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ABSTRACT

Several Bombycoidea caterpillars, renowned for their large size and diverse appearances, possess an intriguing hidden talent – the ability to 'vocalise'. Vocalisation is a rare form of sound production in insects, whereby sounds emanate from the oral cavity by air being forced through the foregut. Here, we report on vocalisation in 10 Bombycoidea species that occur across three families. Sounds in all 10 species are evoked in response to simulated predator attacks. Species were identified as vocalisers based primarily on video evidence of mouthparts being open during sound production. Vocalisations, when considered collectively across all 10 species studied, sound like a train of 'hisses' (sound units) that occur following an attack. Each sound unit comprises a series of pulses (4–104 on average) and is broadband with high dominant frequency (24–49 kHz on average). Given that vocalising species occur in different families across this large superfamily, we asked whether related species shared similar sound features. We found considerable overlap between sound characteristics of different vocalising species, suggesting a shared mechanism overall. However, distinct differences were also noted between families, suggesting that vocalisation may have evolved multiple times within Bombycoidea. The evolutionary origins and specific functions of vocalisation in caterpillars are discussed.

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Introduction

Insect defence sounds are widespread and highly variable in their acoustic characteristics (Low et al. 2021). There are several hypotheses that might explain why and how different sound types and their respective mechanisms evolved. For example, one hypothesis is that variation among defence sounds may have evolved due to differences in function (Corcoran et al. 2010; Low et al. 2021) similar to the way in which some colours are proposed to be aposematic while others are considered cryptic (Higginson and Ruxton 2010). Other factors that could explain variability in sounds include selective pressures posed by different predators with different hearing abilities, or variation in the signalling environment that could scatter or mask noise (Low et al. 2021). However, these hypotheses remain largely untested, creating a critical knowledge gap within the field. To address this gap and gain insights into the function and evolution of communication

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signals in insects, a promising avenue of research involves conducting comparative analyses among extant species that manifest diversity in their signal types (e.g. Scott et al. 2010; Vidal-García et al. 2020).

Bombycoidea (silk and hawk moth) caterpillars are an ideal model group for a comparative approach because their defence sounds are taxonomically widespread, acoustically diverse, and functionally dedicated to defence, unlike sounds of adults which can function in both mating and defence (e.g. tiger moths, Simmons and Conner 1996). Four different types of sounds have been reported to date in larval Bombycoidea: clicking, chirping, whistling, and vocalising (Bura et al. 2016). Clicking and chirping sounds are produced by mandible snapping and scraping respectively, and have been described in several species (Brown et al. 2007; Bura et al. 2009, 2012; Low et al. 2022). Whistling is produced by forcing air from spiracles (Bura et al. 2011; Sugiura and Takanashi 2018; Sugiura et al. 2020). Vocalising is the most unusual sound-producing mechanism and is produced by forcing air through the foregut. This mechanism has only been formally studied in one caterpillar species to date, *Amphion floridensis* (Lepidoptera: Bombycoidea: Sphingidae) (Rosi-Denadai et al. 2018). Sounds of late instar *A. floridensis* are produced in trains consisting of long and short duration sound units (Rosi-Denadai et al. 2018). Based on morphological analyses and numerical modelling, long and short sounds result from air being moved into and out of the foregut, respectively (Rosi-Denadai et al. 2018). The unusual sound-producing mechanism of *A. floridensis* is novel for insects in general, and here we report on this mechanism in nine additional Bombycoidea species.

The focus of this study is to describe vocalisation defence sounds in Bombycoidea caterpillars. The objectives include: 1) describing and characterising vocalisation sounds in 10 species; and 2) conducting a comparative analysis between species and families to assess if and how the sounds differ. Characterising these sounds is important for determining if species are using a mechanism similar to that of *A. floridensis*, or, if there is variation among the sounds, what this variation might mean in terms of how vocalisation evolved in Bombycoidea caterpillars. The results of this study will set the stage to test hypotheses on the evolution of insect defence sounds with comparative methods using Bombycoidea caterpillars as a model group.

Materials and methods

Caterpillar sampling

Ten species of Bombycoidea caterpillars belonging to three different families were included in this study: three Saturniidae (Ceratocampinae: *Citheronia bellavista*, *Citheronia lobesis*, *Citheronia sepulcralis*), six Sphingidae (Macroglossinae: *Aellopos titan*, *Amphion floridensis*, *Erinnyis ello*, *Nyceryx magna*, *Pachygonidia drucei*, *Sphecodina abbottii*), and one Brahmaeidae (*Brahmaea tancrei*). All species were collected and tested by the Yack bioacoustics lab at Carleton University (Ottawa, ON, Canada) as part of an ongoing survey of the diversity of Bombycoidea sound production. Specimens were obtained opportunistically as eggs or larvae from various sources worldwide, including Canada, USA, Europe, and Costa Rica, between 2009 and 2019 (CFIA permits P-2008–02614 and P-2016–02619). Larvae reared from eggs were fed local host plants suitable to each species. All caterpillars were tested as late instars, usually from IV to V. Sample sizes and recording methods varied between species included in this study. Details on sample sizes, sources of larvae, location of voucher specimens, and plants fed to each species are provided in Table S1.

Inclusion criteria

Species included in this study were identified as vocalisers using the following methods. 1) Observation of mouthparts during sound production using video attack trials, close-up mouthpart videos, or observations under a microscope. If mandible movements corresponding to sound production were not observed (i.e. mandibles were not being rubbed together during sound production), the species was included as a vocaliser. This was the primary criterion for inclusion. 2) Relationship to species confirmed by method 1. For example, in *Citheronia lobesis*, where mouthparts could not be observed during sound production, we included it because it is a congener of *C. bellavista* and *C. sepulcralis*, both confirmed species based on method 1. 3) Elimination of spiracle whistling by assessing whether longitudinal body contractions occurred during sound production, as is the case in *Amorpha juglandis*, a known whistler (Bura et al. 2011). If contractions were observed, we covered spiracles with either latex or a water bath to determine if sound production still occurred.

Sound and video recordings

Caterpillars were tested for sound production by simulated attack trials. An individual was allowed to rest on a cutting of host plant for 15 minutes prior to the beginning of a trial. Sound production was induced by pinching the caterpillar with blunt forceps either behind the head capsule or on the posterior end. Recordings conducted in the field were performed in a portable chamber lined with acoustic foam. In the lab, recordings were performed in a walk-in acoustic chamber (Eckel Industries Ltd., Cambridge, MA, USA).

Sounds were recorded using one of three ¼ inch microphones, all with broad frequency responses: Earthworks QTC40 (Milford, NH, USA), B&K Type 4939 microphone (Naerum, Denmark) amplified with a B&K Type 2690 Nexus conditioning amplifier (Naerum, Denmark), and GRAS Type 46BF (Holte, Denmark) connected to a GRAS Type 26TC preamplifier and an Avisoft power module (Avisoft Bioacoustics, Berlin, Germany). Earthworks and B&K were recorded as '.wav' files to a Fostex FR-2 Field Recorder (Gardena, CA, USA) at a sampling rate of 192 kHz. GRAS recordings were obtained at a sampling rate of 192 kHz using Avisoft-RECORDER (Avisoft Bioacoustics, Berlin, Germany). Microphones were placed between 2 and 10 cm from the caterpillar's head and recordings were conducted following simulated attacks. Sound files from these recordings were used to characterise acoustic traits in both temporal and spectral domains.

Sounds from some video recordings of attack trials were also used for temporal analysis of acoustic traits. These were extracted as .wav files from .mp4 files obtained using a SONY HDR XR-500 HD camcorder (Tokyo, Japan) and a SONY ECM-MS907 microphone (Tokyo, Japan) which was placed 5–10 cm from the head of the caterpillar.

Sound analyses

Six temporal and three spectral characteristics were analysed from sound files using Avisoft SASlab Pro (Avisoft Bioacoustics, Berlin, Germany). Temporal characteristics included unit duration (ms), number of pulses per unit (PPU, #), pulse rate (#/s), interpulse interval (IPI) within a unit (ms), train duration (s), and number of units per train (#). We define temporal characteristics as follows: a unit is an individual sound as perceived by the human ear (Broughton 1963); a pulse is a transient waveform with a distinct rise and fall (Broughton 1963) and is a component of a unit; and a train refers to a series of units following an attack. Spectral characteristics included dominant frequency and bandwidths at −3 and −10 dB below dominant frequency. Dominant frequency is the peak with the most energy on the power spectrum, created using 1024-point Fast-Fourier Transform (Hann window). All sound units were sampled randomly from the second train of units produced after pinching. Three units per each individual were measured from up to five individuals per species, or from five trials in situations where five individuals were not available. To illustrate each signal type by species, representative waveforms and spectrograms were generated with R (version 4.2.2, R Core Team 2022) in RStudio (version 2023.0.9.1, RStudio Team 2023) using the packages *tuneR* (Ligges et al. 2023), *seewave* (Sueur et al. 2008), and *phonTools* (Barreda 2015). Graphical illustrations were produced with a Wacom Intuos S creative tablet (Kazo, Saitama, Japan) using CorelDRAW 2021 (Ottawa, ON, Canada).

Statistical analyses

To explore the variation among species and families based on acoustic parameters, we conducted a principal components analysis (PCA). Pearson correlation analysis was first used to calculate pairwise correlation coefficients, and variance inflation factor (VIF) values were computed for each predictor variable. Variables exceeding a VIF threshold of 10 were considered to exhibit significant multicollinearity and excluded from the analysis. Coefficients of variation (CVs) were calculated for all species as a measure of the variation within each acoustic trait. Mann–Whitney U-tests were conducted to test for significant differences between *A. floridensis* and other species. All statistical analyses were implemented with R in RStudio using packages *stats*, *MASS*, and *Factoextra* (Kassambara and Mundt 2020).

Results

All 10 species in this study responded to simulated attacks by producing sounds, and all species met our inclusion criteria as vocalisers. Sound production following simulated attack is shown for two representative species belonging to two different families in Figure 1, and audio for these species is available in Movie S1. In eight species, videos of mouthpart movements during sound production confirmed that mandibles were not interacting during sound production, thus eliminating clicking and chirping sounds. In these species, mandibles were held widely or partially opened during sound production (e.g. Figure 1). In three species, *A. floridensis* (Figure 1d), *C. sepulcralis* (Figure 1h), and *B. tancrei*, close-up videos of the buccal cavity while sound was being produced showed

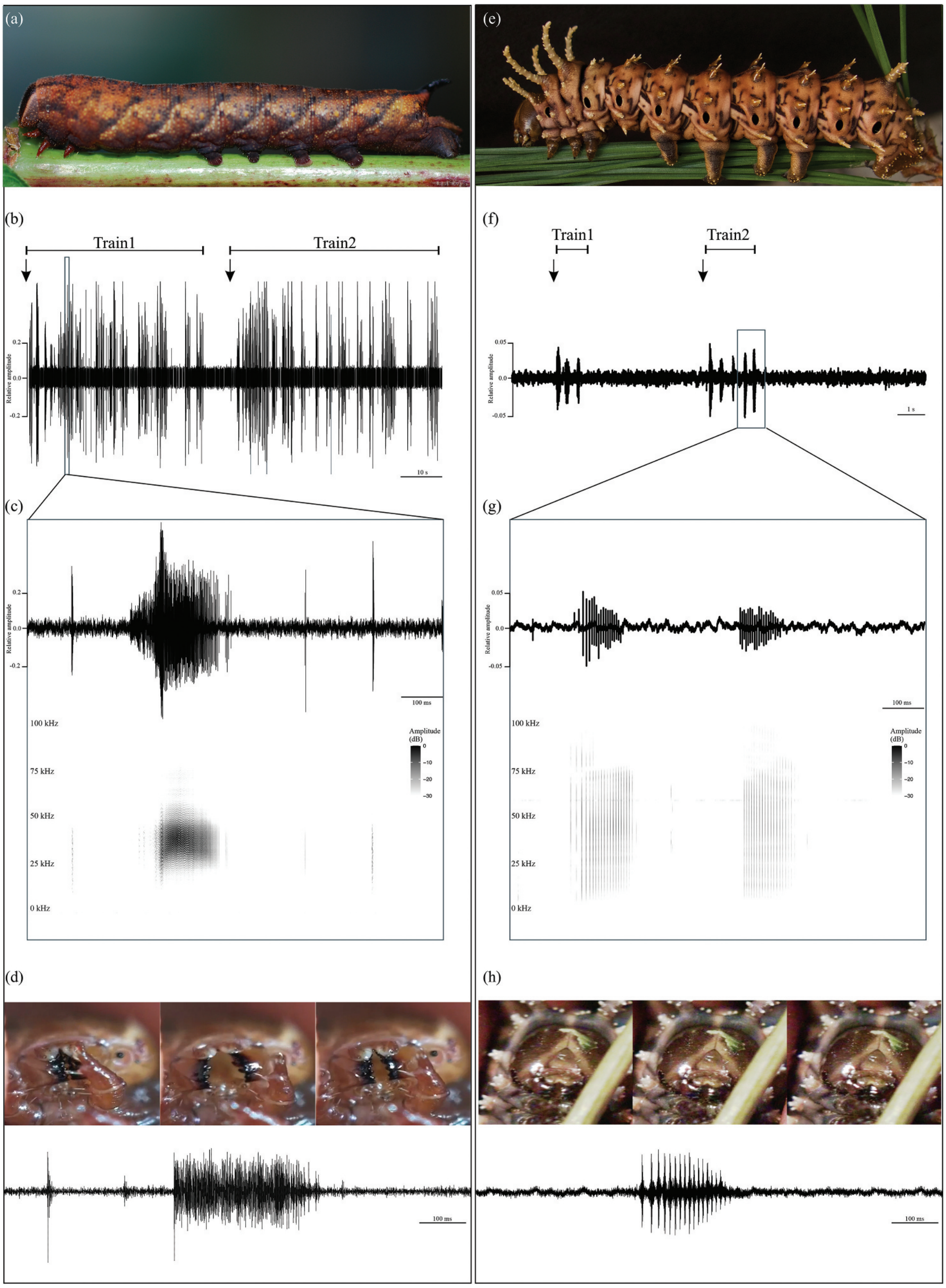

Figure 1. Acoustic characteristics of two vocalising species, *Amphion floridensis* (Sphingidae: Macroglossinae) (a–d) and *Citheronia sepulcralis* (Saturniidae: Ceratocampinae) (e–h). (a) Final (fourth) instar of *A. floridensis* in natural resting position on host plant. (b) Two sound trains resulting from simulated attacks (arrows). Segment marked with a box is expanded in part c. (c) Sound waveform (top) and spectrogram (bottom) of four vocalization units. (d) Top: screenshots from a mandible video demonstrating that sound production occurs while the mandibles are held open. Bottom: corresponding waveforms from audio of mandible video. (e) Final (fifth) instar of *C. sepulcralis* in natural resting position on host plant. (f) Two sound trains resulting from simulated attacks (arrows). Segment marked with a box is expanded in part g. (g) Sound waveform (top) and spectrogram (bottom) of two vocalization units. (h) Top: screenshots from a mandible video demonstrating that sound production occurs while the mandibles are held open. Bottom: corresponding waveforms from audio of mandible video.

movements of the hypopharynx, but these movements did not occur consistently with sound production. The two species lacking videos of mouthparts during sound production are *C. lobesis* and *E. ello*. *Citheronia lobesis* was included as a vocaliser due to the similarity of its sounds compared to its sister species, *C. bellavista* and *C. sepulcralis*, both confirmed to hold mandibles open during sound production. *Erinnyis ello* was also included as a vocaliser due to the similarity of its sounds to other vocalising members of the Macroglossinae (e.g. *N. magna*, Table 1). Furthermore, whistling in *E. ello* was eliminated due to the lack of longitudinal abdominal contractions during sound production.

What are the features of vocalisation sounds? A summary of the acoustic characteristics of each species is shown in Table 1, with representative waveforms from each species shown in Figure 2 and density plots representing the range of acoustic traits by species in Figure 3. Vocalisations vary greatly in unit duration (from 0.3 to 544.7 ms) and PPU (from 1 to 332), but the sound units are on average long (mean 105.4 ± 119.1 ms) with many PPU (mean 36.0 ± 67.0). Pulse rates are high, with 37.0 to 1789.4 pulses per second (mean 324.7 ± 278.7). Vocalisation sounds are broadband (−10 dB, mean 30.3 ± 14.0 kHz) with dominant frequencies tending to be in the ultrasonic range (mean 39.3 ± 9.4 kHz). If we consider the amount of variation in each acoustic trait across species, we see that some traits exhibit a lower range of variation than others based on coefficients of variation (CVs) (Figure 4; a higher CV indicates more variation). Spectral traits and pulse rate are less variable than other traits within and between species, with CVs ≤ 0.5 on average and narrow ranges of values. A vocalisation can therefore be described as a 'hiss' sound with multiple PPU produced at a high pulse rate and broadband properties.

How do vocalising sounds differ? Principal components analysis (PCA) was performed to identify and visualise the similarities and differences among sounds. We excluded the number of units per train from our PCA due to its strong correlation with 'train duration' ($r = 0.98$) and high VIF (>30) to mitigate multicollinearity. Variation among vocalising sounds was partitioned along three axes (eigenvalues >1), accounting for 69.5% of the variation in acoustic characteristics (Table 2 and Figure 5). The first principal component (PC1; accounting for 31.4%) was primarily influenced by unit duration and PPU, while the second principal component (PC2; 22.6%) was most heavily influenced by bandwidths at −3 and −10 dB and the third principal component (PC3; 15.5%) by IPI median. In other words, unit duration, PPU, bandwidths, and IPI median account for a large part of the variation between vocalising sounds of all species. However, there is considerable overlap between the acoustic traits of species (Figure 5a). This is because each species tends to have high variability in some traits and low variability in others (as shown in Figure 4), which makes it difficult to discern patterns between species. The most notable result is that *A. floridensis* differs markedly relative to other species in train duration, number of units, unit duration, and PPU (Table 1 and Figure 3). *Amphion floridensis* train durations are significantly longer than all other species (Mann–Whitney U-tests, $p < 0.005$), and contain significantly more units per train – except for *B. tancrei* (Mann–Whitney U-tests, $p < 0.005$). Unit durations and PPU of *A. floridensis* are also higher and more widely distributed compared to other species. *Amphion floridensis* is reported to produce units with durations that are bimodally distributed, i.e. long and short units (Rosi-Denadai et al. 2018). When comparing long and short units separately to the units of other species, long

Table 1. Acoustic characteristics of all species analysed in this study. Acoustic measurements were obtained from 3 sound units each for up to 5 different individuals per species. All values in this table are provided as 'Mean (SD)'.

	Temporal Traits						Spectral Traits[a]		
FAMILY: Subfamily Species	Unit Duration (ms)	PPU	Pulse Rate (#/s)[a]	IPI Median (ms)[b]	Train Duration (s)	Units/Train	Dominant Frequency (kHz)	-10 dB (kHz)	-3 dB (kHz)
SATURNIIDAE: Ceratocampinae									
Citheronia bellavista	108.1 (46.6)	10.1 (6.1)	102.5 (52.5)	12.4 (8.7)	0.6 (1.1)	1.7 (0.7)	36.5 (10.4)	25.5 (9.7)	7.0 (3.0)
Citheronia lobesis	132.1 (27.6)	21.7 (6.4)	163.9 (30.8)	5.5 (1.3)	1.3 (1.2)	3.2 (1.7)	36.7 (3.8)	47.8 (14.2)	9.5 (5.1)
Citheronia sepulcralis	153.1 (28.4)	29.1 (16.2)	192.1 (95.8)	4.9 (1.9)	0.8 (0.4)	2.4 (0.8)	48.5 (5.6)	27.7 (14.6)	8.3 (5.0)
SPHINGIDAE: Macroglossinae									
Aellopos titan	51.3 (14.2)	8.3 (4.6)	170.3 (109.0)	5.2 (2.7)	1.1 (0.9)	4.0 (2.5)	44.6 (3.6)	43.3 (12.3)	8.4 (1.7)
Amphion floridensis	189.1 (198.0)	103.9 (111.1)	534.6 (314.7)	3.2 (3.3)	36.3 (32.6)	80.5 (68.4)	38.1 (5.2)	26.3 (9.7)	8.9 (5.5)
Erinnyis ello	61.3 (44.6)	19.5 (4.4)	438.5 (269.5)	2.0 (1.1)	0.9 (0.6)	3.2 (1.5)	24.0 (23.2)	17.6 (8.1)	2.7 (1.3)
Nyceryx magna	59.8 (61.6)	47.0 (53.8)	655.0 (324.0)	0.8 (0.1)	2.1 (1.7)	7.0 (4.7)	32.5 (4.0)	24.0 (7.2)	4.9 (2.0)
Pachygonidia drucei	26.9 (29.8)	12.0 (10.2)	622.4 (233.3)	1.9 (0.8)	0.7 (1.5)	2.0 (1.5)	40.9 (15.2)	45.9 (15.0)	9.8 (4.4)
Sphecodina abbottii	59.6 (67.5)	7.9 (9.2)	155.6 (64.7)	7.6 (2.4)	4.7 (2.5)	13.2 (4.8)	37.6 (8.4)	29.6 (9.5)	8.7 (5.1)
BRAHMAEIDAE									
Brahmaea tancrei	17.5 (16.0)	3.8 (1.9)	292.0 (151.4)	4.1 (3.1)	6.3 (3.7)	25.0 (12.0)	40.7 (4.4)	18.6 (5.8)	5.2 (1.9)

[a] Units composed of single pulses were not included in pulse rate calculations or spectral analyses.
[b] Median (rather than mean) IPI values were included as IPI tends to be heavily skewed.

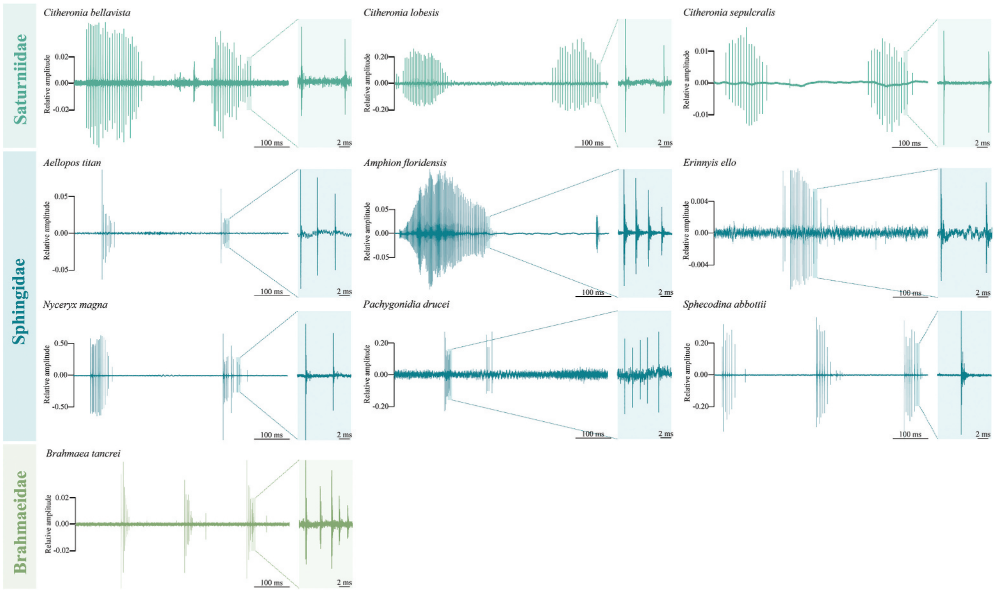

Figure 2. Representative waveforms of each species included in this study. All sound traces are 600 ms in duration and show 1–3 sound units. Shaded box encloses 10 ms and is expanded on the right to show individual sound pulses.

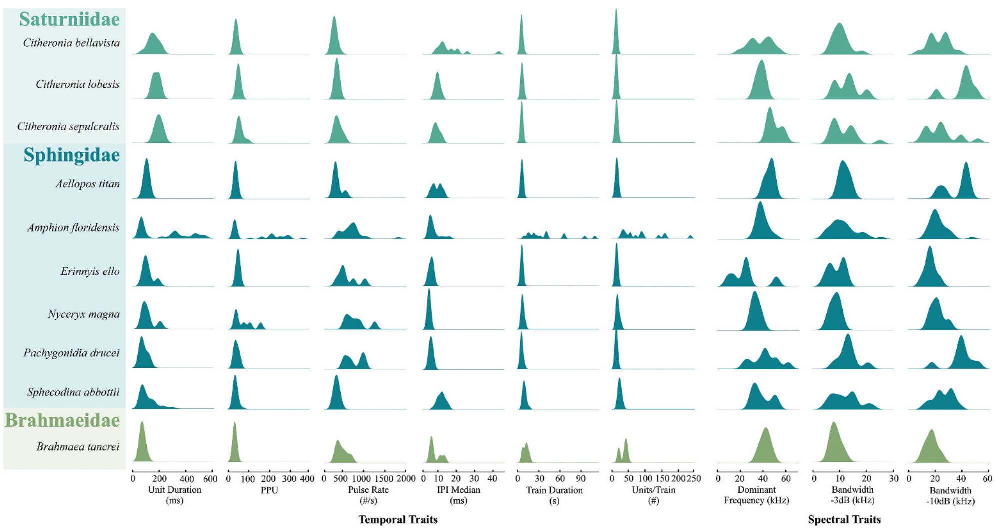

Figure 3. Density plots showing the frequency distribution of temporal and spectral acoustic traits within each species, colour-coded by family.

units are significantly longer with more PPU than all other species (Mann–Whitney U-tests, $p < 0.005$), while short units show some overlap. The pulse rate, however, is less variable, demonstrating that *A. floridensis* long sound units are not produced at a faster pulse rate than other species. No other species were observed to produce long and short units to the same extent as *A. floridensis*, though *N. magna* and *E. ello* did display a small

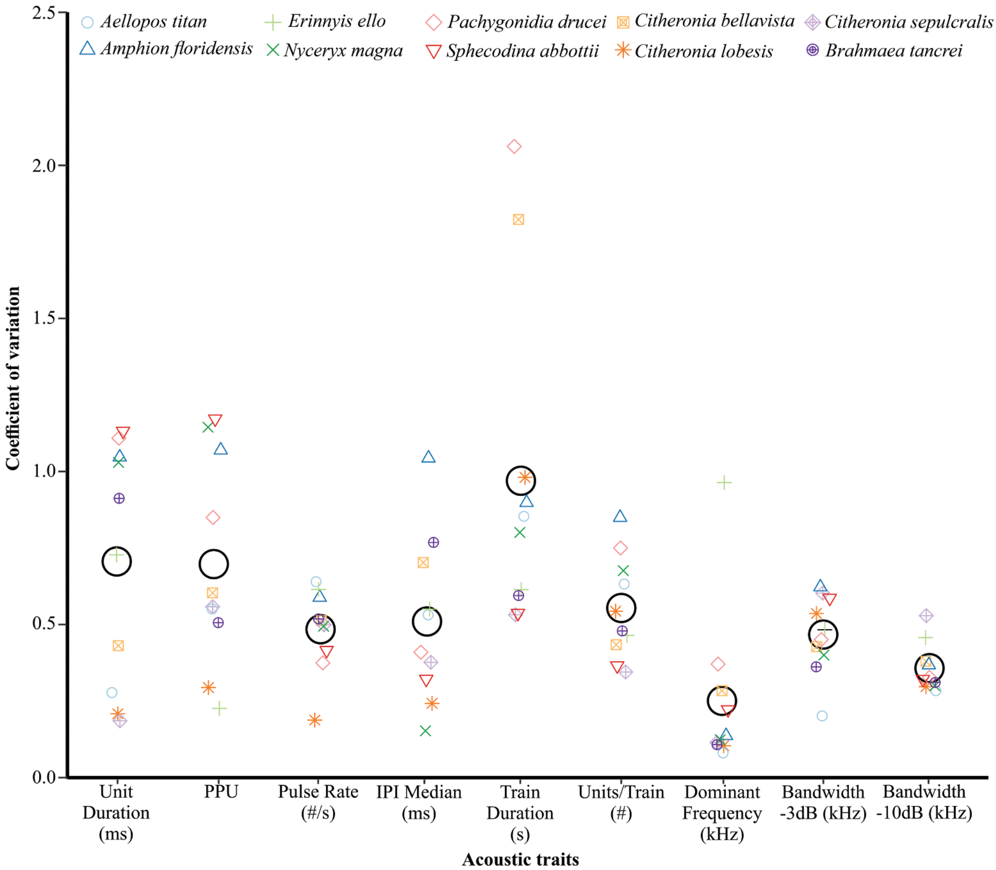

Figure 4. Coefficients of variation (CV) of each species for each acoustic trait. Each coloured symbol represents a different species. Points were jittered slightly along the x-axis to increase visibility of points. Black circles indicate the mean CV for each acoustic trait.

bimodal distribution of sound unit durations (Figure 3). Overall, vocalisation sounds exhibit substantial variation within species, but there is considerable overlap between species' sounds as would be expected with a shared mechanism.

Table 2. Summary of three principal components (PCs) with eigenvalues >1 resulting from a principal components analysis of acoustic characteristics from vocalisations of 10 Bombycoidea caterpillar species. Variables with the largest contributions to each PC are shown in bold.

	Component		
Parameter	PC1 (31.4%)	PC2 (22.6%)	PC3 (15.5%)
Unit Duration	**−0.4878**	0.1138	−0.4721
PPU	**−0.5652**	−0.0657	−0.2738
Pulse Rate	−0.3423	−0.3661	0.4323
IPI Median	0.3938	0.1266	**−0.5535**
Train Duration	−0.2780	−0.3246	−0.1466
Dominant Frequency	−0.1491	0.3324	0.3111
−3 dB	−0.1220	**0.5669**	0.2868
−10 dB	−0.2360	**0.5433**	−0.0910

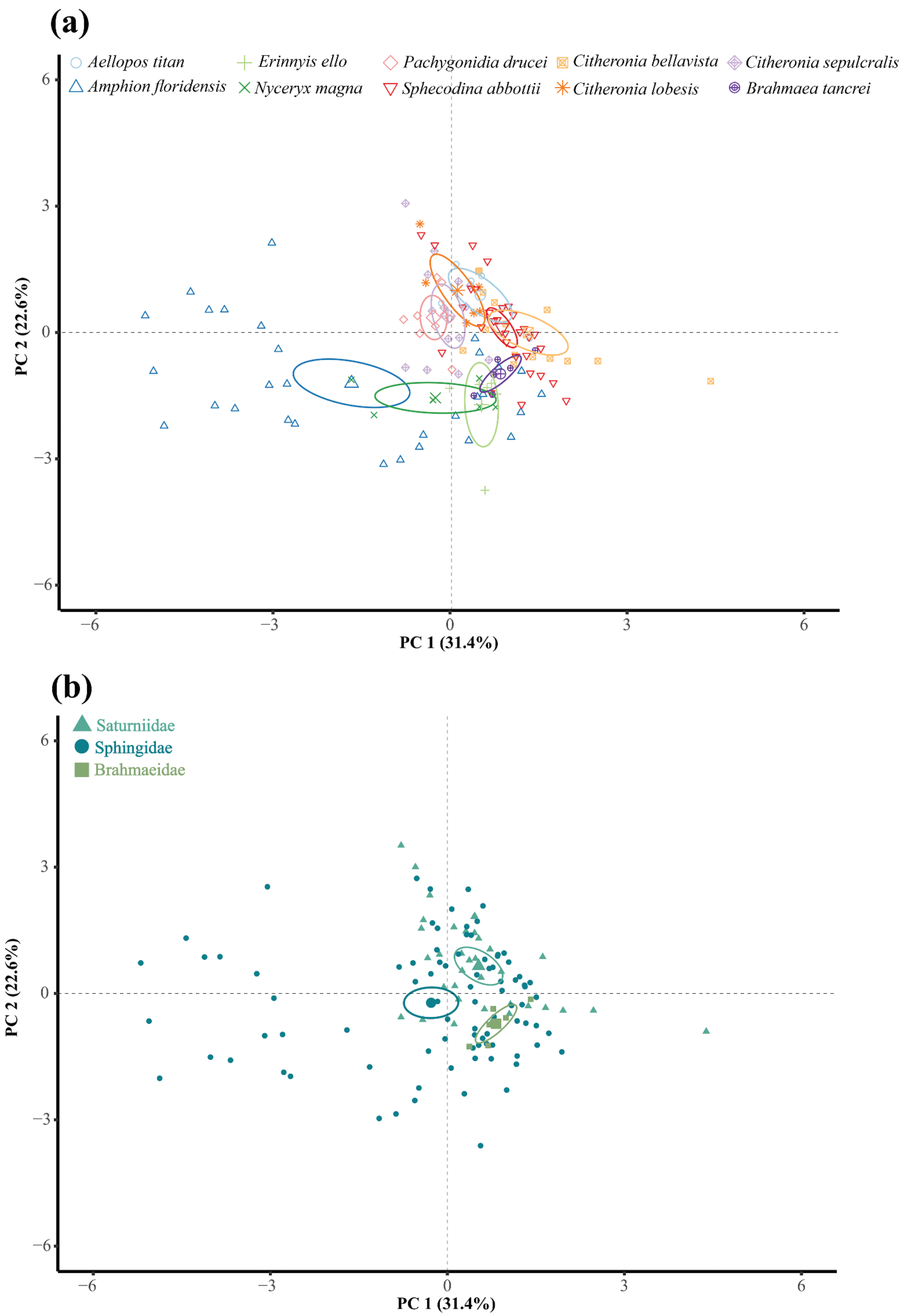

Figure 5. Principal components analysis (PCA) of acoustic characteristics obtained from vocalisations of 10 Bombycoidea caterpillar species. Plot in two-dimensional space defined by the first two axes of the PCA. (a) Colours and symbols represent the 10 species. (b) Colours and symbols represent the three families. Species (a) and families (b) are clustered with centroids (large points) and 95% CIs (ellipses) depicted for each type. For details on PC loadings, see Table 2.

How do vocalisations vary, if at all, between families? Species in Saturniidae have shorter trains with fewer units, and longer units with more PPU and lower pulse rates compared to Sphingidae and Brahmaeidae (Figure 3). When sounds were classified by family in the PCA (Figure 5b), the 95% confidence interval of each family was distinctly separate from each other. However, with only one species in Brahmaeidae and the close relationship among tested Saturniidae species (i.e. *Citheronia* are sister species), we cannot draw conclusions regarding family differences without further sampling.

Discussion

Vocalisation can be broadly defined as sound that emerges from the oral cavity caused by the flow of air (Bradbury and Vehrencamp 2011). While this mechanism of sound production is common in vertebrates, having evolved via modification of airflow through the respiratory system, it is much less common in insects because their respiratory systems are not linked to their oral cavity. Vocalisation has only been reported in two insect orders to date (reviewed in Low et al. 2021), and in each case is proposed to function in a defensive context. One species of Orthoptera, *Mygalopsis marki* (Orthoptera: Tettigoniidae), has been reported to produce sound via forced air from its oral cavity, though further details on the exact mechanism are lacking (Bailey and Sandow 1983). In Lepidoptera, vocalisation-like sounds have only been reported in the Superfamily Bombycoidea, and in adults or larvae depending on the species. In adults, six species of hawk moths (including the Death's-head hawk moth *Acherontia atropos*) produce 'squeaks' from their pharynx (Zagorinsky et al. 2012; Brehm et al. 2015). In *A. atropos*, air is first drawn into the pharynx causing a lobe to vibrate and produce a 'rasp', and then air is expelled without vibrating the lobe to produce a 'whistle' (Brehm et al. 2015). In larvae, the vocalisation mechanism has only been described in the sphingid *A. floridensis* where sounds are produced by the movement of air into and out of the foregut via the mouth (Rosi-Denadai et al. 2018). Air passing between the crop and oesophageal chambers creates ring vortices, and the frequencies of the vortices are amplified within the oesophagus. The 'in and out' hypothesis of *A. floridensis* is similar to that proposed for the hawk moth *A. atropos*, though a vibrating lobe was not found in *A. floridensis*. In this study, we reported on vocalisation sounds of an additional nine caterpillar species that represent three different Bombycoidea families. This discussion focuses on three main questions. Are all vocalising sounds the same? How might the mechanism of vocalisation have evolved in caterpillars? What might be the specific defensive function of these sounds?

Are all vocalising sounds the same? Bombycoidea caterpillars produce defence sounds using one of four different mechanisms: clicking, chirping, whistling, and vocalising (Bura et al. 2016). Qualitatively, vocalisations differ in that they are long hisses, while clicks and chirps are short sounds and whistles are more squeak-like. However, while vocalisations sound different from the other mechanisms, not all vocalisations are identical. The cause of this variation is challenging to speculate on, mainly because the mechanism of sound production is not clearly understood. In *A. floridensis*, long sounds are suggested to occur via inhalation of air into the foregut, and short sounds by expelling air back out (Rosi-Denadai et al. 2018). However, none of the other nine species in this study produce both long and short units to the same extent as *A. floridensis* (Figure 3), though *N. magna* and *E. ello* exhibit small bimodal distributions and the distribution of *S. abbottii* is right-tailed. Perhaps a bimodal distribution suggests a species produces sound during both inhalation and exhalation, while the other species produce sound only during either inhalation or exhalation. It is also possible that the other species possess a vibrating lobe such as is found in the adult hawk moth *A. atropos*. The variation among vocalisation sounds of all species creates considerable overlap between the sounds of different species (Figure 5a), possibly indicating that the sounds are produced via a similar mechanism, with variation explained either by divergence from a shared ancestral state or convergent evolution. Additionally, the fact that all species produce sounds of a similar frequency supports a vocalisation hypothesis given that frequency is determined by foregut size (Rosi-Denadai et al. 2018), and all species examined in this study were of similar size. Future studies are necessary to investigate if sound production only occurs during either inhalation or exhalation, to examine the internal morphology of additional species for the presence of a lobe, and to determine if movements of the hypopharynx (observed in three species) are somehow related to the mechanism of sound production.

How might vocalisation have evolved? We outline three hypotheses that could explain the evolutionary origins of vocalisation in Bombycoidea caterpillars. First, larval vocalisation may have been co-opted from adult vocalisation, meaning the caterpillars produce sound because their adult life stage also produces sound. However, because adults of four species of vocalising caterpillars did not produce sound upon tactile stimulation (*E. ello*, *N. magna*, *S. abbottii*, *A. floridensis*; Kawahara and Barber 2015), and caterpillars of the six squeaking adult hawk moths are not known to vocalise, this hypothesis has little support.

Second, vocalisation may have evolved from larval regurgitation behaviours. Regurgitation behaviour can be subdivided into three types: primary, secondary, or non-regurgitators (Grant 2006). Primary regurgitation involves the ability to re-imbibe regurgitant which could serve as a precursor for imbibing air, as proposed by Rosi-Denadai et al. (2018). Caterpillars that regurgitate more frequently have reduced growth rates compared to non-regurgitators (Bowers 2003), and so switching to forced air rather than regurgitant could be an adaptation to circumvent this cost. Notably, the 10 vocalising species in this study are classified as non-regurgitators (Low, unpublished), meaning they do not regurgitate except as a last resort (i.e. after numerous pinches). If vocalisation evolved from primary regurgitation, we would predict the ancestral condition to be that of a primary regurgitator. We would also predict the presence of incidental sounds while regurgitating in some Bombycoidea species as a possible intermediate step between regurgitation and vocalisation. Another prediction is that vocalising species will have large crops and shortened midguts, as has been shown for primary regurgitators when compared to non-regurgitators (Grant 2006).

Third, vocalisation may have evolved from defensive posturing. Defensive posturing occurs when a caterpillar inflates their anterior section by retracting their head into their thorax so that they appear larger (Hossie and Sherratt 2014). Inflating the thorax in this fashion may cause air to be sucked into the foregut. Under this hypothesis, we would predict the ancestral condition to include defensive posturing without vocalisation, and that vocalising species are capable of expanding their anterior end. However, behavioural data (not shown) indicate that only *A. floridensis* and *P. drucei* retract their heads in this fashion, and not every time they produce sound. Therefore, of the three hypotheses outlined above, vocalisation evolving from regurgitation seems the most promising and is worth investigating in future studies using phylogenetic comparative methods and morphological analyses.

What is the specific defensive function of these sounds? There are several hypotheses that could explain the function of insect defence sounds that are aimed at an attacking predator. These include but are not restricted to sonar jamming, acoustic aposematism, or deimatism (as reviewed by Low et al. 2021). Sonar jamming is a form of interference signal that disrupts the echolocation calls of bats. To effectively disrupt bat calls, jamming sounds require a high duty cycle with many pulses per unit (Corcoran et al. 2009; Kawahara and Barber 2015). However, within vocalising Bombycoidea caterpillars, most of the sounds are not high enough in duty cycle to jam echolocation calls and their pulse rates are much lower than anti-bat moth clicks. Acoustic aposematism describes when a sound advertises a noxious or dangerous attribute of the prey item (Low et al. 2021). If vocalisation is aposematic, we would predict the presence of additional and unpleasant attributes such as regurgitation behaviour, a strong bite, or stinging spines. As mentioned above, however, none of the vocalising species in this study regurgitate readily. Additionally, none of the species possess secretory spines or attempt to bite the attacking forceps during trials (MLL, personal observations). Acoustic aposematism therefore seems unlikely as a function of vocalisation sounds. Deimatic displays are those that startle a predator and cause it to slow down or end its attack (Drinkwater et al. 2022). Deimatic sounds are proposed to be long, loud, and sudden in order to activate a predator's innate startle response (Hill 2007; Kowalski et al. 2014), and can often form part of a multi-component defensive display (Drinkwater et al. 2022). The vocalisations in this study are relatively long in duration, distinctly audible at close-range (i.e. loud), and are produced upon attack (i.e. sudden). They are also broadband with ultrasonic dominant frequencies, which may indicate these sounds are directed at a wide range of predators. Several species combine their sounds with intense thrashing (*N. magna* and *E. ello*), or with eyespots that are revealed (*E. ello* and *B. tancrei*) or emphasised (*S. abbottii*) when attacked (MLL, personal observations), behaviours which may additionally startle a predator. Deimatism is therefore a promising hypothesis for the function of vocalisation sounds, which could be tested by investigating the relationship of vocalisation to other defensive behaviours, as well as by testing the sounds on predators.

Summary

Bombycoidea caterpillars are an ideal model group for exploring large-scale evolutionary questions using comparative methods because they generate a wide range of sound types in the context of defence (Bura et al. 2016). A key step in this research is to characterise the sounds and mechanisms, and how traits vary between species. In this study, we have meticulously documented the vocalisation sounds of 10 species of Bombycoidea caterpillars. Overall, these sounds exhibit considerable variation in their acoustic characteristics, but with sufficient overlap that hints at a shared mechanism. Given the variation in sounds between species, and the occurrence of these species in three separate families, it seems likely that a similar mechanism has evolved independently in several lineages. Future research should investigate potential evolutionary correlates between vocalisation sounds and other caterpillar traits to try to understand how these sounds may have evolved, and in different lineages.

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Data availability statement

For access to acoustic data from this study, please contact the corresponding author at jayneyack@cunet.carleton.ca.

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