

# Flexibility in assessment of prey cues: frog-eating bats and frog calls

Rachel A. Page<sup>1,2,\*</sup> and Michael J. Ryan<sup>1,2</sup>

<sup>1</sup>Section of Integrative Biology C0930, University of Texas, Austin, TX 78712, USA

<sup>2</sup>Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Republic of Panama

Predators use cues associated with their prey to assess prey quality and to avoid consuming poisonous prey. Considerable attention has been given to predators' use of aposematic cues to assess prey quality, but little is known about predators that eavesdrop on prey cues that are not intended for them. Here we investigate the prey-cue/prey-quality associations of a predator that eavesdrops on the sexual advertisement signals of its prey. Stability is expected in prey-cue/prey-quality associations when mistakes in prey assessment are lethal. Conversely, flexibility is possible when mistakes are less costly. Predators that must respond to temporal and spatial fluctuations in prey availability should be more flexible in their assessment of prey quality. Given these predictions, we examined flexibility in the ability of wild-caught bats to reverse prey-cue/prey-quality associations for a preferred prey and a poisonous one. We found that the predatory bat, *Trachops cirrhosus*, has a heretofore undescribed ability to reverse its evaluations of the cues that signal preferred prey.

**Keywords:** behavioural flexibility; predator-prey interactions; reversal learning; eavesdropping; *Trachops cirrhosus*; *Physalaemus pustulosus*

## 1. INTRODUCTION

It is critical that predators do not eat poisonous prey. Towards this end, predators use the cues of their prey to assess prey palatability. The most striking case is aposematism, in which unpalatable prey have evolved conspicuous cues to warn predators of their distastefulness. Many studies of aposematism have investigated predators' associations of prey cue and prey quality (Guilford 1988; Endler 1991; Schuler & Roper 1992; Speed 2000; Sherratt 2002). In comparison, little attention has been given to the associations of predators that eavesdrop on the signals of their prey. In contrast to the case of aposematism, eavesdropping predators use prey cues that are not intended for them. These cues can be incidental, such as movement of the prey through leaf litter. For example, barn owls (*Tyto alba*; Payne 1971), Indian false vampire bats (*Megaderma lyra*; Marimuthu & Neuweiler 1987), and African heart-nosed bats (*Cardioderma cor*; Ryan & Tuttle 1987) all localize prey using such sounds. Prey-localization cues can also be deliberately produced by the prey, such as sexual advertisement signals. For example, the clerid beetle, *Thanasimus formicarius*, is attracted to the pheromones of its prey, the bark beetle, *Ips typographus* (Hansen 1983); the *Photuris* firefly is attracted to light signals of its prey, the *Photinus* firefly (Lloyd & Wing 1983); the Mediterranean house gecko, *Hemidactylus tursicus*, is attracted to the calls of its prey, the decorated cricket, *Gryllodes supplicans* (Sakaluk & Belwood 1984). In the case of predators eavesdropping on advertisement signals, prey are faced with opposing selection pressures acting on different components of fitness. To attract mates they must be

conspicuous to them, but to survive they should be inconspicuous to predators. This conundrum is well known in the frog-eating bat, *Trachops cirrhosus*, and its anuran prey, the túngara frog, *Physalaemus pustulosus* (Ryan *et al.* 1982), and is widespread among taxa and sensory modalities (Zuk & Kolluru 1998).

The selection pressure exerted by eavesdropping predators on their sexually advertising prey is critical to the dynamics of sexual selection, yet it cannot be fully understood without investigating the stability of predators' associations between prey cue and prey quality. Such associations can have a strong genetic basis, they can be learned through a particular formative experience and then fixed, or they can be plastic throughout the lifetime of the predator.

There are several cases of predators that show a strong genetic component in feeding responses. Newborn garter snakes, *Thamnophis elegans*, from a coastal population sympatric with slugs but allopatric with leeches, readily fed on slugs and showed strong feeding responses to slug chemosensory cues. Newborn snakes from an inland population allopatric with slugs but sympatric with leeches (which are similar to slugs in their chemosensory cues but unpalatable), refused to eat slugs and showed no response to slug chemosensory cues (Arnold 1977, 1980). Similarly, the turquoise-browed motmot, *Eumomota superciliosa*, showed innate aversion to coral snake colours and patterns (Smith 1975). Hand-reared motmots, taken from the nest as hatchlings, flew to the opposite corner of test cages and produced alarm notes when presented with wooden models with patterns of yellow and red rings, but readily attacked snake models with patterns of green and blue rings or red and yellow stripes. Several studies show that domestic chicks, *Gallus gallus domesticus*, also have an

\* Author for correspondence (rachelpage@mail.utexas.edu).

innate aversion to colours found in aposematic prey (Schuler & Hesse 1985; Roper & Cook 1989; Gamberale-Stille & Tullberg 2001). Thus, there are many cases in which predators are born with adaptive associations between prey cues and prey quality.

Associations between prey cue and prey quality can also be acquired and mediated through learning, as many predators form such associations through their experience with prey. For example, learned associations can be instilled by a particular formative experience, such as extreme illness following the ingestion of unpalatable prey (for example, Brower *et al.* 1967; jays and butterflies). Such learning tends to be rapid, and difficult to reverse (Garcia *et al.* 1955). Alternatively, selection for predator flexibility can lead to a predator's ability to redefine prey-cue/prey-quality associations easily, and to alter prey assessment decisions throughout life.

The degree of flexibility of predator associations can have a profound effect on the evolution of prey cues. Considerable attention has been given to the effect of predators on prey cues, particularly in studies of visual aposematism. In most cases, however, studies have been theoretical or conducted solely with laboratory animals, and actual measures of predator response to variation in prey cues have been lacking. A recent study of predator response to prey warning cues found that predators learned less quickly than has been assumed in the theoretical models (Rowe *et al.* 2004), stressing the importance of documenting flexibility with real predators. In this study, we test predator flexibility in an eavesdropping predator, *T. cirrhosus*.

The frog-eating bat, *T. cirrhosus*, uses species-specific anuran mating calls to detect and locate prey, and to assess prey quality (Tuttle & Ryan 1981). It exhibits a strong preference for the calls of palatable species over poisonous ones (Tuttle & Ryan 1981). Specifically, *T. cirrhosus* show a strong preference for the calls of its preferred prey, the túngara frog (*P. pustulosus*), over the calls of local poisonous species (Tuttle & Ryan 1981). The bats also generalize these preferences to novel frog calls: given the calls of two novel species, the bats will preferentially approach calls that sound to humans more like resident palatable frogs over calls that sound more like resident poisonous frogs (Ryan & Tuttle 1983).

Several factors suggest that *T. cirrhosus* is specially adapted for frog predation. Like other echolocating bats, *T. cirrhosus* is highly sensitive to ultrasound and less sensitive to sounds of lower frequencies. Unlike other bats, *T. cirrhosus* shows an additional sensitivity peak below 5 kHz, making it sensitive to the low-frequency sounds that characterize anuran mating calls (Ryan *et al.* 1983). *T. cirrhosus* has three peaks of cochlear neuron density (two more than most mammals; one more than most other species of bat). The third peak is located in the apical portion of the cochlea, which is thought to detect low-frequency sounds (Bruns *et al.* 1989). In addition to its unusual low-frequency hearing, *T. cirrhosus* has unique salivary glands that may serve to neutralize toxins to allow the consumption of anuran prey (Tandler *et al.* 1997).

Although *T. cirrhosus* appears to be specialized for the detection and consumption of anuran prey, *T. cirrhosus* is an opportunistic forager, feeding on many species of insects, frogs and lizards. The ability to eat prey items

other than túngara frogs is not surprising: túngara frogs are seasonal breeders. The interaction between the unique sensory adaptations of *T. cirrhosus* on the one hand and its wide foraging breadth on the other raises a number of interesting questions. Given the consistency of prey-cue/prey-quality associations by *T. cirrhosus*, its generalization of these associations to novel acoustic stimuli, and its specialized adaptations for frog consumption, it seems probable that associations between prey cue and prey quality in this predator would be fixed. Here we ask, given the task of assessing prey quality, how flexible are the associations eavesdropping predators form between prey stimulus and prey quality?

Reversal studies are one means to quantify this type of behavioural flexibility (Pavlov 1928; Bitterman 1972). In a reversal study, responses to one stimulus (A) are rewarded while those to another stimulus (B) are not (A+/B-). When preference for A over B is established, the rewards are reversed, such that B is rewarded and A is not (A-/B+). The experimenter measures latency for the subject to reverse its preference: to now prefer B over A. Reversal learning has been demonstrated in a wide variety of taxa including mammals, birds, reptiles, fishes and insects (Bitterman 1975; Davey 1989). In most of these studies, the experimenter does not reverse a pre-existing preference, but rather establishes and then reverses a preference in the laboratory, usually with novel, arbitrary stimuli with which the subject has had no experience.

Although numerous laboratory studies have shown that learned preferences can be reversed, it is difficult to extrapolate from these studies to the wild. In our study we test flexibility in the predatory bat, *T. cirrhosus*, in response to natural stimuli that have most probably been reinforced in the wild for the individual's entire lifetime, as well as during the species' past evolutionary history. We use reversal experiments to assess plasticity in prey-cue/prey-quality associations to the calls of two potential prey species: the palatable túngara frog, *P. pustulosus*, and the poisonous marine toad, *Bufo marinus* (Chen & Kovarikova 1967; Bagrov *et al.* 1993). We assess the initial response of wild-caught bats to frog calls and toad calls, and then conduct a series of conditioning tests to reverse the initial response. Our results show flexibility in predator ability for prey-cue/prey-quality reversal.

## 2. MATERIALS AND METHODS

Experiments were conducted on Barro Colorado Island, Panama, from February to May 2003. Bats were captured in mist nets set along streams and ponds and were tested in a 4.5 m × 4.5 m × 2.5 m outdoor flight cage. Calls of *P. pustulosus* and *B. marinus* were broadcast from a Dell Inspiron 8100 laptop computer, a SA-150 Realistic amplifier, and 40-1040 Radio Shack speakers. Calls were broadcast at 75 dB SPL (re. 20 µP) at a distance of 1 m from each speaker, to approximate typical intensities of *P. pustulosus* and *B. marinus* calls in the wild. Ten bats were tested in total, and each was tested individually. In each trial, the bat began at a perch in a fixed position in one corner of the flight cage. In single speaker tests, the speaker was concealed beneath a 1.5 m × 1.5 m screen covered in leaf litter and situated on the side of the flight cage opposite the perch. In two-speaker choice tests, each speaker was concealed beneath a separate 1.5 m × 1.5 m screen covered in leaf litter, and the two

screens were situated in opposite corners of the flight cage. The bat's flight to the speaker was videotaped with two cameras. The first video camera (Sony DCR-TRV340) was focused on the bat at the perch, and was used to measure flight latency: the time between the onset of the acoustic stimulus and the bat's flight from its perch. The second video camera (Panasonic WV-BP330) was fixed to the ceiling of the flight cage, and was used to record the bat's approach to the speaker. We defined phonotaxis as flight to and landing on a speaker. Because frogs are protected on Barro Colorado Island, small bait fishes were purchased, frozen, thawed and offered as food rewards on the speakers. The bats readily accepted fishes as food rewards. We carefully regulated the bats' food intake and tested only when the bats were sufficiently hungry to be motivated. Tests were conducted in near darkness, with a Sony HVL-IRH2 infrared light to illuminate the bat at the perch and a 25 W red light bulb to illuminate the bat's approach to the speaker for the video camera recording from above.

(a) *Controls for experimental design*

In trials conducted with rewards, multiple rewards were placed in random positions on the leaf litter screens as well as on each speaker, to ensure that the bat approached the speaker in response to the acoustic stimuli presented and not in response to olfactory, echolocation, visual or other possible cues associated with the reward. In addition to the speaker broadcasting the frog or toad calls, several dummy speakers were positioned under the screens, and all speakers were repositioned between trials. This ensured that the bat's approach to the speaker was associated with the acoustic stimulus broadcast from the speaker, and not with the location of the speaker or with properties of the speaker itself. Speakers were interchanged between trials to control for potential speaker biases, and in two-speaker tests, the presentation of the acoustic stimuli was alternated between sides to control for potential side biases. The screens under which the speakers were concealed were marked in 10 cm intervals in the  $x$  and  $y$  dimensions. Each speaker position corresponded to a specific ( $x,y$ ) coordinate on the screen, and speaker coordinates were assigned with a random number generator using Microsoft EXCEL. In all tests, speakers were repositioned randomly between trials.

(i) *Initial tests*

Once a bat was captured, we tested its initial responses to frog and toad calls. All tests were conducted in approximately 15 min intervals only when bats were motivated to feed. No food rewards were offered in the initial tests.

*Preference tests.* In preference tests, we tested the null hypothesis that bats show no preference for one acoustic stimulus over another. We simultaneously broadcast calls from two speakers positioned a minimum of 2 m apart. One speaker broadcast frog calls and one speaker broadcast toad calls. The calls were broadcast for a maximum of 60 s or until the bat landed on a speaker, whichever came first. We recorded a choice when the bat flew to and landed on one of the two speakers. We conducted three initial preference tests.

*Recognition tests.* It is possible to prefer one stimulus to another when presented with both (preference), but to respond to the less preferred stimulus in the absence of the preferred stimulus. We thus conducted tests to determine whether the calls presented simultaneously in the preference

tests were recognized as cues signalling prey when presented in isolation (recognition). We tested the null hypothesis that there would be no difference between recognition of the test stimulus and recognition of a silent speaker. We broadcast either frog or toad calls from a single speaker for a maximum of 30 s or until the bat landed on the speaker, whichever came first. We presented each call for only 30 s to avoid habituation to the stimulus and recorded a response only if the bat flew to and landed on the speaker within the 30 s of call presentation. To ensure that lack of response to toad calls was not owing to satiation, sensory fatigue or habituation, we followed presentation of the first stimulus immediately with presentation of the second stimulus, and only scored trials in which the bat responded to at least one of the paired stimuli. We compared these results to the number of times the same bat would fly to and land on a silent speaker (bats never landed on silent speakers in any of our observations in the flight cage). We conducted six initial recognition tests in total: three initial recognition tests with toad calls and three initial recognition tests with frog calls.

(ii) *Acquisition of response to toad calls*

Because pilot tests showed no response to toad calls, even when calls were broadcast repeatedly with food rewards on the speaker (Tuttle & Ryan unpublished data; Page unpublished data), we elected to use a transfer-across-a-continuum fading procedure (Terrace 1963) rather than using a trial-and-error technique to condition bats to respond to toad calls. In a trial-and-error procedure, responses to the one stimulus ( $S+$ ) are rewarded, responses to another stimulus ( $S-$ ) are either punished or not reinforced, and subjects learn to discriminate between the two stimuli from their mistakes. In contrast to trial-and-error learning, fading procedures involve the gradual introduction or removal of a stimulus, such that the subject learns to discriminate between two stimuli without the necessity of mistakes (Terrace 1963).

To create the acoustic stimuli used in this conditioning procedure, we faded the first stimulus (frog calls) into the second stimulus (toad calls) in a series of five steps by gradually decreasing the amplitude of the first stimulus and increasing the amplitude of the second stimulus. We synthesized the fading stimuli and equalized the average RMS (root mean square) power of all stimuli with COOL EDIT sound-editing software. The following fading stimuli were used (figure 1):

- (i) frog call at 100% amplitude, toad call at 0% amplitude;
- (ii) frog call at 75% amplitude, toad call at 25% amplitude;
- (iii) frog call at 50% amplitude, toad call at 50% amplitude;
- (iv) frog call at 25% amplitude, toad call at 75% amplitude;
- (v) frog call at 0% amplitude, toad call at 100% amplitude.

We conducted all tests with a single speaker, and rewarded all trials with a food item on the speaker. The acoustic stimulus was presented for a maximum of 60 s or until the bat flew to the speaker, whichever came first. The criterion for progressing from one step of the conditioning procedure to the next was immediate flight to and landing on the speaker. Once the criterion for one stimulus was met, the next stimulus was presented, until the bat had progressed through all five acoustic stimuli. Trials were conducted in approximately 15 min intervals while the bat was motivated to feed.



Figure 1. Oscillograms of the five acoustic stimuli used in the fading procedure. (a) Frog call at 100% amplitude, toad call at 0% amplitude. (b) Frog call at 75% amplitude, toad call at 25% amplitude. (c) Frog call at 50% amplitude, toad call at 50% amplitude. (d) Frog call at 25% amplitude, toad call at 75% amplitude. (e) Frog call at 0% amplitude, toad call at 100% amplitude.

### (iii) Extinction of response to frog calls

We used trial-and-error methods for the next step of the conditioning programme. For ethical reasons, we did not use punishment (the association of the stimulus with a negative outcome such as an electric shock or a noxious taste) to extinguish the bat's response to frog calls. Instead, we used the lack of a positive reinforcement: we repeatedly broadcast frog calls without offering a reward, while continuing to offer rewards in response to toad calls. An extinction trial consisted of broadcasting frog calls for 60 s without a food reward. We conducted trials in approximately 15 min intervals while the bat was motivated to feed. We broadcast toad calls with a food reward once in each interval between the frog-call extinction trials. We continued this procedure until the bat showed no response to frog calls in three consecutive trials. We counted the number of trials necessary to reach this extinction criterion.

### (iv) Final tests

As in initial tests, no rewards were offered in final tests. Again, tests were conducted in approximately 15 min intervals when the bat was motivated to feed.

**Preference tests.** As in the initial preference tests, we tested the null hypothesis that bats show no preference for one acoustic stimulus over another. Final preference tests were identical to the initial preference tests: the bat was presented with one speaker broadcasting frog calls and one speaker broadcasting toad calls, and we recorded latency to flight and phonotaxis to the speaker in the manner described above. Three final preference tests were conducted for each bat.

**Recognition tests.** As in the initial recognition tests, we tested the null hypothesis that bats would show no difference between recognition of the test stimulus and recognition of a silent speaker. As in initial recognition tests, we broadcast either frog or toad calls alone from a single speaker for a maximum of 30 s or until the bat landed on the speaker, whichever came first. We recorded latency to flight and phonotaxis to the speaker in the manner described above. For each bat, three final recognition tests were conducted for toad calls and three final recognition tests were conducted for frog calls.

Following testing, each bat was reconditioned to its original preference and recognition state, and released at the site of capture. All experiments were licensed and approved by the Smithsonian Tropical Research Institute.

## 3. RESULTS

### (a) Controls for experimental design

In all tests the bats flew to the speakers broadcasting the acoustic stimulus and never to the dummy speakers. The bats never found food rewards that were not placed directly on top of the speaker broadcasting the acoustic stimulus. We found no speaker or side biases.

#### (i) Initial tests

**Preference tests.** As expected, bats showed strong initial preference for frog calls over toad calls ( $p=0.001$ , exact binomial test; figure 2a). All 10 bats flew to frog calls over toad calls in each of the three initial preference tests. Although bats were given up to 60 s of call presentation per trial, all subjects flew to and landed on the speaker within 10 s of call presentation (mean latency to flight  $\pm$  s.e.m.:  $2.38 \pm 0.50$  s).

**Recognition tests.** The bats consistently recognized frog calls as signals of potential prey ( $p=0.000\ 01$ , Fisher's exact test; figure 2b), but did not show significant recognition of toad calls ( $p=0.5$ , exact binomial test; figure 2b). All 10 bats flew quickly to frog calls in each of the three initial recognition tests. Eight bats showed no response at all to toad calls in any of the recognition tests, and two bats responded to toad calls in one of the three recognition tests. The bats that flew to toad calls responded with long latencies to flight (13.25 s for one bat, 23.87 s for the other). In comparison, the mean latencies to flight ( $\pm$  s.e.m.) of these two bats in response to frog calls were  $3.03 \pm 1.02$  s and  $1.52 \pm 0.64$  s, respectively. Mean latency to flight ( $\pm$  s.e.m.) for all bats in response to frog calls was  $2.33 \pm 0.38$  s.

#### (ii) Acquisition of response to toad calls

Each of the 10 bats tested rapidly completed the five-step

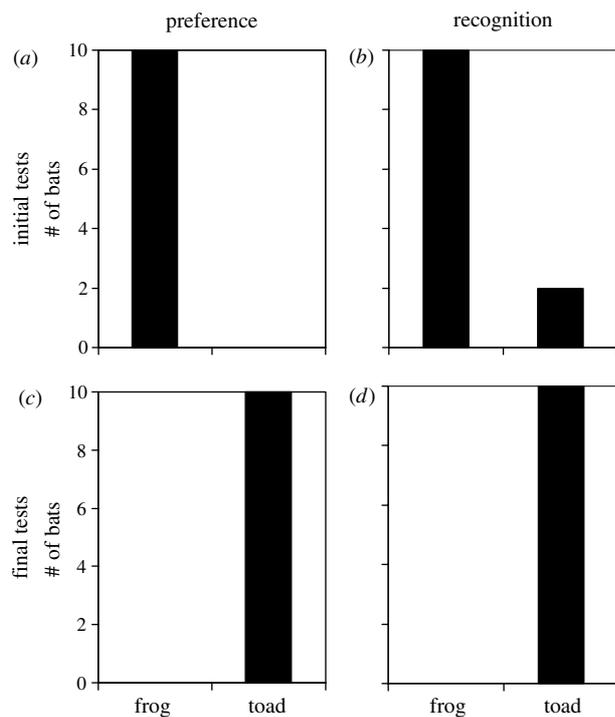


Figure 2. Response of bats to frog and toad calls before and after conditioning. (a) Initial preference tests.

(b) Initial recognition tests. (c) Final preference tests.

(d) Final recognition tests. Bats show strong initial preference for frog over toad calls ( $p=0.001$ , exact binomial test), strong initial recognition for frog calls ( $p=0.000\ 01$ , Fisher's exact test), and no significant initial recognition of toad calls ( $p=0.5$ , Fisher's exact test). Following conditioning, bats show strong preference for toad over frog calls ( $p=0.001$ , exact binomial test), strong recognition for toad calls ( $p=0.000\ 01$ , Fisher's exact test) and no recognition of frog calls ( $p=1.0$ , Fisher's exact test). The differences between initial and final trials are highly significant (for preference:  $p=0.000\ 01$ ; for recognition:  $p=0.000\ 1$ , Fisher's exact test).

fading programme. All responded to each fading step with immediate and direct flight to the speaker, such that only one trial was necessary per step. Trials were conducted in approximately 15 min intervals. All bats completed this stage of the conditioning programme in approximately 1 h.

#### (iii) Extinction of response to frog calls

Extinguishing the response to frog calls required more time, and there was greater variation among bats. For all bats, response to frog calls was extinguished within the course of a night. The mean number of trials ( $\pm$ s.e.m.) required to reach extinction criterion was  $13.7 \pm 2.26$ . The quickest bat learned in five trials; the slowest bat required 29 trials.

#### (iv) Final tests

**Preference.** Following the conditioning programme, bats showed a strong preference for toad calls over frog calls ( $p=0.001$ , exact binomial test; figure 2c). Results from final preference tests were significantly different than results from initial preference tests ( $p=0.000\ 01$ , Fisher's exact test). The mean latency to flight ( $\pm$ s.e.m.) was  $1.54 \pm 0.24$  s.

**Recognition.** Final tests showed strong recognition of toad calls ( $p=0.000\ 01$ , Fisher's exact test; figure 2d) and

no recognition of frog calls ( $p=1.0$ , Fisher's exact test; figure 2d). All 10 bats responded to toad calls with flight to and landing on the speaker; no bats flew in response to frog calls. The differences in recognition between pre-conditioning and post-conditioning responses are highly significant ( $p=0.000\ 1$ , Fisher's exact test). Mean latency to flight ( $\pm$ s.e.m.) to toad calls was  $1.91 \pm 0.28$  s.

## 4. DISCUSSION

In this study, we have shown flexibility in an eavesdropping predator's ability to redefine prey-cue/prey-quality associations.

There has been recent interest in the correlation of learning ability and ecological niche (Galea *et al.* 1996; Hampton & Shettleworth 1996; Sherry & Duff 1996). The pliancy hypothesis (Day *et al.* 1999) suggests that species that specialize on seasonally variable prey, patchy resource availability, or otherwise variable conditions, tend to do well on reversal learning and other tasks that require animals to respond flexibly to environmental cues. Species that must learn to associate a specific cue with high food quality at one time, and subsequently redefine this association when that resource becomes depleted or is no longer available, may be predisposed to rapidly form and re-form associations between food cue and food quality. Several reversal learning studies support this hypothesis. A comparison of actively foraging lizards (*Acanthodactylus boskianus*) and lizards that are sit-and-wait predators (*Acanthodactylus scutellatus*) shows that active foragers perform better on visual reversal learning tasks than sit-and-wait predators (Day *et al.* 1999). Honeybees (*Apis mellifera*) must assess and update information on the location and quality of floral food resources, and respond readily to temporal and spatial fluctuation in resource distribution (Seeley 1986), tasks that suggest a selective advantage for foraging plasticity. Numerous studies show that honeybees are skilled at reversal learning (Ben-Shahar *et al.* 2000; Chandra *et al.* 2000; Ferguson *et al.* 2001; Komischke *et al.* 2002).

Like honeybee foragers, *T. cirrhosus* also relies on a temporally variable food resource. On Barro Colorado Island, frogs are abundant and call conspicuously during the wet season, which lasts approximately eight months each year. In the dry season few frogs call. *T. cirrhosus* is known to feed opportunistically on other prey, including insects, small vertebrates, and even smaller species of bats (Bonato & Facure 2000; Cramer *et al.* 2001). While it may be advantageous for *T. cirrhosus* to respond flexibly to its foraging environment on a seasonal timescale, we were surprised to find this degree of flexibility on the scale of hours rather than weeks or months. It was also unexpected that this degree of flexibility would be exhibited in response to palatable versus poisonous stimuli. In our experiments, *T. cirrhosus* did not choose between two palatable species that differ in temporal availability (as in the case of honeybees, choosing between flower resources that are or are not depleted), but rather between a palatable species and a toxic one. There are many cases in which predators show rapid, even one-trial, learning to avoid the consumption of poisonous prey (Garcia *et al.* 1955). This type of learning, however, is most often fixed through this experience, and is difficult to reverse.

Stability in associations between prey cues and prey

quality is expected when mistakes in assessment are costly, and especially when they are lethal. When prey is assessed by many sensory modalities, however, a mistake at one level can be corrected at another level, and the possibility of ingesting lethal prey becomes small. In these cases, there is the possibility for flexibility in predator assessment of prey.

In *T. cirrhosus*, there are probably several steps at which prey quality can be assessed and toxic prey rejected. The first is the use of prey-emitted acoustic cues, the mating calls produced by male frogs. *T. cirrhosus* can assess prey palatability on the basis of these acoustic cues alone (Ryan & Tuttle 1983). Next, *T. cirrhosus* can use echolocation and/or vision to assess prey size and shape. There is evidence for limited use of echolocation in hunting approaches (Barclay *et al.* 1981). *T. cirrhosus* also has tubercles on its chin and lips whose function is unknown but might allow chemosensory assessment of prey. If *T. cirrhosus* bites the prey, it may recognize unpalatable prey by taste, and for frog species that are only mildly poisonous, *T. cirrhosus* has specialized salivary glands that may reduce some of the toxins found in the frog's skin (Tandler *et al.* 1997). The fact that *T. cirrhosus* does not have to rely solely on acoustic cues, but may also use additional means of prey assessment, increases the opportunity for flexibility in its assessment of prey cues. Predators that can afford to make mistakes should be more flexible in their associations between prey cue and prey quality.

Greenberg (1983) suggested that there is a relationship between a predator's predisposition for learning and the degree to which it is a specialist or generalist in foraging. He found that generalist species of warblers were quick to approach novel objects and flexible in their assessment of potential prey, while specialists were more likely to be neophobic and stereotyped in their foraging strategies. Thus if *T. cirrhosus* is indeed a specialized frog predator, extreme flexibility in foraging would be unexpected in this species.

Our results suggest that on a continuum of specialists and generalists, *T. cirrhosus* may lie somewhere in between, combining the physiological adaptations of a specialist with the cognitive and behavioural flexibility of a generalist. The ability to hear low-frequency sounds may allow *T. cirrhosus* to exploit a largely unoccupied acoustic foraging niche, and within this niche it may be a neophilic generalist.

Our study shows that predators that eavesdrop on the signals of their sexually advertising prey can be highly flexible in their associations between prey cue and prey quality. Predator flexibility can have conservation implications (Schlaepfer *et al.* 2002), affecting the ability of an animal to respond to rapid environmental change, the decline of populations of important prey, and the introduction of novel prey species. Predator flexibility can also affect the dynamics of prey cue evolution. The effect of predator learning on prey cues has been studied in the case of aposematism, but has been largely ignored in the case of eavesdropping predators. For prey that are caught between the necessity of producing conspicuous advertisement calls to attract mates while at the same time attempting to minimize detection by eavesdropping predators, predator flexibility can have a substantial effect on signal evolution. For example, if

genetic changes in frog advertisement calls that made them less recognizable to predators could be quickly matched by non-genetic changes (i.e. learning) in predator response, predator flexibility could constrain prey signal evolution. A less flexible predator, on the other hand, might be slow to match changes in prey advertisement signals, and as such, might impose a lesser selective constraint on prey signal evolution. A thorough theoretical analysis is required for a full understanding of how signal evolution would proceed under selection generated by more and less flexible predators.

We are grateful to M. Domjan for suggesting the use of fading in the conditioning experiment. For comments and discussion we thank M. Dawkins, A. C. Kamil, E. K. V. Kalko, E. G. Leigh, M. D. Tuttle and A. S. Rand, and two anonymous reviewers. For assistance with fieldwork and video analysis we thank D. Bethel, T. Beng, M. K. Clark, K. Klymus, D. K. Marks, K. Sheldon, D. Temple and C. Ting. Funding was provided to R.A.P. by the Smithsonian Tropical Research Institute, the National Science Foundation, the Theodore Roosevelt Memorial Fund of the American Museum of Natural History and the University of Texas at Austin.

## REFERENCES

- Arnold, S. J. 1977 Polymorphism and geographic variation in the feeding behavior of the garter snake *Thamnophis elegans*. *Science* **197**, 676–678.
- Arnold, S. J. 1980 The microevolution of feeding behavior. In *Foraging behavior: ecological, ethological, and psychological perspectives* (ed. A. Kamil & T. Sargent), pp. 409–453. New York: Garland Press.
- Bagrov, A. Y., Roukoyatkina, N. I., Fedorova, O. V., Pinaev, A. G. & Ukhanova, M. V. 1993 Digitalis-like and vasoconstrictor effects of endogenous digoxin-like factor(s) from the venom of *Bufo marinus* toad. *Eur. J. Pharmacol.* **234**, 165–172.
- Barclay, R. M. R., Fenton, M. B., Tuttle, M. D. & Ryan, M. J. 1981 Echolocation calls produced by *Trachops cirrhosus* (Chiroptera: Phyllostomatidae) while hunting for frogs. *Can. J. Zool.* **59**, 750–753.
- Ben-Shahar, Y., Thompson, C. K., Hartz, S. M., Smith, B. H. & Robinson, G. E. 2000 Differences in performance on a reversal learning test and division of labor in honey bee colonies. *Anim. Cogn.* **3**, 119–125.
- Bitterman, M. E. 1972 Comparative studies of the role of inhibition in reversal learning. In *Inhibition and learning* (ed. R. A. Boakes & M. S. Halliday), pp. 153–176. London: Academic Press.
- Bitterman, M. E. 1975 The comparative analysis of learning. *Science* **188**, 699–709.
- Bonato, V. & Facure, K. G. 2000 Bat predation by the fringe-lipped bat, *Trachops cirrhosus* (Phyllostomidae, Chiroptera). *Mammalia* **64**, 241–243.
- Brower, L. P., van Brower, J. & Corvino, J. M. 1967 Plant poisons in a terrestrial food chain. *Proc. Natl Acad. Sci. USA* **57**, 893–898.
- Bruns, V., Burda, H. & Ryan, M. J. 1989 Ear morphology of the frog-eating bat (*Trachops cirrhosus*, Family: Phyllostomidae): apparent specializations for low-frequency hearing. *J. Morphol.* **199**, 103–118.
- Chandra, S. B. C., Hosler, J. S. & Smith, B. H. 2000 Heritable variation for latent inhibition and its correlation with reversal learning in honeybees (*Apis mellifera*). *J. Comp. Psychol.* **114**, 86–97.
- Chen, K. K. & Kovarikova, A. 1967 Pharmacology and toxicology of toad venom. *J. Pharm. Sci.* **56**, 1535–1541.
- Cramer M. J., Willig M. R. & Jones, C. 2001 *Trachops cirrhosus*. *Mammalian Species*. **656**, 1–6.

- Davey, G. 1989 *Ecological learning theory*. London: Routledge.
- Day, L. B., Crews, D. & Wilczynski, W. 1999 Spatial and reversal learning in congeneric lizards with different foraging strategies. *Anim. Behav.* **57**, 393–407.
- Endler, J. A. 1991 Interactions between predators and prey. In *Behavioural ecology: an evolutionary approach* (ed. J. R. Krebs & N. B. Davies), pp. 169–196. Oxford: Blackwell Scientific Publications.
- Ferguson, H. J., Cobey, S. & Smith, B. H. 2001 Sensitivity to a change in reward is heritable in the honeybee, *Apis mellifera*. *Anim. Behav.* **61**, 527–534.
- Galea, L. A. M., Kavaliers, M. & Ossenkopp, K.-P. 1996 Sexually dimorphic spatial learning in meadow voles *Microtus pennsylvanicus* and deer mice *Peromyscus maniculatus*. *J. Exp. Biol.* **199**, 195–200.
- Gamberale-Stille, G. & Tullberg, B. S. 2001 Fruit or aposematic insect? Context-dependent colour preferences in domestic chicks *Proc. R. Soc. B*, **268**, 2525–2529. (doi:10.1098/rspb.2001.1814).
- Garcia, J., Kimeldorf, D. J. & Koellino, R. A. 1955 Conditioned aversion to saccharin resulting from exposure to gamma radiation. *Science* **122**, 157–158.
- Greenberg, R. 1983 The role of neophobia in determining the degree of foraging specialization in some migrant warblers. *Am. Nat.* **122**, 444–453.
- Guilford, T. 1988 The evolution of conspicuous coloration. *Am. Nat.* **131**(Suppl.), S7–S21.
- Hampton, R. R. & Shettleworth, S. J. 1996 Hippocampus and memory in a food-storing and in a nonstoring bird species. *Behav. Neurosci.* **110**, 946–964.
- Hansen, K. 1983 Reception of bark beetle pheromone in the predaceous clerid beetle, *Thanasimus formicarius* (Coleoptera: Cleridae). *J. Comp. Physiol.* **150**, 371–378.
- Komischke, B., Giurfa, M., Lachnit, H. & Malun, D. 2002 Successive olfactory reversal learning in honeybees. *Learn. Mem.* **9**, 122–129.
- Lloyd, J. E. & Wing, S. R. 1983 Nocturnal aerial predation of fireflies by light-seeking fireflies. *Science* **222**, 634–635.
- Marimuthu, G. & Neuweiler, G. 1987 The use of acoustical cues for prey detection by the Indian false vampire bat, *Megaderma lyra*. *J. Comp. Physiol. A* **160**, 509–516.
- Pavlov, I. P. 1928 *Lectures on conditioned reflexes*. New York: International Publishers.
- Payne, R. S. 1971 Acoustic location of prey by barn owls (*Tyto alba*). *J. Exp. Biol.* **54**, 535–573.
- Roper, T. J. & Cook, S. E. 1989 Responses of chicks to brightly coloured insect prey. *Behaviour* **110**, 276–293.
- Rowe, C., Lindström, L. & Lytinen, A. 2004 The importance of pattern similarity between Müllerian mimics in predator avoidance learning *Proc. R. Soc. B*, **271**, 407–413. (doi:10.1098/rspb.2003.2615).
- Ryan, M. J. & Tuttle, M. D. 1983 The ability of the frog-eating bat to discriminate among novel and potentially poisonous frog species using acoustic cues. *Anim. Behav.* **31**, 827–833.
- Ryan, M. J. & Tuttle, M. D. 1987 The role of prey-generated sounds, vision, and echolocation in prey localization by the African bat *Cardiaderma cor* (Megadermatidae). *J. Comp. Physiol.* **161**, 59–66.
- Ryan, M. J., Tuttle, M. D. & Rand, A. S. 1982 Bat predation and sexual advertisement in a neotropical anuran. *Am. Nat.* **119**, 136–139.
- Ryan, M. J., Tuttle, M. D. & Barclay, R. M. R. 1983 Behavioral responses of the frog-eating bat, *Trachops cirrhosus*, to sonic frequencies. *J. Comp. Physiol.* **150**, 413–418.
- Sakaluk, S. K. & Belwood, J. J. 1984 Gecko phonotaxis to cricket calling song: a case of satellite predation. *Anim. Behav.* **32**, 659–662.
- Schlaepfer, M. A., Runge, M. C. & Sherman, P. W. 2002 Ecological and evolutionary traps. *Trends Ecol. Evol.* **17**, 474–480.
- Schuler, W. & Hesse, E. 1985 On the function of warning coloration: a black and yellow pattern inhibits prey attack by naïve domestic chicks. *Behav. Ecol. Sociobiol.* **16**, 249–255.
- Schuler, W. & Roper, T. J. 1992 Responses to warning coloration in avian predators. *Adv. Stud. Behav.* **21**, 111–146.
- Seeley, T. D. 1986 Social foraging by honeybees: how colonies allocate foragers among patches of flowers. *Behav. Ecol. Sociobiol.* **19**, 343–354.
- Sherratt, T. N. 2002 The coevolution of warning signals *Proc. R. Soc. B*, **269**, 741–746. (doi:10.1098/rspb.2001.1944).
- Sherry, D. F. & Duff, S. J. 1996 Behavioural and neural bases of orientation in food-storing birds. *J. Exp. Biol.* **199**, 165–172.
- Smith, S. M. 1975 Innate recognition of coral snake pattern by a possible avian predator. *Science* **187**, 759–760.
- Speed, M. P. 2000 Warning signals, receiver psychology and predator memory. *Anim. Behav.* **60**, 269–278.
- Tandler, B., Nagato, T. & Phillips, C. J. 1997 Ultrastructure of the unusual accessory submandibular gland in the fringe-lipped bat, *Trachops cirrhosus*. *Anat. Rec.* **248**, 164–175.
- Terrace, H. S. 1963 Discrimination learning with and without “errors”. *J. Exp. Anal. Behav.* **6**, 1–27.
- Tuttle, M. D. & Ryan, M. J. 1981 Bat predation and the evolution of frog vocalizations in the neotropics. *Science* **214**, 677–678.
- Zuk, M. & Kolluru, G. R. 1998 Exploitation of sexual signals by predators and parasitoids. *Q. Rev. Biol.* **73**, 415–438.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.