

The evolution of behaviour, and integrating it towards a complete and correct understanding of behavioural biology

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Abstract—Tinbergen suggested there are four major aims or questions in ethology. All of these contribute to the larger single question of why animals behave as they do. Here, I emphasise one aim, to understand the evolution of behaviour. Using studies of sexual communication in túngara frogs (*Physalaemus pustulosus*) I attempt to illustrate how an analysis of the past evolution of behaviour can contribute to our understanding of its current function and the details of the mechanisms guiding it. I argue that integration of Tinbergen's four questions not only give us a more complete understanding of the biology of behaviour, it might be necessary to give us a correct understanding.

Keywords: behaviour; evolution; *Physalaemus*; sexual selection; túngara frogs.

EVOLUTION AND BEHAVIOUR

In his classical discourse on aims and methods in ethology, Niko Tinbergen (1963) first posed a single, central (and what Tinbergen referred to as “admittedly vague”) question: *Why do animals behave the way that they do?* (p. 411). He suggested four aims, questions, approaches, or levels of analysis that can be used to address this question: causation, survival value, ontogeny, and evolution. This special issue celebrates the anniversary of that paper which was of such important heuristic value for our field. My purpose is to consider aspects of the evolution of behaviour.

In this paper, Tinbergen stresses more than once that the central question in ethology, mentioned above, addresses the general biology of behaviour, and he gives praise to Konrad Lorenz's insistence in stressing this notion. Tinbergen then specifies an integrative analysis of behaviour that addresses several important aspects of its biology: the physiological mechanisms regulating the behaviour, the

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current adaptive significance of the behaviour, the acquisition of the behaviour by the individual, and the past evolutionary history of the behaviour.

The virtues of integration

There is no doubt that Tinbergen appreciated that an integrative approach to animal behaviour would result in a more complete understanding of the main question that motivates us — why do animals behave as they do? Consider, for example, how less complete our understanding, and perhaps our appreciation, of the vocal acrobatics of song birds would be if we did not understand how that richness in repertoire was derived by the neural mechanisms in the sound control nuclei in the brain (Nottebohm, 1984; Brenowitz and Kroodsma, 1996), how the details of the songs were learned during an early critical period (Marler, 1997), how these sounds were produced by the biomechanical details of the syrinx (Greenwalt, 1968; Podos, 1996; Fee, 1998; Suthers, 2001), as well as understanding the fitness benefits that accrued from complex song (Searcy and Andersson, 1986).

Without information on Tinbergen's four main questions, our interpretation of the biology of any behaviour is incomplete. But I would like to make a further argument: without integration our interpretations might be inaccurate. A virtue of an integrative approach is that data and interpretations drawn from one level of analysis can inform data and interpretations drawn from another level. This view is antithetical to some, who maintain that there should be hard boundaries between levels of analysis so as not to confuse them (Reeve and Sherman, 1993, 2001). And there is no question that there has been confusion (Sherman, 1988; Alcock and Sherman, 1994). When we ask why song birds sound as they do, we must specify whether we refer to how the syrinx generates sounds, what fitness benefits the males derive from their serenades, how they are able to acquire such a song, or from what kinds of ancestral sounds these melodies evolved. But to pretend that these boundaries of scientific interest are anything more than human categorical constructs to assist in perceiving an otherwise incomprehensibly large subject is to risk real answers to the real question of why animals behave as they do.

In this paper I will use studies my colleagues and I have conducted on acoustic communication in frogs to demonstrate how an integrative approach to the biology of communication behaviour, which encompasses three of Tinbergen's four questions, helps us to avoid interpretations that are not only incomplete but also inaccurate. This integrative approach will hopefully and eventually lead to a complete and correct understanding of why these animals communicate as they do.

Evolution of behaviour, then and now

The study of the evolution of behaviour has a history that was firmly planted within what we would now call the field of phylogenetics, and which presages much of what is being done today by students of behavioural evolution. Influenced by the earlier work of Heinroth (1909) and Whitman (1898), Lorenz (1941) argued that the

analysis of similarities and differences in behaviour could elucidate phylogenetic relationships among species. This argument was initially rejected because some thought behaviour too flexible and inconsistent to be a reliable taxonomic variable (Atz, 1970; Aronson, 1981), but there is now a consensus that behavioural data can contribute importantly to phylogenetic analysis, and might even be as reliable as other data sets (DeQueiroz and Wimberger, 1993; Wimberger and DeQueiroz, 1996).

A number of ethologists, including Darwin, were also interested in the historical patterns that gave rise to the behaviour of extant species. Darwin (1871), for example, posited that facial expression in humans were shared with other primates by descent through a common ancestor. He even suggested that in mate attraction “the same sounds are often pleasing to widely different animals, owing to the similarity of their nervous systems” (p. 91, 1872 [in 1965 reprint]). Huxley’s (1914) hypothesis of ritualisation (see also Tinbergen, 1951), which describes how complex displays are derived from behaviour patterns functional in other contexts, might be the classical account of the historical patterns by which behaviour evolves.

The study of animal behaviour underwent a revolution initiated by W.D. Hamilton’s (1964) papers on the genetical evolution of social behaviour and G.C. Williams’ (1966) book, *Adaptation and Natural Selection*. This revolution was sustained by a number of important papers, and especially by a spasm of creativity by R.L. Trivers, which we are unlikely to see again in our field for some time. In a period of 6 years he published five classical papers that addressed reciprocal altruism (Trivers, 1971), parental investment and sexual selection (Trivers, 1972), sex ratio evolution (Trivers and Willard, 1973), parent offspring conflict (Trivers, 1974), and haplodiploidy and insect sociality (Trivers and Hare, 1976). When E.O. Wilson codified many of the principles of sociobiology in 1975, a new discipline, much more closely allied with theoretical population genetics and focused on individual fitness was born. Much of animal behaviour was divided into studies of underlying neural mechanisms and sociobiology. Some thought that ethology was the glue that would maintain these two disparate concentrations in the field. Wilson, however, made a bold prediction (Wilson, 1975, p. 6):

The conventional wisdom also speaks of ethology, which is the naturalistic study of whole patterns of animal behaviour, and its companion enterprise comparative psychology, as the central unifying fields of behavioural biology. They are not; both are destined to be cannibalised by neurophysiology and sensory physiology from one end and sociobiology and behavioural ecology from the other.

When the dust settled, most studies of naturalistic animal behaviour, now called behavioural ecology, concentrated on studies of current adaptive significance, Tinbergen’s question of survival ability. But a parallel revolution was taking place in the field of systematics (Hull, 1988). Willi Hennig (1950) published a work in German in 1950, in which he argued that taxonomic relationships should reflect

phylogenetic ones and offered a method based on shared derived characters for uncovering such relationships. The impact of this new approach of cladistics increased substantially with the English publication of *Phylogenetic Systematics* (Hennig, 1966). The controversy that cladistics engendered in the systematics community brought it to the more general attention of evolutionary biologists and resulted in several important books that introduced phylogenetic methods to the study of ecology, evolution and behaviour (Ridley, 1983; Brooks and McLennan, 1991; Harvey, 1991; Brooks and McLennan, 2002).

The reintroduction of phylogenetics (Tinbergen's question of evolution) to animal behaviour was in a narrower context than its initial exposition in the works of Darwin, Huxley, and Lorenz. The interaction of the new phylogenetics and behaviour was not to derive behavioural characters for phylogenetic analyses, molecular characters were beginning to reign supreme in phylogenetic reconstruction, nor was it to uncover past patterns of the evolution of behaviour. Phylogenetic tools were used almost exclusively to test hypotheses of adaptation. This emphasis was especially due to Felsenstein's (1985) seminal contribution on phylogenies and the comparative method. Many tests of behavioural adaptation make predictions about the associations of traits within species (e.g., testes size and mating system). Felsenstein pointed out that in such comparisons individual species are not necessarily independent data points. Taxa exhibiting similar traits might do so because they share them through evolution of a common ancestor rather than independently evolving them in response to a similar selection pressure. He outlined a method, Independent Contrasts, which can be used to estimate the degree to which traits of species have diverged from a common ancestor.

Since the reintroduction of phylogenetics to animal behaviour, there have been other uses of these techniques besides testing hypothesis of adaptation (reviewed by Martins, 1996; Ryan, 1996). In the remainder of this paper, I will examine how we use a phylogenetic analysis, Tinbergen's aim of understanding evolution, to compliment our studies of survival value and causation of behaviour. I will emphasise how including an evolutionary analysis in our studies can change interpretations of behaviour based only on studies of function and causation. This is why I will emphasise that integrating Tinbergen's four questions is not only needed for a complete understanding of the biology of behaviour, but it might be necessary for a correct understanding.

SEXUAL COMMUNICATION IN TÚNGARA FROGS: SEXUAL SELECTION AND SENSORY EXPLOITATION

We have taken an integrative approach to attempt a deep understanding of the sexual communication system of the túngara frog, *Physalaemus pustulosus*. These studies began with the behavioural ecology approach and attempted to understand the selection pressures responsible for the call complexity series in this species. Later, we added a neuroethological perspective to detail some of the neural

mechanisms underlying female preferences for mating calls. Finally, we used recent phylogenetic approaches to uncover patterns of signal-receiver evolution to contrast sexual selection hypotheses of good genes, runaway sexual selection and sensory exploitation and, more recently, to explore how historical contingency can influence current brain function.

A main point of this paper is to consider the varied uses we can make of phylogenetics to understand behavioural evolution. A subtext is that integration is necessary for a complete and correct understanding of behavioural biology. I will illustrate this by demonstrating how our interpretations of behavioural and neural data changed when we interpreted them in a phylogenetic context.

Behaviour

There are nearly 5000 species of frogs and most of them produce a species-specific advertisement call. This call is used by males to mediate male-male interactions and to make their presence known to females. Females assess these calls and decode the information that leads to assortative mating among species and selective mating within the species.

The focus of our studies is the túngara frog, and reviews of this system summarising earlier and more recent work in this system can be found in Ryan (1985) and Ryan and Rand (2003).

Túngara frogs, *Physalaemus pustulosus*, are small (ca 30 mm snout-vent length), and are common throughout much of the lowland tropics in Middle America and northern and western South America. This species is unusual in that it produces an advertisement call of facultative complexity with acoustically distinct components. The basic component of the call is referred to as a whine (fig. 1). It is a frequency sweep that begins at about 900 Hz and in about 300 ms descends to approximately 400 Hz. This component is necessary and sufficient to elicit phonotaxis from females; it will also elicit calling from males, although males are permissive and will respond to a wider variety of sounds. All other closely related species produce whines. The second component of the túngara frog call is the chuck. It is typically a short sound of ca. 30 ms in duration with a fundamental frequency of 250 Hz and 15 or so harmonics. Most of the call energy is in the higher harmonics.

Males usually produce a simple call, whine-only, when calling by themselves. In larger choruses most, if not all, of the males produce complex calls, whines followed by up to six chucks, although a whine plus two chucks seems to be normative in most situations. In phonotaxis experiments females are attracted to a simple call but prefer complex calls (Rand and Ryan, 1981). Given this selective advantage to producing complex calls, this raised the question of why males do not always do so, and suggested a cost to offset the benefit. Although calling is energetically costly, adding chucks does not increase oxygen consumption or lactate concentrations (Bucher et al., 1982; Ryan, 1983). However, there is a predation cost. The frog-eating bat, *Trachops cirrhosus*, locates frogs by passively orienting to the advertisement call (Tuttle and Ryan, 1981). The bats respond to male túngara calls in much the same

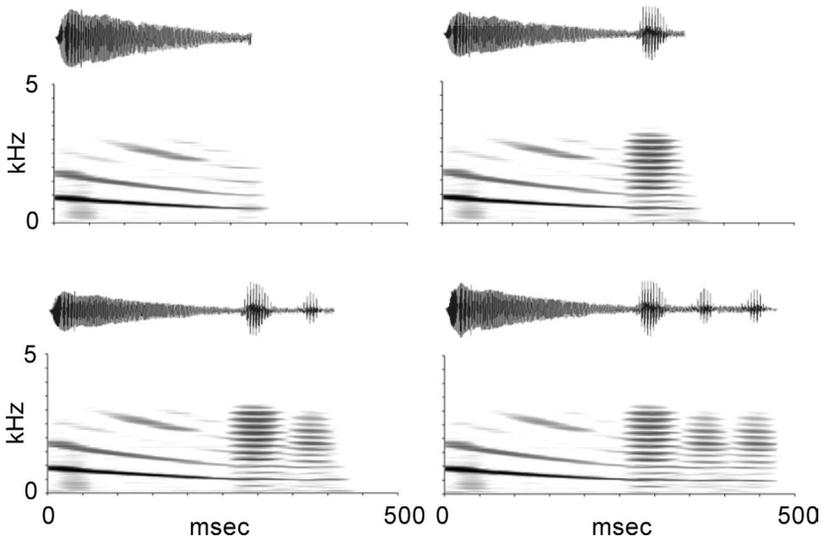


Figure 1. Sonograms (bottom of each panel) and oscillograms (top of each panel) of a call complexity series of a male túngara frog. Calls contain a whine and from 0-3 chucks (proceeding clockwise from top left).

manner as female túngara frogs do. The bats are attracted to simple calls but, when given a choice between calls with and without chucks, they prefer complex ones. The difference is that the bats eat rather than mate with the signaller (Ryan et al., 1982).

In túngara frogs, as with a number of other species of frogs, females prefer to mate with larger males. This mate choice is mediated to a large extent by variation in the chuck of the male's call. Larger males produce chucks with a lower fundamental frequency, probably because their larynges are bigger and therefore vibrate at a lower frequency. Females prefer lower frequency chucks and this results in them choosing larger males, and generating sexual selection for larger males, lower frequency chucks, and larger larynges (Ryan, 1980, 1983a; Wilczynski et al., 1995).

Is the female preference for lower frequency chucks adaptive? It appears so. In these frogs, as in most others (Shine, 1979), females are larger than males. On average, when a female chooses a larger male she reduces the size difference between herself and her mate. The larger the size difference between the two, the more eggs are unfertilised (Ryan 1983, 1985). This seems to result from a mismatch in the position of the cloaca of the male, from where the sperm is released, and the cloaca of the female, from where the eggs are released. The larger the size difference between the male and female the less likely that eggs and sperm come into contact during external fertilisation. The same phenomenon seems to be true in other frogs (Davies and Halliday, 1978; Bourne, 1993). Regardless of the details influencing the fertilisation effect, it appears that female choice of larger males is adaptive in this species.

These studies of the behavioural aspects of the túngara frog's reproductive communication system lead to some basic conclusions. The male call complexity series evolved under the contrasting selection pressures of sexual selection and predation. From the female perspective, females prefer lower frequency chucks which results in their mating with larger males. By mating with larger males they gain a reproductive advantage through having more eggs fertilised due to the smaller difference in body size between the mates. It would thus seem logical that females evolved a preference for lower frequency chucks as a response to selection for increased fecundity.

Neurobiology

Capranica and his colleagues (Capranica, 1977; Wilczynski and Capranica, 1984) had shown that the frog auditory system was a valuable model demonstrating how animals decoded biologically relevant acoustic pattern — in this case the species recognition calls. We extended this neuroethological paradigm for species recognition to sexual selection in order to begin to understand how the auditory system results in certain female preferences among conspecifics.

Much of the frog's initial processing of auditory cues takes place at the periphery. There are two auditory end organs in the inner ear of the frog that are responsive to sonic frequencies. Among a suite of differences between these end organs, the most apparent is the frequency range to which each organ is maximally sensitive. The amphibian papilla (AP) is more sensitive to lower frequencies, usually below 1500 Hz, and the basilar papilla (BP) is more sensitive to higher frequencies, usually above 1500 Hz. Capranica's matched filter hypothesis (1977) suggested that the emphasised frequencies in the species advertisement call matches the sensitivities of the two end organs. Gerhardt and Schwartz (2001) have reviewed the literature and confirmed this hypothesis. Although many species of frogs have advertisement calls with most energy restricted to the frequency range of only one peripheral end organ, there is a strong correlation between the emphasised frequencies of the call and the tuning of the end organs among species.

In túngara frogs the two call components have energy that is mostly distributed within the sensitive range of either the AP or the BP. The whine has a dominant frequency of about 700 Hz, which is close to the maximum sensitivity of the AP, and the chuck emphasises the energy in the upper harmonics, with an average dominant frequency of about 2500 Hz, close to the sensitivity of the BP (Ryan et al., 1990). Thus, whereas some species exhibit a match between the advertisement call's emphasised frequencies and tuning of one or both of the peripheral end organs, in túngara frogs each component matches the tuning of one of the end organs.

Guided by the neurophysiological data, we conducted a series of phonotaxis experiments in which the whine and the chuck were deconstructed to determine the salient features necessary to elicit phonotaxis from females (Rand et al., 1992; Wilczynski et al., 1995; fig. 2). Given the distribution of energy in natural calls, only the fundamental frequency of the whine is necessary to influence phonotaxis; the

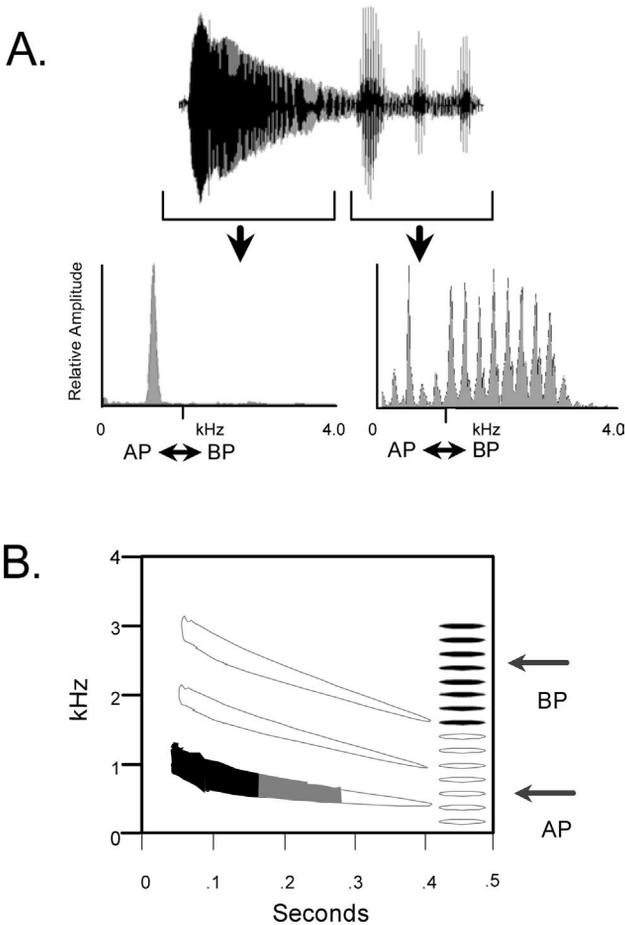


Figure 2. A: An oscillogram of a typical whine plus three chucks, and power spectra of the whine (left) and the chucks (right). On the x axis of each power spectrum we indicate the general range to which the AP (<1500 Hz) and the BP (>1500 Hz) of most anurans are maximally sensitive. B: A sonogram of the whine and chuck. Arrows indicate the frequencies to which the AP and the BP of the túngara frogs are most sensitive. The sonogram also illustrates the results of signal deconstruction experiments. The sounds that are not shaded have no effect on female phonotaxis. Those that are shaded in black are sufficient to elicit phonotaxis, while those in grey add to the attractiveness of the signal but by themselves are not sufficient to elicit it.

upper harmonics do not influence female behaviour. Of the fundamental frequency, there are three parts: one is necessary and sufficient to elicit phonotaxis; one adds to the attraction of the whine but by itself will not attract females; and one has no influence on the female. In the chuck, only the higher-half harmonics, >1500 Hz, increased the attractiveness of the chuck, and that effect could be mimicked with a pure tone near the most sensitive frequency of the BP. Combining the neurophysiological results with the behavioural experiments, we concluded that in túngara frogs the AP is the end organ primarily responsible for the initial decoding

of the whine, while the BP is primarily responsible for initial decoding of the chuck. These data also offer strong support, and a different kind of support, for the matched-filter hypothesis Capranica.

There is a slight mismatch between the average tuning of the BP and the dominant frequency of the chuck. The average dominant frequency is about 2500 Hz while the average BP tuning is about 2200 Hz. We constructed a computer model of the tuning of the BP and used natural calls to determine if chucks with lower dominant frequencies passed more energy through this filter. These simulations confirmed the obvious expectation that lower frequency calls elicit more neural stimulation from the BP than higher frequency calls do. The matched-filter hypothesis is also confirmed by behavioural studies that show single tones within the sensitivity range of the BP can mimic the effects of the entire chuck, and within this same frequency range a lower frequency tone that matches the tuning is preferred over a higher frequency tone (Ryan et al., 1990; Wilczynski et al., 1995).

We can add our interpretations from the neurobiology studies to behavioural studies. Females prefer larger males because they prefer lower frequency chucks, and they prefer lower frequency chucks because these calls better match the tuning of their BP. Since the females gain a reproductive advantage from mating with larger males, we assume that both the preference for complex calls, the preference for lower frequency chucks, and the tuning of the BP evolved to maximise female fecundity.

Evolution

We compared the evolution of the male call in concert with that of female preference. A comparative approach allows hypotheses about the evolution of this communication system to be tested. *Physalaemus pustulosus* is a member of the *Physalaemus pustulosus* species group. This group, as defined by Cannatella and Duellman (1984) and Cannatella et al. (1998), contains six species (fig. 3). Three species are found in Middle America (*P. pustulosus*) or in South America east of the Andes (*P. pustulosus*, *P. petersi*, *P. freibergeri*; the taxonomic status of *P. petersi* and *P. freibergeri* is uncertain, and here we treat them as single species), and three are found west of the Andes (*P. coloradum*, *P. pustulatus*, and an undescribed species, species B). It is likely that other new species will be described. The remainder of the genus, ca. 35 species, are in South America east of the Andes.

As described in detail above, *P. pustulosus* produces an advertisement call of varying complexity. *P. petersi* can also make complex calls (Boul and Ryan, 2004). Unlike *P. pustulosus*, these males add only one, and not multiple, secondary components, called a squawk, and not all populations produce complex calls. None of the species on the western side of the Andes produces complex calls, and our studies of the other ca. 30 congeners not in the species group also reveal a lack of complex calls. The most parsimonious assumption is that the complex call evolved in the ancestor of the eastern clade of the *Physalaemus pustulosus* species group.

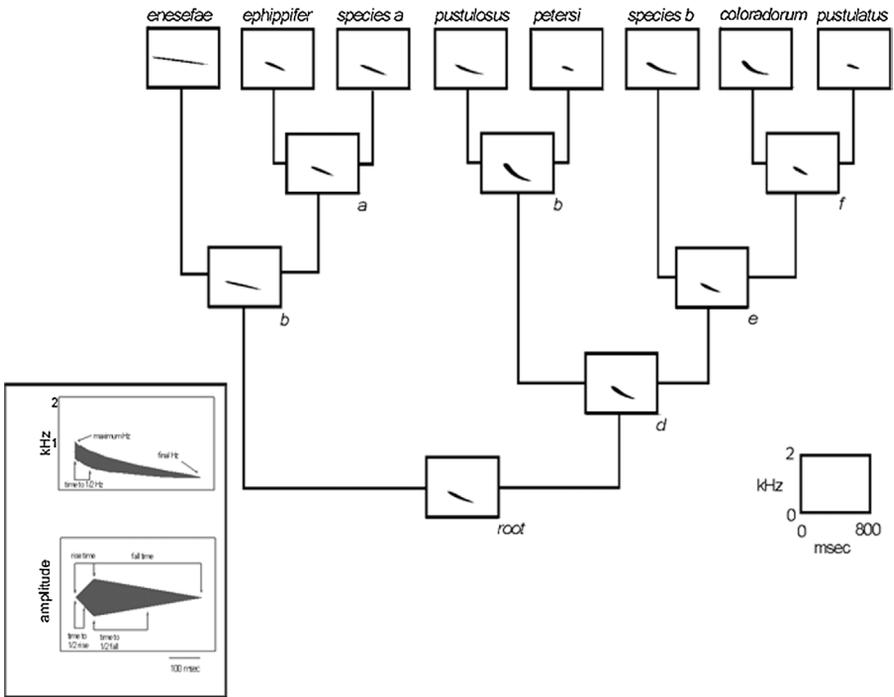


Figure 3. The phylogeny of the *Physalaemus pustulosus* species group (all of the descendants of node d) and three other congeners (all of the descendants of node b) used in outgroup analysis. The synthetic advertisement call for each species is shown at the tips of the phylogeny. The insert shows the parameters of the fundamental frequency measured for the call of each species. These are the variables that were used for estimating and synthesising the ancestral calls, which are shown on the nodes of the phylogeny.

One of the conclusions about the communication system of the túngara frog was that preference for complex calls was adaptive because preference for chucks allowed females to choose larger males, who tended to fertilise more eggs (Ryan, 1983, 1985). It would thus seem logical that the preference for chucks evolved in concert with the chucks. This would also be consistent with the two most popular hypotheses for the evolution of female mating preferences: runaway sexual selection, and selection for good genes. In the former, Fisher (Fisher, 1930) suggested that once a genetic correlation, or linkage disequilibrium, is established between genetic variation for traits and genetic variation for preferences, the preference would quickly ‘run away’ to fixation in a population. This is not a result of direct selection on the preference, but a correlated response of the preference to evolution of the male trait which is under direct selection generated by female choice. The evolution of preference for good genes comes about in a similar way (Kirkpatrick, 1982). In this scenario genetic variation for the preference becomes correlated with the good genes, and again the preference runs away and becomes established in the population.

In the two scenarios described above, runaway sexual selection and good genes, the traits and preferences, or more generally, the signals and receivers co-evolve. This hypothesis can be tested using phylogenetic techniques to ascertain if the evolutionary pattern of trait-preference evolution does suggest that these characters are linked in a manner that results in tight co-evolution (Ryan, 1990). A simple example is one in which there is a derived trait, such as a chuck, found in one taxon but absent in other close relatives. The species with the chuck also have a preference for the chuck. Since the chuck is only present in one taxon the most parsimonious assumption is that it evolved in that lineage. What about the preference? We must know something more about the preference in other species to speculate about its pattern of evolution. Not surprisingly, most studies of sexual selection have not been concerned about preferences for traits that do not exist. If the preference exists only in the species (or the lineage) with the chuck, we would conclude that the chuck and the preference for chuck coevolved. If instead, the preference for chuck existed in other species, even though those species were lacking the derived trait, we might conclude that the preferences of these species were shared through a common ancestor.

We tested these contrasting hypotheses of tight co-evolution (good genes and runaway) versus sensory exploitation in *Physalaemus* by determining if a species in the western clade of the *Physalaemus pustulosus* species group has a preference for chucks added to their own call, even though the evidence strongly suggests that the chuck evolved in the eastern clade of the species group after these two clades diverged. We added chucks digitally excised from a *P. pustulosus* call to the call of *P. coloradorum*. When female *P. coloradorum* were given a choice between the simple call typical of their species, a whine with no chucks, versus an artificial complex call, a *P. coloradorum* whine with *P. pustulosus* chucks, females showed a preference for the latter (Ryan and Rand, 1993). This study shows that the preference for complex calls exists in species lacking such calls. The most parsimonious interpretation about the evolution of preference for chucks is that it is shared by *P. coloradorum* and *P. pustulosus* through a common ancestor. If true, then our conclusions about the pattern of evolution is that the preference for chucks existed prior to the evolution of chucks, and that males evolved chucks to exploit these pre-existent preferences. An alternative hypothesis that the ability to produce the chuck has been lost in some taxa, is less parsimonious but, of course, could still be correct. At this point, however, the data seem to support the sensory exploitation hypothesis.

It might seem odd that a preference for such a specific acoustic trait as a chuck could exist before the chuck evolved, but it appears that the preference for the chuck is just one expression of a more general preference that includes white noise in the chuck's amplitude envelope, various pure tones, and even artificial "bells and whistles", although there certainly are sounds that do not enhance call attractiveness, and none of these other stimuli is more attractive than a chuck (Ryan and Rand, unpubl.).

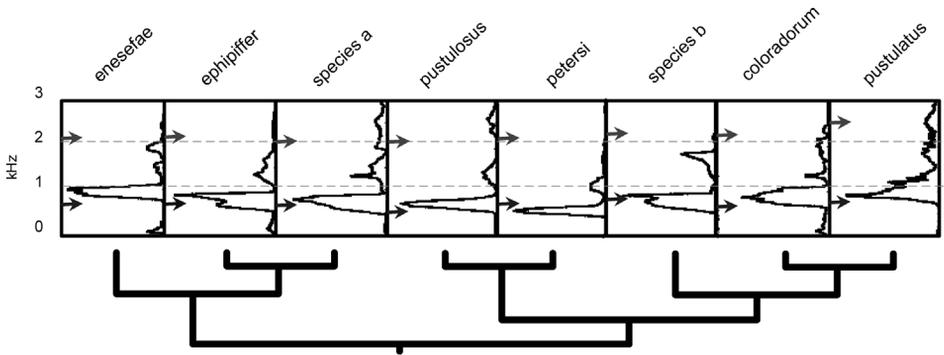


Figure 4. Tuning of peripheral end organs in the *Physalameus pustulosus* species group. For each species we show the mean peak sensitivities of the amphibian papillae (lower arrow) and the basilar papillae (upper arrow). We also show a power spectrum of a typical advertisement call. For *P. pustulosus* the power spectrum is of a simple call. (Redrawn from Wilczynski et al., 2001).

We can apply this phylogenetic approach to data on the tuning of the auditory periphery that is involved in decoding of the call. The most sensitive frequency of the AP matches the dominant frequency of the whine in túngara frogs. The most sensitive frequency of the BP is a close match to the dominant frequency of the chuck, and the slight mismatch between them might explain in part the female túngara frog preference for lower-frequency chucks. With the exception of *P. petersi* and *P. freibergeri*, other species in the *Physalameus pustulosus* species group lack a secondary component although they all produce whine-like advertisement calls. We examined the tuning of the auditory periphery of the other members of the species group to determine if the BP tuning that matches the chuck is a result of co-evolution of signal and receiver, or if the tuning is a property of the species group which males exploited when they evolved chucks.

The results support the hypothesis of sensory exploitation (Ryan et al., 1990; Wilczynski et al., 2001; fig. 4). For all species examined, the tuning of the AP is within a region of substantial energy in the species' whine. AP tuning varies significantly among species, although it is not significantly correlated with the whine's dominant frequency. In the BP, however, the tuning is statistically similar among all species, with one exception. *P. pustulatus* is the only species in which the BP tuning shows a significant difference from that of other species group members; its BP tuning is significantly higher. The rest of the species, including *P. pustulosus*, are not significantly different. Thus the BP tuning is a conserved property of the auditory system of these frogs. We must reject the hypothesis that BP tuning in *P. pustulosus* coevolved with the chuck. Instead, it appears that the relationship between the chuck's spectral characters and this neural property is a result of signal and not receiver evolution. Therefore, sensory exploitation seems to explain both the behavioural preference for the chuck as well as some of the tuning properties of the peripheral auditory system.

Summary

In our studies of sexual communication in túngara frogs we have attempted to understand the role of sexual selection in the evolution of the complex advertisement call. Behavioural studies have documented a selective advantage as well as the selective trade-offs for males making complex calls. The neurobiology studies identify some of the underlying mechanisms that enable females to decode the whine and the chuck and also suggest a mechanism to explain the behavioural bias of the female towards lower frequency chucks. Initially, the interpretation was that females evolved a preference for complex calls and low-frequency chucks because it allows them to choose larger males and thus maximise reproductive success. Thus trait and preference, signal and receiver, must have coevolved to bring about such benefits. The addition of a phylogenetic perspective, however, changes this interpretation. Neither the preference for chucks nor the auditory tuning that guides females to lower-frequency chucks are restricted to species with chucks; both of these receiver traits are found in species that produce only simple calls. Thus it appears that there was a pre-existing preference for chucks and that in evolving chucks males exploited this.

Since we first suggested the hypothesis of sensory exploitation (Ryan, 1990; Ryan et al., 1990), a number of other studies have suggested similar results, including studies on swordtails, auklets, spiders, water mites, and song birds (summarised in Ryan, 1998). These findings have an impact on our understanding of sexual selection, but are applicable to the more general field of animal communication (e.g., Kilner et al., 1999), and are readily applicable to other fields such as cognitive psychology (Enquist, 1998) and conservation biology (Schlaepfer et al., 2002).

SEXUAL COMMUNICATION IN TÚNGARA FROG: SPECIES RECOGNITION AND HISTORICAL CONTINGENCY

Until now we have been addressing how females use variation in male signals within the species to choose mates, and the evolutionary pattern by which the current relationships between signal and receiver came into being. Females also use advertisement calls to ensure conspecific matings. There are about 5000 species of frogs; most of them call, and all known advertisement calls differ among species. Furthermore, in phonotaxis experiments females almost always show a preference for the conspecific call over a heterospecific call, even if the females are allopatric with the heterospecific. There are innumerable strategies that a receiver (human, frog, or computer) can use to discriminate between pairs of stimuli. This is because most animal signals are composites of suites of characters, and different weighting schemes (i.e., the value of individual parameters in decision making) can produce the same results; the study of feature weighting, or heterogeneous summation (Tinbergen, 1951; Baerends et al., 1965) is an important part of animal communication (Nelson and Marler, 1990; Ryan and Getz, 2000). This

has been shown in a study by Gerhardt (1981) of two species of tree frog: *Hyla gratiosa* and *H. cinerea*. The two species have similar advertisement calls, and are both sympatric and synchronic with one another. For each species, the most challenging reproductive decision is decoding the differences between this pair of sounds. By painstakingly varying individual signal parameters, Gerhardt showed that each species weighted call parameters differently when making the same discrimination. In terms of natural selection and adaptation, this difference in recognition strategies might seem trivial; the end result is that females prefer conspecifics over heterospecifics. But clearly, the way in which the two species achieve the same function differs; their brains work differently and we would like to know why.

We have recently been investigating the evolution of species recognition in túngara frogs. Again we have utilised an integrative approach in which studies of behaviour and neurobiology (and ‘artificial’ neurobiology) are performed in a phylogenetic context. The specific question is: “How does past history influence species recognition decisions”; the more general question is “How does historical contingency influence brain function”.

Behaviour

The túngara frog is allopatric with all member of the *Physalaemus pustulosus* species group, and with all members of the genus with one exception: túngara frogs and *P. enesefae* overlap in a small region of the Llanos in Venezuela. Nevertheless, the expectation is that female túngara frogs should prefer the conspecific advertisement call over that of other species.

We conducted a series of phonotaxis experiments in which we synthesised the average call of each species of the species group as well as those of three congeners not in the species group. We asked if females would discriminate between the two calls, showing a preference for the conspecific call over each of the heterospecific calls. These calls contained only the fundamental frequency of the whine, since in túngara frogs information in the upper harmonics of the call does not influence female phonotaxis. In all cases female túngara frogs showed a statistically strong preference for the conspecific call.

We also asked if females would recognise the calls of the other species by presenting them the heterospecific call paired with a white noise stimulus. This answer cannot be garnered from the discrimination experiments between conspecific and heterospecific calls. For example, we know that female túngara frogs prefer a whine-chuck to a whine-only. These results do not reveal whether the whine alone is recognised by females as an advertisement call.

When we conducted the recognition tests we found that females showed statistically significant recognition for a number of heterospecific signals. One reason is that there has been no selection to avoid such calls since túngara frogs are allopatric with these heterospecifics. In the absence of selection, we might expect that calls

sounding similar to that of the túngara frog might elicit a response from female túngara frogs. Thus overall call similarity might explain the cases of false recognition.

Another possible explanation is that of historical contingency. Túngara frogs share recent ancestors with the heterospecifics we tested. Common ancestry explains why species in the group share similar call characters, such as frequency-modulated whines. Shared ancestry could also suggest that females of different species share some similar auditory biases. If females of one species find a certain call appealing it should not be surprising that closely related females might as well, especially when the species are allopatric and there has been no selection for avoiding these heterospecifics (Ryan et al., 2003). We attempted to disentangle the roles of overall acoustic similarity and phylogenetic relatedness. Our phylogeny of the *Physalaemus pustulosus* species group was derived from several data sets: gene sequences, allozyme variation, morphological characters and advertisement calls. Analysis of each data set yields a similar set of phylogenetic relationships with one exception: calls. A phylogeny based on call variation alone little resembles the consensus tree or any of the other phylogenetic hypotheses (Cannatella et al., 1998).

We used various techniques to reconstruct ancestral calls for the species group (fig. 3). The value of each call variable was estimated at the ancestral nodes of the tree using Felsenstein's Independent Contrast method (Felsenstein, 1985), as well as other methods of estimation. We then used the estimates of each of the call variables at each node to synthesise the ancestral call. This exercise, we realise, does not guarantee a good estimate of what ancestors sounded like. We believe, however, that these estimates give us some idea of the acoustic landscape traversed by ancestors in the species group during the evolution of call recognition.

We repeated the discrimination and recognition tests with the ancestral calls, and a pattern began to emerge. Although females mostly still discriminate in favour of the conspecific call, the strength of the discrimination shows some variation among calls. In particular, females do not show statistically significant discrimination between the conspecific call and the call at the ancestral node with *P. petersi*. In the recognition experiments there are a large number of calls, ancestral and heterospecific, that females falsely recognise as indicating an appropriate mate.

Because there is no significant correlation between acoustic similarity and phylogeny, we can partition the effects of each in explaining how female responses vary among calls. In the discrimination experiments phylogenetic relatedness explains 45% of the variation in female phonotactic responses, while call similarity only explains 18%. In the recognition experiments phylogeny (38%) and call similarity (31%) contribute similarly to explaining why females do what they do (Ryan and Rand, 1995, 1999).

We interpret these results as evidence that historical contingency influences the strategies that female túngara frogs use to decode species-specific calls; that is, to decide if a call indicates an appropriate mate. This historical influence might result from the fact that close relatives inherit from a common ancestor aspects of their mating call as well as the mechanisms used to decode these calls. This interpretation

generates a prediction. If one were to manipulate the kinds of calls that ancestors had to decode, then extant species would still be able to decode the conspecific call, but the details of how they do it would differ.

Artificial neural networks

The hypothesis that brain evolution influences decoding schemes seemed doomed to be evaluated by the strength of its logic rather than experimental testing. Artificial neural networks, however, offer a way to test this hypothesis.

In a previous study we had trained populations of artificial neural networks to recognise túngara frog calls (Phelps and Ryan, 1998, 2000; Phelps et al., 2001). We showed that the response of the networks to novel stimuli predicted the response of female túngara frogs to the same novel stimuli, suggesting that there might be some similarities in how the two systems decode the target stimulus and generalise to other stimuli. We used this approach to test the hypothesis of historical contingency described above.

We trained a population of artificial neural networks to recognise calls with a genetic algorithm, which mimics the process of natural selection. Each network in the population was tested with the target call and noise. The degree to which the network discriminated between the two was a measure of its 'fitness'; the larger the difference in response to the two stimuli, the higher its fitness. Once each network was assigned a fitness value, networks would be chosen from the population with replacement; networks of higher fitness were more likely to be selected to be in the next 'generation'. This process was continued until a certain fitness criterion was reached for the population; that is, until the networks in this populations had 'evolved' to recognise the target call.

We used three populations of artificial neural networks. Each had a different 'history' in that they were trained to a different sequence of calls. In the final training session, however, they all were trained to recognise the túngara frog call. Networks in one population, the 'mimetic history' treatment, were trained to first recognise the call at the root of the species group phylogeny ('root'; fig. 3). Once they evolved to recognise the root call, they were trained to recognise the immediate descendent of the root (call d; fig. 3). Once the networks achieved recognition of that call, they were trained to recognise the immediate descendent of the node ancestral to *P. pustulosus* — *P. petersi* (call c; fig. 3). After recognition of this call was achieved, the networks were trained to recognise the túngara frog call. A second population of networks was trained to a random history of calls. Three calls were chosen at random from the species group, including both ancestral and heterospecific calls. After being trained to recognise each of the three calls in sequence the networks were trained to recognise the túngara frog call. Networks in a third population were trained to recognise calls in a treatment referred to as a 'mirror history'. Here, the three ancestral calls used in the mimetic history were rotated 180 degrees in multivariate space creating 'mirror images' of the calls. Although to the human ear these calls did not sound very different from the other ancestral calls, they

occurred in a portion of the acoustic landscape not utilised by the species group, heterospecifics or ancestors. We used this history because the calls used in training had the same degree of acoustic differences as in the mimetic history, but the calls themselves were different.

The first result of these neural network simulations is that, regardless of the treatment (mimetic, random or mirror history), all networks were able to be trained to recognise the túngara frog call. The interpretation is that the network's past history did not constrain its ability to evolve call recognition. We then asked if the network history influenced the manner in which the networks decoded the call. We did this by determining the degree to which each population of networks could predict the behaviour of females. As in our earlier studies, the response of the trained networks and the female túngara frogs to a variety of novel stimuli was determined. Our results showed that only the networks with the mimetic history showed a significant correlation between their response and the response of real females. A maximum likelihood analysis also showed that the response of the mimetic history networks was a significantly better predictor of female preference than were responses of either the networks with the random history or the mirror history.

The neural network simulations suggest several conclusions. History does not constrain the evolution of recognition strategies. History does, however, influence the decoding strategy used. And more specifically, the neural networks with a mimetic history must have utilised a computational strategy in some ways similar to the one used by female túngara frogs.

We hope that we have not been seduced by the elegance of artificial neural networks; we realise artificial intelligence machines are not animal brains. We feel, however, that we offer an approach to testing hypotheses that can be added to an arsenal of other techniques. We think they are especially useful for investigating historical contingency because they capture some of the complexity and the unpredictability as to how brains come about to solve problems.

CONCLUSIONS

Tinbergen (1963) outlined an important heuristic for studying the biology of behaviour. The four questions categorised different approaches that can be taken to derive an understanding of behaviour, and it is obvious that all of these questions must be addressed for a complete understanding of behaviour. But there is more to it. I argue that all of these questions must be addressed for a correct understanding of behaviour, and I illustrate this point with our studies of sexual communication in túngara frogs. Our studies of sexual selection and neural mechanisms, more generally, survival ability and causation, have led to a logically consistent interpretation of how this communication system evolved: trait and preference co-evolve to maximise reproductive success. It was logical but was it true? The addition of a phylogenetic component to our studies showed it was not. Trait and preference did not

co-evolve but, instead, traits evolved to exploit pre-existing preferences. Furthermore, our studies showing female recognition of ancestral and heterospecific calls could easily have been interpreted as females generalising to calls that were similar in their overall acoustic structure, but again by including Tinbergen's question of evolution we see that the vagaries of past history influence the way the frog brain works today.

My goal in this paper was two-fold. First, to illustrate some of the ways ethologists are currently addressing the question of evolution. Second, to argue that employing the four aims and methods of Tinbergen in isolation is not sufficient, but these aims and methods need to be integrated to have an understanding of the biology of behaviour that is both correct and complete.

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REFERENCES

- Alcock, J.S. & Sherman, P.W. (1994) The utility of the proximate-ultimate dichotomy in ethology. *Ethology*, 96, 58-62.
- Aronson, L.R. (1981) Evolution of telencephalic function in lower vertebrates. In: P.R. Laming (Ed.), *Brain Mechanisms of Behaviour in Lower Vertebrates*, pp. 33-58. Cambridge University Press, Cambridge.
- Atz, J.W. (1970) The application of the idea of homology to behaviour. In: L.R. Aronson, E. Tobach, D.S. Lehrman & J.S. Rosenblatt (Eds.), *Development and Evolution of Behaviour: Essays in Honor of T.C. Schneirla*, pp. 53-74. W.H. Freeman, San Francisco.
- Baerends, G.P., Brill, K.A. & Bult, P. (1965) Versuche zur Analyse einer erlernten Reizsituation bei einem Schweinsaffen (*Maccaca nemestrina*). *Z. Tierpsychol.*, 22, 394-411.
- Boul, K.E. & Ryan, M.J. (2004) Population variation of complex advertisement calls in *Physalaemus petersi* and comparative laryngeal morphology. *Copeia*, 2004, 624-631.
- Bourne, G.R. (1993) Proximate costs and benefits of mate acquisition at leks of the frog *Oloolygon rubra*. *Anim. Behav.*, 45, 1051-1059.
- Bucher, T.L., Ryan, M.J. & Bartholomew, G.W. (1982) Oxygen consumption during resting, calling and nest building in the frog *Physalaemus pustulosus*. *Physiol. Zool.*, 55, 10-22.
- Brenowitz, E.A. & Kroodsma, D.E. (1996) The neuroethology of birdsong. In: D.E. Kroodsma & E.H. Miller (Eds.), *Ecology and Evolution of Acoustic Communication in Birds*, pp. 285-304. Cornell University Press, London.
- Brooks, D.R. & McLennan, D.A. (1991) *Phylogeny, Ecology, and Behavior*. University of Chicago Press, Chicago.

- Brooks, D.R. & McLennan, D.A. (2002) *The Nature of Diversity*. University of Chicago Press, Chicago.
- Cannatella, D.C. & Duellman, W.E. (1984) Leptodactylid frogs of the *Physalaemus pustulosus* group. *Copeia*, 1984, 902-921.
- Cannatella, D.C., Hillis, D.M., Chippinendale, P., Weigt, L., Rand, A.S. & Ryan, M.J. (1998) Phylogeny of frogs of the *Physalaemus pustulosus* species group, with an examination of data incongruence. *Syst. Biol.*, 47, 311-335.
- Capranica, R.R. (1977) Auditory processing in anurans. *Fed. Proc.*, 37, 2324-2328.
- Darwin, C. (1871) *The Descent of Man and Selection in Relation to Sex*. Murray, London.
- Davies, N.B. & Halliday, T.R. (1978) Deep croaks and fighting assessment in toads *Bufo bufo*. *Nature*, 274, 683-685.
- DeQueiroz, A. & Wimberger, P.H. (1993) The usefulness of behaviour for phylogeny estimation: Levels of homoplasy in behavioural and morphological characters. *Evolution*, 47, 46-60.
- Enquist, M. & Arak, A. (1998) Neural representation and the evolution of signal form. In: R. Dukas (Ed.), *Cognitive Ecology*, pp. 21-87. University of Chicago Press, Chicago.
- Fee, M.S., Mitra P.P. & Kleinfeld (1998) The role of nonlinear dynamics of the syrinx in the vocalizations of a songbird. *Nature*, 395, 67-71.
- Felsenstein, J. (1985) Phylogenies and the comparative method. *Am. Nat.*, 125, 1-15.
- Fisher, R.A. (1930) *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.
- Gerhardt, H.C. (1981) Mating call recognition in the barking treefrog (*Hyla gratiosa*): Responses to synthetic calls and comparisons with the green treefrog (*Hyla cinerea*). *J. Comp. Physiol.*, 144, 17-26.
- Gerhardt, H.C. & Schwartz, J.J. (2001) Auditory tuning and frequency preferences in anurans. In: M.J. Ryan (Ed.), *Anuran Communication*, pp. 73-85. Smithsonian Institution Press, Washington DC.
- Greenwalt, C.H. (1968) *Bird Song: Acoustics and Physiology*. Smithsonian Institution Press, Washington DC.
- Hamilton, W.D. (1964) The genetical evolution of social behaviour (I and II). *J. Theor. Biol.*, 7, 1-16, 17-52.
- Harvey, P.H. & Pagel M.D. (1991) *The Comparative Method in Evolutionary Biology*. Oxford University Press, Oxford.
- Heinroth, O. (1909) Beobachtungen bei der zucht des zigenmelkers (*Caprimulgus europaeus* L.). *J. Ornithol.*, 57.
- Hennig, W. (1950) *Grundzuge einer Theorie der phylogenetischen Systematik*. Deutscher Zentralverlag, Berlin.
- Hennig, W. (1966) *Phylogenetic Systematics*. Univeristy of Illinois Press, Urbana.
- Hull, D.L. (1988) *Science as Progress*. University of Chicago Press, Chicago.
- Huxley, J.S. (1914) The courtship habits of the Great crested grebe; with an addition to the theory of sexual selection. *Proc. Zool. Soc. Lond.*, XX, 491-562.
- Kilner, R.M. & Davies, N.B. (1999) Signals of need in parent-offspring communication and their exploitation by the common cuckoo. *Nature*, 397, 667-672.
- Kirkpatrick, M. (1982) Sexual selection and the evolution of female choice. *Evolution*, 36, 1-12.
- Lorenz, K. (1941) Comparative studies of the motor patterns of the Anatinae (translated by R. Martin, 1971). *Studies in Animal and Human Behaviour*, 2, 14-18, 106-114.
- Marler, P. (1997) Three models of song learning: Evidence from behaviour. *J. Neurobiol.*, 33, 501-516.
- Martins, E. (1996) *Phylogenies and the Comparative Method in Animal Behaviour*. Oxford University Press, Oxford.
- Nelson, D.A. & Marler, P. (1990) The perception of birdsong and an ecological concept of signal space. In: W.C. Stebbins & M.A. Berkeley (Eds.), *Comparative Perception: Complex Signals*. pp. 443-478. John Wiley & Sons, New York.

- Nottebohm, F. (1984) Birdsong as a model in which to study brain processes related to learning. *Condor*, 87, 227-236.
- Phelps, S.M. & Ryan, M.J. (1998) Neural networks predict response biases in female túngara frogs. *Proc. R. Soc., Lond. Ser. B*, 265, 279-285.
- Phelps, S.M. & Ryan, M.J. (2000) History influences signal recognition: neural network models of túngara frogs. *Proc. R. Soc., Lond. Ser. B*, 267, 1633-1639.
- Phelps, S.M., Ryan, M.J. & Rand, A.S. (2001) Vestigial preference functions in neural networks and túngara frogs. *Proc. Nat. Acad. Sci. (USA)*, 98, 13161-13166.
- Podos, J. (1996) Motor constraints on vocal development in a songbird. *Anim. Behav.*, 51, 1061-1070.
- Rand, A.S., Ryan, M.J. & Wilczynski, W. (1992) Signal redundancy and receiver permissiveness in acoustic mate recognition by the túngara frog *Physalaemus pustulosus*. *Am. Zool.*, 32, 81-90.
- Rand, A.S. & Ryan, M.J. (1981) The adaptive significance of a complex vocal repertoire in a neotropical frog (*Physalaemus pustulosus*). *Z. Tierpsychol.*, 57, 209-214.
- Reeve, H.K. & Sherman, P.A. (1993) Adaptation and the goals of evolutionary research. *Quart. Rev. Biol.*, 68, 1-32.
- Reeve, H.K. & Sherman, P.A. (2001) Optimality and phylogeny: A critique of current thought. In: S. Orzack & E. Sober (Eds.), *Adaptationism and Optimality*. Cambridge University Press, Cambridge.
- Ridley, M. (1983) *The Explanation of Organic Diversity, the Comparative Method and Adaptations for Mating*. Clarendon Press, Oxford.
- Ryan, M.J. (1980) Female mate choice in a Neotropical frog. *Science*, 209, 523-525.
- Ryan, M.J. (1983) Sexual selection and communication in a Neotropical frog, *Physalaemus pustulosus*. *Evolution*, 39, 261-272.
- Ryan, M.J. (1985) *The Túngara Frog, A Study in Sexual Selection and Communication*. University of Chicago Press, Chicago.
- Ryan, M.J. (1990) Sensory systems, sexual selection, and sensory exploitation. *Oxf. Surv. Evol. Biol.*, 7, 157-195.
- Ryan, M.J. (1998) Receiver biases, sexual selection and the evolution of sex differences. *Science*, 281, 1999-2003.
- Ryan, M.J. (1996) Phylogenetics and behaviour: Some cautions and expectations. In: E. Martins (Ed.), *Phylogenies and the Comparative Method in Animal Behaviour*, pp. 1-21. Oxford University Press, Oxford.
- Ryan, M.J., Tuttle, M.D. & Rand, A.S. (1982) Sexual advertisement and bat predation in a Neotropical frog. *Am. Nat.*, 119, 136-139.
- Ryan, M.J., Bartholomew, G.A. & Rand, A.S. (1983) Energetics of reproduction in a Neotropical frog, *Physalaemus pustulosus*. *Ecology*, 64, 1456-1462.
- Ryan, M.J., Fox, J.H., Wilczynski, W. & Rand, A.S. (1990) Sexual selection for sensory exploitation in the frog *Physalaemus pustulosus*. *Nature*, 343, 66-67.
- Ryan, M.J. & Rand, A.S. (1993) Sexual selection and signal evolution: the ghost of biases past. *Phil. Trans. R. Soc. Ser. B*, 340, 187-195.
- Ryan, M.J. & Rand, A.S. (1999) Phylogenetic influences on mating call preferences in female túngara frogs (*Physalaemus pustulosus*). *Anim. Behav.*, 57, 945-956.
- Ryan, M.J. & Getz, W. (2000) Signal decoding and receiver evolution: An analysis using an artificial neural network. *Brain, Behav. Evol.*, 56, 45-62.
- Ryan, M.J., Rand, W., Hurd, P.L., Phelps, S.M. & Rand, A.S. (2003) Generalization in response to mate recognition signals. *Am. Nat.*, 161, 380-394.
- Ryan, M.J. & Rand, A.S. (2003) Mate recognition in túngara frogs: A review of some studies of brain, behaviour, and evolution. *Acta Zool. Sin.*, 49, 713-726.
- Schlaepfer, M.A., Runge, M.C. & Sherman, P.W. (2002) Ecological and evolutionary traps. *Trends Ecol. Evol.*, 17, 474-480.

- Searcy, W.A. & Andersson, M. (1986) Sexual selection and the evolution of song. *Ann. Rev. Ecol. Syst.*, 17, 507-533.
- Sherman, P.W. (1988) The levels of analysis. *Anim. Behav.*, 36, 616-618.
- Shine, R. (1979) Sexual selection and sexual dimorphism in the amphibia. *Copeia*, 1979, 297-306.
- Suthers, R.A. (2001) Peripheral vocal mechanisms in birds: Are songbirds special? *Neth. J. Zool*, 51, 217-242.
- Tinbergen, N. (1951) *The Study of Instinct*. Oxford University Press, Oxford.
- Tinbergen, N. (1963) On aims and methods in ethology. *Z. Tierpsychol.*, 20, 410-433.
- Trivers, R.L. (1971) The evolution of reciprocal altruism. *Q. Rev. Biol.*, 35-57.
- Trivers, R.L. (1972) Parental investment and sexual selection. In: B. Campbell (Ed.), *Parental Investment and Sexual Selection*. Aldine Pub. Co., Chicago.
- Trivers, R.L. (1974) Parent-offspring conflict. *Am. Zool.*, 14, 249-264.
- Trivers, R.L. & Hare, H. (1976) Haplodiploidy and the evolution of the social insects. *Science*, 191, 249-263.
- Trivers, R.L. & Willard, D.E. (1973) Natural selection of parental ability to vary the sex ratio of offspring. *Science*, 179, 90-92.
- Tuttle, M.D. & Ryan, M.J. (1981) Bat predation and the evolution of frog vocalizations in the Neotropics. *Science*, 214, 677-678.
- Whitman, C.O. (1898) *Animal Behaviour*. Woods Hole, Massachusetts.
- Wilczynski, W. & Capranica, R.R. (1984) The auditory system of anuran amphibians. *Progr. Neurobiol.*, 22, 1-38.
- Wilczynski, W., Rand, A.S. & Ryan, M.J. (1995) The processing of spectral cues by the call analysis system of the túngara frog, *Physalaemus pustulosus*. *Anim. Behav.*, 49, 911-929.
- Wilczynski, W., Rand, A.S. & Ryan, M.J. (2001) Evolution of calls and auditory tuning in the *Physalaemus pustulosus* species group. *Brain Behav. Evol.*, 58, 137-151.
- Williams, G.C. (1966) *Adaptation and Natural Selection*. Princeton University Press, Princeton, New Jersey.
- Wilson, E.O. (1975) *Sociobiology*. Belknap Press, Cambridge, Massachusetts.
- Wimberger, P.H. & DeQueiroz, A. (1996) Comparing behavioural and morphological characters as indicators of phylogeny. In: E. Martins (Ed.), *Phylogenies and the Comparative Method in Animal Behaviour*. Oxford University Press, Oxford.