

THE ABILITY OF THE FROG-EATING BAT TO DISCRIMINATE AMONG NOVEL AND POTENTIALLY POISONOUS FROG SPECIES USING ACOUSTIC CUES

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Abstract. The bat *Trachops cirrhosus* eats frogs and uses the frogs' vocalizations to locate them. It is able to discriminate between poisonous and palatable prey species on the basis of acoustic cues alone. In this study we presented bats with pairs of novel frog calls (i.e. calls with which the bats had no prior experience) which, to our ears, differed in the degree to which they were similar to the call of a local, poisonous species, *Bufo typhonius*. In all cases the bats were more often attracted to the call that was least similar to the call of *B. typhonius*. One call which was preferred when it was the member of one stimulus pair was avoided when it was the member of another pair: therefore, bats seemed to classify the calls in a continuous fashion. This has interesting implications for the potential of frog vocal mimicry complexes to evolve under the influence of bat predation. Acoustic analysis of the calls suggests that discrimination was primarily based on temporal properties of the calls.

Our discovery that the frog-eating bat, *Trachops cirrhosus* (Phyllostomatidae), uses frog advertisement calls for localizational cues has important implications for the evolution of anuran vocalizations (Ryan et al. 1981, 1982; Tuttle & Ryan 1981, 1982; Tuttle et al. 1982). Not only do *T. cirrhosus* use the calls to localize frogs, they also rely on acoustic cues to discriminate among species. For example, when given a choice between the calls of the small tungara frog, *Physalaemus pustulosus*, which has a snout-vent length (SVL) of 30 mm, and the large South American bullfrog, *Leptodactylus pentadactylus* (180 mm SVL), the bats preferred the call of the former; presumably because the bullfrog is too large to be a prey item for the bat (Tuttle & Ryan 1981).

T. cirrhosus also discriminate between poisonous and palatable prey species. The skin of toads (family Bufonidae) contains a variety of venoms (Low 1972; Flier et al. 1980): consequently, they are poisonous to most predators. The ability to recognize the calls of toads would be of obvious importance to the bat. We have shown that in a simultaneous choice test, *T. cirrhosus* will avoid the call of the poisonous toad *Bufo typhonius*, a species that is found in the same area as *T. cirrhosus* (Tuttle & Ryan 1981).

Thus in at least some comparisons, *T. cirrhosus* can discriminate between frogs of different sizes and palatabilities on the basis of the frogs' vocalizations alone. A number of studies have investigated visual cues used in prey recognition (reviewed by Curio 1976; see especially Robinson 1970), but to our knowledge no one has addressed the analogous use of acoustic cues by predators, although studies have shown the manner in which predators use acoustic cues to locate their prey (e.g. Konishi 1973; Fiedler 1979).

In this study we investigated the response of *T. cirrhosus* to pairs of novel frog calls: that is, calls with which we were certain that the bats had no prior experience. These were calls of frogs from Florida, U.S.A. To our ears, these calls differed in their degree of similarity to the call of *B. typhonius*. The purpose of this study was twofold. First, we wished to determine whether bats assessed the calls of potentially poisonous prey categorically (e.g. *B. typhonius* calls = poisonous, all others = non-poisonous) or continuously (e.g. calls more similar to *B. typhonius* = more poisonous, calls less similar = less poisonous; see Green & Marler 1979 for a discussion of animal perception of signals). The mode of assessment will not only influence prey preference by the bat, but also should influence the potential for vocal mimicry, since it will dictate the amount of convergence necessary between calls of models and mimics. The

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second goal of this study was to gain some understanding of the call characteristics used by *T. cirrhosus* to discriminate prey. The results of these experiments using natural frog calls can be used to direct studies with synthetic stimuli to assess the ability of bats to discriminate biologically meaningful call parameters in the sonic frequencies (i.e. < 20 kHz). These results are especially interesting because most studies of signal processing by microchiropteran bats have concentrated on ultrasonic frequencies (e.g. Henson 1970; Suga et al. 1978; Pollak & Bodenhamer 1981; but see Poussin & Simmons 1982; Ryan et al., in press).

Methods

Novel Frog Calls

We determined the response of *T. cirrhosus* to pairs of novel frog calls, which to our ears formed a continuum of increasing similarity to the call of *B. typhonius*. Novel calls were used to eliminate the possibility that the bats had prior experience with any of the frog calls tested. There is no overlap in the geographic range of the frog species whose calls were used and the range of *T. cirrhosus*.

The responses of individual bats to pairs of advertisement calls of the following species were determined: *Bufo terrestris*, *Pseudacris nigrita* and *Hyla squirella*. These calls were selected because of their similarity to the *B. typhonius* call, and were recorded by Dr A. Stanley Rand in Florida, U.S.A. We ranked the advertisement calls on the basis of their similarity to the *B. typhonius* call before the playback experiments were conducted. After the playback experiments, we analysed the *B. typhonius* call and the stimulus calls with a Nicolet 444A spectrum analyser.

Playback Experiments

Bats used in the playback experiments were mist-netted along streams at the Smithsonian Tropical Research Institute's field station on Barro Colorado Island, Republic of Panama. The bats were kept, one at a time, in a large (4.5 × 4.5 × 2.3 m) outdoor screened flight cage in the forest on the island, and were tested individually in the same flight cage. During their captivity the bats were fed recently-thawed minnows. This avoided any possibility of the bat associating a food item in the flight cage with a frog species. Most feeding was done each night after the bat was tested, because bats did not respond to frog calls when satiated. After testing, the bats were returned to their original sites of capture.

At the start of testing, the bat was on a perch in one corner of the flight cage and the observer sat in the opposite corner. There was one small extension speaker in each of the other two corners. Calls were broadcast simultaneously from the speakers with two Stellavox tape recorders that were located in the observer's corner, opposite the bat. The distance from the bat to either speaker was ca 4 m. Frog calls were broadcast at an intensity of 75 dB SPL at 1 m from the speaker.

A choice was noted if the bat flew directly from the perch to within 1 m of a speaker during the 60 s of call presentation. In most choices, the bats flew within several inches of the speaker or landed on it (see Table I in Ryan et al. 1982 for typical approach distances of bats to speakers during playbacks). A 'no-response' was noted if the bat did not fly within 1 m of a speaker during the 60 s that calls were broadcast. A bat was tested with a given stimulus pair on one night only, and only one stimulus pair was tested on a given night. Trials were conducted until the bat did not respond in several consecutive trials. In most simultaneous choice tests with *T. cirrhosus*, a bat would not respond more than 10 times in one night. Bats were tested with the following stimulus pairs: *B. terrestris* versus *P. nigrita*, *B. terrestris* versus *H. squirella*, and *P. nigrita* versus *H. squirella*.

Results

Acoustic Analysis of Frog Calls

Spectral and temporal characteristics of the advertisement calls of the local toad, *B. typhonius*, and the novel frog calls used in the playback experiments, are shown in Fig. 1. The *B. typhonius* call has energy concentrated in a frequency band from 1035 Hz to 1925 Hz, with peak energy concentration at 1825 Hz (Fig. 1A). The temporal pattern of the call is characteristic of most toads studied, consisting of a train of pulses with extreme amplitude modulation of a carrier frequency (Fig. 1A; Martin 1972). The *B. typhonius* call has a fast rise time followed by a slower decay, and might be classified a Type-II call according to the scheme of Martin (1972).

The stimulus calls are shown as we ranked them, from most similar to the *B. typhonius* call (*B. terrestris*) to least similar (*H. squirella*), with a power spectrum (frequency versus relative amplitude, left of figure) and oscillogram (time versus relative amplitude, right of figure)

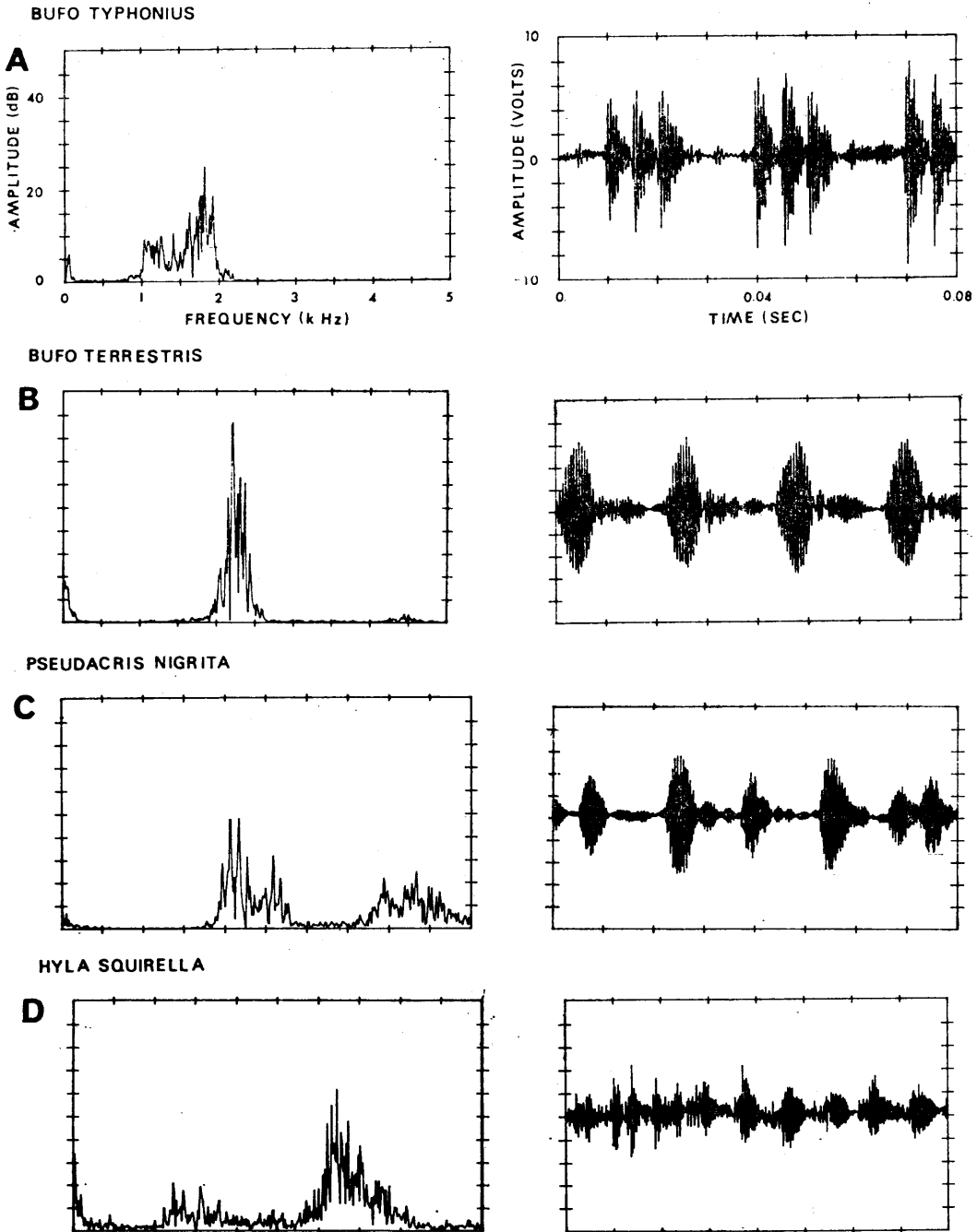


Fig. 1. A power spectrum (frequency versus relative amplitude, left of figure) and an oscillogram (time versus relative amplitude, right of figure) of the *Bufo typhonius* advertisement call, and the calls of the 'novel species' used in the bat preference experiments. The novel calls are ranked from most similar to the *B. typhonius* call to least similar, with *B. terrestris* being most similar and *H. squirella* least similar.

of each call (Fig. 1B–D). The *B. terrestris* call encompasses a narrower frequency range than the others, with most of its energy concentrated around 2212 Hz (Fig. 1B). Single pulses are regularly spaced within the call, as opposed to the occurrence of groups of pulses in the *B. typhonius* call. *B. terrestris* does not have arytenoid valves (Martin 1972), which may result in the pulse having a slower rise time than the *B. typhonius* pulse (Fig. 1B). The *P. nigrata* call has two major bands of spectral energy concentration, from 1975 Hz to 2675 Hz, and from 3937 Hz to 4625 Hz; the peak energy concentration is at 2175 Hz (Fig. 1C). Like the *Bufo* calls, the *Pseudacris* call consists of a series of pulses. Each pulse has a rise time which is faster than *B. terrestris* but slower than *B. typhonius*. The decay time of the *Pseudacris* pulse is slower than that of the *B. terrestris* pulse, but faster than the *B. typhonius* pulse (Fig. 1C). The *H. squirella* call is 'noisier' (i.e. the energy is distributed over a larger range of frequencies) than the three other calls. Most energy occurs from 1225 Hz to 1787 Hz, and from 3000 Hz to 3850 Hz, with a peak of energy at 3225 Hz (Fig. 1D). The call appears to be amplitude-modulated, but not as extremely as the previous three calls. The call also differs from the *Bufo* and *Pseudacris* calls in having a much longer duty cycle (i.e. the duration of the pulse divided by the time from the beginning of one pulse to the next; Fig. 1D).

Playback Experiments

The results of the playback experiments show that when given a choice between calls that are most similar (*B. terrestris*) and second most similar (*P. nigrata*) to the *B. typhonius* call, the bats preferred the less similar call (Table I). They also preferred the less similar call when given a choice between the first and third most similar calls (*B. terrestris* versus *H. squirella*), and between the second and third most similar calls (*P. nigrata* versus *H. squirella*; Table I).

Discussion

Discrimination of the Calls by Men and Bats

Acoustical analysis of the calls reveals a variety of spectral and temporal properties that could result in the calls being perceived as similar to each other (Fig. 1). We ranked the calls as sounding relatively more or less similar to the *B. typhonius* call. An examination of the spectral properties of the calls does not reveal any obvious patterns. Although the *Bufo* calls seem to have the highest overlap of frequencies, it is not clear how the spectral properties of the other calls would have resulted in our ranking.

Many herpetologists, and even some non-herpetologists, readily recognize a toad call when they hear one—even if they have not heard that particular species before. The fundamental frequencies of toad calls can vary greatly (e.g. 450 Hz in *B. regularis* versus 2570 Hz, in *B.*

Table I. Responses of *Trachops cirrhosus* to Pairs of Novel Frog Calls in Simultaneous Choice Tests*

No. of responses			
Bat no.	<i>Bufo terrestris</i> (1)	<i>Pseudacris nigrita</i> (2)	No response
1	0	2	8
2	0	8	0
Total	0	10	8 $\chi^2 = 10.0, P < 0.005$
Bat no.	<i>Bufo terrestris</i> (1)	<i>Hyla squirella</i> (3)	No response
1	0	8	2
2	1	7	0
3	1	8	0
Total	2	23	2 $\chi^2 = 17.7, P < 0.001$
Bat no.	<i>Pseudacris nigrita</i> (2)	<i>Hyla squirella</i> (3)	No response
1	1	7	0
2	2	6	0
3	3	6	7
Total	6	19	7 $\chi^2 = 6.8, P < 0.01$

*The null hypothesis of no preference predicts an equal probability, 0.5, of a response to each stimulus. Trials with no response were not used in calculating the chi-squared values. Numbers in parentheses refer to our subjective ranking of the similarity of stimulus calls to the *Bufo typhonius* call; 1 = most similar.

cognatus; Martin 1972). So the trait that makes most toad calls easy to identify (at least for humans) is not the peak energy distribution alone. With one possible exception (*B. quercicus*), toad calls consist of a train of pulses with extreme amplitude modulation of the carrier frequency. Variation in the temporal distribution of pulses within the call, and the rise and decay times of individual pulses, are due to the action of the thoracic muscles and the nature (or absence) of the arytenoid valves (Martin 1972).

Our classification of call similarity seems to have been based primarily on the temporal properties of the call. The *H. squirella* call is a pulse train, but it differs from the *Bufo* and *Pseudacris* calls by not having the same extreme degree of amplitude modulation and by having a much longer duty cycle. The differences between the *Pseudacris* and *Bufo* calls are less obvious, and perhaps in this discrimination we were aided by the spectral properties of the calls. The calls of *B. terrestris* and *B. typhonius* probably seem very similar because of the extreme amplitude modulation of the pulses. The decay times of the pulses are also similar, but the rise times are quite different. Owing to the action of arytenoid valves in the larynx of *B. typhonius*, the air passes from the larynx to the vocal sac plosively, thus producing the fast rise time which is characteristic of Type-I and II toad calls (Martin 1972). *B. terrestris*, however, do not have arytenoid valves and thus the pulse is characterized by the slower rise time of Type-III calls (Martin 1972).

If the results of the playback experiments are to suggest how the bats discriminated among these calls, two assumptions are necessary: (1) the call preference of the bats is due to the differing degrees of similarity of the test calls to the *B. typhonius* calls; and (2) the bats are discriminating the sounds in a manner similar to humans. Given these assumptions and the results of the playback experiments, it appears that the bats might use predominantly a variety of temporal properties to discriminate the calls. Potential temporal properties include the degree of amplitude modulation, the length of the duty cycle, and the rise and decay times. Furthermore, in some discriminations (e.g. *B. terrestris* versus *P. nigrita*), the spectral patterns of the call might be used as well.

Bat Prey Recognition and Anuran Vocal Mimicry

This study shows that when presented with pairs of novel calls, *T. cirrhosus* tends to avoid

calls that are relatively more similar to the call of the local and poisonous toad *B. typhonius*. This has several implications for prey recognition by *T. cirrhosus* and, consequently, leads us to some speculations about the potential for an anuran vocal mimicry complex to evolve under the influence of bat predation. The first conclusion about the recognition by the bats of potentially poisonous prey is that the perceptual association between the poisonous prey item and its call is not uniquely associated with a local species: that is, novel calls can elicit an avoidance response. The second conclusion is that the bats do not merely classify calls as being poisonous or non-poisonous. Instead, they seem to employ a continuous rather than a categorical mode of classification—calls seem to be classified as more or less poisonous. This continuous mode of perception results in the bat's response being dependent upon the context of the call (i.e. the other calls being tested).

The fact that bats will avoid the novel calls of palatable frogs that sound more similar to the calls of poisonous species suggests that an anuran mimicry complex could exist. The results of this study demonstrate a degree of similarity, not necessarily the minimum degree, which might result in *T. cirrhosus* avoiding the calls of mimics. These results also show that the degree of similarity between the calls of models and mimics is not the only factor that would influence the response of *T. cirrhosus*: the degree of spatial and temporal overlap of the calling species should also play a role. For example, our results (Table I) suggest that if *P. nigrita* and *H. squirella* were calling at the same time, *T. cirrhosus* would be attracted to the call of *H. squirella*. But if *P. nigrita* were calling with *B. terrestris*, it would now be the species more prone to predation by *T. cirrhosus*.

Wickler (1968) has discounted arguments suggesting that models and mimics must overlap in geographic range in order for mimicry to evolve. The results of this study indicate that the evolution of mimicry might actually be enhanced if the model and mimic do not overlap, thus prohibiting simultaneous comparisons of potential prey by the predator.

The mode of prey recognition used by the bats will obviously influence whether or not a vocal mimicry complex can evolve. These results suggest that it might be possible. But they also indicate that the efficacy of a vocal mimicry complex is strongly dependent on the context in which the call is produced.

Mechanisms of Frog Call Discrimination by Bats

Microchiropterans are well known for their ability to use echoes of ultrasonic signals for acoustical imaging of prey items (e.g. Henson 1970; Suga et al. 1978; Pollak & Bodenhamer 1981). But it is now clear that, when hunting, at least some of these bats also rely on acoustic cues in the sonic frequency range emitted by the prey (Fiedler 1979; Tuttle & Ryan 1981; Bell 1982; Poussin & Simmons 1982; Ryan et al. 1982, and in press). *T. cirrhosus* extensively uses acoustic cues in the sonic range not only to localize but also to distinguish among prey items. We have shown in another study that *T. cirrhosus* is quite sensitive to pure-frequency tones in the sonic range (Ryan et al., in press). In fact, the behavioural sensitivity of the bats in the sonic range tested increases in the frequency range where most frog calls occur. Therefore, we would expect that bats could rely on spectral properties to distinguish among a majority of frog species, given the distinctive spectral features of most frog calls (Ryan et al., in press). This study indicates that the similarity of toad and toad-like calls to our ears is largely due to their shared temporal characteristics. The bats apparently classified the calls as relatively more or less similar to *B. typhonius* calls as we did, and we suggest that their discrimination is also based primarily on the temporal properties of the calls. Therefore, *T. cirrhosus* appears to be well-equipped to discriminate frog calls in both the spectral and temporal domains. Future experiments using synthetic stimuli will investigate the extent to which *T. cirrhosus* can discriminate spectral and temporal properties of sonic signals. We predict that these bats are not unique among microchiropterans in their ability to process these types of information, and that acoustic cues in the sonic range emitted by prey probably aid a number of species of microchiropterans in both localizing and identifying their prey.

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