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Sound strategy: acoustic aposematism in the bat–tiger moth arms race

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Abstract The night sky is the venue for an ancient arms race. Insectivorous bats with their ultrasonic sonar exert an enormous selective pressure on nocturnal insects. In response insects have evolved the ability to hear bat cries, to evade their hunting maneuvers, and some, the tiger moths (Arctiidae), to utter an ultrasonic reply. We here determine what it is that tiger moths “say” to bats. We chose four species of arctiid moths, *Cygnia tenera*, *Euchaetes egle*, *Utetheisa ornatrix*, and *Apantesis nais*, that naturally differ in their levels of unpalatability and their ability to produce sound. Moths were tethered and offered to free-flying naïve big brown bats, *Eptesicus fuscus*. The ability of the bats to capture each species was compared to their ability to capture noctuid, geometrid, and wax moth controls over a learning period of 7 days. We repeated the experiment using the single arctiid species *E. egle* that through diet manipulation and simple surgery could be rendered palatable or unpalatable and sound producing or mute. We again compared the capture rates of these categories of *E. egle* to control moths. Using both novel learning approaches we have found that the bats only respond to the sounds of arctiids when they are paired with defensive chemistry. The sounds are in essence a warning to the bats that the moth is unpalatable—an aposematic signal.

Introduction

Nocturnal insects fly at great risk. Insectivorous bats hunt them using sophisticated ultrasonic sonar (Schnitzler and Kalko 2001). Insects have countered with the evolution of ears to detect the echolocation cries of bats (Fullard and Yack 1993) and by using evasive aerobatic maneu-

vers if and when they are detected (Roeder 1967; Miller and Surlykke 2001). Nevertheless, individual bats capture hundreds of insects each foraging night. Tiger moths (Lepidoptera: Arctiidae) fly and mate at night and are thus exposed to this severe predation pressure, but tiger moths have an additional ploy. They answer the echolocation cries of bats with trains of high-frequency clicks beamed from a pair of cuticular blisters—tymbals—on the ventro-lateral aspects of the thorax (Fullard and Fenton 1977; Fullard and Heller 1990; Fullard et al. 1994).

Although it is generally agreed that the sound production of arctiids is a defensive strategy, there are different hypotheses regarding the mechanism through which the clicks exert their effects—the startle hypothesis, the jamming hypothesis, and the acoustic aposematism hypothesis (Miller and Surlykke 2001). Proponents of the startle hypothesis suggest that the moth sounds trigger the mammalian startle reflex (Humphries and Driver 1970; Bates and Fenton 1990). They attach special significance to the fact that moths emit their clicks in the terminal stages (feeding buzz) of a bat’s attack (Miller 1991). This timing is expected to promote disruption late in the attack sequence and not allow sufficient time for the bat to reassess the situation. The main lines of evidence in support of the jamming hypothesis are the spectral similarity of the moth-produced sounds and bat echolocation cries and again the timing of the moth signals relative to the cries (Fullard et al. 1979, 1994). The moths’ clicks are proposed to interfere with the bat’s perception of the returning echoes of its own vocalizations. One possibility is that the moth clicks, by their temporal proximity and resemblance to echoes, create the illusion of multiple targets (Fullard et al. 1979, 1994). A second possibility is that the moths’ clicks disrupt the neural mechanism for encoding the time of arrival of the echo (Tougaard et al. 1998). Either would again cause confusion on the part of the bat at a critical moment. Finally the aposematic hypothesis suggests a connection between the presence of noxious chemicals, common defenses in arctiids, and their ability to produce ultrasonic clicks (Dunning and Roeder 1965; Eckrich and Boppré 1990; Acharya and Fenton 1992;

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Dunning et al. 1992; Dunning and Krüger 1995). Bats may learn to associate sound with unpalatable prey and avoid sound-producing species—that is, the clicks are a warning.

The bat–moth story shaped the field of neuroethology and it is one of the most sophisticated predator–prey interactions known. Earlier work has shown that all three hypotheses—jamming, startle, and acoustic aposematism—can function in any given interaction. We feel, however, that it is highly unlikely that the three hypotheses have had the same selective value in nature and it is more likely that one has provided the main impetus driving the evolution of the tymbal and sound production in the Arctiidae. In keeping with the roots of neuroethology it is important to ask this question in the most natural context possible with bats and moths free to interact in space and time.

We here approach the question from a fresh perspective. We investigate how free-flying naïve big brown bats (*Eptesicus fuscus*) learn to handle categories of tiger moths that occur naturally or have been experimentally manipulated to dissociate the sound signal and the chemical signal. Two tiger moth characteristics are of critical importance in pursuing this idea: their ability to produce sound and the presence of a chemical defense. The strength of our experimental design is in its ability to differentiate between the effects of sound and chemical defense on the hunting strategies of bats. When the presence (C+) or absence (C–) of a chemical defense and the ability (S+) or inability (S–) to produce sound are combined independently four groups of moths are formed. Exposing different naïve bats to each of the four groups and recording their interaction over time allowed us to compare which single characteristic or combination of moth characteristics leads to the avoidance of sound-producing moths observed in nature.

Materials and methods

Multispecies experiments

Using a multispecies approach we chose four species of tiger moths that fall naturally into discrete categories. The dogbane tiger *Cychnia tenera* was shown to be unpalatable and sound producing, C+S+, whereas the milkweed tussock moth *Euchaetes egle* is C–S+, the ornate tiger *Utetheisa ornatrix* is C+S–, and the nais tiger moth *Apanthesis nais* is C–S– (Fig. 1a). Adult moths were either captured in the field in blacklight traps (*A. nais*) or reared in the lab on the natural host plants: *C. tenera*—*Apocynum cannabinum*; *U. ornatrix*—*Crotalaria spectabilis*; *E. egle*—*Asclepias tuberosa*. Palatabilities were determined in a separate series of laboratory feeding assays using big brown bats and silenced tiger moths (Hristov and Conner 2005). Sound production capability was determined in preliminary experiments utilizing tactile stimulation, measuring responses to ultrasonic sources such as jingling keys, and by presenting each species with the echolocation cries of big brown bats. For the learning experiments moths were offered on a light-weight monofilament tether that allowed the insect limited evasive maneuvering (flight). Moths were

attached using a light-weight surgical microclip. Bats were trained with wax moths (with sound-producing tegula removed) and geometrid moths from the tether for 2 weeks. The wax moths were the staple diet, and geometrids were added to introduce variety in size and wing shape.

For the 3–7 consecutive days of each experiment individual bats were offered 16 moths per day sequentially and in random order: 4 wax moths, 4 geometrids, 4 noctuids (novelty controls), and 4 experimental arctiids. We recorded the percent of each category captured and how these percentages change over time—learning. C+S+ arctiids were offered to three bats (one male and two females), the C–S+ arctiids were offered to two bats (one male and one female), the C+S– arctiids were offered to two bats (two females), and the C–S– arctiids were offered to three bats (one male and two females). The total number of bat–moth interactions on which our analysis is based is 946.

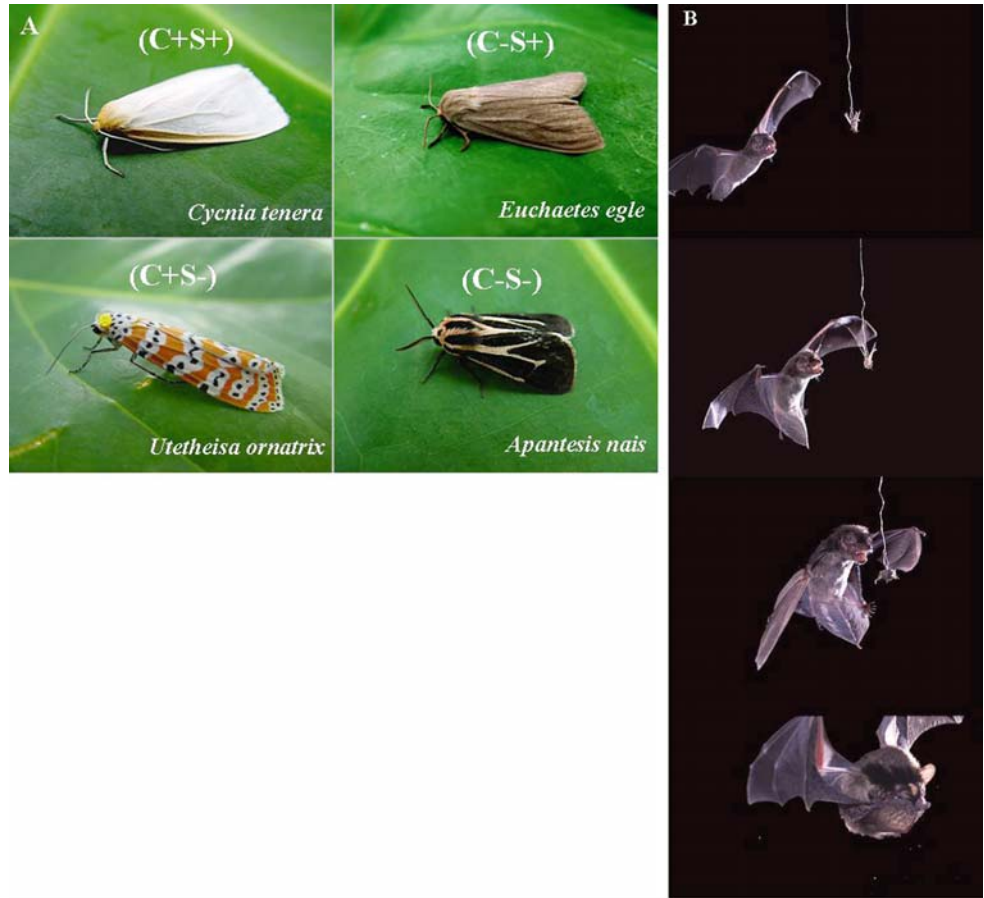
Big brown bats are known to include moths in their diet (Black 1972). Nursing big brown bats were captured with their preflight young at local maternal roosts in and near Winston-Salem, North Carolina. Experiments were carried out in a 5.8×4.0×3.0-m bat facility in the Department of Biology at Wake Forest University. Bats were maintained in accordance with the animal care and use committee protocols A98-080 and A01-186. The descriptor “naïve” used throughout this article refers to bats that have been hand raised and had never heard arctiid sounds prior to the start of the experiment.

Observations and recordings were made using a pair of digital, high speed, infrared-sensitive video cameras (Photron FastCam PCI 500, Photron USA, San Diego, Calif.). Infrared (IR) illumination was provided by a pair of 120 W heat lamps equipped with IR filters (U.S. Army surplus vehicle headlight IR filter). This illumination was supplemented with low-intensity deep red light sufficient to reveal the silhouette of bat and moth to a light-adapted eye. The acoustic behavior of the bats and moths was monitored using a set of 900 MHz wireless headphones (Model 33-1186, Radio Shack, Fort Worth, Tex.) connected to a QMC S200 bat detector (Ultrasound Advice, London, UK) and recorded in BatSound Pro v3.2 (BatSound, Pettersson Electronics, Uppsala, Sweden) installed on an A21 IBM laptop (Pentium III, 1.3 MHz; 64 MB RAM). The computer was connected to the bat detector through a NI-16-IE National Instruments high-speed A/D PCMCIA card sampling at 250 KHz.

Single-species experiments

These experiments used the same methods described above except all the categories of moths were produced using the same moth species *E. egle*. The presence or absence of chemical defenses in *E. egle* was controlled by the diet on which the larvae were raised. Unpalatable individuals (C+) were fed the leaves of cardenolide-rich *Apocynum cannabinum* (Cohen and Brower 1983) while palatable individuals (C–) were fed the leaves of cardenolide-poor *Asclepias tuberosa* (Brower 1969). Palatabilities were verified in a

Fig. 1 **a** Arctiid species and their representative categories used in the multispecies approach. **b** Approach and capture sequence of a big brown bat (*Eptesicus fuscus*) with a control wax moth (*Galleria mellonella*) as prey. Sequence illustrates multiple points at which a decision to reject the prey item can be made



separate series of laboratory feeding assays (Hristov and Conner 2005). The ability or inability to produce sound was controlled by carefully slitting the tymbals of the naturally S+ moths to silence them. Since the tymbal is an air-filled surface structure the operation has little effect on the mobility of the moth. The effectiveness of the procedure was verified using a hand-held bat detector (Ultrasound Advice Mini-2) and it was also verified by reviewing the audio tracks of each experiment. C+S+ individuals were offered to one bat (a male) and the C-S- individuals were offered to one bat (a male). The C-S+ data is the same as the data used in the multispecies analysis; C+S- data are not available. The total number of additional bat-moth interactions on which this analysis is based is 208.

Statistical methods

We did not attach special significance to the shapes of the learning curves or their y intercepts. We analyzed only the signs of the trends. Each learning curve was fitted by least squared regression and a slope was determined to be significantly positive, negative, or zero. Each relationship from the multispecies and single-species studies was compared to its a priori prediction (increasing, decreasing, or zero). The exact probability of obtaining a series of matches between the signs of the slopes for the re-

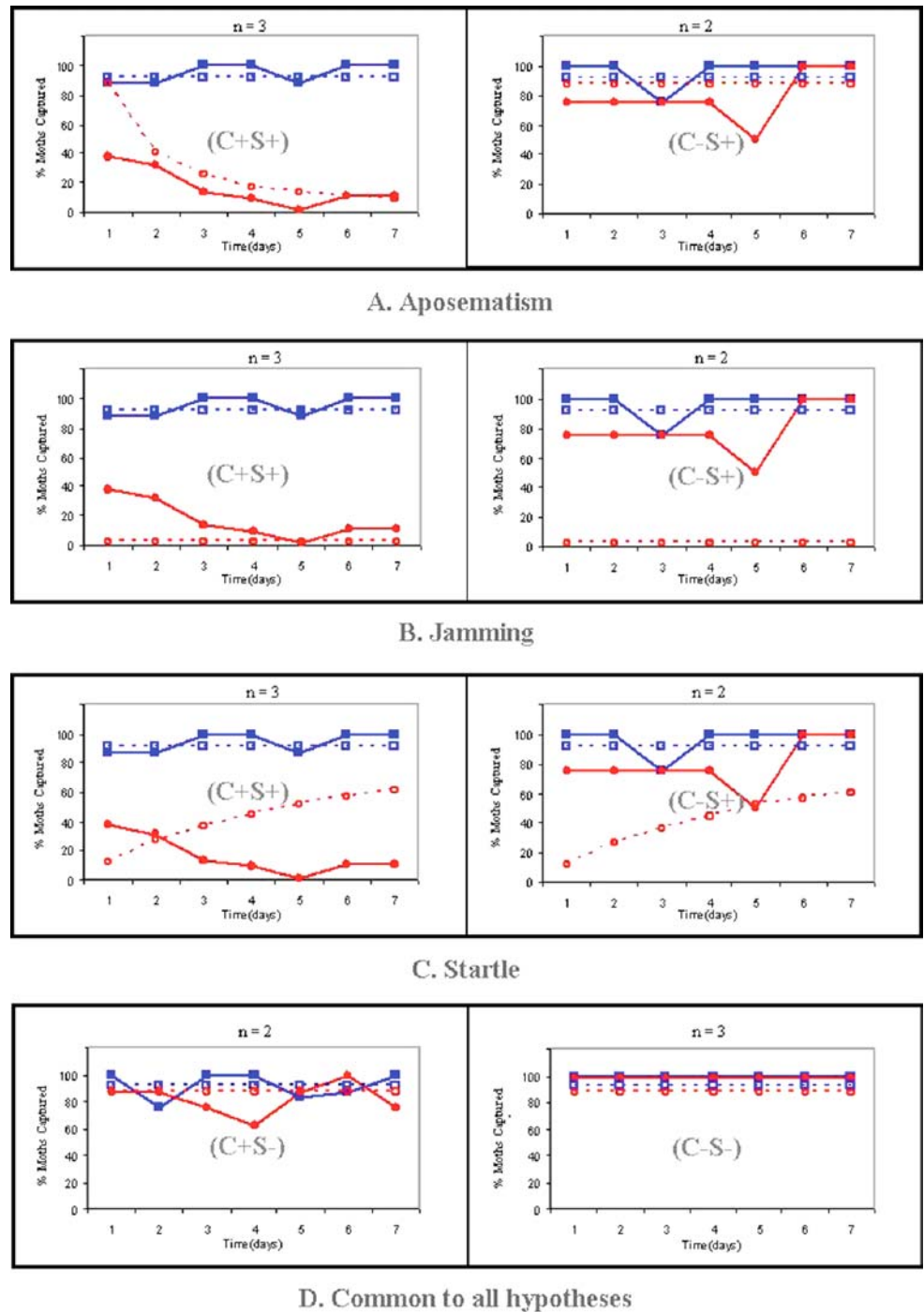
sults and predictions was calculated using a multinomial distribution (Siegel and Castellan 1988). This calculation gives a conservative estimate of the matches occurring by chance.

Results

The S+ tiger moths invariably responded to the echolocation cries of the attacking bats with trains of intense ultrasonic clicks during the approach and terminal phases of the echolocation-cry sequences as described previously (Fullard et al. 1994; Barber and Conner 2004). The naïve bats readily captured tethered moths in their tail or wing membranes and transferred them quickly to their mouths (Fig. 1b; to view action see Supplemental Material 1—video). Palatable moths (C-) were stripped of their wings and eaten while unpalatable moths (C+) were quickly dropped. The three-dimensional kinematic analyses of the recorded bat-moth interactions will be presented elsewhere.

A priori predictions on the direction of the learning curves for jamming, startle, and aposematism differ (Fig. 2, dotted lines) and allowed us to determine which hypothesis underlies the behavior. According to the acoustic aposematism hypothesis bats can be predicted to capture C+S+ moths initially and after tasting the moths the bats should learn

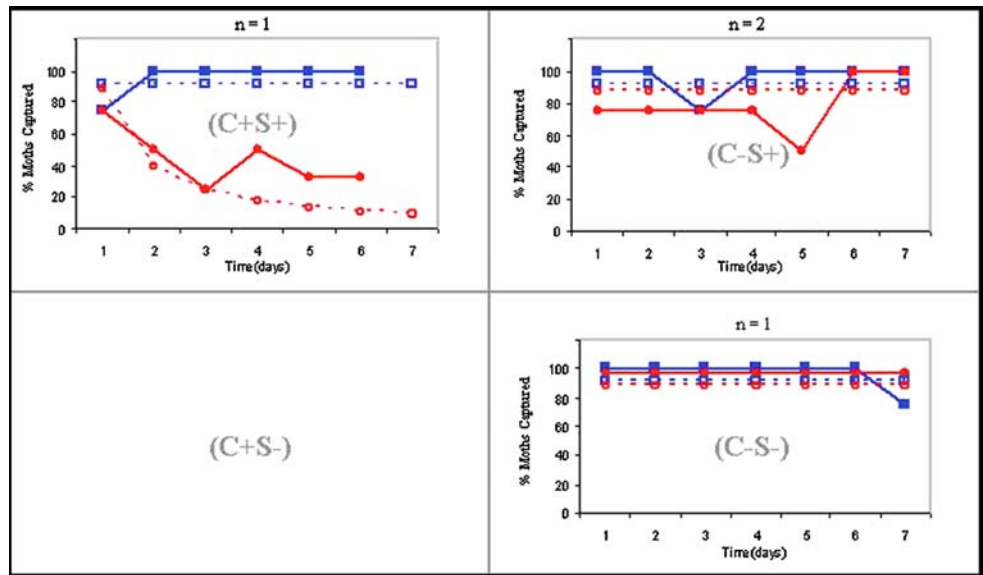
Fig. 2 Multispecies predictions and results for the interactions of big brown bats with control and experimental moths under the acoustic aposematism hypothesis, jamming hypothesis, and startle hypothesis. *Dotted lines* indicate predictions for experimental moths (*red*) and control moths (*blue*). *Solid lines* indicate empirical results for experimental moths (*red*) and control noctuid moths (*blue*). Control geometrids and control wax moths were handled like noctuids and are not shown



to avoid clicking individuals (Fig. 2a, left, red dotted line). Control moths should be captured and eaten with regularity (Fig. 2, blue dotted lines). With the jamming hypothesis bats should have great difficulty capturing clicking moths regardless of their palatability (Fig. 2b, red dotted line) and based on the startle hypothesis bats should be unable to capture clicking moths initially but should rapidly overcome the startling effect (Fig. 2c, red dotted line). We do not attach special significance to the exact shapes of the predictions but rather to the trends (increasing, decreasing, or zero). The bats learned very quickly (in some cases single-

trial learning) to avoid C+S+ moths (Fig. 2a–c, left, red solid line; to see and hear an example view Supplemental Material 2—video). The data point for the first day (38%) is lower than might be expected but this can be explained by the finding that the bats learned to avoid the C+S+ moths within the first two of the four total arctiid offerings on that day. The average for the first two offerings is actually much higher (67%). Bats did not avoid or learn to avoid clicking moths in the absence of a chemical defense (Fig. 2a–c, right, red solid line). In fact, the bats did not distinguish between clicking moths (C–S+) and silent controls (Fig. 2, blue

Fig. 3 Single-species predictions and results for the interactions of big brown bats with different categories of *Euchaetes egle*. Dotted lines indicate predictions of experimental moths (red) and control moths (blue). Solid lines indicated empirical results for experimental moths (red) and control noctuid moths (blue)



dotted lines, only noctuids shown); both learning curves have a slope not significantly different from zero.

The experiment was repeated using a single-species approach in which the natural C–S+ milkweed moth *Euchaetes egle* was manipulated experimentally to render it suitable for the C+S+ and C–S– categories (Fig. 3). The categories of moths were offered to naïve bats as described above. Again tymbalar sound production only had an effect when paired with unpalatability (Fig. 3, upper left, red solid line). C–S+ individuals were captured with ease and consumed (Fig. 3, upper right, red solid line). C–S– *Euchaetes* were handled like controls. Only the a priori predictions for acoustic aposematism were met. When combined the multispecies and single-species results (signs of slopes) showed striking consistency with the predictions (signs of slopes) according to the acoustic aposematism hypothesis, allowing a direct calculation of the multinomial probability of our outcomes as $P=(1/3)^6$ or 0.0014 (Supplemental Material 3—table). Although the acoustic aposematism predictions are the least probable of the three hypotheses by chance, they are the results that we obtained.

Discussion

The results of these experiments consistently point to acoustic aposematism as the *raison d'être* of sound production in species of tiger moths that we studied. In both experiments the bats quickly learned to avoid clicking moths if and only if the clicks were associated with noxious taste. However the results of any learning experiment depend on the rate at which the predator experiences the clicking moths and has the opportunity to associate the sounds with a noxious food item (Domjan and Grau 2003). Our arctiids represented 25% (4 of 16) of the moths offered per day. This value compares favorably with the encounter rates inferred from ultraviolet trap catches reported by Dunning

et al. (1992) and from our own observations in a variety of habitats (unpublished).

Our results are in agreement with field observations on the behavior of the bats *Lasiurus borealis* and *L. cinereus* and the arctiids *Hypoprepia fucosa* and *Halysidota tessellaris* (Acharya and Fenton 1992; Dunning et al. 1992). In these studies muted arctiids were attacked more often than sound-producing arctiids and moths successfully captured were frequently rejected presumably due to bad taste. These bats were undoubtedly quite experienced with sound-producing species and their behavior would correspond most closely to what we saw on day 7 of our experiments.

The acoustic warning system in tiger moths could be selected for in either of two ways. First a sound-producing moth might survive the encounter with a bat and leave offspring that share the ability to respond to bats—an individual selection argument. Second a sound-producing moth could be killed in the process of “educating” the bat and yet its sound production legacy may live on through the moth’s kin—a kin selection argument (Mallet and Joron 1999). Since the tymbal organ is a synapomorphy for the family Arctiidae (Weller et al. 1999) the evolution of acoustic aposematism likely evolved as an early family characteristic in combination with defensive chemistry.

Our experiments do not preclude the jamming hypothesis by different species in different environments. We feel, however, that jamming would represent a novel embellishment of an already existing aposematic signal. The requirements for an acoustic signal to function as a warning are less stringent than those necessary for jamming—that is, precise timing, the filling of acoustic time, and spectral matching. Startle may be marginally effective (Bates and Fenton 1990; Miller 1991) but our studies indicate that it is exceedingly ephemeral and unlikely to affect a persistent predator particularly at the high encounter rates that appear to occur in nature (Dunning et al. 1992). If in certain environments the encounter rates are lower the significance of startle may increase.

If acoustic aposematism is a common basis for sound production in tiger moths, the occurrence of acoustic mimics is a logical evolutionary outcome. Unpalatable species may function in large Müllerian mimicry rings including other sound-producing arctiids and nonarctiids. Alternatively palatable species may gain Batesian benefit from the education of predators by unpalatable sound-producing species (Dunning 1968). Both are predictably sound strategies for dealing with eared predators such as bats.

The arms race between insects and bats continues. Bat-detecting ears and evasive maneuvers have been found in beetles, crickets, locusts, lacewings, and mantids (Miller and Surlykke 2001; Yager et al. 2000). Any insect that flies at night must have a strategy for dealing with bats. Ultrasonic sound production appears rare, perhaps because of the necessary link between defensive chemistry and sound production that we have described here.

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Electronic Supplementary Material

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