a complex vocal repertoire in a Neotropical frog, *Z. Tierpsychol*., 57:209-14, 1981. [6]

What Kind of Calls Do the Males Like to Produce?

Stanley Rand who had worked with túngara frogs on BCI and other places had observed that solo frogs generally preferred to produce the simplest call (whine only) but tended to increase the complexity of their calls by adding one or more chucks when in the company of other calling conspecific males. Stanley Rand and Michael Ryan now set out to use their bioassay to carefully test the hypothesis that males increase the complexity of their calls as the size of the chorus of calling frogs increases. This time their bioassay needed no phonotaxis and simply involved counting the number of chucks that the male frogs added to their whines in response to the calls of other frogs. The calls of test male frogs were recorded without any playback or after playing back the calls of other males, varying the complexity of the playback calls and their loudness (by placing the speakers at different distances from the test frog). In each case, they counted the number of chucks in the calls of the test frog. I have graphed a sample of their results in *Figure* 6 which clearly support the hypothesis that male frogs prefer to give simple calls when no one else is calling but make their calls more complex if they hear the calls of other males [6].

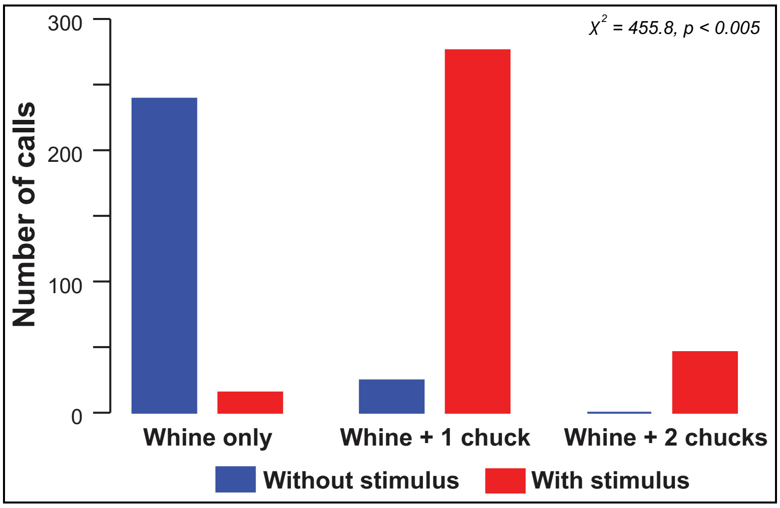

Figure 6. Males túngara frogs give simple calls (mostly whines without chucks and very few calls containing both whines and chucks) when they do not hear other males calling (no stimulus) but switch to more complex calls (adding one or two chucks to their whines) when they hear other males calling (with stimulus). Figure drawn by the author, based on data in A. S. Rand, and M. J. Ryan. The adaptive significance of a complex vocal repertoire in a Neotropical frog, *Z. Tierpsychol.*, 57:209-14, 1981 [6].

Why Don't Males Do Their Best?

Although the results of these two experiments are very clear, they create a paradox. If female frogs prefer males who give complex calls why don't male frogs always do so? Why are they so reluctant to add chucks to their whines and appear to do so only when faced with competition from other males? In attempting to answer such why questions, we must always remember that animal behaviour evolves in nature and not in the laboratory. We must also remember that natural selection is expected to maximise lifetime reproductive success and not short-term gains. Michael Ryan and his colleagues, therefore, wondered if there is a cost to giving complex calls that would actually serve to reduce the lifetime fitness of the male frogs even though such calls are attractive to the female frogs. They did not consider it likely that the energetic cost of the complex call was the problem. Calls are energetically expensive, but the frogs seem to spend most of the energy in producing a whine and adding one or two additional chucks seems to cost very little extra energy, as we can also see from the upper part of *Figure* 4.

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So, the culprit must lie outside the frog rather than inside. That immediately suggests that there must be something in the environment of the frogs that renders complex calls costly. If we now think of the male frogs' calls as signals directed towards the female frogs, we can point a finger at potential eavesdroppers. But why should someone eavesdrop on the male frog calls, and only on the complex calls, and why should that reduce the fitness of the calling frogs? Michael Ryan writes "There is another cost of chucks, one that remained hidden to me for more than a year but that had been influencing the evolution of sexual beauty in túngara frogs for millennia: the cost of eavesdroppers."

But who is this elusive eavesdropper? Enter Merlin Tuttle (*Figure* 7), a world-famous bat researcher and even more famous bat conservationist. Every bat researcher and every conservation biologist, passionate about bats or some other animal, knows about Tuttle's *Bat Conservation International*. Given STRI's attractiveness to scientists, it is perhaps not surprising that Merlin Tuttle was visiting. But, surprisingly, he had photographed a local fringe-lipped bat *Trachops cirrhosus* with a túngara frog in its mouth (*Figure* 8). Surprising because up until this time, a frog was considered a most unlikely item in a bat's diet. Moreover, at that time it seemed most unlikely that the bat could hear the frogs and use their calls to locate them. This is because the well-known echolocation of bats works in the ultrasonic region (50,000 to 100,000 Hz) while the frog calls (700 to 2200 Hz) are well within our hearing range. Merlin Tuttle was eager to know how the fringe-lipped bats on BCI were able to catch túngara frogs, and indeed, whether they were doing so routinely. Michael Ryan and Stanley Rand were equally eager to know if the threat of predation by the fringe-lipped bat was the reason why túngara frog males were so reluctant to add chucks to their calls.

Hot on the trail of the culprit, the three scientists joined forces and conducted new experiments involving the frogs as well as the bats. First they simply set up nocturnal observation stations and, to their surprise, they saw that the fringe-lipped bats were catching six túngara frogs per hour of the night. Were the bats catching the frogs by actually listening to them rather than by some other means? An obvious possibility was that the bats were using their

Figure 7. Merlin D Tuttle (1941–). With over 60 years of in-depth knowledge and experience as a renowned bat expert, educator and wildlife photographer, Merlin Tuttle founded Bat Conservation International (BCI) and Merlin Tuttle Bat Conservation (MTBC), to teach the world how to understand and appreciate the vital contributions bats make to human beings and the world we live in (www.MerlinTuttle.org). His unique vision of winning friends instead of battles has led to amazing success in addressing one of the world's greatest conservation challenges. He has turned countless bat haters into protectors by diplomatically showing them how helping bats help people. His classic research on population ecology and behaviour of bats has been published in leading journals, including a cover story in *Science*. And his conservation work has been featured in five National Geographic articles, as well as in *The New Yorker*, *The Wall Street Journal*, and numerous other leading publications worldwide.

well-known echolocation to bounce ultrasound off the frog's bodies, rather than listening to the frog calls. However, preliminary experiments playing back túngara frog calls from speakers indicated that the bats were indeed attracted to the speakers. But as Michael Ryan says, the acid test was to repeat the experiment that he and Stanley Rand had performed to discover that female túngara frogs preferred the complex rather than the simple calls of the male frogs. They did this in two different ways.

First, they caught some fringe-lipped bats and offered them two speakers, one playing the túngara frog simple calls and the other the complex calls, in their flight cages. It is thrilling to imagine the experiment in progress—the observer in one corner, the bat in the opposite corner and the two speakers in the two other corners. The bats significantly preferred to fly toward or land near the speaker emitting the complex calls (*Figure* 9, upper panel). In a second set of experiments, speakers playing the simple or complex calls were installed simultaneously in the field, at five different sites. Once again, the bats flew past the speakers playing the complex call significantly more often than they did for the speakers playing the simple calls (*Figure* 9, lower panel) (see the

Figure 8. A fringe-lipped bat *Trachops cirrhosus* that has just captured its favourite prey, the túngara frog Physalaemus pustulotus. (Photo: Merlin Tuttle).

figure legends and suggested reading [7] for more details). You will have to agree that Michael Ryan was justified in proclaiming "paradox resolved!", or more poetically that "The males are at the tipping point between sex and survival: more chucks tilt the balance one way; fewer chucks, the other." So, yes, male frogs sing for sex, but they cannot do their best. To be more precise, they cannot always sing their best as far as the females are concerned, but they achieve the best possible compromise as far as their lifetime reproductive success is concerned. Recall that males do produce complex calls when they face competition from other calling males. [6, 7].

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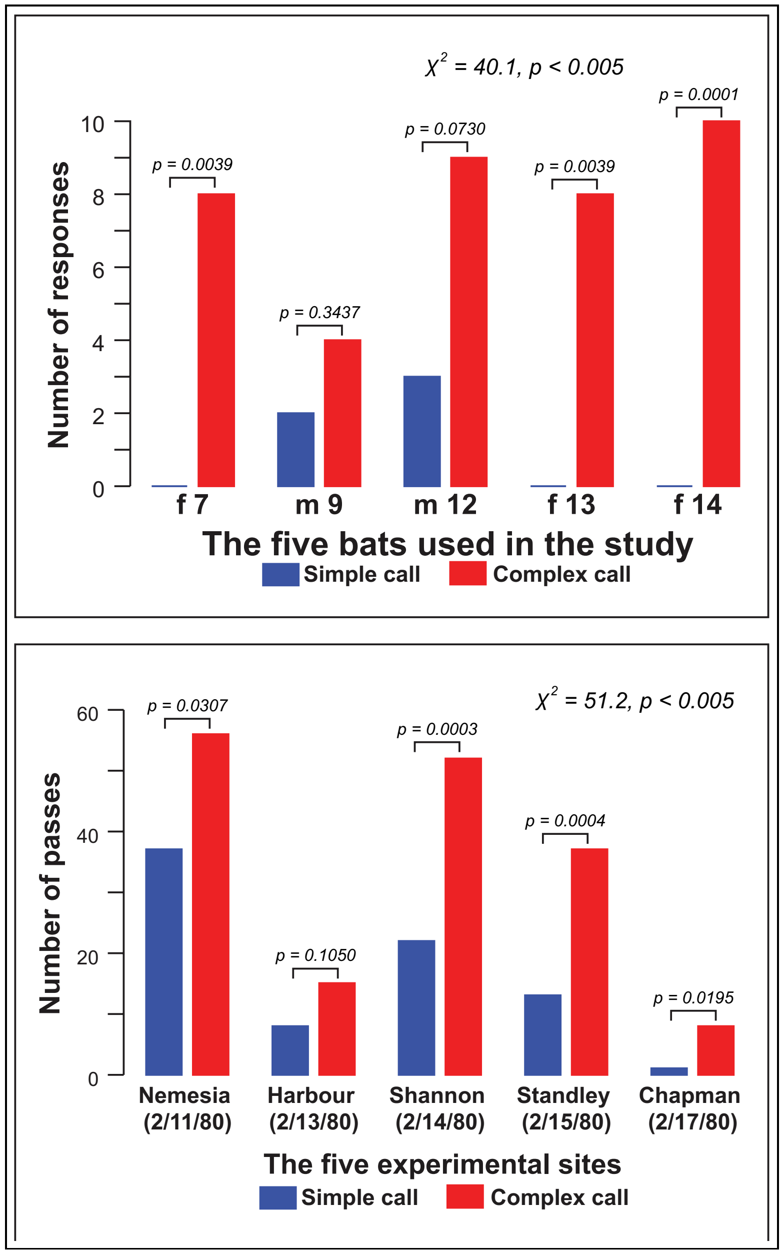
What is Beauty?

During the next four decades, Michael Ryan enlisted the collaboration of scores of students, postdocs, technicians, and colleagues to cross the t's and dot the i's as it were, and gave us the most complete understanding yet, of how the (female túngara frog's) brain perceives beauty (in the male túngara frog's call). We now know why the female túngara frog likes calls with chucks and why the fringe-lipped bat also prefers calls with chucks, and also how frog-eating bats have special adaptations in their inner ears

Figure 9. The predatory bat *Trachops cirrhosus* is more attracted to frogs giving complex calls (whines followed by chucks) rather than simple calls (whines only). Three out of five bats tested (upper panel) and at four out of five sites tested (lower panel) bats made significantly more passes (responses) at speakers playing complex calls as compared to passes made at speakers playing simple calls. Because sample sizes of the number of trials for different bats were not equal and because the numbers of bats making passes in the field experiments were not known, the null hypothesis of no preference was tested and was rejected in both cases (upper panel: $\chi^2 = 40.1, P < 0.005$; lower panel; $\chi^2 = 51.2, P < 0.005$). *Figure* drawn by the author, based on data in M. J. Ryan, M. D. Tuttle, and A. S. Rand. Bat predation and sexual advertisement in a Neotropical frog, *Amer. Natur.*, 119: pp.136–39, 1982 [7].



to permit them to hear frog calls even while remaining sensitive to ultrasonics they use for echolocation. All this required that he studied the anatomy of frog and bat brains, their larynges and ears, electrical activity and gene expression in their brains in response to various stimuli. The big picture concerning why natural selection has favoured this particular sexual aesthetic in the frogs, and this particular culinary aesthetic in the bats make a fascinating story [3].

Biologists are well aware that animals may use not just vision, but many other sensory modalities including smell, sound, touch and substrate vibration to perceive their world. We, therefore, rewrite the poet's claim that 'beauty is in the eyes of the beholder' and proclaim, in the words of Michael Ryan, that 'beauty is in the brain of the beholder'. But, as scientists, we do not stop further inquiry, assuming that 'love is blind'. We are interested in and confident about understanding how and why certain sensory inputs but not others make the brain of the receiver perceive beauty. As Professor Michael Ryan's life-time research, summarized so beautifully in *A Taste for the Beautiful: The Evolution of Attraction* [3] shows, such inquiry and understanding do not take the beauty out of beauty—they take the mystery out of beauty and thus make it more beautiful. Indeed, that is how the scientist's brain perceives beauty.

Reflections

As the readers of this series would be familiar by now, it is time to reflect on these experiments, to glean more general lessons for the inclusive, democratic pursuit of science. The experiments I have described in this article abundantly illustrate all the qualities of simplicity, curiosity, and unsophisticated, low-cost research that are the hallmarks of the experiments running throughout this series. Nevertheless, there are at least three rather unique points to which I would like to draw the attention of my readers.

The first point to reflect upon is that the experiments described in this article were indeed very simple and required rather little by way of sophistication. Notwithstanding the need to record, analyse and playback frog calls, I can easily see high school kids, undergraduate students, and amateur lay persons performing these experiments with no difficulty. This is largely true of most of the experiments described in all the articles in this series. What is perhaps unique however is that these simple experiments laid the foundation of knowledge that permitted the very same Michael Ryan to go on to perform many more complicated, sophisticated and expensive experiments to complete the story, and provide a fairly complete understanding of how female choice works, what the females find beautiful and why all males cannot always be as 'beautiful' as the female would like. These sophisticated experiments included the study of frog and bat anatomy, recording electrical activity from the frog brains, measuring gene expression in brain cells, constructing neural network models and designing robots to mimic the frogs. But, these more sophisticated experiments would not have been thinkable, nor would they have been meaningful without the foundational knowledge that (1) males can make simple and complex calls, (2) females prefer complex calls, (3) males are reluctant to make complex calls and (4) a local predatory bat also prefers males that make complex calls. By first performing simple, unsophisticated and low-cost experiments, Michael Ryan was able to produce the foundational knowledge that was so essential for all his subsequent work.

These sophisticated experiments included the study of frog and bat anatomy, recording electrical activity from the frog brains, measuring gene expression in brain cells, constructing neural network models and designing robots to mimic the frogs.

The lesson I draw from this is that research enterprises should start simple and unsophisticated, and if the researcher is asking the right questions and doing clever-enough experiments, much more is likely to follow. In many cases, as was true in the case of Michael Ryan, the same researcher who started small would have made a name for herself good enough to attract the funding and facilities needed for the next steps. I worry that there is a greater danger of not starting research due to lack of money or, of having too much money in the beginning and willy-nilly neglecting natural history and basic biology, and thus building a grand castle on loose sand. But if a particular researcher is indeed unlucky for some reason, someone else can take the next steps, and the credit for producing the foundational knowledge still stays with the original researcher who will forever be recognized for this.

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The second point worth reflecting upon is Michael Ryan's decision to work on the túngara frog [2–4]. Studying bullfrogs in the

Figure 10. The red-eyed treefrog *Agalychnis callidryas*. Yes, frogs can be beautiful, and yet they may be unsuitable to answer certain kinds of scientific questions—a pity! Source: https://commons.wikimedia.org/wiki/File:Red_eyed_tree_frog_edit2.jpg

1970s, the young Michael Ryan "became interested in the mechanisms by which females selected mates and the factors that influenced the evolution of this behaviour." In the hope of studying these phenomena, he began looking for a suitable frog species that had a long breeding season to enable long-term observations and the gathering of large amounts of data, and in which there was not the confounding effect of males defending territories. As it happens far too often in biology, even to this day, inadequate natural history knowledge makes it difficult to identify species suitable for answering specific questions. Based on other people's inadequate natural history observations, Michael Ryan had to initially content himself in choosing the red-eyed treefrog *Aglychnis callidryas* as his model organism (*Figure* 10).

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Arriving in person on Barro Colorado Island and pursuing his own natural history studies, he soon discovered the disadvantages for his purpose, of this "incredibly beautiful species"—males called infrequently and usually high up in the canopy, coming down only after having secured a mate for which visual cues were likely to have been more important. In a telling comment, Michael Ryan writes, "As I would gaze into the night time canopy, trying to discern the form of one of the treefrogs, there was always a great commotion at my feet. It was the cacophony of a túngara frog chorus.” The obvious thing to do was to ditch the beautiful treefrog and embrace the ugly little ‘toad’, and so he did. To quote him again, “To study animal behaviour in the field, one needs much fortitude. ... Extreme fortitude and patience may produce great benefits later on. However, a time may come when the researcher must decide that for conducting a particular study, a certain species is not appropriate, after all. Science tends to popularise the Jane Goodall studies, but probably more numerous cases exist in which dedicated and intense research paid few or no dividends because of an initial error in choosing a species to study.”

As researchers, we do not always pay adequate attention to deliberately and consciously choose our research questions, our study species and our research methods. If we honestly introspect on the choices we have made in the past, we will find that chance, arbitrariness, and external constraints have played a more important role than we would like to admit. Even worse, the desire and presumed safety in choosing all three—question, species and method—from among the most common and fashionable ones around, play a no small part.

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Reviewing a PhD thesis recently, I wrote that: "First, I must commend the choice of the study animal. Ethologists and behavioural ecologists often tend to choose unusual, endangered or otherwise glamorous animals for their study, even at the cost of many constraints in the availability of samples for study as well as the feasibility of observation and experimentation. Free-ranging dogs, especially in India, are abundantly available, easy to observe and experiment and also of great practical importance to society. Dogs are thus ideally suited both from the point of view of basic research in ethology and behavioural ecology as well as producing knowledge relevant to society, especially in the context of human-animal conflict". On a personal note, I myself began my research career with bacteria, switched to bacteriophages and then to geckos before falling permanently in love with the evolution of social behaviour and with the Indian paper wasp *Ropalidia marginata*.

The final point I want to reflect on concerns scientific etiquette, cooperation and generosity. When Michael Ryan was thinking of abandoning the red-eyed treefrog in the canopy and succumbing to the túngara frog chorus at his feet, Stanley Rand, who was studying the túngara frog on Barro Colorado Island, not only encouraged Michael Ryan to use it for his studies on sexual selection but gave him the unpublished manuscript that he had written in the late 1960s and early 1970s and in which he had described their simple and complex calls, "a manuscript ... filled with incredibly interesting and detailed natural history as well as experimental studies of female phonotaxis" [5]. On the other hand, we have all seen far too often that scientists are resentful of colleagues encroaching on their 'territories' of species, study sites and equipment. It is now history that Michael Ryan and Stanley Rand became lifelong friends and collaborators (until Stan's death in 2005), prompting Michael Ryan to write that "Stan was always very generous with his time, his ideas, his equipment, and his immense knowledge of tropical biology" dedicate his *A Taste for the Beautiful* to Stan with the words "In memory of Stan Rand, fellow traveller" and begin his obituary of Stan with the words "There is a hole in my chest where my heart used to be and a chasm in tropical biology the size of the Panama Canal".

Acknowledgements

I thank Michael Ryan, Sujata Deshpande, Maria Modanu and TNC Vidya for many helpful comments on this article.

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