

What's the buzz? Ultrasonic and sonic warning signals in caterpillars of the great peacock moth (*Saturnia pyri*)

Veronica L. Bura · Alan J. Fleming · Jayne E. Yack

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Abstract Caterpillars have many natural enemies and, therefore, have evolved a diversity of antipredator strategies. Most research focuses on those strategies (crypsis, countershading, and warning coloration) targeting visually guided predators. In contrast, defensive sounds, although documented for more than a century, have been poorly studied. We report on a novel form of sound production—chirping—in caterpillars of the common European Great Peacock moth (*Saturnia pyri*). Chirps are broadband, with dominant peaks ranging between the sonic (3.7 kHz) and ultrasonic (55.1 kHz) and are generated by a rapid succession of mandibular “tooth strikes.” Chirp trains are induced by simulated predator attacks and precede or accompany the secretion of a defensive chemical from integumental bristles, supporting our hypothesis that these sounds function in acoustic aposematism. We propose that these caterpillars generate multimodal warning signals (visual, chemical, and acoustic) to target the dominant sensory modalities of different predators, including birds, bats, and invertebrates.

Keywords Caterpillar · Acoustic · Aposematism · Chemical defense · Ultrasound

Introduction

Acoustic communication in Lepidoptera has been extensively researched, with more than 200 published reports on the subject (Minet and Surlykke 2003). The vast majority of these studies focus on adults (moths and butterflies), where hearing and sound production have evolved multiple times and function in a variety of social and defensive contexts. Comparatively, little is known about the role of acoustic communication in caterpillars, and there is a conspicuous absence of this important life stage in reviews on lepidopteran bioacoustics (e.g., Scoble 1995; Minet and Surlykke 2003). There is, however, a growing body of literature suggesting that acoustic communication is widespread in larval Lepidoptera. Vibrational communication mediates mutualistic relationships with ants (DeVries 1991; Travassos and Pierce 2000), facilitates territorial interactions (Yack et al. 2001; Fletcher et al. 2006; Bowen et al. 2008), and functions in predator detection (Meyhoffer et al. 1997; Castellanos and Barbosa 2006). Airborne sound production in caterpillars on the other hand has received less experimental attention. Brown et al. (2007) demonstrated that a common North American silk moth, *Antheraea polyphemus* produces short mandibular clicks to warn predators of a regurgitant chemical defense. This has been the only experimental study of caterpillar sound production, even though the phenomenon has been reported for more than a century (reviewed in Brown et al. 2007). It is believed that sound production is not only common in the large superfamily Bombycoidea (silk and hawk moths) but that there exists a wide diversity of sound production mechanisms and functions. However, these sounds have not been formally characterized, and the functions remain unexplored.

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V. L. Bura · A. J. Fleming · J. E. Yack (✉)
Department of Biology, Carleton University,
1125 Colonel By Drive,
Ottawa, ON, Canada, K1S 5B6
e-mail: jyack@ccs.carleton.ca

The focus of this study is on caterpillars of the Great Peacock moth (*Saturnia pyri*; Fig. 1a): a large, cryptic species that ranges throughout southern Europe, Asia Minor, and Northern Africa (Rougeot 1971). During a survey of larval defensive behaviors, we noted that *S. pyri* produced long, repeated, high-pitched “chirping” sounds that were distinct from the short clicking sounds reported by Brown et al. (2007). Also, unlike *A. polyphemus*, *S. pyri* did not regurgitate. Previous studies on *S. pyri* show that they use phenolics and related compounds secreted from scoli (bristle bearing outgrowths) in combination with body thrashing as their defense strategy (Deml 2001; Deml and Dettner 1993, 1995; Fig. 1b). Interestingly, there was no reference to sound production in these studies. We hypothesize that sound production in *S. pyri* functions to warn predators of defensive chemical secretions. Our goals were to characterize these novel sounds and their mode of production and to determine whether they likely function in acoustic aposematism.

Methods

Study organisms *Saturnia pyri* Schiffermüller eggs were obtained from wild caught females in Kloten and Mohlin, Switzerland (Import Permits #P-2007-03105 and #P-2008-

02614). Larvae were reared on cuttings of poplar (*Populus sp.*) housed in an insect rearing facility at Carleton University. All experiments were performed on late instars.

Sound production mechanism Larval mouthparts were videotaped using a Sony HDR-HC7 HD Handycam (Tokyo, Japan) equipped with a Sony ECM-MS957 microphone and a macrolens. Videos were analyzed using iMovie 3.0.3 on an Apple computer. To confirm their involvement in sound production, the outer edges of both mandibles were ablated. Larvae were anesthetized using carbon dioxide and mandibles ground with a Vogue Professional 6700 nail drill (Woodland Hills, CA, USA) equipped with diamond dental burs. In experimental trials, the edges of both mandibles were ground in succession and the larvae tested for sound production. Controls were performed using a smooth bit that did not change the mandible structure. Mandibles from known sound producers were dissected, sputter coated with gold-palladium, and examined using a JEOL JSM-6400 scanning electron microscope (Tokyo, Japan).

Sound recording and analysis Recordings were performed in an acoustic chamber (Eckel Industries Ltd., Cambridge, MA, USA) or in an enclosure lined with acoustic foam. Temporal characteristics of chirp trains were measured from

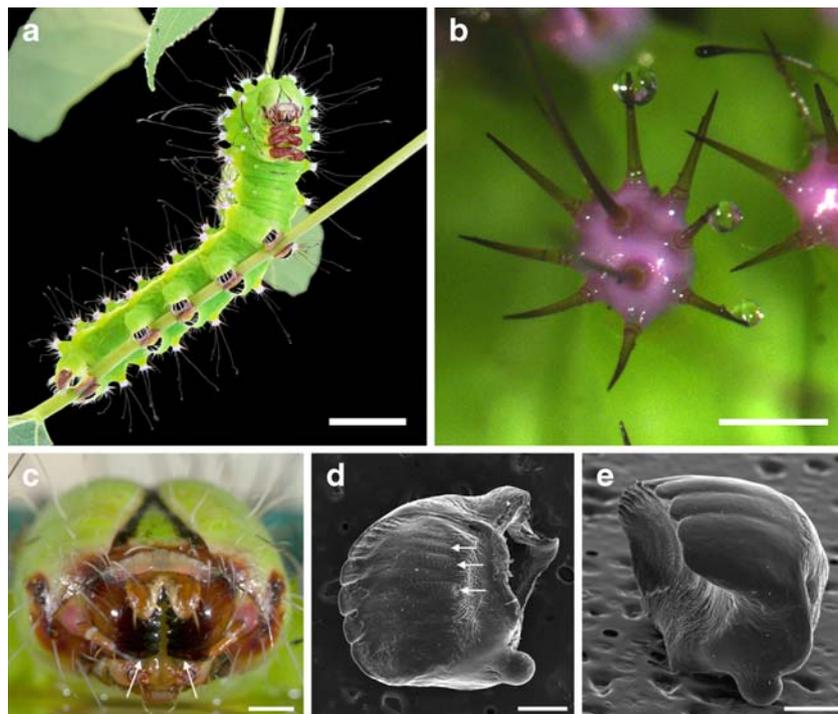


Fig. 1 **a** A late instar *Saturnia pyri* larvae. Scale bar, 1 cm. **b** Higher magnification of a scoli showing the bristle secretions. Scale bar, 1 mm. **c** Light micrograph of the mouthparts with arrows showing the mandibles. Scale bar, 1 mm. **d** Scanning electron micrograph of a

right mandible with arrows indicating “track marks” from the opposite mandible. **e** Scanning electron micrograph of the left mandible showing the serrated outer edge. Scale bars, 0.5 mm

the first three trains of 14 recordings using Raven Bioacoustics Research Program 1.2 (Cornell Laboratory of Ornithology, Ithaca, NY, USA). Spectral characteristics were examined from five randomly chosen chirps for multicomponent chirps, and all single component chirps (see “Results”) from five animals. Sounds were recorded with a Brüel & Kjær (Naerum, Denmark) 1/4 in. microphone type 4939 (grid off), placed approximately 4 cm away from the head capsule, amplified with a Brüel & Kjær Nexxus conditioning amplifier type 2690, and recorded onto a Fostex FR-2 Field Memory Recorder (Gardena, CA, USA) at a sampling rate of 192 kHz. Spectra were produced using a 512-point Fast Fourier Transform (Hanning window). Sound pressure levels were determined by comparing peak-to-peak voltages to the amplitude of a calibrated pure tone.

Attack trials Attacks were performed by pinching the animal with blunt forceps, a technique commonly used to simulate an attack by a bird or the mandible bite of a predaceous insect (e.g., Bowers 2003, Grant 2006). Larvae were isolated on sprigs of poplar at least 30 min prior to experimentation. Five attacks were delivered to either the head-capsule or the posterior end of the larva, with approximately 5 s between attacks. In an additional trial set, one pinch was delivered to the posterior end of the caterpillar. Defensive behaviors were videotaped and analyzed as described above to determine (1) the mean number of chirps in 60 s following the first attack, (2) the occurrence of sound production and chemical release throughout the attack sequence, and (3) the temporal relationship between defensive behaviors.

Results

When disturbed, *S. pyri* caterpillars generated distinct and audible chirp trains. Caterpillars responded acoustically to a variety of disturbances, including touching the body, or agitating the plant sprigs, but the most reliable method to induce signaling was to grasp the larva’s posterior end or head capsule (Supplementary material, movies 1 and 2), both of which induced different responses (see “Attack” section below).

Sound-producing mechanism Video analysis and ablation experiments confirmed that sounds are produced by the mandibles (Supplementary material, movie 3). Each chirp is produced by either the right or left mandible (Fig. 1c) sliding against the inner surface of the opposite mandible, and each chirp train results from repeated movements of one particular mandible against the other. Once a chirp train

is completed, the larva may or may not switch sides between trains, but switching mandibles was never observed within a particular train. Both mandibles have serrated outer edges (Fig. 1c–e), and we propose that the edge of one mandible scrapes against the inner surface of the other, where this is evidenced by distinct “track marks” (Fig. 1d). These tracks occur on both mandibles, validating our observations that at least some individuals are “ambidextrous.” The tracks are slightly textured (Fig. 1e), suggesting that the outer mandible ridges rub against these inner “teeth” to produce individual components or “tooth strikes” within a chirp.

Sound characteristics Temporal patterns of sounds were analyzed from the first three trains of multiple attack trials (42 trains from 14 animals), where attacks were directed towards the posterior body region. Chirp trains ranged from 0.05 to 4.65 s (mean 1.70 ± 1.12 s) and contained on average 5.74 ± 3.45 chirps per train (Fig. 2a). A chirp was defined as a series of tooth strikes separated by a minimum distance of 100 ms. Chirps occurring at the beginning of a train tended to be longer (mean duration 67.48 ± 23.15 ms, $n=21$) and contained a greater number of tooth strikes (mean 5.38 ± 1.53) than those that occurred at the end (mean duration 9.14 ± 10.17 ms, mean number of tooth strikes 1.29 ± 0.46 , $n=21$).

Spectral analysis was performed on the first three chirp trains from five individuals where attacks were delivered to the posterior region. Sounds were broadband, with most energy between 3 and 90 kHz (Fig. 2b, c). The energy distribution of multi- and single-component chirps differed (Fig. 2c), with the former having more energy in the ultrasonic range (median dominant frequency 36.00 kHz, mean dominant frequency 34.45 ± 11.93 , 88% >20 kHz, $n=25$) and the latter having more energy in the sonic range (median dominant frequency 14.63 kHz, mean dominant frequency 21.57 ± 15.19 , 70% <20 kHz, $n=23$).

Sound levels were measured from ten chirp trains obtained from four late instar larvae attacked from the posterior end. Maximum amplitudes, measured from the loudest tooth strike in a chirp, ranged from 52 to 66 dB SPL (median 57 dB SPL), measured at 10 cm from the source.

Attack experiments A simulated predator attack induced a variety of defensive behaviors, including directed thrashing, head hiding/curling-in, secretion of chemicals from the scoli accompanied by a foul smelling odor, and sound production. Attacks to the head region typically resulted in the larva curling its head inward while producing sound and releasing chemical from the anterior scoli (Supplementary material, movie 1). In contrast, attacks to the posterior region caused the caterpillar to thrash towards the site of attack while signaling continuously (Supplementary

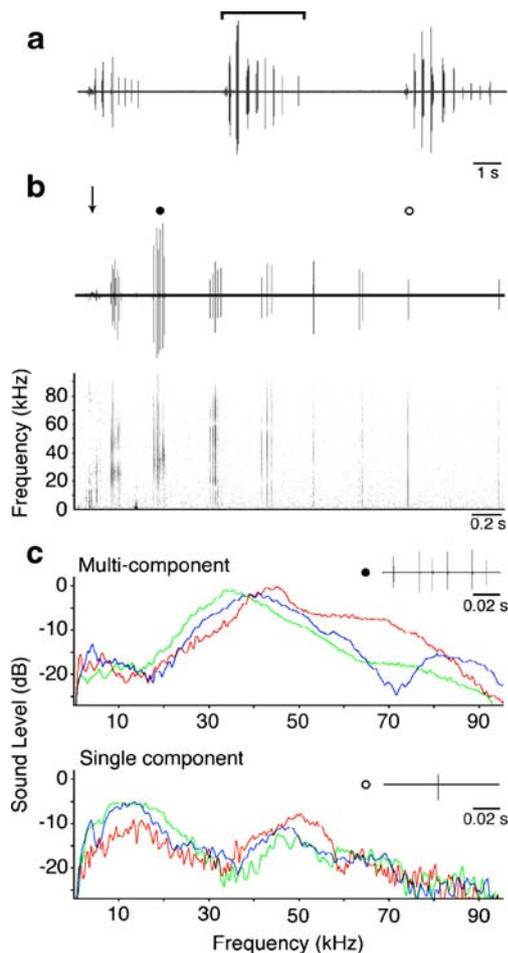


Fig. 2 Oscillograms of *Saturnia pyri* sounds recorded from fifth instar larvae. **a** Three chirp trains following three consecutive attacks. **b** Time expansion of the bracketed train from part (a), showing typical multicomponent (closed circle) and single component (open circle) chirps. The arrow indicates the time of attack. The accompanying spectrogram shows the frequency distribution of each chirp. **c** Power spectra of multicomponent and single component chirps taken from three individuals. Time expanded chirps from (b) are inset for each type

material, movie 2) and producing secretions from both the anterior and posterior scoli. During a 60-s period, chirp rates following attacks to the posterior region (24.95 ± 19.04 , $n=20$) were significantly higher than those following attacks to the head (12.20 ± 16.79 , $n=20$; $P=0.03$ two-tailed t test, $t_{38}=2.25$; Fig. 3a, b).

Overall, both acoustic signaling and chemical release increased with the degree of disturbance (Fig. 3a–c). During a 60-s period, larvae attacked five consecutive times signaled significantly more (24.95 ± 19.04 chirps) than did those attacked once (3.50 ± 2.78 chirps; $P=0.0006$ two-tailed t test, $t_{30}=3.85$). The relationship between sound production, chemical secretion, and number of attacks is illustrated in Fig. 3c. Following a single attack, some caterpillars generated sound without secreting chemical (S+C–), but when attacked multiple times, sound

production was always accompanied by chemical secretion (S+C+). In all five-pinch trials resulting in both sound production and chemical secretion, the first chirp preceded or accompanied the first droplet of chemical significantly ($P=0.001$, $\chi^2=10.9$, $df=1$; Fig. 3d).

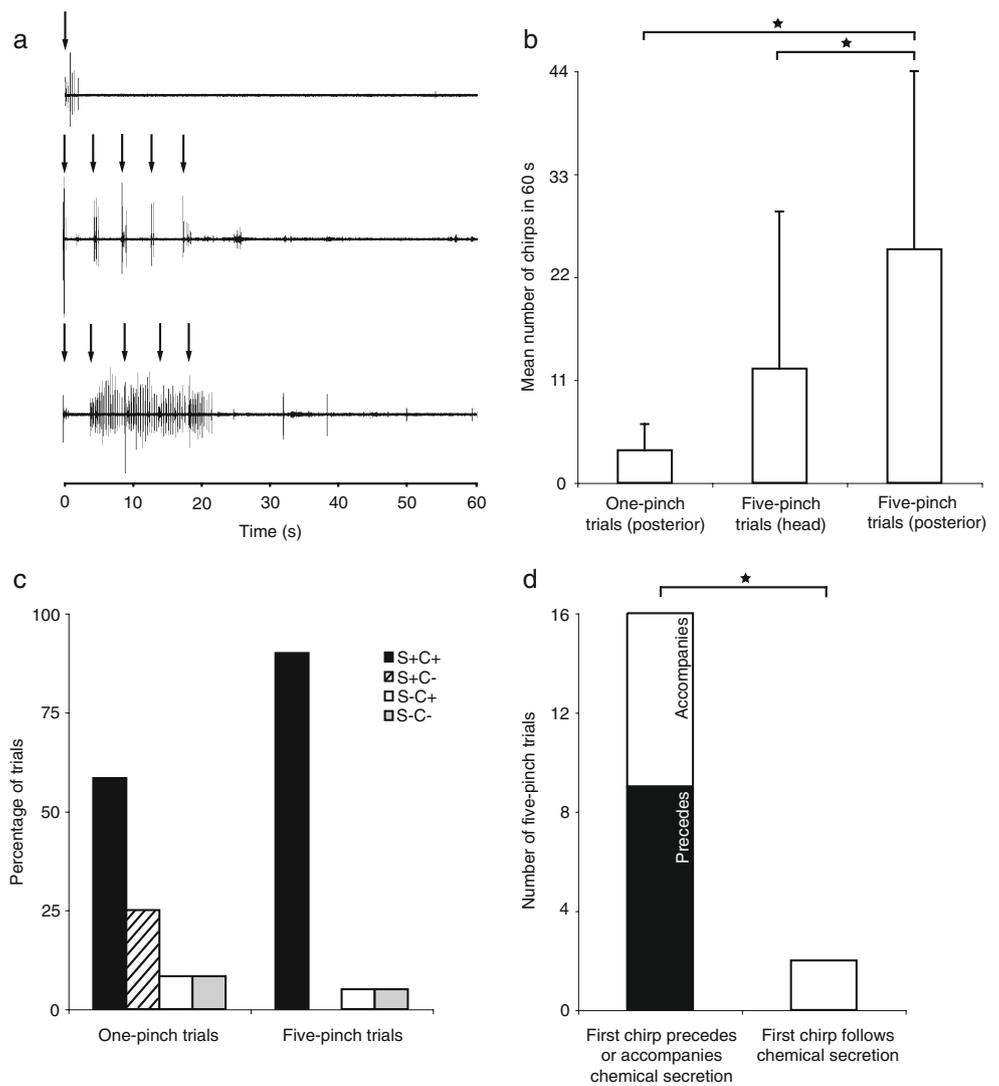
Discussion

Caterpillars have many natural predators and consequently have evolved a variety of defenses. The majority of studies on antipredator mechanisms have focused on those targeting visual predators (e.g., crypsis, countershading, and visual aposematism). However, it is believed that the focus on visual cues is a reflection of our own sensory bias (Lederhouse 1990), and it is reasonable to assume that caterpillars employ a range of signal types (airborne sounds, solid-borne vibrations, visual cues, and volatile chemicals) to communicate with a diversity of predators. Here, we introduce a novel acoustic signal for caterpillars—mandibular “chirping”—and provide experimental evidence for acoustic aposematism.

Individual chirps are generated by stridulation, whereby the serrated outer edge of one mandible slides against the textured ridges or “teeth” on the inner surface of the opposite mandible to produce a series of “tooth strikes.” This is a novel form of sound production in caterpillars. Unlike most examples of stridulation in arthropods, whereby the two body parts are specifically adapted for sound production (e.g., file and scraper) (Ewing 1989), the mandibles of *S. pyri* are not clearly differentiated. The variability in the inner surface of the mandibles, and the amount of pressure against the two parts may account for the diversity in the number of tooth strikes per chirp.

We hypothesize that stridulation in *S. pyri* is an aposematic display, warning predators of a chemical defense. Although the term aposematism is most often associated with visual warning displays, there is a growing number of examples of acoustic warning signals, particularly when the predator–prey interaction takes place under low light conditions (e.g., moths warning bats: Hristov and Conner 2005, Ratcliffe and Nydam 2008; bees warning mice: Kirchner and Röscher 1999) or when sound is part of a multimodal display to reinforce learning (Rowe and Guilford 1999). Several predictions satisfy the acoustic aposematism hypothesis. First, sound production is strongly associated with a physical disturbance simulating a predator attack. Second, the number of acoustic signals generated is significantly correlated to an increase in the number of attacks. A third prediction is that natural predators of *S. pyri* should be capable of hearing these sounds. *Saturnia pyri* chirps resemble other insect disturbance sounds (Masters

Fig. 3 **a** Oscillograms showing typical responses to one-, five- (head), and five- (posterior) pinch attack trials, with *arrows* indicating when larvae were attacked. **b** Mean number of chirps produced over 60 s following one-, five- (head), and five- (posterior) pinch attack trials. **c** Behavioral responses to increasing levels of disturbance (one and five pinch attacks, to the posterior region). S+C+ both sound and chemical production; S-C- neither sound nor chemical production. **d** The temporal relationship between sound production and chemical release in five-pinch trials



1980) in that they are spectrally broadband and temporally simple, permitting them to be perceived by a wide range of predators. But what are the predators of *S. pyri*? Birds are believed to be an important predator (Deml and Dettner 1993), and the sonic components of the chirps overlap with the optimal frequency range of most avian predators (Schwartzkopff 1955; Dooling 1991). The ultrasonic components of *S. pyri* chirps suggest that they could be directed towards bats. There is substantive evidence that caterpillars are common prey of gleaning bats and large caterpillars can form up to 60% of the diet of some species (Kalka and Kalko 2006, Wilson and Barclay 2006). Finally, ants are thought to be one of the main arthropod predators of *S. pyri* (Deml and Dettner 1995), and although ants do not possess ears (Yack 2004), they are sensitive to solid borne vibrations (Kirchner 1997). Although we did not test for vibratory stimuli directly, stridulations may be transmitted through the plant and function as a warning to ants. Indeed, wolf spiders are deterred by vibrational signals

generated by stridulating insects (Masters 1979). A final prediction is that the sounds are associated with an honest defense. Our results demonstrate that stridulation most often precedes or accompanies scoli secretions, which in previous studies have been shown to contain compounds that function in deterring birds, ants, fungi, and microorganisms (Deml and Dettner 1993, 1995; Deml 2001). Interestingly, sound production is more pronounced during posterior attacks, corresponding to our observation that more chemical is secreted during these attacks. Overall, our predictions support the acoustic aposematism hypothesis.

Despite the lack of formal study on the subject of caterpillar sound production, there is abundant inferential evidence that the phenomenon is widespread in the large silk and hawk moth (Bombycoidea) caterpillars and diverse with respect to the mechanisms of sound production. Earlier reports and preliminary studies have variously described these sounds as “singing” (Reed 1868), “the crack of an electric spark” (Bethune 1868),

“crackling–rasping” (Heinrich 1979), squeaking, and clicking (Yack lab, unpublished). The two formal studies to date have revealed two different types of sound production (chirping and clicking) that warn of different forms of chemical defenses (toxic spines and regurgitation), representing an interesting example of convergent evolution. Given the purported ubiquity and diversity of sound production in this large superfamily (comprising more than 3,600 species; Regier 2008), future studies should focus on the evolutionary origins, mechanisms, and functions of different sounds in protecting caterpillars against predators.

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