

Vibration detection and discrimination in the masked birch caterpillar (*Drepana arcuata*)

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Abstract Leaf-borne vibrations are potentially important to caterpillars for communication and risk assessment. Yet, little is known about the vibratory environment of caterpillars, or how they detect and discriminate between vibrations from relevant and non-relevant sources. We measured the vibratory ‘landscape’ of the territorial masked birch caterpillar *Drepana arcuata* (Drepanidae), and assessed its ability to detect and respond to vibrations generated by conspecific and predatory intruders, wind and rain. Residents of leaf shelters were shown to respond to low amplitude vibrations generated by a crawling conspecific intruder, since removal of the vibrations through leaf incision prevented the resident’s response. Residents did not respond to large amplitude, low frequency disturbances caused by wind and rain alone, but did respond to approaching conspecifics under windy conditions, indicating an ability to discriminate between these sources. Residents also responded differently in the presence of vibrations generated by approaching predators (*Podisus*) and conspecifics. An analysis of vibration characteristics suggests that despite significant overlap between vibrations from different sources, there are differences in frequency and amplitude characteristics that caterpillars may use to discriminate between sources. Caterpillars

live in a vibration-rich environment that we argue forms a prominent part of the sensory world of substrate bound holometabolous larvae.

Keywords Sensory · Vibration · Communication · Territorial · Caterpillar

Introduction

Vibrational communication is widespread in insects, and the number of reported examples continues to increase as methods for recording low amplitude vibrations become more readily available (reviewed in Bennet-Clark 1998; Greenfield 2002; Virant-Doberlet and Čokl 2004; Cocroft and Rodríguez 2005; Hill 2008). Solid-borne vibrations used by insects range from simple incidental cues generated by predators and prey to complex communication signals. Despite the purported importance of vibrations among insects, little is known about how they detect vibrations, and how they distinguish between relevant signals or cues, and non-relevant background noise. This information is particularly lacking for the soft-bodied larvae of holometabolous insects.

Holometabolous insects are those exhibiting complete metamorphosis with a conspicuous and relatively abrupt change in form or structure from the larva to pupa and finally the adult stage. These include major groups of economic and environmental importance such as ants and wasps (Hymenoptera), beetles (Coleoptera), and butterflies and moths (Lepidoptera). Accumulating evidence suggests that vibrations play important roles in obtaining food (e.g., Ishay et al. 1974), detecting predators (e.g., Castellanos and Barbosa 2006), garnering protection against predators (e.g., Travassos and Pierce 2000), and defending resources (e.g.,

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Yack et al. 2001; Fletcher et al. 2006). Since larvae of beetles, moths and butterflies, among other holometabolous insects, are typically bound to their host plant, the ability to detect and discriminate between plant-borne vibrations is likely key to their survival. At present, little is known about the vibration ‘landscape’ of larvae on their natural substrate, whether larvae are capable of detecting vibrations, and how they discriminate between vibrations. In this study we will use the masked birch caterpillar, *Drepana arcuata*, as a model system to address some of these topics.

The masked birch caterpillar is common throughout deciduous woodlands of northeastern North America, building and residing inside silk leaf shelters on birch and alder trees. Residents of leaf shelters respond to approaching conspecifics by generating complex vibrations (Yack et al. 2001; Scott et al. 2010) and purportedly respond to the vibration cues generated by the intruder. In addition, *D. arcuata* should also be subjected to vibratory cues from natural enemies, as well as abiotic interference from wind and rain. There were three primary objectives for this study: (1) to record and characterize vibrations available to a resident caterpillar on its leaf to assess what cues are available and what features caterpillars may use to discriminate between them; (2) to test the hypothesis that *D. arcuata* are sensitive to vibrations, which was done by selectively removing vibratory cues of an approaching conspecific; and (3) to test the hypothesis that *D. arcuata* discriminate between vibrations, which was done by recording vibrations from different sources (conspecific intruder, predator, and simulated wind and rain) and examining behavioral responses of the resident in the presence of these vibrations.

Materials and methods

Insects

Drepana arcuata Wlk. (Lepidoptera: Drepanidae) were collected as gravid females from May to August between 2004 and 2011 near Ottawa, ON, Canada. Females laid eggs and larvae were reared on cuttings of paper birch (*Betula papyfera*) maintained in indoor enclosures ($25 \pm 2^\circ\text{C}$, $70 \pm 5\%$ relative humidity, and 12:12 LD). All caterpillars used in the trials were 4th or 5th instars (residents were 29.75 ± 5.42 mg and intruders were 25.61 ± 3.95 mg). Adults (29.71 ± 5.78 mg) of the generalist predator *Podisus maculiventris* Say (Heteroptera: Pentatomidae) were field-collected from birch trees in the Ottawa region during August 2007. They were maintained in indoor enclosures and fed caterpillars of *D. arcuata*.

Experimental set up

Leaf vibrations and insect behaviors were recorded while a resident caterpillar was exposed to an approaching conspecific or predator, an abiotic factor (simulated wind or rain), or a combination of a conspecific and abiotic factor. The set-up for all trials consisted of a resident caterpillar that was placed on a birch leaf attached to a 10 to 15-cm long twig placed in a water-filled plastic vial (Fig. 1). Leaves were pre-selected to be within 5–7 cm wide and 6–9 cm in length. The resident was left undisturbed for at least 6 h on the isolated leaf to allow it to build its shelter. At least 1 h prior to an experiment, reflective tape used for laser recordings, or in some cases an accelerometer, was

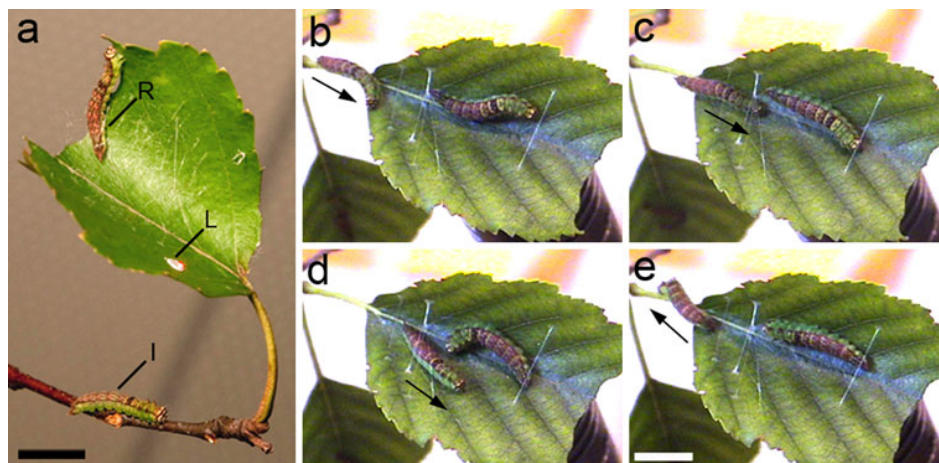


Fig. 1 Experimental set up used to stage an encounter between conspecifics. **a** An intruder (*I*) caterpillar crawls up the birch twig leading to a partially constructed leaf shelter occupied by a resident (*R*). The laser spot (*L*) is positioned ~1 cm of the resident's shelter. Scale bar 15 mm. **b–e** Photo sequence of an interaction whereby the

intruder enters the leaf at the petiole (**b**), and crawls into the leaf shelter while the resident begins to signal (**c**). The resident turns around to face the intruder (**d**) while continuing to signal, and the intruder then turns around to exit the leaf (**e**). Arrows show the direction of the intruder's movement. Scale bar 10 mm

affixed to the leaf (see below for details on vibration recordings). All trials were videotaped simultaneously using two cameras: one for close-ups of the leaf shelter (Handycam HDV 1081i/MiniDV, Sony), and one for full-trial visualization (Handycam DCR-TRV19/MiniDV, Sony). Video clips were imported to a Power Macintosh (G4) as Imovie files, saved as Quicktime Pro files, and analyzed using Image J (NIH, Bethesda, MD, USA). All trials were carried out inside an acoustic chamber (C-14A MR, Eckel, Morrisburg, ON, Canada).

In trials where a resident was exposed to an intruder, the recordings were initiated 1–2 min prior to introducing the intruder to establish baseline levels. In trials with conspecifics, the intruder was isolated on a birch twig without leaves for 15–20 min before the trial. In predator trials, the intruder was individually held in a plastic vial and food deprived for at least 12 h prior to the trial. Following the baseline recording, the intruder was introduced with a paintbrush to the twig, which it crawled up and onto the leaf. Interactions were recorded for at least 5 min after the onset of the resident caterpillar signaling or until one of the contestants left the leaf, or the predator attacked the resident.

The resident's response to two simulated environmental factors, wind and rain, was recorded under the same conditions described above except an accelerometer was attached to the underside of the leaf to record the vibrations instead of using the laser. An accelerometer was used during these trials, since wind and rain often caused large movements of the leaf that displaced the laser beam from its target. Leaf vibrations during wind and rain exposure were also recorded with a laser for measuring vibration characteristics (see next section). Artificial 'gusts' of wind were generated using a domestic fan (Sears 564-42-28002, Sears Canada Inc.) at a velocity of 1.0 m/s (measured with a Hotwire Anemometer, VWR 21800-024). Wind gusts lasted approximately 4 s and were presented at a rate of about 8 per minute. A moderate rain was simulated using a manual atomizer, delivering multiple and simultaneous water droplets to the leaf surface at a rate of about 1 spritz per second and a volume of ~ 0.15 ml per spritz. Trials combining the simultaneous exposure of a conspecific intruder and either wind or rain simulation were also carried out following the same methods described above, but the conspecific intruder was added 1 min following the onset of the abiotic stimulus.

An additional experiment was conducted to assess the vibration properties of a birch leaf following a similar procedure described in Casas et al. (1998). A resident was set up for laser recordings as described above, but the reflective disc was placed 3 cm from the tip of the leaf. A small plastic ball (65 mg, 3 mm in diameter) was dropped onto the leaf at a distance of 1 cm from the leaf tip.

Vibration recording and analysis

Leaf vibrations produced by resident caterpillars, intruding conspecifics and predators, wind, rain and ball dropping events were recorded with a laser vibrometer (PVD-100, Polytec Inc., Ann Arbor, MI, USA), and these recordings were used to characterize vibrations. Reflective tape (0.25 cm diameter) serving as the laser target was attached to the leaf surface as close as possible to the leaf shelter. Because during the course of a trial, the resident can move between its shelter and leaf edge where it feeds, placement of the reflective tape outside of the shelter allowed us to record vibrations typically within 2 cm of the resident caterpillar (see Table 1). The laser output was low-pass filtered at 22 kHz (no high-pass filter), digitized at 48.0 kHz and recorded to a data recorder (FR-2, FOSTEX America, Norwalk, CT, USA). Temporal and spectral characteristics of the vibrations were analyzed using Raven Pro v. 1.2 (Cornell Laboratory of Ornithology, Ithaca, NY, USA). Power spectra were produced using an 8192-point Fast Fourier Transform (Hann window, 50% overlap). Velocities were obtained by measuring voltages on the oscilloscope directly from the analogue output of the laser vibrometer.

An accelerometer was used to record vibrations over the course of the entire trial under conditions of wind and rain. A PE accelerometer (0.5 g) (2222 C, ENDEVCO, San Juan Capistrano, CA, USA) was attached to the lower leaf surface within 1–3 cm from the resident caterpillar. Vibrations were amplified (NEXUS 2690, Bruel and Kjaer, Nærum, Denmark) and recorded as described above.

Cut-leaf experiment

The cut-leaf experiment tested the hypothesis that the caterpillars detect intruders using vibration cues. The experimental set-up was as previously described for conspecific interactions, except in all treatments two alligator clips were positioned on either side of the cut line, or proposed cut line. Each pair of caterpillars (resident and intruder) was subjected to three treatments: whole leaf, cut leaf, and rejoined leaf. During the whole leaf trial, the resident was left undisturbed for 15–20 min before introducing the intruder to the leaf twig, and the trial was carried out until the resident began signaling. The intruder was then removed, and placed back in its container for 15–20 min, at which time the resident settled and resumed normal activities. In the next treatment (cut leaf), the resident's leaf was severed between the shelter and the petiole, creating a 0.5–1.0 mm gap so that vibrations from the approaching intruder would not be transmitted through the substrate until the intruder crossed the gap. The final treatment involved rejoining the separated leaf sections using small strips of paper tape on the underside of the leaf.

Table 1 Characteristics (mean $\pm$ SD^a) of leaf vibrations recorded with a laser vibrometer

	Source of vibration	Periodicity (events/s)	Dominant frequency (Hz)	Velocity (mm/s)	Distance from laser spot (mm)
^a $N = 10$ individual trials with three measurements per trial ^b Rates reported in this table are sampled from the first 10 s of a conspecific encounter ^c Oscillations above background levels within a single wind 'gust' or rain 'spritz'	Resident				
	Chewing (bites)	1.69 $\pm$ 0.24	7.94 $\pm$ 2.77	2.47 $\pm$ 1.03	29.82 $\pm$ 1.54
	Anal scraping	0.84 $\pm$ 0.13 ^a	38.3 $\pm$ 9.40	5.28 $\pm$ 2.84	15.40 $\pm$ 6.73
	Mandible drumming	1.94 $\pm$ 0.14 ^a	56.70 $\pm$ 26.30	49.91 $\pm$ 29.25	16.71 $\pm$ 6.60
	Mandible scraping	0.68 $\pm$ 0.42 ^a	11.7 $\pm$ 5.60	29.02 $\pm$ 17.23	19.33 $\pm$ 4.51
	Intruder				
	Caterpillar crawling	10.70 $\pm$ 5.77	7.47 $\pm$ 4.38	2.08 $\pm$ 1.78	21.5 $\pm$ 9.71
	Predator walking	9.40 $\pm$ 6.70	11.7 $\pm$ 6.80	3.26 $\pm$ 2.02	23.10 $\pm$ 5.87
	Abiotic				
	Wind	4.14 $\pm$ 1.56 ^b	5.02 $\pm$ 1.18	198.4 $\pm$ 28.40	n/a
	Rain (droplets)	56.60 $\pm$ 5.80 ^b	55.29 $\pm$ 47.50	127.2 $\pm$ 25.90	n/a
	Ball dropping	n/a	8.46 $\pm$ 6.51	n/a	20 mm

Videotapes of trials were subsequently analyzed for the location of the intruder with respect to the leaf incision (or lack thereof) when the resident signaled.

Statistical analyses

The dichotomous results (e.g., resident signaling $\times$ non-signaling) of each set of trials were subjected to the randomness G test using William's procedure to correct the G values (Sokal and Rohlf 1995). In the cut-leaf experiment, the distance between resident and intruder caterpillars at the onset of signaling was subjected to analysis of covariance with leaf manipulation as the independent variable (leaf left whole, cut, or rejoined) and distance between the resident and leaf incision as covariate (PROC GLM; SAS Institute 2002). This analysis was subsequently complemented by regression analyses between the resident-intruder distance (dependent variable) and the resident-leaf incision distance (independent variable) (PROC REG; SAS Institute 2002). The results of rate of signaling, and distance between resident and intruder at the onset of signaling (for each type of resident caterpillar signal) were subjected to Student's t test with the type of intruder (conspecific and predator) as the independent variable (Sokal and Rohlf 1995). The results from rate of signaling and distance were tested for assumptions of normality and homogeneity of variances (PROC UNIVARIATE, SAS Institute 2002) and no data transformation was required.

A canonical variate analysis (CVA) of the signal characteristics of caterpillar chewing, predator walking, and caterpillar crawling was performed to see how they might be differentiated (PROC CANDISC with Distance statement; SAS Institute 2002). Such results were subsequently subjected to complementary analysis of variance and Fisher's LSD test at $p < 0.05$ (PROC GLM, SAS Institute 2002).

Results

Vibrations during conspecific trials

When a conspecific larva crawls upon an occupied leaf, the resident typically begins signaling before the intruder reaches the shelter. If residents use vibration cues to detect intruders, those cues should be available to the resident and distinguishable from background noise generated by the resident's own activities or abiotic disturbances. The stages of a typical conspecific encounter along with associated leaf vibrations are shown in Figs. 1 and 2 (see also Online Resource 1). Prior to introducing the intruder, the resident rests, feeds, or works on its shelter. Resident's chewing generates low amplitude, evenly spaced vibrations with a bandwidth of 100 Hz at -30 dB, and frequencies extending to 8 kHz (Figs. 2, 7; Table 1). When the intruder is introduced to the twig with a paintbrush, a large amplitude disturbance is usually generated (see Fig. 2, Online Resource 1), but this disturbance does not cause the resident to discontinue normal activities. Intruder crawling produced a continuous low amplitude waveform with a bandwidth of 150 Hz at -30 dB, and frequencies extending to around 2 kHz (Figs. 2, 7; Table 1). Crawling vibrations were generally detectable by our laser only after the resident ceased chewing, yet, the resident 'noticed' the intruder (i.e., stopped chewing and remained still) above its' own chewing. Therefore, either the resident is capable of detecting crawling vibrations over its own chewing, or it is cueing in on visual or chemical stimuli generated by the intruder. This question is addressed in the 'cut leaf' experiment (see below). Further approach of the intruder triggered large amplitude, stereotyped and repeated vibration signals caused by the resident [i.e., anal scraping—scraping specialized anal setae (oars) on the leaf surface; mandible scraping—scraping mandibles laterally against

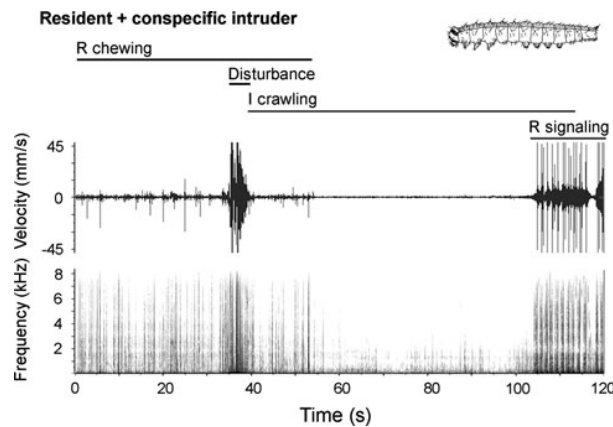


Fig. 2 Waveform and corresponding spectrogram during an encounter between a resident (*R*) and conspecific intruder (*I*) recorded with the laser vibrometer positioned 1 cm from the resident's shelter. The trace begins with the resident chewing, followed by a disturbance caused by adding the intruder to the twig. As the intruder crawls onto the leaf and toward the resident, the resident stops chewing, is motionless for a while, and then begins signaling

leaf surface; mandible drumming—striking the mandibles vertically on the leaf surface] (Figs. 2, 7; Table 1; Online Resource 1). These signals had broader bandwidths and higher frequencies than did other vibrations recorded in this study. General characteristics of residents' signals are presented in Table 1. Since the focus of this study was on the resident's detection of and responses to leaf vibrations, further analyses of these signals were not part of this study.

Removal of vibration cues (cut-leaf experiment)

A leaf incision between the resident and proximal part of the leaf prevents the transmission of vibration between the approaching intruder and resident, but these cues are available when the leaf is initially intact (whole leaf) and rejoined. When the leaf was initially intact, the resident initiated signaling before the intruder reached the point of eventual incision in 85% of the trials (Fig. 3). When the leaf was cut, signaling by the resident did not occur in 81% of the trials, until the intruder crossed the gap in the leaf. This trend was reversed when the leaf was rejoined (Fig. 3).

The distance at which the resident initially began signaling differed significantly with leaf manipulation (Fig. 4). The analysis of covariance indicates a significant effect not only for leaf manipulation ($F_{2,24} = 8.14$, $p = 0.002$), but also for the distance between the resident and the leaf incision once the intruder has crossed the incision point ($F_{2,24} = 2.35$, $p = 0.03$), as well as a marginally significant interaction between them ($F_{23,24} = 1.94$, $p = 0.05$). The regression model between conspecific distance at the onset of signaling and distance between

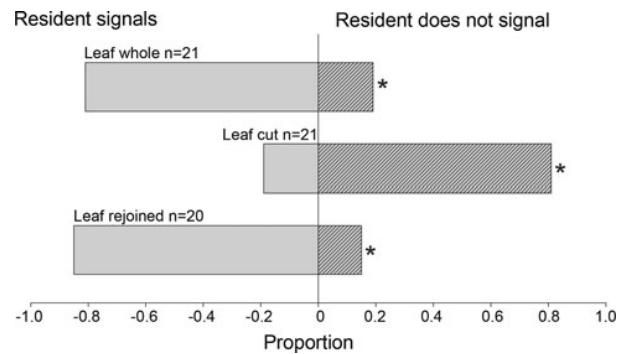


Fig. 3 Proportion of resident caterpillars signaling in response to conspecific intruders under different leaf manipulations (i.e., whole, cut, and rejoined). Asterisks indicate significant departure from the random expectation by the adjusted *G* test ($p < 0.05$)

resident and leaf incision was not significant when the leaf was intact and when rejoined after incision ($p > 0.05$), with the onset of signaling taking place predominantly before the intruder reached the incision (Fig. 4). In contrast, the onset of resident signaling takes place with the intruder crossing the point of incision with cut leaf (slope of the distance between conspecifics and distance between intruder and leaf incision is 0.96 ± 0.16 , not differing from one) (Fig. 4).

Vibrations during predator, wind and rain trials, and ball dropping

An approaching predator produced low amplitude vibrations with an irregular waveform, a bandwidth of 100 Hz at -30 dB, and frequencies extending to around 3 kHz (Figs. 5, 7; Table 1). Residents responded by producing three types of signals upon 'detecting' the intruder (see details below). Artificial wind produced high amplitude, low frequency vibrations with a bandwidth extending to 40 Hz at -30 dB, and most energy around 5 Hz (Figs. 6a, d, 7; Table 1). Artificial rain produced a rapid series of high amplitude events (corresponding to individual water droplets) with regular oscillations and a bandwidth of 110 at -30 dB, and frequencies extending up to 20 kHz (Figs. 6c, d, 7; Table 1). The impact of a ball dropping on the leaf produced high amplitude regular oscillations of the leaf, not unlike those seen for raindrops (Fig. 7).

Residents' responses to conspecifics, predators, wind and rain

Residents were exposed to conspecifics, predators, wind, rain, and combinations of a conspecific with wind or rain. Residents signaled in response to both types of intruders (Figs. 2, 5, 8), but when presented with a wind or rain

Fig. 4 a Photo sequence representing stages of the cut-leaf experiment (whole, cut, and rejoined). ‘*D*’ represents the distance between the resident and intruder when the resident first signals, and ‘*d*’ represents the distance between the resident and the leaf cut, or would-be leaf cut. *Thick black lines* indicate the position of clamps holding the leaf in place. **b** Distance relations between caterpillars at the onset of resident signaling (*D*) and resident-leaf incision distances (*d*) when the leaf was cut (*left panel*) and when left intact and rejoined after the cut (*right panel*)

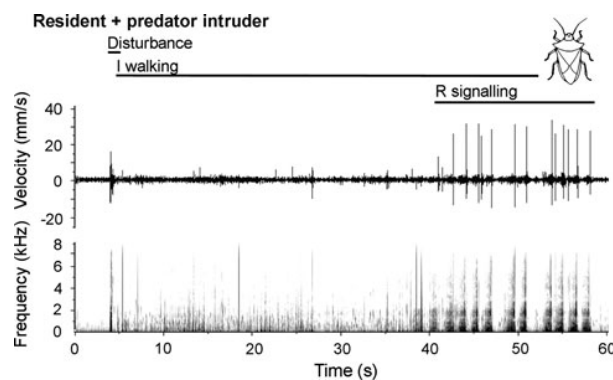
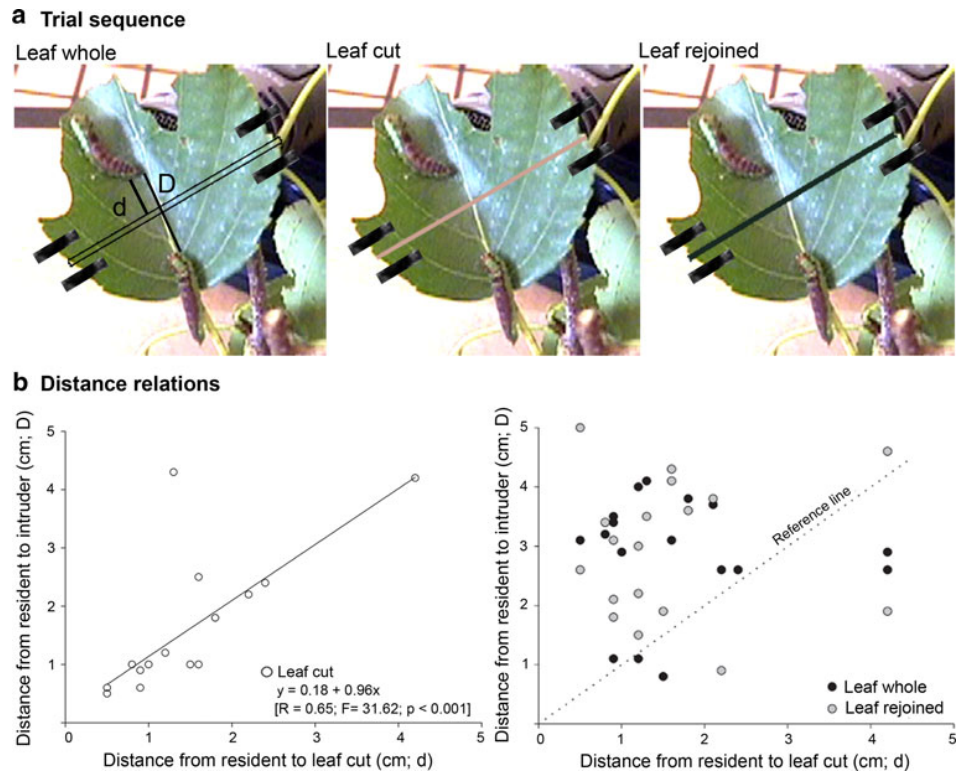


Fig. 5 Waveform and corresponding spectrogram recorded with the laser vibrometer during an encounter between a resident (*R*) and predator intruder (*I*) with the laser positioned ~2 cm from the resident. The trace begins with the resident resting, followed by a disturbance caused by adding the intruder to the twig. As the intruder walks onto the leaf and toward the resident, the resident begins signaling

stimulus alone residents did not signal (Figs. 6a, 8). Interestingly, staging a conspecific interaction in the presence of a wind disturbance did evoke a signaling response in the resident (Figs. 6b, c, 8). Rain prevented conspecific interactions from occurring since the intruder did not move on the twig when simulated rain was applied and therefore these data are not presented in Figs. 6 and 8.

The response of the resident was significantly different depending on the type of intruder (Fig. 9). A conspecific intruder takes longer to reach the resident's shelter than does the predator, which usually reaches the shelter within few seconds (<20 s). Residents began anal scraping and mandible drumming at significantly greater resident-intruder distances when approached by a conspecific as opposed to a predator ($t_{27} > 3.3$ and $p < 0.0001$ for both signals) (Fig. 9). There was no significant difference between distances for the onset of mandible scraping ($t_{14} > 1.7$ and $p = 0.10$) (Fig. 9), which is the last and most vigorous signaling event in staged interactions between caterpillars of *D. arcuata*. The rate of signaling by the resident during the first 30 s of signaling also differed significantly, with higher rates of all signals occurring in response to the predator ($t_{31} > 2.0$ and $p < 0.05$) (Fig. 9).

How do vibration characteristics of crawling, walking and chewing vary?

Our results that residents detect intruder vibrations over their own chewing and respond differently to the two types of intruders led us to perform a CVA analysis of the signal characteristics of caterpillar chewing and crawling in addition to predator walking to see how they might be differentiated. The CVA analysis indicated significant

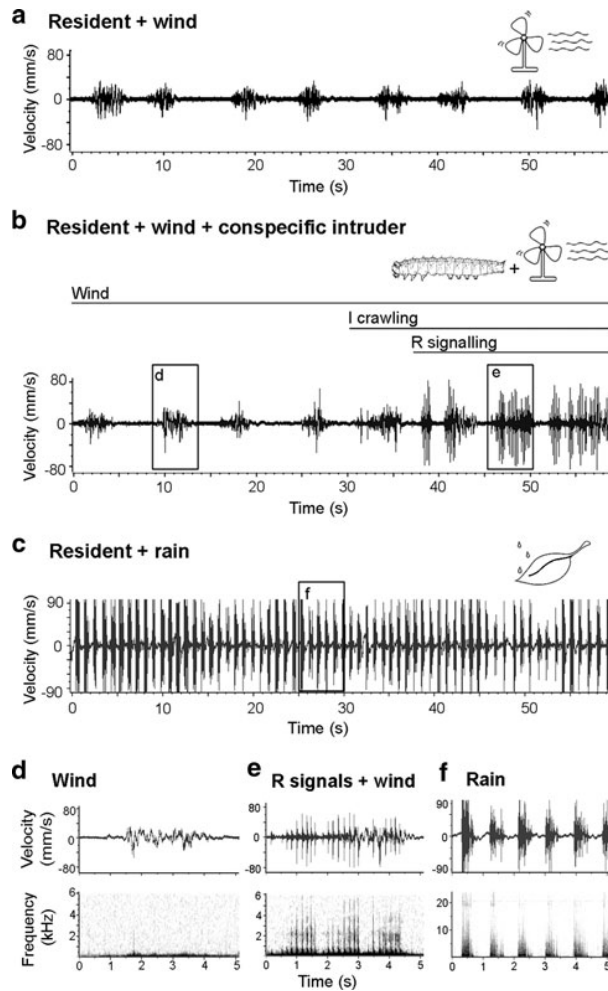


Fig. 6 Vibrations recorded using an accelerometer attached to a leaf while a resident caterpillar is exposed to simulated wind alone (a), wind and a conspecific intruder (b), or rain alone (c). Enclosed boxes are enlarged in d–f below, showing waveforms and corresponding spectrograms of wind, resident signaling + wind, and rain, respectively

differences between these activities (Wilks' lambda = 0.077, $F_{\text{apr.}} = 3.13$, $df_{\text{num;den}} = 10, 12$; $p = 0.03$), and only the 1st ordination axis generated was significant ($p = 0.03$) explaining 84% of the observed variance. Dominant frequency was the main contributor of this canonical axis and therefore the most important characteristic allowing discrimination among vibrations produced by these activities. This was confirmed with subsequent analysis of variance for this characteristic (Fig. 10). There is significant difference between caterpillar crawling and predator walking, which were easily distinguished. However, caterpillar chewing exhibits intermediate results and was not distinguishable from either (Fig. 10). This finding suggests that chewing interruption improves detection (and discrimination) of intruder caterpillar crawling and

predator walking based mainly on the dominant frequency of these cues.

Intruders' responses to resident signaling

Although monitoring intruders' responses during trials was not an original goal of our study, we include preliminary results, which have implications for understanding the function of the vibratory signals of residents. Intruders left the leaf in the presence of resident signaling in about 80% of the trials with the predator alone, conspecific alone, or conspecific + wind (Online Resource 2). In all cases, residents were signaling while the intruder left the leaf. Predators usually left the leaf with a low attack success rate (9% of the trials resulted in attack and kill), and frequently left without even attempting the attack. When the predator attempted an attack, the resident shelter structure and particularly the mandible scraping signal seemed to greatly impair the attack, which tended to be more successful with a poor shelter structure.

Discussion

Despite burgeoning evidence that holometabolous larvae use solid-borne vibrations for communication purposes, little is known about their ability to detect or discriminate between different signals, cues, and background noise. Our results show that *D. arcuata* larvae use vibratory cues to detect approaching conspecific intruders, and that they respond differently in the presence of vibrations produced by conspecifics, predators, wind and rain.

Vibration detection

Previously, it was demonstrated that resident *D. arcuata* larvae respond to an approaching intruder by discontinuing their normal activity (shelter building, eating, etc.) and then signaling. It was inferred that incidental vibration cues generated by an approaching intruder caused the resident to signal, but this was not tested (Yack et al. 2001). Here, we show that approaching conspecifics produce vibrations that are both sufficient and necessary for triggering a resident's response in the majority of trials. In two trials, residents responded to the intruder prior to the latter crossing the leaf cut. This could indicate that the resident was already 'excited' from the previous whole leaf trial, or that residents could use other cues in addition to vibration, to detect intruders. Also, residents produced distinctive vibratory signals, and only in the presence of these signals do intruders leave the leaf. We conclude that *D. arcuata* is able to detect and recognize vibrations produced by different sources. Vibration receptors have not been

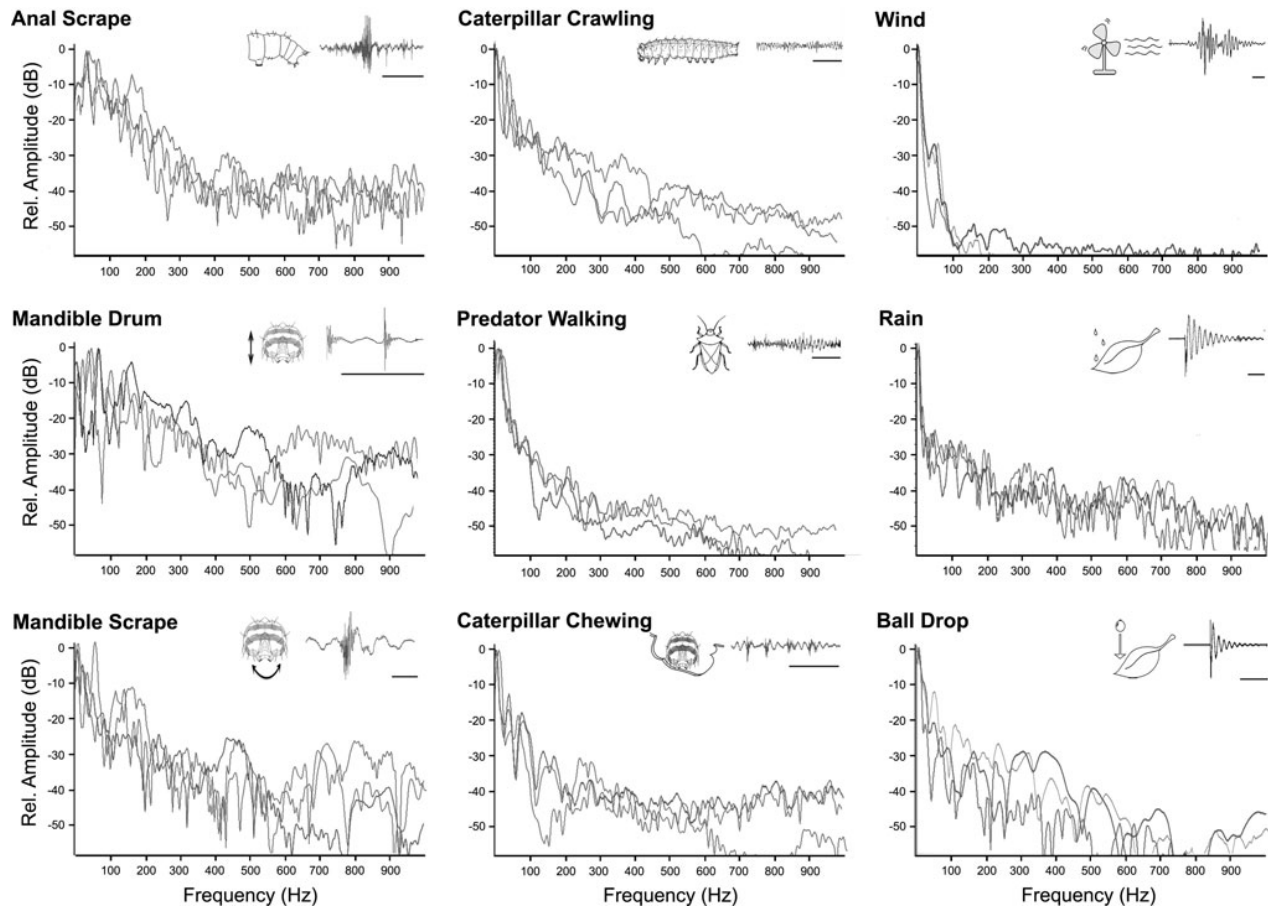


Fig. 7 Frequency spectra and oscillograms for vibrations produced by various biotic and abiotic sources on birch leaves, recorded using a laser vibrometer. Three representative spectra recorded during

separate trials using different animals and leaves are displayed. All spectra are normalized to their peak power. All scale bars for waveforms are 1 s, except for the mandible drum, which is 500 ms

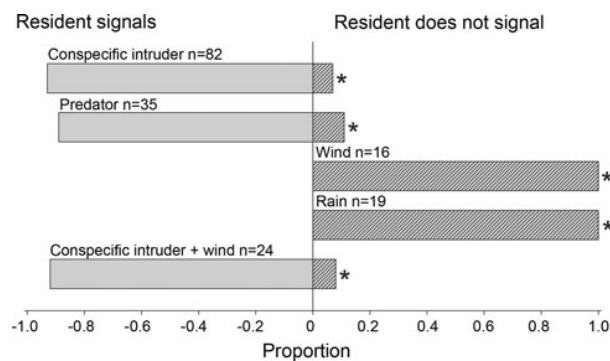


Fig. 8 Proportion of resident caterpillars signaling in response to different environmental factors (conspecific intruder, predator, wind, rain, or a combination of conspecific intruder and wind). Asterisks indicate significant departure from the random expectation by the adjusted G test ($p < 0.05$)

confirmed in the larvae of any holometabolous insects, although various structures including antennal filiform sensillae (Meurgey and Faucheux 2006), abdominal and

thoracic chordotonal organs associated with setae (Hasenfuss 1993) and chordotonal organs associated with ‘pleural discs’ (Hess 1917; Saliba 1972) have been implicated as vibration detectors based on their morphology. Vibration receptors have not yet been identified in *D. arcuata*, but preliminary investigations suggest that proleg setae and chordotonal organs are involved (Yack, unpublished).

Vibration discrimination

An ability to discriminate between leaf vibrations from different sources would allow a caterpillar to assess risk and make informed behavioral decisions. We show that residents respond differently in the presence of different vibration sources, and while we cannot rule out the possibility that cues other than vibrations (e.g., visual, chemical) are also being used, we argue that vibrations are potentially key contributors to decision making.

Vibrations caused by abiotic sources such as wind and rain are common sources of background noise for

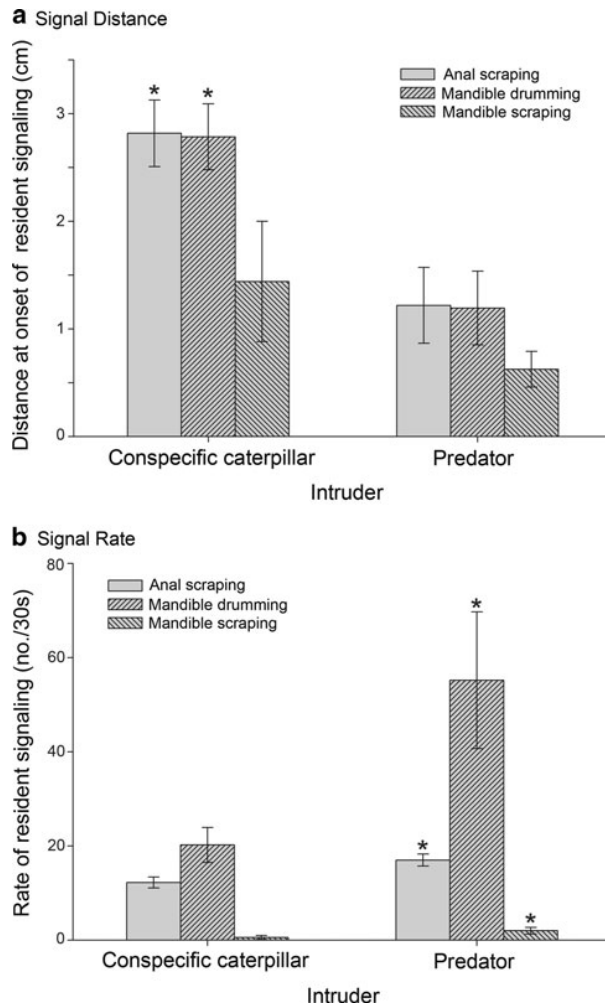


Fig. 9 **a** Distance between the resident and intruder at the onset of each type of resident acoustic signal. **b** Rate of each signal type produced by the resident during the first 30 s of signaling. The vertical bars indicate standard error of the means and the asterisks indicate significant differences between intruders for each type of signal by Student's *t* test ($p < 0.05$)

plant-borne insects (Cocroft and Rodríguez 2005; McNett et al. 2010). In addition, vibrations produced by a caterpillar's own activities, such as chewing and crawling, are also potential sources of interference that could impair an individual's ability to detect an intruder. We show that resident caterpillars did not signal in response to vibrations caused by simulated wind and rain alone. Similar examples of invertebrates and vertebrates ignoring non-relevant vibratory 'noise' have been reported in caterpillars (Castellanos and Barbosa 2006), frog embryos (Warkentin 2005), and spiders (Barth et al. 1988). In our study, we went a step further and combined abiotic with biotic vibrations. Interestingly, the large amplitude vibrations caused by wind did not interfere with the intruder caterpillar's approach or the resident's ability to detect the low

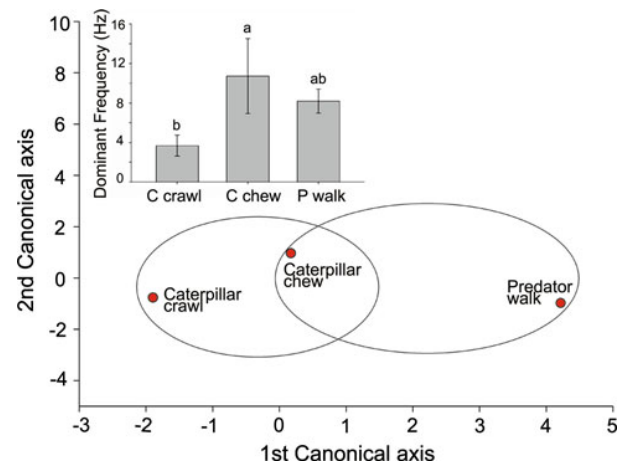


Fig. 10 Diagram of canonical variate analysis (CVA) showing the discrimination between vibrations generated by caterpillar crawling and chewing, and predator walking. The symbols are centroid of treatments and represent the class mean canonical variates. Ovals indicate treatments that were not significantly different (approximated F-test at $p < 0.05$), based on the Mahalanobis distance (D^2) between class means. The main signal characteristic was dominant frequency, which was subjected to (univariate) analysis of variance and subsequent Fisher's LSD test ($p < 0.05$) to discriminate between signals, as represented in the inserted histogram plot. Histogram bars ($\pm$ SEM) followed by the same letter are not significantly different

amplitude vibrations of the approaching intruder. Given that leaf vibrations caused by wind are characteristically of low frequency, with most energy below 20 Hz (c.f. Barth et al. 1988; Casas et al. 1998; Caldwell et al. 2009; this study), it is possible that vibration receptors in the caterpillars are tuned to higher frequencies, as seen in spiders (Barth 1997). Caterpillars also seemed to detect the low amplitude crawling vibrations of an approaching intruder despite the potential interference of their own chewing. Our results suggest such recognition is frequency mediated. Our finding that the dominant frequencies of chewing and crawling (and predator walking) partially overlap are consistent with the result that when an intruder approached a resident, the resident typically stopped chewing, and then remained silent for a period before beginning to signal. This period perhaps allows the caterpillar to 'listen' to the approaching intruder as it draws closer to better assess the threat. Similar to trials with wind, rain did not cause residents to signal. However, we could not assess if residents could detect intruders over rain, since rain prohibited the intruder caterpillar from moving, an expected result given the risk of being dislodged by rain.

Residents seem to discriminate between the vibrations made by the two different types of intruders. There were significant differences in the distance at which the resident began signaling, and the rate of signaling. In response to conspecifics, signaling onset occurs at a greater distance,

usually when the intruder crawls onto the leaf petiole, while signaling in response to predators was delayed until the intruder reached the shelter. There are two possible explanations for this delayed response to predators. One is that the resident may not detect the predator until it is close. An alternative explanation is that the caterpillar may have detected the predator by cueing in on the vibration frequency generated with its walking, but did not respond until the predator was very close. Such a delay is likely to impair prey location by *P. maculiventris*, which allegedly uses prey-produced vibrations to locate its prey (Pfannenstiel et al. 1995).

It is worth noting that all leaf vibrations recorded in this study had overlapping frequency distributions, with most energy falling below 100 Hz. This is explained by the fact that all sources excite the natural resonance properties of the birch leaf to varying degrees, as demonstrated by the ball dropping experiments. Despite the similarities, vibrations generated by different sources do show differences in their spectral properties, and this is particularly evident in the drumming and scraping signals, which typically contain much higher frequencies. In addition, caterpillars may use temporal and amplitude properties, or a combination of all these traits to discriminate between sources. More detailed analyses of the physical properties of these vibrations, in combination with playback studies, will provide insights into the vibration discrimination capabilities of these caterpillars.

Why do caterpillars signal?

Residents responded by signaling more vigorously to a predator than to a conspecific over the first 30 s from the beginning of signaling. Signaling in response to the predator occurs at a much higher rate with a quick escalation of the signaling sequence from mandible drumming to scraping. In order to understand the significance of the increased rate in response to a predator, one needs to understand the functional significance of signaling. A previous study (Yack et al. 2001) supported the hypothesis that signaling functioned in territorial defense. Signals are produced primarily by residents, and conspecific intruders responded by leaving the leaf in the majority of trials, although sometimes intruders ‘won’ the contest to take over the shelter. Also, since no biting or other physically aggressive behavior is associated with contests, vibrations are considered to be ritualized displays to warn another caterpillar to not waste time pursuing an occupied territory (Yack et al. 2001; Scott et al. 2010). Our result in this study, that residents also signaled in response to a predator, was surprising, since in the previous study, a simulated attack (probing with a paintbrush) evoked minimal signaling. In the previous study, either the stimulus used did not represent a natural predator or perhaps represented a

different type of predator (e.g., wasp), which the resident may respond to differently (see Castellanos and Barbosa 2003). Regardless, the hemipteran predator in the current study appeared to respond to the signaling behavior of the resident by discontinuing its hunt. Possible explanations for why signaling is deterrent to predators could be that the signals mimic those of predator spiders, that they advertise unprofitability, or that the signal may be inviting unwanted secondary predators to the intruder, while the resident is more secure in its shelter. Interestingly, the rapid mandible drumming signals of the resident resemble to some extent raindrops in their temporal, amplitude and spectral features, and it is possible that rapid mandible drumming mimics rain.

Our study demonstrates that masked birch caterpillars use vibratory cues to detect an approaching conspecific intruder, and supports the hypothesis that they can discriminate between relevant and non-relevant vibrations in their environment. Exciting prospects for future studies in this model system include identifying the vibration receptor organs, and testing hypotheses on the adaptive significance of caterpillars’ generating complex vibratory signals in the presence of con- and heterospecific intruders.

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