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(Citation)

Ecology, 101(10):e03112

(Issue Date)

2020-07-23

(Resource Type)

journal article

(Version)

Version of Record

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(URL)

<https://hdl.handle.net/20.500.14094/90008664>



The Scientific Naturalist

Ecology, 101(10), 2020, e03112
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Squeaking caterpillars: independent evolution of sonic defense in wild silkmoths

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Manuscript received 12 February 2020; revised 27 April 2020; accepted 5 May 2020. Corresponding Editor: John Pastor.

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Citation: Sugiura, S., T. Takanashi, W. Kojima, and Z. Kajiura. 2020. Squeaking caterpillars: independent evolution of sonic defense in wild silkmoths. *Ecology* 101(10):e03112. 10.1002/ecy.3112

Key words: abdominal spiracles; acoustic aposematism; anti-predator defenses; Lepidoptera; Saturniidae; startle displays.

Some caterpillars produce sounds to startle or warn predators by rubbing the mandibles together or forcing air through the abdominal spiracles/oral cavity (Bura et al. 2016). Sound production through larval abdominal spiracles (i.e., whistling) has been reported in two closely related moth groups (Bura et al. 2016), hawkmoths (Sphingidae) and wild silkmoths (Saturniidae), which share a common ancestor (Hamilton et al. 2019). Although the sound characteristics and sound-producing spiracles of hawkmoth larvae have been clarified (Bura et al. 2011, 2016, Sugiura and Takanashi 2018), those of wild silkmoth larvae remain unclear. Revealing how wild silkmoth larvae whistle would contribute to understanding the evolutionary origin of sound production in Lepidoptera.

Larvae of the wild silkmoth *Rhodinia fugax* feed on the leaves of diverse plant families, such as Betulaceae, Fagaceae, Rosaceae, Sapindaceae, and Ulmaceae (Fig. 1; Mitamura 1998). The larvae produce a sound similar to a whistle when they are disturbed (Video S1, Audio S1). Although the unique sounds produced by *R. fugax* larvae were reported more than 100 yr ago (Packard 1904), the sound characteristics and sound-producing mechanisms remain unexplored. To clarify how *R. fugax* larvae produce sounds, we investigated the frequency of sound production, analyzed the sounds, and located the specific sound-producing structure.

For the following experiments, *R. fugax* larvae were collected from natural forests or reared from eggs in Japan. Larvae were reared on *Quercus acutissima* (Fagaceae) leaves under laboratory conditions (25°C).

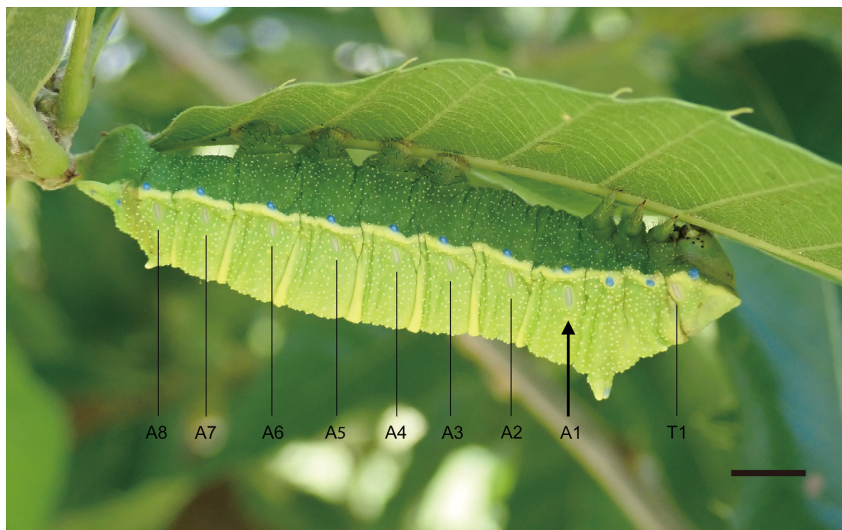


FIG. 1. Fifth instar of *Rhodinia fugax* with sound-producing spiracles on A1 indicated (arrow). Caterpillars have one pair of spiracles on the thorax (T1) and eight on the abdomen (A1–A8). Scale bar: 5 mm. Photo credit: Shinji Sugiura.

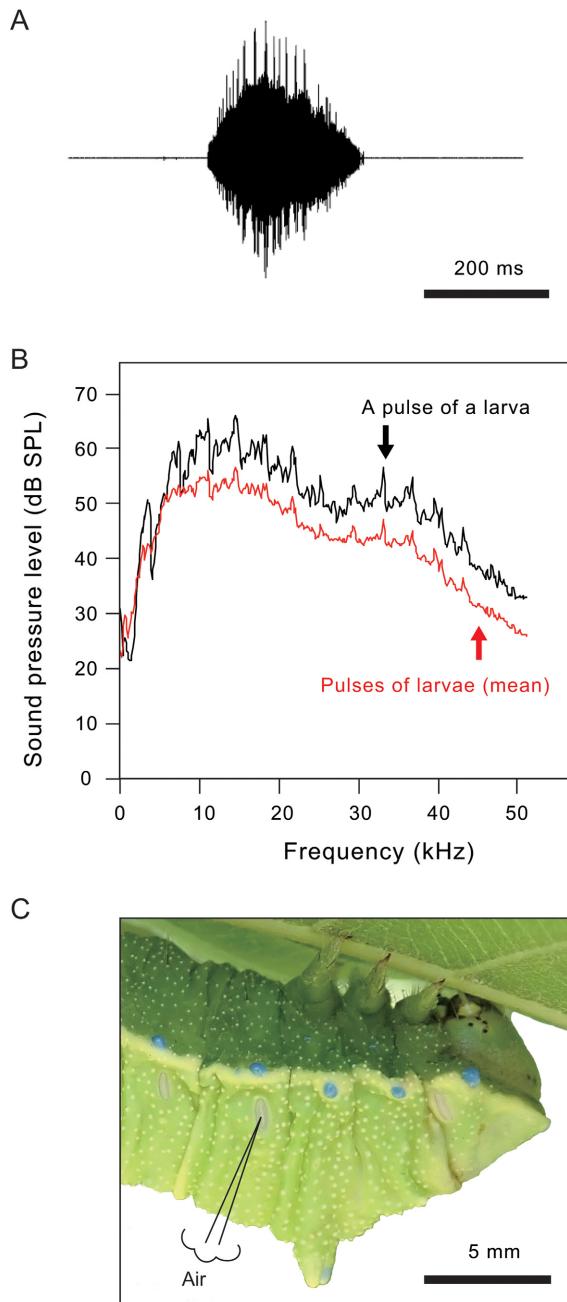


FIG. 2. Sound characteristics and sound-producing mechanisms in *Rhodinia fugax* larvae. (A) An oscillogram of the sound pulse produced by a last instar (Audio S1). (B) Power spectra of larval pulses. The red and black lines are derived from the pulses of 11 larvae (i.e., mean of $n = 11$) and the pulse of one larva (shown in the oscillogram), respectively. (C) Sound production by expulsion of air from the first abdominal (A1) spiracles. Photo credit: Shinji Sugiura.

Rhodinia fugax passes through five instars (Appendix S1: Table S1). We used forceps to stimulate the abdomens of 10 individuals per instar ($n = 50$) to

investigate whether the larvae could produce sounds. Each larva was used in only one experiment.

No first, second, or third instars generated audible sounds. Sounds were produced by 10% of fourth instars and 90% of fifth (last) instars (Appendix S1: Table S1). The rate of sound production increased with body size (logistic regression, $P < 0.0001$; Appendix S1: Table S2).

We recorded the sounds of 11 fifth instars (body length, mean \pm SE, 49.5 ± 2.3 mm, $n = 11$) using a procedure from Sugiura and Takanashi (2018; see Appendix S1 for details). Eight larvae produced a single sound pulse in response to artificial stimuli, and three produced multiple sound pulses (range 7–10 in 10 s). The pulse duration ranged from 108 to 448 ms (mean \pm SE, 296.5 ± 31.7 ms; $n = 11$; Fig. 2A). Spectral analyses revealed that the dominant frequency ranged from 5.6 to 14.5 kHz ($n = 11$; Fig. 2B), and the maximum sound pressure ranged from 47.5 to 66.1 dB SPL (mean, 56.5 dB SPL, $n = 11$; Fig. 2B). The dominant frequency was higher than the highest note on a piano (C8: 4.2 kHz). The sound characteristics of *R. fugax* larvae did not differ appreciably from those of hawkmoth larvae (pulse duration 44–2,060 ms, dominant frequency 5.0–22.1 kHz, and maximum sound pressure 44–82 dB; Bura et al. 2011, Sugiura and Takanashi 2018).

The mandibles remained open during sound production, indicating that *R. fugax* does not use the mandibles to produce sounds. When a fifth instar was submerged in a plastic container ($115 \times 85 \times 55$ mm³) filled with water (400 mL) and stimulated with forceps, it produced air bubbles from a pair of spiracles on the first abdominal segment, accompanied by sounds that were audible to the human ear (Video S2). This implies that *R. fugax* produces sounds by forcing air through the first abdominal (A1) spiracles. To test this, we experimentally obstructed the abdominal spiracles of fifth instars using silicone oil (HIVAC-G, Shin-Etsu Silicone). When one spiracle on the first abdominal segment was obstructed, all of the larvae produced sounds ($n = 20$). However, none of them produced any sounds when both first abdominal spiracles were blocked ($n = 20$). Sound production was not lost in the larvae when both eighth abdominal spiracles (A8) were obstructed ($n = 20$). The rate of sound production differed significantly between treatments (blocked A1 vs. A8; Fisher's exact test, $P < 0.0001$).

Larvae of the sphingid species *Amorpha juglandis* reportedly generate sounds by forcing air out of the A8 spiracles during successive contractions of the anterior dorso- and ventro-longitudinal muscles while maintaining all other spiracles in a closed position (Bura et al. 2011). Our experiments demonstrate that *R. fugax* larvae also force air through a pair of abdominal spiracles to generate the sounds (Fig. 1B). The sound characteristics and sound-producing mechanism of *R. fugax* are similar to those of *A. juglandis* larvae. However, the location of sound production (A1 spiracles) in *R. fugax* is different

from that in sphingid larvae, which generate sound from the last pair of abdominal spiracles (on A8; Bura et al. 2011, Sugiura and Takanashi 2018). Our findings demonstrate that producing sounds through the abdominal spiracles has evolved independently in the families Sphingidae and Saturniidae.

Caterpillar sounds function as anti-predator defenses (Bura et al. 2016). Whistling caterpillars are known only in the lepidopteran families Sphingidae and Saturniidae (Bura et al. 2016), although the insect order Lepidoptera has 133 recognized families (Mitter et al. 2017). Why did whistling caterpillars evolve only in these two groups? We hypothesized that the evolution of whistling is associated with large body size in Lepidoptera. Many species of Sphingidae and Saturniidae are large, and the largest caterpillars are recorded in these groups. We showed that audible sounds were not generated in small instars of *R. fugax*, but only in penultimate and especially last instars (Appendix S1: Table S1). Sound production is limited to penultimate and last instars in sphingids, although the pattern has not been quantified (Bura et al. 2011, Sugiura and Takanashi 2018). As with other types of defense (e.g., caterpillar eyespots; Janzen et al. 2010, Hossie et al. 2015), sonic defenses may also be more effective as anti-predator defenses in larger caterpillars. Large bodies may be more capable of producing sounds than small bodies because a strong force is needed to generate audible sounds through the abdominal spiracles. Furthermore, large caterpillars have bodies tough enough to survive predator attacks, such as bird pecks, as caterpillars generally produce sounds while being attacked. Fragile caterpillars are easily injured or killed by initial predator attacks, likely before sound would startle a would-be predator. Such being the case, we hypothesize that large-bodied caterpillars are more likely to evolve sonic defenses. Curiously, only a small fraction of sphingid and saturniid species are known to produce sounds. Future studies should examine why so few species have evolved sonic defenses and others have not.

ACKNOWLEDGMENTS

We thank K. Sakagami for his assistance. This study was partly supported by a Grant-in-Aid for Scientific Research

(17K08158). Insects were donated by Shinshu University through a Grant-in-Aid from the “National Bio Resource Project (NBRP, RR2002), Silkworm Genetic Resources” for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

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