

# Acoustic mimicry in a predator–prey interaction

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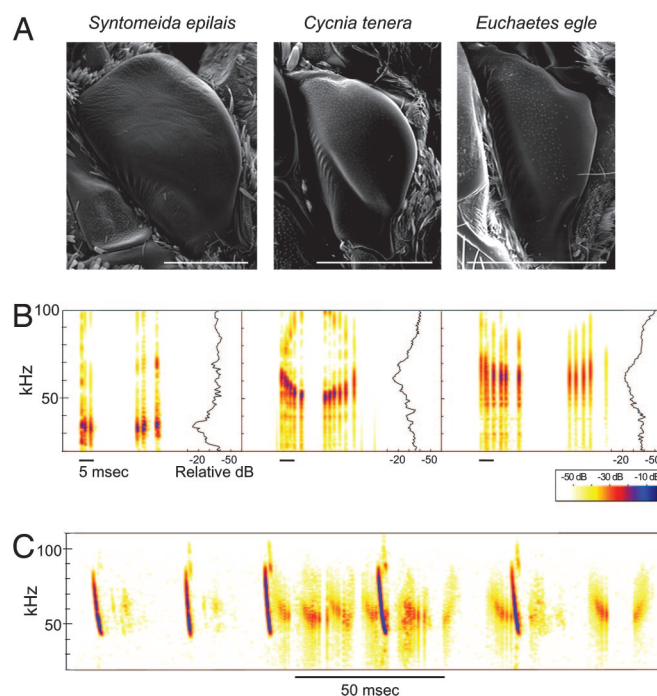
Mimicry of visual warning signals is one of the keystone concepts in evolutionary biology and has received substantial research attention. By comparison, acoustic mimicry has never been rigorously tested. Visualizing bat–moth interactions with high-speed, infrared videography, we provide empirical evidence for acoustic mimicry in the ultrasonic warning sounds that tiger moths produce in response to echolocating bats. Two species of sound-producing tiger moths were offered successively to naïve, free-flying red and big brown bats. Noctuid and pyralid moth controls were also offered each night. All bats quickly learned to avoid the noxious tiger moths first offered to them, associating the warning sounds with bad taste. They then avoided the second sound-producing species regardless of whether it was chemically protected or not, verifying both Müllerian and Batesian mimicry in the acoustic modality. A subset of the red bats subsequently discovered the palatability of the Batesian mimic, demonstrating the powerful selective force these predators exert on mimetic resemblance. Given these results and the widespread presence of tiger moth species and other sound-producing insects that respond with ultrasonic clicks to bat attack, acoustic mimicry complexes are likely common components of the acoustic landscape.

aposematism | Arctiidae | bats

Visual mimicry has played an important role in evolutionary theory (1, 2) since Bates (3) and Müller (4) first proposed that mimics benefit through deception if they are palatable or through spreading the cost of educating predators if they are also noxious. Recent reviews of warning signals and mimicry (5, 6) make no mention of the acoustic domain despite the widespread use of sound as an aposematic signal in animals (7). Decades of anecdotal observations (8–12) have suggested acoustic mimicry among groups ranging from viperid snakes (12) to honey bees and droneflies (9). Perhaps the best studied of these is the purported model/mimic complex involving rattlesnakes and burrowing owls (13).

Here, we report definitive experimental evidence for acoustic mimicry. Tiger moths answer the echolocation attack of bats with ultrasonic clicks broadcast from bilateral metathoracic structures called tymbals (Fig. 1) [to view the tymbal in action, see supporting information (SI) Movie 1]. Vigorous debate (14) over the functions of these sounds has produced three non-mutually exclusive hypotheses: startle, jamming, and warning. Although some evidence exists for both startle (15) and jamming (16, 17) effects, recent work (18) confirmed one critical assumption of the warning model: naïve big brown bats (*Eptesicus fuscus*) failed to learn to avoid chemically protected moths unless those moths also provided an acoustic warning. Acoustic aposematism is a defensive strategy that is clearly open to mimicry.

We trained naïve, lab-raised bats to hunt tethered moths, on the wing, in view of two high-speed video cameras, allowing three-dimensional visualization of interactions that occurred in fractions of a second (see SI Movie 2). The two subject bat species varied in the extent of their ecological association with tiger moths. Red bats (*Lasiurus borealis*) eat primarily Lepidoptera across both seasons and locations (19, 20), whereas big brown bats (*Eptesicus fuscus*) eat mainly beetles, occasionally including moths in their diet (21).



**Fig. 1.** Tiger moth acoustic mimicry complex. (A) Scanning electron micrographs of the tiger moths' sound-producing structures (tymbals) used in this study. Some scales were removed for clarity. (Reference bars: 1 mm.) (B) Example spectrograms (kilohertz  $\times$  time) and power spectra (kilohertz  $\times$  amplitude) of each species call. See ref. 34 for species averages. Note that each call comprises two groups of clicks. The first group is produced as the tymbal is actively pulled inward along the striated band. The second group is produced as the tymbal passively returns to its resting state (see SI Movie 1). (C) A *C. tenera* tiger moth responding to an *L. borealis* echolocation attack. Notice that the tiger moth calls start just after the third echolocation cry. The moth calls appear to be different from B because of overlap created by asynchronous activity between the paired tymbals.

## Results and Discussion

To address Müllerian mimicry, we offered a noxious model tiger moth to five *E. fuscus* and two *L. borealis* bats for five nights, then substituted a second noxious tiger moth species on night 6 (Fig. 2). Each night, four sound-producing tiger moths were randomly presented along with 12 other palatable, silent control moths: eight pyralids (*Galleria mellonella*; the moths initially used to train the bats to capture prey) and four noctuid novelty controls size-matched to the experimental tiger moths presented. Three *E. fuscus* and one *L. borealis* were presented with *Cycnia tenera* as the model and *Syntomeida epilais* as the mimic, two *E. fuscus*

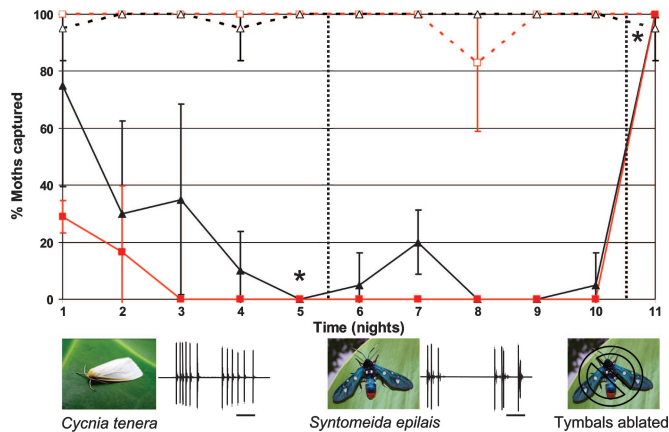
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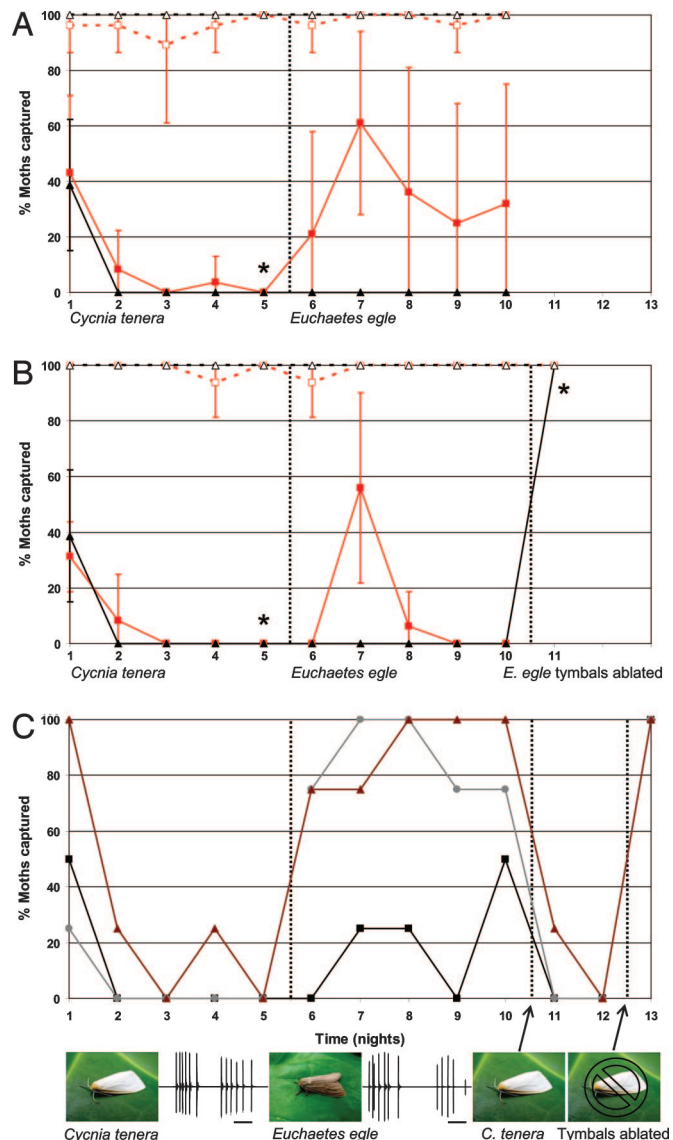
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**Fig. 2.** Müllerian mimicry comparison. Black lines are *E. fuscus* ( $n = 5$ ), and red lines are *L. borealis* ( $n = 2$ ). Solid lines graph the percentage of tiger moths captured. Dashed lines chart the percentage of noctuid novelty controls captured. See *Materials and Methods* for details. Vertical dotted lines indicate tiger moth changes illustrated below the figure. Beside each moth's image are oscillogram (time  $\times$  relative amplitude) traces of its call. (Scale bars: 10 msec.) Asterisks above day 5 indicate statistical significance when comparing day 1 with 5, and those above day 11 specify a significant difference between days 10 and 11. Data are mean  $\pm$  SD. It is important to note that the low percentage of moths captured on night 1, in both Müllerian (this figure) and Batesian (Fig. 3) comparisons, was due both to an initial startle response in which the first sound-producing moth was often avoided and to frequent one-trial learning of the aposematic signal.

and one *L. borealis* were presented with the same moths in reverse order. Both groups learned to avoid the noxious model during the course of the first five nights. As the results for both orders of moth presentation were nearly identical, the data were pooled for analysis. *C. tenera* caterpillars sequester cardiac glycosides from their hostplant, *Apocynum cannabinum*, and the resulting adults are thoroughly unpalatable to bats (22). *S. epilais*, which relies on similar cardiac glycoside chemistry for its unpalatability, consumes *Echites umbellata* as its principal indigenous hostplant and currently relies heavily on *Nerium oleander* (23). The distributions of all of the moths, bats, and hostplants used in the Müllerian experiments overlap in central and northern Florida. By night 5, all bats had learned to avoid the model completely (Fig. 2; for avoidance behavior, see [SI Movie 3](#); Friedman nonparametric ANOVA,  $\chi^2 = 43.02$ ;  $df = 10$ ,  $p < 0.001$ ; Wilcoxon post hoc test comparing night 1 with 5;  $Z = -2.388$ ; two-tailed  $P = 0.017$ ,  $Q = 0.04$ ). On night 6, when the presumed Müllerian mimics were introduced, only one big brown bat captured a single moth (*S. epilais*). Müllerian mimicry clearly works in this acoustic system (Wilcoxon test comparing nights 5 and 6,  $Z = -1.0$ ; two-tailed  $P = 0.317$ ,  $Q = 0.423$ ). The *E. fuscus* showed some catching behavior on night 7, but it quickly decreased and avoidance of the mimic continued for five nights of presentation (Wilcoxon test comparing nights 6 and 10,  $Z = 0.0$ ; two-tailed  $P = 1.0$ ,  $Q = 1.0$ ). To determine whether the bats were generalizing on the basis of the clicks, on night 11 we removed the moths' tymbals and presented the silenced moths to the bats. The percentage of tiger moths caught returned to control levels (Wilcoxon test comparing nights 10 and 11,  $Z = -2.333$ ; two-tailed  $P = 0.017$ ,  $Q = 0.04$ ), but all of these silent noxious moths were subsequently dropped. It is the pre-generated sounds that are driving the mimicry; olfactory cues, wingbeat frequency, and other information from the echolocation stream do not appear to be important.

Batesian mimicry was investigated by again training naïve bats (three *E. fuscus* and seven *L. borealis*) for five nights to avoid a model, *C. tenera* (Fig. 3A; Friedman nonparametric ANOVA,  $\chi^2 =$



**Fig. 3.** Batesian mimicry comparison. See Fig. 2 legend for graph details. (A) All bats used in the Batesian contrast (*E. fuscus*,  $n = 3$ ; *L. borealis*,  $n = 7$ ). The vertical dotted line indicates a tiger moth change from *C. tenera* to *E. egle*. (B) Bats that were deceived by the Batesian mimic (*E. fuscus*,  $n = 3$ ; *L. borealis*,  $n = 4$ ). Vertical dotted lines indicate tiger moth changes from *C. tenera* to *E. egle* and then to *E. egle* with tymbals ablated. (C) Three *L. borealis* that discovered the Batesian mimic, graphed individually. Vertical dotted lines indicate tiger moth changes illustrated below the panel. Data are means  $\pm$  SD.

66.41;  $df = 10$ ,  $p < 0.001$ ; Wilcoxon post hoc test comparing nights 1 and 5,  $Z = -2.871$ ; two-tailed  $P = 0.004$ ,  $Q = 0.01$ ). On night 6, *Euchaetes egle* was introduced. *E. egle* larvae feed on milkweeds, including *Asclepias tuberosa*, but apparently do not sequester the plants' cardiac glycosides in their adult tissues; the moths are totally palatable (22). It is noteworthy that we have often found the hostplants of *C. tenera* and *E. egle* in the same fields, and the adult moths resting on each other's hostplants. Fig. 3A shows that while statistically supporting Batesian mimicry (Wilcoxon test comparing nights 5 and 6,  $Z = -1.414$ ; two-tailed  $P = 0.157$ ,  $Q = 0.18$ ), the variance in capture behavior increased markedly when *E. egle* was introduced. Partitioning the data into bats that were deceived by the Batesian mimic (Fig. 3B) and those that discovered its palatability (Fig. 3C) reveals the cause.

Three *E. fuscus* and four *L. borealis* did not capture *E. egle* when it was introduced on night 6 (Fig. 3B; Friedman nonpara-

metric ANOVA,  $\chi^2 = 66.41$ ;  $df = 10$ ,  $p < 0.001$ ; Wilcoxon post hoc test comparing nights 5 and 6,  $Z = 0.0$ ;  $P = 1.0$ ,  $Q = 1.0$ ). On night 7, all of the *L. borealis* showed some degree of capture behavior, but as in the Müllerian experiments, the behavior quickly terminated, and avoidance of the mimic was not different between the first and last night of its presentation (Wilcoxon test comparing nights 6 and 10,  $Z = 0.0$ ;  $P = 1.0$ ,  $Q = 1.0$ ). Most of the moths taken during these captures were dropped and not eaten, suggesting that other signals, such as chemical cues or vibration in the bats' catching membranes, induced caution. On night 11, when *E. egle* with tymbals removed were introduced, all of the silenced moths were caught and eaten (Wilcoxon test comparing nights 10 and 11,  $Z = -2.646$ ; two-tailed  $P = 0.008$ ,  $Q = 0.028$ ). Our results demonstrate that mimetic generalization was driven by the moth sounds.

Three *L. borealis* discovered the palatability of *E. egle* (to view a discovered Batesian mimic being captured, see [SI Movie 4](#)). Two of them (Fig. 3C, red triangles and gray circles) avoided only the first *E. egle* presented and subsequently captured the next three tiger moths offered on day 6. Interestingly, most of these captured moths were dropped; it was not until night 7 that these two bats ate all of the *E. egle* they captured. The other red bat in this group took substantially longer to discover the palatability of *E. egle* (Fig. 3C, black squares). This animal did not capture any tiger moths on night 6 when they were introduced, but by night 10 it captured and ate 50% of the *E. egle* offered. On night 11, noxious sound-producing *C. tenera* were reintroduced. With the exception of one capture, the bats did not touch any of these *C. tenera*, thus discriminating between the palatable mimic and the unpalatable model. Starting on night 13, silenced *C. tenera* were presented to these three bats for one, two, and five nights, respectively (only the first night of muted *C. tenera* presentation is graphed in Fig. 3C). All of these silenced, noxious moths were caught and dropped, showing once again that the prey-generated sounds drive discrimination. Although an omnibus test of planned comparisons for this group revealed statistical differences (Friedman nonparametric ANOVA,  $\chi^2 = 30.09$ ;  $df = 12$ ,  $p < 0.01$ ), no planned pairwise comparisons were significant (Wilcoxon tests comparing nights 1 and 5, 5 and 6, 6 and 10, 10 and 11, and 12 and 13; all two-tailed  $P = 0.061$ – $0.069$ , all  $Q = 0.18$ ).

It is tempting to hypothesize that the close ecological relationship between red bats and moths can explain why only *L. borealis* discovered the acoustic deception of *E. egle*. Assaying more *E. fuscus* might reveal that some big brown bats are capable of discovering the Batesian mimics. Regardless, sound-producing tiger moths, by means of mimicry, enjoy survival benefits from two very different acoustic predators: bats that specialize on moths and bats that are infrequent moth predators. There are >11,000 species of tiger moths worldwide (24) and numerous sympatric species in any single tropical location (25). This diversity and recent discoveries of both tiger beetles (26) and hawkmoths (unpublished work) responding to bats with ultrasonic sounds suggest that acoustic mimicry complexes are likely to be common and rich components of the natural world.

## Materials and Methods

**Animals.** All vertebrate care was in accordance with Wake Forest University's Animal Care and Use Committee guidelines (ACUC #A04-188). Tiger moths were collected in North Carolina (*C. tenera* and *E. egle*) and Florida (*S. epilais*) and reared in the lab on their natural hostplants: *Cycnia tenera* Hübner, *Apocynum cannabinum* L.; *Syntomeida epilais* (Walker), *Nerium oleander* L.; *Euchaetes egle* Drury, *Asclepias tuberosa* L. Preflight big brown bat (*Eptesicus fuscus* Beauvois) juveniles were obtained from roosts in Forsyth County, North Carolina, and brought into the lab at Wake Forest University. Preflight red bat (*Lasiurus borealis* Müller) pups were obtained from wildlife rehabilitation clinics in central Texas and transported to the laboratory. Bats were housed in an outdoor flight facility (16 ×

6 × 4 m) that was entirely covered in fine insect netting to keep the bats' insect-catching experience under experimental control. During their development and before experiments, the bats were maintained on a diet of mealworms (*Tenebrio* larvae) supplemented with blended meat (Gerber baby food) and high-calorie dietary supplement (Nutri-Cal) fed by syringe (27). Bats were given free access to water, and *L. borealis* juveniles were given an additional 1-ml s.c. injection of lactated Ringers daily. As the bat pups learned to fly and began to hunt, they were trained to capture tethered moths by using a commercially available pyralid moth, *Galleria mellonella*. Male *G. mellonella* use ultrasound in sexual communication when not flying, but they do not acoustically respond to bat attack (28). To eliminate any insectborne ultrasound from the naïve bat pups' environment, however, only female moths were used for training and experimentation. Once the bats were proficient at capturing moths, geometrid moths were introduced to expose them to variations in moth size and wing shape. Geometrid and noctuid moths were captured in the field at UV lights. Noctuid novelty controls used in the experiments included but were not restricted to the following genera: *Anagrapha*, *Anaplectoides*, *Cerma*, *Heliothis*, *Himella*, *Metaxaglaea*, and *Spodoptera* (29).

**Equipment.** Experiments were conducted in an anechoic foam-lined indoor flight facility (5.8 × 4.0 × 3.0 m). Each bat-moth interaction was captured at 250 frames/sec with a pair of digital, high-speed, infrared-sensitive video cameras (Photron FastCam PCI 500) recorded with Photron FastCam Viewer v.1.3 installed on an R40 IBM laptop. Infrared illumination was provided by four Wildlife Engineering LED arrays. This illumination was supplemented with a low-intensity deep red light for behavioral observation. The video was synchronized to a Pettersson Elektronik D940 bat detector and recorded in BatSound Pro v.3.3 installed on an A30 IBM laptop connected to the bat detector via a National Instruments 6062E PCMCIA A/D sampling at 250 kHz. The acoustic behavior of the bats and moths was monitored by the experimenter via a set of Sony 900-MHz wireless headphones connected to the bat detector. For the purposes of the data reported here, this equipment was used to confirm behavioral observations and assess tiger moth response to bat attack.

**Experimental Design.** For the 11–17 consecutive days of each experiment, individual bats were allowed to hunt 16 moths per day sequentially and in random order. The moths were tethered to a fine monofilament line with a small surgical microclip. The tether was attached to a weighted mobile that, coupled with the moth's own erratic flight, allowed for random prey movement within a defined interaction space. Eight *G. mellonella* were presented each night, along with four silent, palatable noctuids that served as size-matched novelty controls and four experimental sound-producing tiger moths. Each moth was presented for 1 min or 10 flight passes. The learning of any aposematic signal depends on the rate at which the animal experiences the stimulus and reinforcer (30). The 25% tiger moth presentation rate we offered compares favorably with UV trap catches reported by other workers (31) and our own observations in a variety of habitats.

**Statistical Analysis.** The heteroscedasticity in our data and multiple measurements made of each bat across several nights dictated the use of paired, nonparametric statistics. Analysis was performed in SPSS v.14.0 (SPSS, Chicago, IL). Each data set (Figs. 2 and 3) was first analyzed with a Friedman's ANOVA. *A priori* post hoc comparisons of percentage of tiger moths captured between two nights (see text for specifics) were performed by using Wilcoxon pairwise tests. All alpha levels were set at 0.05. To control for multiple comparison errors, we computed an adjusted *P* value (*Q* value) using the false discovery rate (32) method in QVALUE (33) (bootstrap method;  $\lambda = 0$ ). We

report both the unadjusted  $P$  value (false positive rate) and the  $Q$  value (false discovery rate) for each test.

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