

Vibration-mediated territoriality in the warty birch caterpillar *Drepana bilineata*

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Abstract. The warty birch caterpillar *Drepana bilineata* produces two distinct types of vibrational signals (mandible drumming and anal scraping) during interactions with conspecifics. Vibrational signalling is characterized using standard and high-speed videography synchronized with laser-doppler vibrometry, and behavioural experiments test the hypothesis that signalling functions to advertise occupancy of birch (*Betula*) leaves. Drumming involves raising the head and striking the leaf with the sharp edges of the open mandibles. Anal scraping involves dragging a pair of specialized oar-shaped setae against the leaf surface. Staged encounters between leaf residents and conspecific intruders result in the resident signalling, with rates increasing as the intruder moves closer. Intruders signal significantly less often than residents. Conflicts are typically resolved within a few minutes, with the resident winning in 61% of the trials, and the intruder winning in 6%. Contests that last more than 30 min are deemed ‘ties’ and comprise the remaining 33% of trials. The results support the hypothesis that vibrational signals function to advertise leaf occupancy. Vibrational communication is believed to be widespread in Drepanoidea caterpillars, but has only been described in two species to date: *D. bilineata* (present study) and *Drepana arcuata*. It is proposed that differences in territorial behaviour and signalling between these species are related to their relative investments in silk leaf mats and shelters. The proximate and ultimate bases for the evolution of vibrational communication in caterpillars are discussed.

Key words. acoustic, caterpillar, communication, competition, evolution, spacing, territory.

Introduction

Larval Lepidoptera are highly successful constituents of many terrestrial ecosystems and include some of the most effective pests of economically important plants (Stamp & Casey, 1993). Key to their success is an ability to communicate with other individuals in their environment. Caterpillars rely on communication during their development to facilitate behaviours associated with foraging, defence, aggregation, shelter building, or competition for resources (Costa & Pierce, 1997; Fitzgerald & Costa, 1999; Cocroft, 2001; Costa, 2006).

Despite the importance of communication, surprisingly little is known about the mechanisms used to broadcast and receive signals (Costa & Pierce, 1997). There is evidence that several species, particularly those travelling in processions, use chemical and tactile cues for communication (Fitzgerald, 1995; Ruf *et al.*, 2001; Fitzgerald & Pescador-Rubio, 2002; Fitzgerald, 2003). Vision is unlikely to play an important role in communication because caterpillars have fairly simple eyes capable of discerning only crude images (Warrant *et al.*, 2003). One sensory modality that remains relatively unexplored in caterpillars is an acoustic sense and, in particular, vibrational communication.

Acoustic signals communicated through plants (= substrate-borne vibrations) are widespread in small herbivorous insects (Cocroft, 2001; Virant-Doberlet & Cokl, 2004; Cocroft & Rodriguez, 2005). Most of the work performed to

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date has focused on adult insects and nymphs of hemimetabolous insects. However, there is mounting experimental, and abundant inferential evidence that larvae of holometabolous insects including moths and butterflies, flies, beetles and wasps, rely on vibrational signals to communicate with both con- and heterospecifics (Cocroft, 2001; Virant-Doberlet & Cokl, 2004; Cocroft & Rodriguez, 2005; Drosopoulos & Claridge, 2006). In Lepidoptera, experimental evidence for vibrational communication is currently limited to a few examples. Lycaenidae and Riodinidae butterfly larvae employ vibrations to maintain mutualistic relationships with ants (DeVries, 1990, 1991; Travassos & Pierce, 2000) and larvae of three moth species (*Sparganothis pilleriana*, *Drepana arcuata* and *Caloptilia serotinella*) belonging to different superfamilies use vibrations in territorial encounters with conspecifics (Russ, 1969; Yack *et al.*, 2001; Fletcher *et al.*, 2006). Beyond these examples, there are several anecdotal references to vibrational communication in caterpillars (Federley, 1905; Dumortier, 1963; Hunter, 1987) and the phenomenon is thought to be widespread.

The superfamily Drepanoidea, a large assemblage of moths containing more than 600 described species (Minet & Scoble, 1999), provides an excellent model system for studying the function and evolution of vibrational communication in Lepidopteran larvae. In *D. arcuata* Walker, larvae use vibrational signalling to resolve territorial disputes with conspecifics over silk leaf shelters (Yack *et al.*, 2001). Solitary late-instar caterpillars occupying shelters produce three distinct signals (mandible drumming, mandible scraping and anal scraping), which escalate as the intruder approaches the resident. This is the first experimental study to demonstrate that caterpillars employ vibrational signals to advertise ownership of a territory. Although vibrational signals have only been studied in this species to date, there is abundant suggestive evidence from descriptive morphological reports (Nakajima, 1970, 1972; I. Hasenfuss, personal communication), and behavioural observations (Dyar, 1894; Federley, 1905; Bryner, 1999; Sen & Lin, 2002; I. Hasenfuss, personal communication; J. Bowen, unpublished data) that vibrational signalling and territorial behaviour are widespread but variable in the Drepanoidea. Some species produce less vibrational signalling and instead use physical contact to defend their nests (I. Hasenfuss, personal communication; J. Bowen, unpublished data). Other species, such as *D. arcuata*, do not attack physically, but resolve conflicts using mostly ritualized signalling (Yack *et al.*, 2001). This system provides an excellent opportunity for understanding the evolution of signalling at both ultimate and proximate levels of analysis. The first step is to characterize vibrational signalling and behaviours associated with vibrational signalling in different Drepanoidea species.

The present study focuses on the warty birch caterpillar, *Drepana bilineata* Packard (Drepanoidea: Drepanidae) (Fig. 1), a sympatric congener of *D. arcuata*. Reports on the life-history traits of *D. bilineata* have included brief accounts of adult and larval morphology (Dyar, 1894; Beutenmuller, 1898; Davicault, 1935). However, to the authors' knowledge, there are no reports on territorial behaviour or vibrational signal

production. The life histories of *D. bilineata* and *D. arcuata* share many similarities, including laying eggs in rows, feeding on the same host plants (primarily birch (*Betula*) and alder (*Alnus*)), laying silk mats, and having five larval instars, with the first two skeletonizing the leaf and the final three eating whole leaves (Packard, 1890; Dyar, 1894; present study). However, there are two notable differences that have implications for territorial behaviour. First, *D. arcuata* constructs silken leaf shelters throughout their development, a behaviour that is absent in *D. bilineata*, and presumed to be costly in terms of time and energy expenditure (Berenbaum *et al.*, 1993; Fitzgerald & Clark, 1994; Yack *et al.*, 2001). Second, *D. bilineata* is solitary throughout all five instars whereas *D. arcuata* lives gregariously until the third instar. *Drepana arcuata*, like many other animals that invest in a resource, is thought to defend a leaf due to the investment of building a shelter (Yack *et al.*, 2001). Because *D. bilineata* does not have a nest to defend, and it disperses earlier in development, it is predicted that it will be less territorial than *D. arcuata*, and will not use vibrational signals to the degree observed in *D. arcuata*. The present study aims to: briefly document the life-history and behavioural traits of *D. bilineata* that may be relevant to spacing/territoriality; test for and characterize vibrational signals; experimentally test the function of vibrational signalling; and compare the vibrational signals with those of *D. arcuata* to gain insight into factors that may have influenced the evolution of vibrational communication in the Drepanoidea.

Materials and methods

Animals

Drepana bilineata moths were collected from the wild at 15-W ultraviolet collecting lights (Bioquip, Rancho Dominguez, California) between May and August 2003 and 2004 throughout the National Capital Region of Eastern Ontario, Canada, and at the Queen's Biological Station, near Kingston, Ontario, Canada. Females oviposited on cuttings of paper birch (*Betula papyrifera*) and the larvae were reared indoors under an LD 16 : 8 h photoperiod at 21–26 °C. Early- (first and second) and late- (third to fifth) instar larvae were used for life-history and behavioural observations, morphological studies of sound producing mechanisms, and laser vibrometry recordings.

General behaviour and life history

The intention of this part of the study was to document behaviours and life-history traits relevant to communication and spacing. Larvae were reared and observed under three different conditions. To confirm the number of developmental instars and document the general anatomy and behaviours associated with each instar, 36 hatchlings were reared separately in glass jars on cuttings of *B. papyrifera*. Each day, the

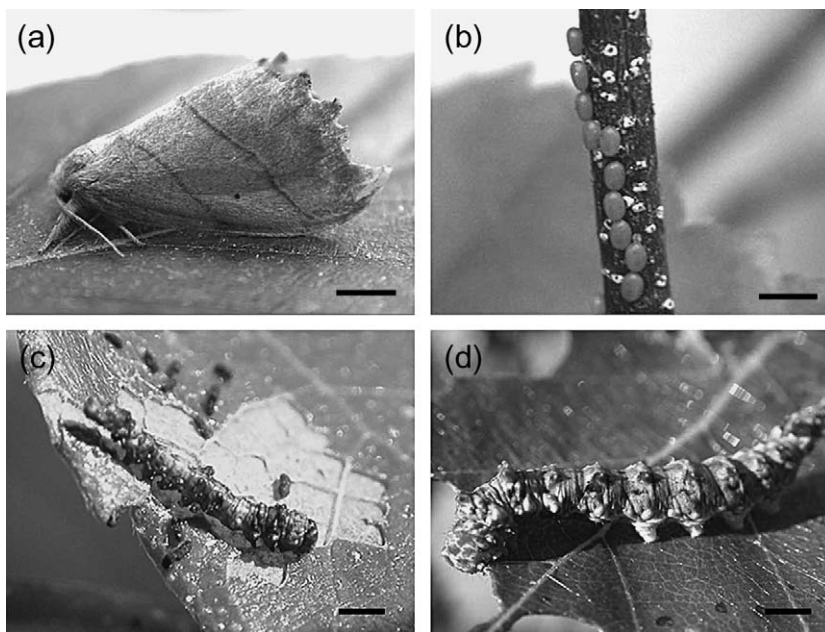


Fig. 1. *Drepana bilineata* at various life stages. (a) The adult moth in its resting position, showing the hook-tip wings, characteristic of the Drepaninae subfamily (scale bar = 3 mm). (b) Eggs laid in a row of ten along a twig of paper birch (*Betula papyrifera*, scale bar = 2 mm). (c) An early (second)-instar larva that has skeletonized a paper birch leaf (scale bar = 1 mm). (d) A late (fifth)-instar larva that has laid a silk mat on the leaf surface (scale bar = 2 mm). Note the absence of anal prolegs in both the larval instars.

developmental stage, the position on the leaf, the presence of silk on the leaf, the mode of feeding, and any other notable behaviours were recorded. Four or more individuals of each instar were preserved in 80% ethanol for later morphological study. Photographs of eggs and larvae were obtained with an Olympus dissection microscope (SZX12; Olympus, Japan) equipped with a PixelINK Megapixel firewire camera (PL-A642), or with a Nikon Coolpix camera (4500; Nikon, Japan).

Behavioural observations under semi-natural conditions were made by rearing three broods of caterpillars on potted tree saplings of *B. papyrifera* placed inside screened enclosures in the greenhouses at Carleton University. Wild-caught females were released in the enclosure, and the trees were then monitored daily to record the number of eggs laid per row and their general positions on leaves and twigs. Larvae were monitored daily for their general locations on the leaves, feeding patterns, silk laying habits and notable interactions between individuals.

To obtain an understanding of daily activity patterns, four late-instar larvae were placed on fresh leaf cuttings and videotaped using a time-lapse camera during the daylight hours (11.30–18.00 h) for periods in the range 2–5 h per individual. Tapes were analyzed for the time each individual engaged in any particular behaviour (e.g. laying silk, feeding, resting). A total of 15 h of footage was recorded from four individuals, and the mean amount of time caterpillars spent performing each behaviour was calculated.

Signal characteristics

Signalling was monitored and characterized using two recording methods. The first involved recording the signals of late-instar resident caterpillars using a videocamera and

microphone during staged encounters with intruders (see below). These recordings were used to initially describe structures and movements associated with signalling, and to measure temporal characteristics of signalling. Only late-instars were recorded using this method because early-instar signals are not detectable with a microphone. Videos were imported to an Apple eMac (G4; Apple Corp., Cupertino, California) as Apple iMovie 3.03 files, and video clips were saved as Apple Quicktime Pro 7.2.0 files, where sounds were subsequently extracted as 'aiff' files. Temporal characteristics, including signal durations, complex and bout durations, and number of signals per complex/bout, were measured using Canary or Raven Bioacoustics Research Programs (Cornell Laboratory of Ornithology, Ithaca, New York). Signal durations were obtained from 16 fourth- and fifth-instar larvae that were selected at random throughout the summer from the broods of five wild-caught females. Durations of 70 mandible drums (five drums per individual) were measured from 14 individuals, and 65 anal scrapes (five scrapes per individual) were measured from 13 individuals. Means of signal durations were calculated per individual and then used to calculate a grand mean. Using high-speed video analysis (see below), it was found that, although the two signals sometimes occurred separately, they are most often partially overlapping. Because it is often difficult to distinguish where an anal scraping signal ends, anal scrapes were measured from the beginning of the signal to the end of the mandible drum. Temporal analysis of signal bouts and complexes were analyzed from ten residents signalling in the presence of a conspecific intruder. Bouts were defined as any combination of signals that are flanked by feeding, walking, or at least 1 s of inactivity. Complexes were defined as a combination of an anal scrape immediately followed by one or more mandible drums, or a single signal. Mean complex and bout

durations, mean number of complexes per bout, and mean number of signals per complex were calculated from 18 bouts (one to three bouts per individual), comprising 104 complexes (number of complexes per bout varied).

The second method for characterizing signals employed laser-doppler vibrometry (LDV) in conjunction with high-speed video, and was used for describing the mechanisms and spectral components of signalling. All five instars were recorded using this method. Vibrations were recorded using a LDV (Polytec OFV 511 sensor head and OFV 3001 controller; Polytec GmbH, Germany) at the University of Toronto at Scarborough. The laser was reflected by a piece of reflective tape (approximately 1 mm²) positioned 1.5–2.5 cm from the resident caterpillar. Vibrations perpendicular to the leaf surface were measured at the location of the reflective tape. Signals were digitized (PCI-6023E; National Instruments, Austin, Texas; 20 or 50 kHz sampling rate) simultaneously with the capture of digital high-speed video (500 frames s⁻¹; PCI 1000; RedLake Motionscope, San Diego, California), using Midas software (Xcitex, Cambridge, Massachusetts). All recordings were made on a vibration-isolated table. Spectral characteristics of 17 mandible drums and 17 anal scrapes were measured from six individuals (two to three signals per individual) in Raven Bioacoustics Research Program using a 1024-point Fourier transform (DFT, Rectangular window).

Morphology

Structures associated with signal production were examined in larvae of all developmental stages preserved in 80% ethanol. Drawings of the last abdominal segments (A8–A10) were made using a drawing tube, and setae were identified according to Stehr (1987) and I. Hasenfuss (personal communication). For scanning electron micrographs, mandibles and anal segments were dissected, mounted on aluminum stubs and air-dried. Specimens were sputter-coated with gold-palladium and examined using a JOEL scanning electron microscope (JSM-6400; Joel, Japan).

Signal function

To test the hypothesis that signalling functions to advertise occupancy of leaves, 56 encounters were staged between a resident larva and an introduced conspecific intruder of approximately the same size. Larvae chosen for experiments were fourth or fifth instars selected at random from broods of five wild-caught females. In preparation for a trial, a larva (designated as 'resident') was placed on a birch leaf that was attached to its original twig. The twig, stripped of all leaves except one, was cut to a length of 8–12 cm, and its cut end placed in a water filled vial through a hole in the lid. Leaves were selected based on their size (mean $\pm$ SD: $8.2 \pm 1.5 \times 5.6 \pm 1.0$ cm), and the absence of feeding scars, or other types of leaf damage. A fresh twig was used for each experiment. Prior to each experiment, the

vial containing the twig and occupied leaf was held in position with a clamp such that the larval interaction could be viewed with a video camera. The larva was left undisturbed for 30–60 min prior to the trial. All trials were videotaped with a Sony Digital Handicam (TR7000; Sony Corp., Japan) and a remote Sony audio microphone (ECM-MS907) placed 1–2 cm behind the leaf. Before the intruder was introduced, the resident larva was videotaped for 5 min. 'Intruders' were isolated in a container with birch twigs (containing no leaves) for 15–20 min before each trial. Using a paintbrush, intruders were gently transferred to the twig, a few centimetres below the point where the petiole attaches to the twig. The beginning of the trial was considered the point at which the intruder's head crossed the junction of the petiole and the base of the leaf. The interaction was videotaped until 5 min after one contestant left the leaf (i.e. when one contestant 'won' the encounter). If there was no winner within 30 min, the trial was deemed a 'tie'. This time was chosen based on previous trials with another species, *D. arcuata* (Yack *et al.*, 2001). After each trial, the weight of each caterpillar was recorded and individuals were isolated in a separate container so they would not be reused in another trial. In four of the 56 trials, the weights of individuals were deemed significantly different between contestants and these trials were omitted from the analysis.

Videotapes from 52 trials were analyzed to measure the durations and outcomes of contests, and to monitor changes in signalling rates in both residents and intruders throughout each trial. In 35 trials where a winner was decided, the mean durations of contests were calculated. Durations of trials that the resident won were compared with those that the resident lost using a Wilcoxon rank sum test. Mean signalling latencies (i.e. the time from the beginning of the trial to the first resident signal) were assessed for each trial. The distance between individuals (based on the distance between the resident and the closest point of the intruder) was also recorded at the time of the first signal. To compare signalling rates of residents and intruders during encounters, both signal types (mandible drumming and anal scraping) were counted at 5-s intervals during the first and last 80 s of each trial for 20 trials (chosen at random from the 52 trials), and the minimum distance between contestants was measured at each interval. Overall signalling rates were calculated by taking the mean of all signalling types for all distance categories. The total number of signals between residents and intruders was compared using a paired *t*-test.

Another subset of trials was analyzed to assess how signals escalated with respect to decreasing distances between individuals. In 21 of the 52 trials where the intruder came within at least 0.5 cm of the resident, the rates of signalling and head contact were measured at three stages (far, mid and close) of the intruder's approach. The far stage comprised the 20-s interval after the beginning of the trial. Mid-distance comprised the 20-s period midway between the far and close distances. Signals at the close stage were recorded for 20 s from the point when the intruder first made contact with the resident or, in trials where contact was not made,

when the intruder came within 0.5 cm from the resident. Signal escalation was analyzed by calculating the mean number of signals at each distance category (far, mid and close) for each individual. The data were square-root transformed and the means were compared using analysis of variance (ANOVA). *Post hoc* analyses were conducted using a Tukey–Kramer HSD. A grand mean of signalling rates per signal type (mandible drumming, anal scraping) and head contact at each distance category was calculated to create a histogram. Overall signalling rates were calculated by taking the mean of all signalling types for all distance categories for comparison with *D. arcuata* (see below).

Comparison with *Drepana arcuata*

Signalling rates of *D. arcuata* were obtained from 16 staged encounters from a previous study using similar methodology as described above (Yack *et al.*, 2001). In particular, trials selected for comparison included residents and intruders of similar size, and residents and intruders were treated the same way prior to the experiment. Signalling patterns were compared between species. Signal escalation and overall signalling rates were calculated and compared as for *D. bilineata* (see above). Overall signalling rates were compared between species using a two-tailed Student's *t*-test.

Control experiments

Two control experiments were carried out. The first tested whether the intruder would vacate a leaf regardless of whether the leaf was occupied by a resident larva. Twenty residents were removed from a leaf after being allowed to settle for 1 h to ensure that a silk mat had been established. Intruders were then placed on the petiole of the leaf and their behaviour was videotaped. A second experiment tested whether signalling could be provoked by another type of disturbance, such as an attack by a parasitoid. Parasitoid attacks were simulated on 19 resident larvae by poking their anterior ends with a paintbrush consisting only of two hairs (to simulate the ovipositor of a parasitoid; Stamp, 1986; Cornell *et al.*, 1987). Larvae were videotaped for 2 min after the 'attack'.

Results

Behaviour and life-history traits relevant to signalling

Females lay eggs in rows of 2–10 on the upper leaf surface or on small twigs adjacent to a leaf (Fig. 1b). The caterpillar has five instars, and all live solitarily on the leaf. First and second instars occupy individual feeding regions at leaf edges where they skeletonize the upper leaf surface (Fig. 1c). When approached by a conspecific wandering along the leaf edge, an occupant will move its head and body in response. These movements were later confirmed to be vibrational signals.

When not feeding, a larva rests in an arched position such that its body is in line with the curvature of the leaf edge. Upon reaching the third instar, a larva moves to a new leaf and lays down a mat of silk on the upper leaf surface (Fig. 1d). It will consume almost the entire leaf from the outer edge, and then chew off the naked petiole at its base, possibly to remove visual evidence of feeding from avian predators (Heinrich, 1979). All late instars (three to five) feed in this manner, and a single individual will consume 10–12 leaves before pupating. Time-lapse videos show that, on average, late-instar larvae spend $8.7 \pm 0.9 \text{ min h}^{-1}$ ($n = 4$) laying down silk, $34.0 \pm 9.6 \text{ min h}^{-1}$ feeding, and $13.7 \pm 7.4 \text{ min h}^{-1}$ resting. When approached by a conspecific, leaf occupants will initiate head and abdominal movements that are associated with vibrational signals.

Signal characteristics

Drepana bilineata larvae produce two types of vibrational signals: mandible drumming and anal scraping. Signalling by both early- and late-instar larvae is initiated when a resident of a feeding spot (early-instar) or leaf (late-instar) is approached by a conspecific. In describing the temporal and spectral characteristics of signals, the present study focused on fourth- and fifth-instar larvae.

Mandible drumming (Fig. 2) is produced by striking the leaf with the serrated edges of open mandibles (Fig. 3) to create a short, percussive signal. The entire head and thoracic region of the body are lifted by approximately twice the length of the head and then rapidly struck down against the leaf surface. The mean $\pm$ SD duration of a single drum is $37.9 \pm 13.1 \text{ ms}$ (range = 17–75 ms, $n = 70$ signals from 14 individuals). Spectral analyses reveal that the drums are broadband with a dominant frequency of $97 \pm 73 \text{ Hz}$ ($n = 17$ signals from six individuals).

Anal scraping (Fig. 2) is produced by dragging the modified setae (Fig. 3) across the leaf surface. The anal scraping movement begins with an extension of the posterior end of the abdomen, followed by lowering the terminal abdominal segment so that the setae make contact with the leaf, and concludes with an anterior dragging motion along the surface. On average, anal scrapes are $125 \pm 26.7 \text{ ms}$ (range = 60.0–211 ms, $n = 65$ signals from 13 individuals) in duration, and are broadband with a dominant frequency of $50 \pm 23 \text{ Hz}$ ($n = 17$ signals from six individuals).

Signalling typically occurs in bouts (Fig. 2), each comprising 8.2 ± 7.6 complexes, and lasting $6.4 \pm 10.2 \text{ s}$ ($n = 18$ bouts from ten individuals). Each complex is $223 \pm 315 \text{ ms}$ ($n = 104$ complexes from ten individuals) in duration, and typically comprises one or two signals. When a mandible drum and anal scrape occur together, the anal scrape almost always precedes the mandible drum (Fig. 2).

Structures associated with signalling

Video analysis reveals two primary structures used for producing signals: modified anal setae and mandibles (Fig. 3). The anal scrape is produced by a pair of chitinous

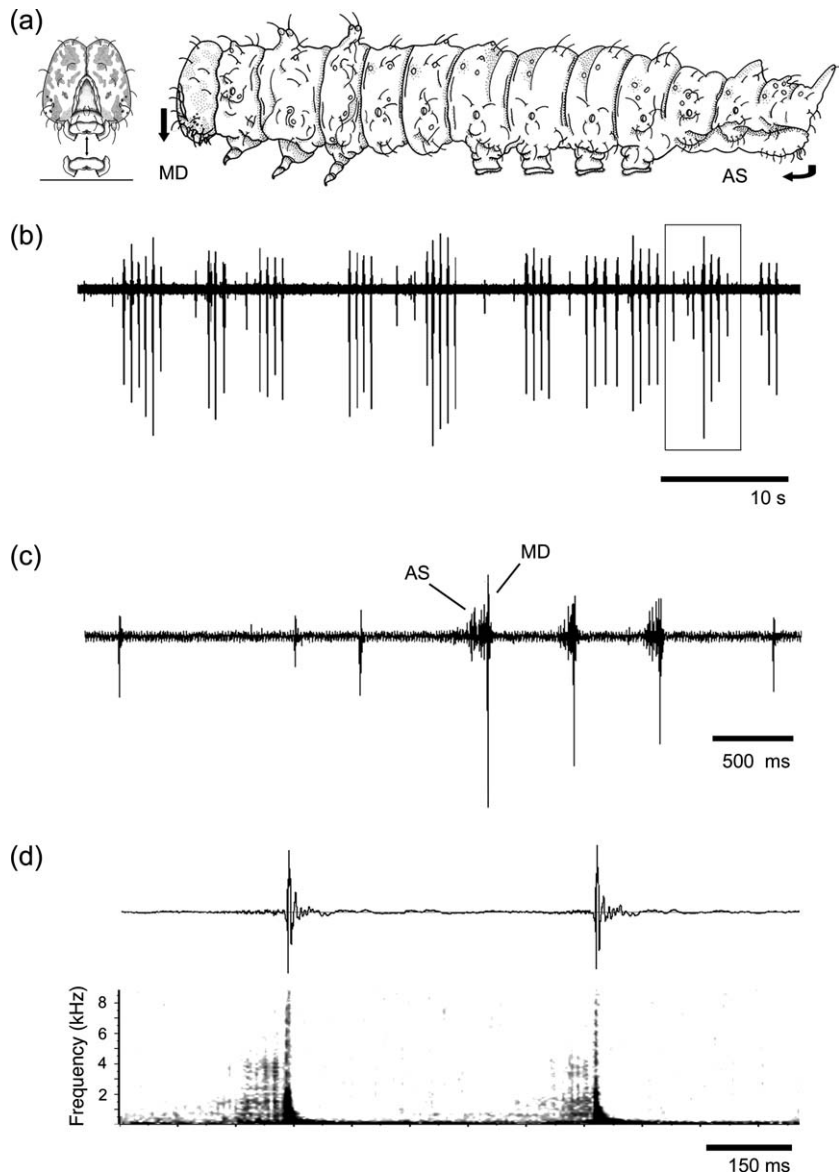


Fig. 2. Typical vibrational signals of late instar *Drepana bilineata* larvae. (a) Schematic drawing of a late instar larva showing the movements associated with mandible drumming (MD), and anal scraping (AS) with the specialized oar-shaped setae. (b) Oscillogram illustrating a series of bouts generated by a resident caterpillar when a conspecific enters the leaf. (c) Bout expanded from (b) to show typical complexes comprising single mandible drums, or a single mandible drum after an anal scrape. (d) Oscillogram and corresponding spectrogram of two consecutive anal scrape/mandible drum complexes.

oar-shaped setae located on the last abdominal segment (A10) (Fig. 3a,b). Comparative analysis with larvae that lack these modifications show that this feature is a modification of the PP1 seta (Stehr, 1987; I. Hasenfuss, personal communication). The PP1 setae are oar-shaped in instars two to five but, in first instars, they lack the broad 'paddle' shape and look more like pegs. The mandibles (Fig. 3c,d) are highly sclerotized with a serrated edge on the outer surface.

Function: experimental trials and signalling rates

A total of 52 encounters were staged between a resident and an intruder of similar weights. Weights of the contestants

(recorded after each trial) in 52 ($n = 104$) encounters were in the range 7.7–210.0 mg (mean = 84.8 ± 51.5 mg), but were similar between contestants in a given trial (mean difference = 5.1 ± 20.1 mg, paired t -test, $t = -1.84$, $P = 0.072$). Residents won 61.5% of the trials, intruders won 5.8% of the trials and 32.7% of the contests were ties. Contests lasted 385.8 ± 329.7 s in trials where a winner was decided ($n = 35$) and contests won by residents were not significantly different in duration than those won by intruders (resident = 383.4 ± 344.0 versus intruder = 411.3 ± 114.6 s; range: resident = 66–1603 versus intruder = 279–480 s; Wilcoxon rank sum test, $Z = 0.68$, $P = 0.41$).

Residents were silent until they detected an intruder (Figs 4, 5), and signalled at a latency of 49.2 ± 52.3 s ($n = 43$) from the beginning of the trial, and at a mean distance of

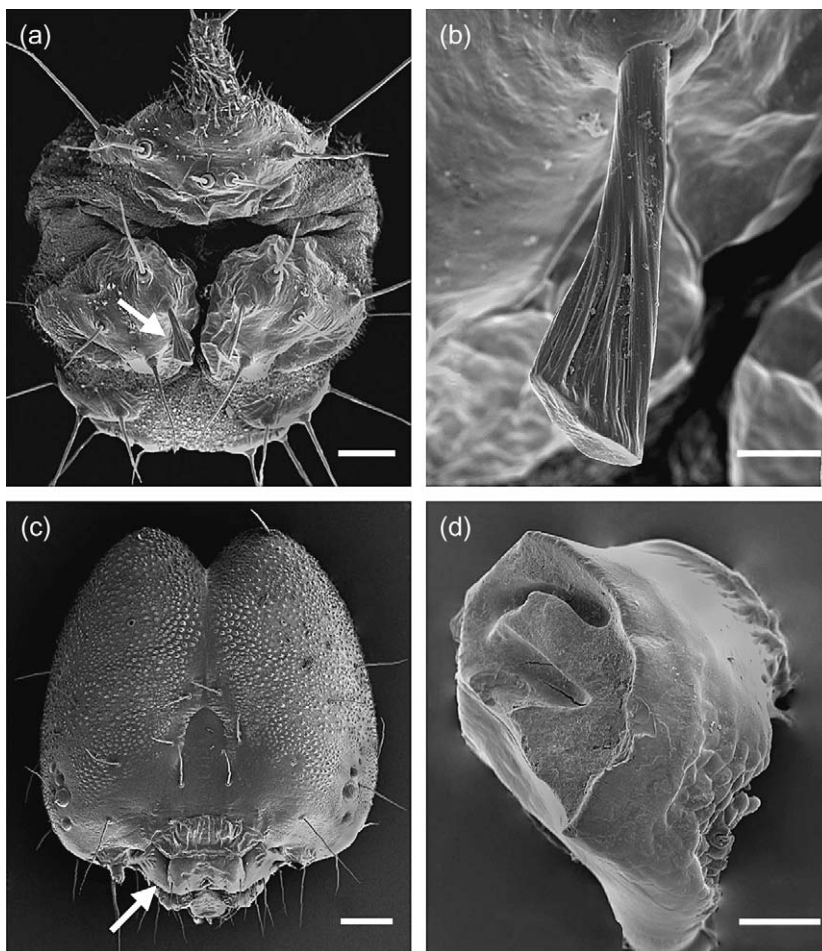


Fig. 3. Scanning electron micrographs of sound-producing structures of *Drepana bilineata*. (a) A ventral view of a late-instar larva showing the position of the anal oar-shaped setae (arrow, scale bar = 200 μ m). (b) Higher magnification of a single oar-shaped seta (scale bar = 50 μ m). (c) The head capsule of a late-instar larva showing the position of the mandibles (arrow, scale bar = 200 μ m). (d) Higher magnification of a single mandible (scale bar = 100 μ m).

2.4 $\pm$ 1.6 cm ($n = 41$) from the closest point of the intruder (head or tail). Residents remained in the same approximate position on the leaf during trials (Fig. 4a). Residents signalled overall more than intruders (Fig. 5), signalling significantly more in the first 80 s (paired t -test: $t = -5.066$, $P < 0.001$, $n = 20$) and last 80 s of each trial (paired t -test: $t = -5.178$, $P < 0.001$, $n = 20$) and were the first to signal in 43 of the 52 trials.

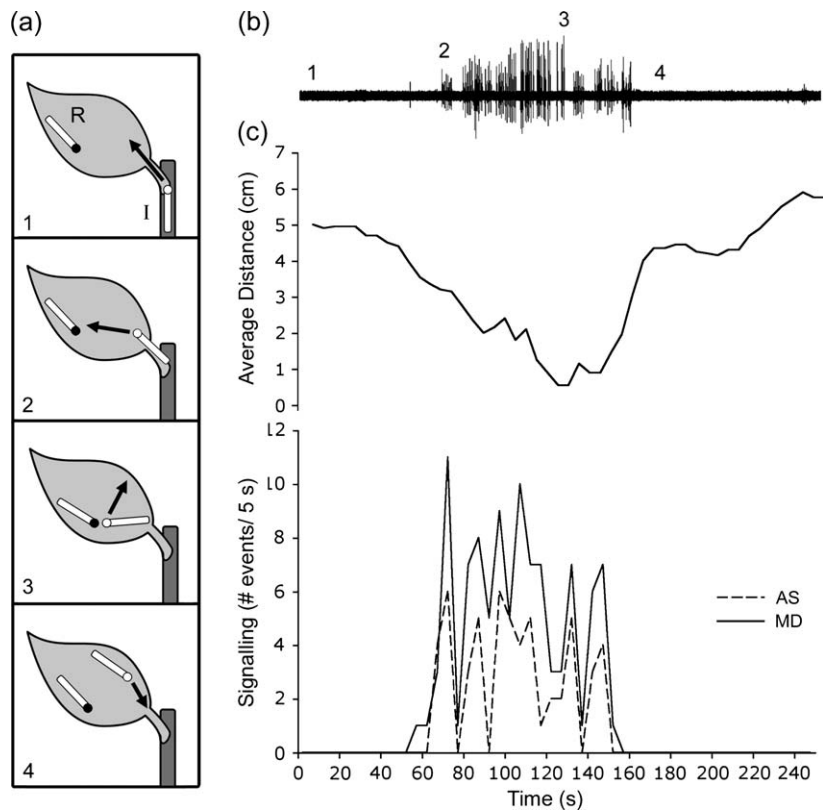
The rate of signalling in residents escalated as the intruder got closer (Figs 4–6). Mandible drumming and anal scraping did not change from far to mid distances but increased significantly from mid to close distances (ANOVA: $F = 17.0$, $P < 0.001$ and $F = 14.7$, $P < 0.001$, respectively). Residents ceased signalling within a few seconds after the intruder left the leaf (Fig. 5b). When the intruder came within 0.5 cm or less of the resident, the resident sometimes hit the intruder with its head (in 52.4% of trials where contact was made, the resident hit the intruder with its head at least once, $n = 21$) or posterior end (in 28.6% of trials where contact was made, the resident hit the intruder with its posterior end at least once, $n = 21$), depending on where it was touched. Head contact increased significantly from

far to close distances (ANOVA: $F = 6.0$, $P = 0.005$). Biting was never observed.

Comparison with *Drepana arcuata*

When comparing signalling between *D. arcuata* and *D. bilineata* larvae, some key differences were noted in the types of signals and rates of signalling. Both species produce mandible drumming and anal scraping signals, but only *D. arcuata* produces mandible scraping (Yack *et al.*, 2001; Fig. 6). Overall, *D. arcuata* larvae signalled significantly more than *D. bilineata* (t -test, $t = 5.902$, $P < 0.001$; Fig. 6c). Signal patterns were also quite different. *Drepana bilineata* typically produces an anal scrape followed by a single mandible drum whereas, in *D. arcuata*, an anal scrape can be followed by up to eight mandible drums (Yack *et al.*, 2001; Fig. 6). Similar to *D. bilineata*, signalling rates of anal scraping and mandible drumming increase as the distance between individuals decreases (in *D. arcuata*, mandible drumming and anal scraping increased significantly from far to mid distances (ANOVA: $F = 5.1$, $P = 0.015$ and $F = 7.5$, $P = 0.033$,

Fig. 4. Resident signalling during a single agonistic encounter (238 s). (a) Schematic of the different stages of the encounter. Frame 1: the resident (R) is feeding as the intruder (I) moves along the twig toward the leaf before the trial. Frame 2: the resident begins to signal as the intruder enters the leaf. Frame 3: the resident signals continuously as the intruder makes contact. Frame 4: the resident stops signalling as the intruder walks away and eventually leaves the leaf. Arrows indicate the path of the intruder across the leaf. (b) Oscillogram illustrating the vibrational signals made by the resident throughout the encounter. Numbers correspond with frames from (a) and timescale corresponds to (c). (c) Mean distance between resident and intruder at the beginning of each 5-s interval and the number of mandible drums (MD) and anal scrapes (AS) in consecutive 5-s intervals, including 1 min before the trial, and 1 min after intruder departure. Time scale is the same for both distance and signalling graphs.



respectively)) but, in *D. arcuata*, mandible scraping replaces mandible drumming during the last stages of the encounter (mandible scraping increases significantly from mid to close distances (ANOVA: $F = 30.5$, $P < 0.001$)).

Control trials

In the 20 control trials where intruders were introduced to an empty leaf that had been previously occupied, intruders entered the leaf in all trials except one. In these 19 trials, they remained on the leaf for the full 20 min that were recorded, and began to lay silk and feed on the leaf. No signalling occurred by the 'new resident' in all 19 trials.

Experiments using a simulated parasitoid suggest that late-instar *D. bilineata* larvae do not use vibrational signalling to deter parasitoids. In all 19 trials where larvae were poked with a two-haired paint-brush (simulating a parasitoid attack), not one larva responded by signalling.

Discussion

The present study describes novel vibrational signals in the warty birch caterpillar *D. bilineata* and contributes toward our understanding of the diversity and evolution of vibrational signalling in the Drepanoidea. *Drepana bilineata* produces two types of substrate-borne signals during interactions with

conspecifics: mandible drumming and anal scraping. The results are discussed with respect to the function and evolution of signalling in *D. bilineata* and other Drepanoidea.

Vibrational signals

Mandible drumming in *D. bilineata* is a percussive signal easily distinguished from any other vibrations detected as side effects of chewing, walking or laying silk. Drumming with the head or mandibles is reported in other insects (termites: Röhrig *et al.*, 1999; Rosengaus *et al.*, 1999; death-watch beetles: Birch & Keenlyside, 1991; carpenter ants: Fuchs, 1976). In Lepidoptera larvae, drumming is only described formally in one other Drepanoidea species (*D. arcuata*, Yack *et al.*, 2001), and in one species of Tortricidae (*Sparganothis pillariana*, Russ, 1969), although it is implicated from behavioural observations in other Drepanoidea (*Drepana falcata*: Bryner, 1999, I. Hasenfuss, personal communication; *Drepana lacertinaria*, *Watsonalla binaria* and *Watsonalla uncinula*: I. Hasenfuss personal communication; *Nordstromia lilacina* and *Tridrepana arikana*: Sen & Lin, 2002). In studying the mandible morphology of *D. bilineata* and *D. arcuata*, no distinguishing features are observed that may be adapted for sound production. Rather, these caterpillars appear to make use of their sharp mandibles, which are common to most caterpillars that use them for chewing (Scoble, 1995). However, in producing these

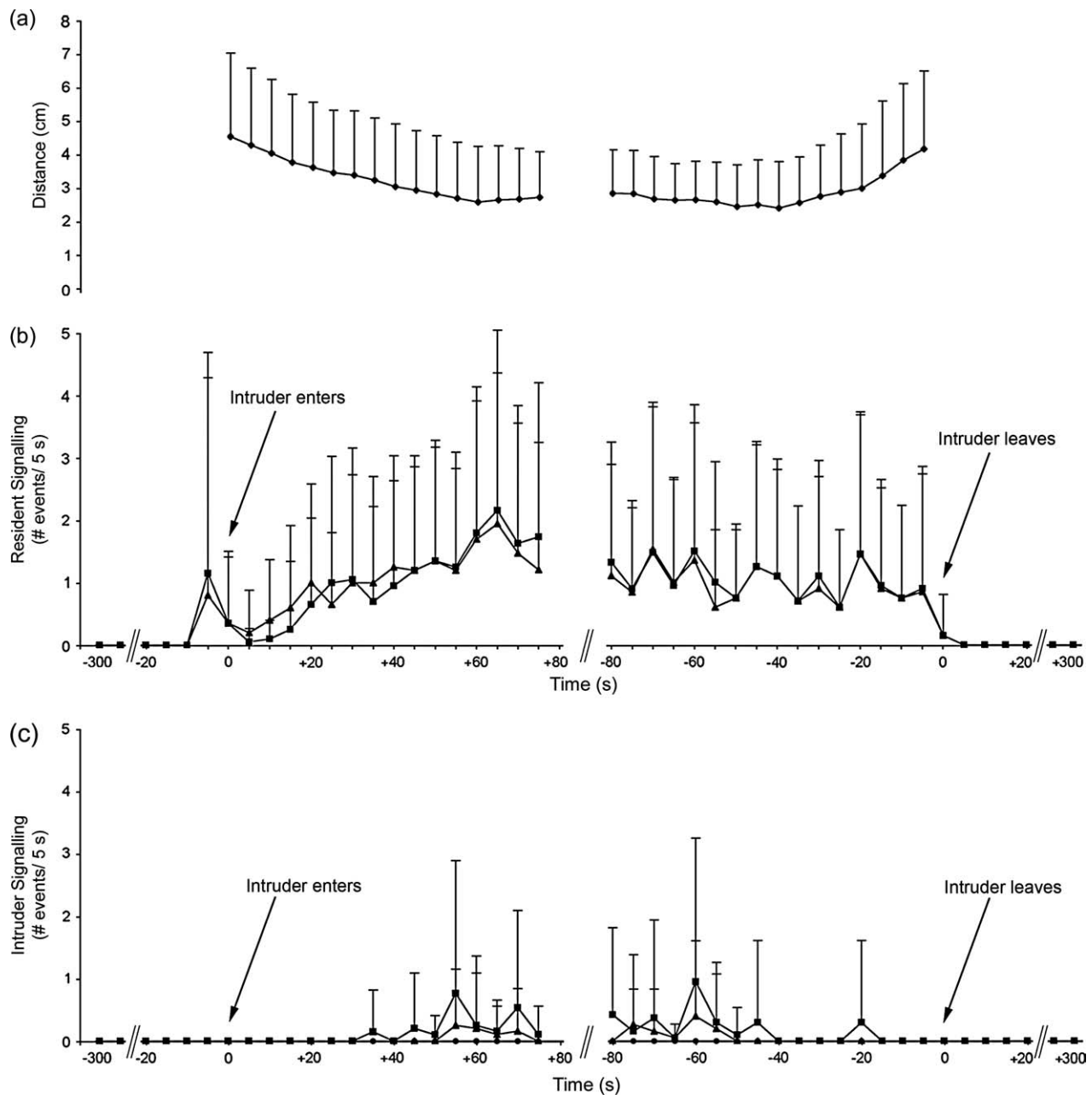


Fig. 5. Resident and intruder signalling over 20 encounters. (a) Mean distance (+SD) between resident and intruder larvae at the beginning of each 5-s interval. Signalling rate of residents (b) and intruders (c) before and after trials, and for the first 80 s and last 80 s of each trial. Squares denote average mandible drum rate per 5-s interval, and triangles denote average anal scrape rate per 5-s interval.

rapid percussive signals, the caterpillar must incorporate behavioural adaptations, including raising its head with its mandibles open, and then rapidly striking the leaf. Members of the Drepanoidea subfamily Thyatirinae, lunge forward and attack an intruder with opened mandibles in attempt to inflict injury by biting (J. Bowen, unpublished data; I. Hasenfuss, personal communication). Based on preliminary evidence, however, it is hypothesized that these movements associated with physical attacks

in Thyatirinae became ritualized as mandible drums in Drepaninae.

Anal scraping in *D. bilineata* is performed by dragging modified PP1 setae against the leaf surface in a stridulatory manner. These modified setae are described in several other Drepaninae, varying widely in shape and size between species (Nakajima, 1970, 1972; Yack *et al.*, 2001; I. Hasenfuss, personal communication). They appear to be specialized for sound production and, to the authors' knowledge, are unique

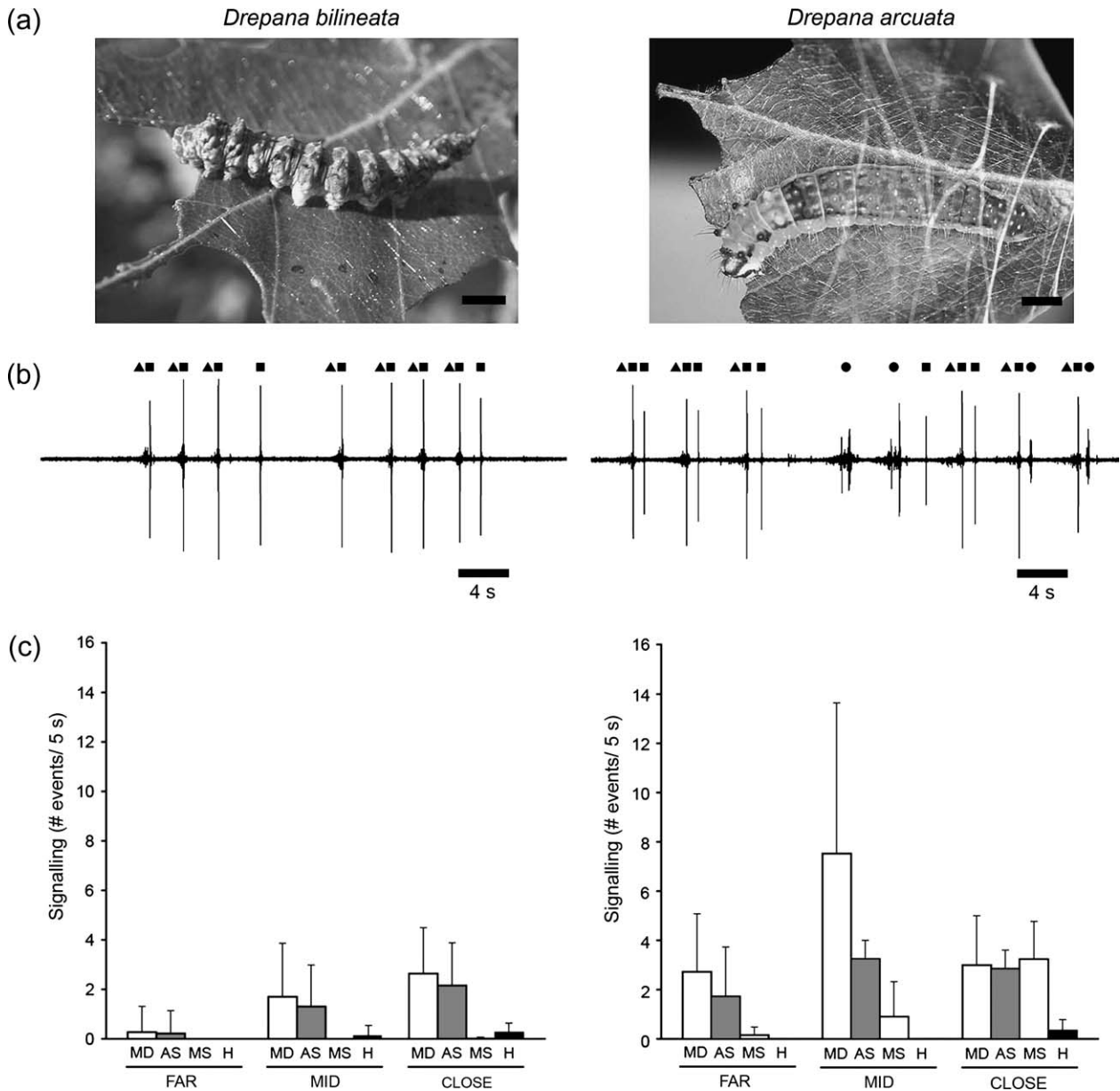


Fig. 6. Comparison of signalling between *Drepana arcuata* and *Drepana bilineata*. (a) Photographs of *D. bilineata* (left, scale bar = 4 mm) and *D. arcuata* (right, scale bar = 4 mm) late-instar larvae demonstrating differences in silk investment. (b) Oscillograms illustrating differences in signals and signalling patterns between the two species. (c) Graphs showing mean $\pm$ SD signal rates by residents with direct contact (or body-to-body distance of less than 0.5 cm in *D. bilineata*) at stages of intruder approach (*D. bilineata*: left, $n = 21$; *D. arcuata*: right, $n = 16$). For *D. bilineata*, mandible drumming (MD) and anal scraping (AS) increased significantly only from MID to CLOSE distances, whereas head contact (H) increased significantly from FAR to CLOSE distances. Mandible scraping (MS) did not differ significantly by distance. For *D. arcuata*, MD and AS increased significantly from FAR to MID distances, MD then returned to FAR levels and AS did not change from MID to CLOSE distances. MS and H increased significantly only from MID to CLOSE distances. When all signalling rates were combined, *D. arcuata* signalled significantly more than *D. bilineata*.

to this subfamily. Anal scraping movements have been implicated from behavioural observations in other Drepanoidea (*D. falcataria*: Federley, 1905; Bryner, 1999, I. Hasenfuss, personal communication; *D. curvatula*: Federley, 1905; *D. lacertinaria*, *W. binaria*, and *W. uncinula*: I. Hasenfuss

personal communication, Federley, 1905; *N. lilacina* and *T. arikana*: Sen & Lin, 2002). How might this unique behaviour of anal scraping have evolved at the proximate level? In *D. bilineata* and *D. arcuata*, anal scraping is produced by the last abdominal segment, which lacks anal prolegs. The basal

condition, characteristic of the Thyatirinae (and most other Lepidoptera) is to possess anal prolegs, which are used for walking (J. Bowen, unpublished data; I. Hasenfuss, personal communication). During attacks, Thyatirinae walk toward an intruder, and prepare to lunge and bite. Therefore, it is hypothesized that motor patterns associated with anal scraping evolved from those associated with walking.

Function of signalling in Drepana bilineata

The results from the staged encounter experiments support the hypothesis that vibrational signalling in *D. bilineata* functions in territorial disputes over occupied leaves. This conclusion is based upon several observations. First, signalling is produced most often in the context of conspecific interactions and acoustic displays are generally restricted to a territory. Second, resident caterpillars are usually the first to signal during an encounter, and they signal significantly more than intruders. Third, resident larvae win significantly more encounters than do intruders. Finally, signalling rates increase as the intruder gets closer to the resident. These characteristics are typical of signals associated with territorial displays in many animals, where ritualized signalling allows contestants to resolve conflicts without fighting (Baker, 1983; Huntingford & Turner, 1987). Although the hypothesis that signalling functions in spacing/territoriality is supported by these results, it is prudent to consider alternative hypotheses. Perhaps these signals function as aposematic warning signals, as seen in some Bombycoidea caterpillars that produce clicking noises to warn predators that they are unpalatable (Brown *et al.*, 2007). However, this function is unlikely for *D. bilineata* because they have no obvious noxious defences (e.g. spines, regurgitation). Also, in the simulated parasitoid trials *D. bilineata* larvae stop signalling when 'attacked'. A second alternative hypothesis is that signalling functions to enhance mutualistic relationships with ants, whereby the ants defend the caterpillars in exchange for sweet secretions (DeVries, 1990, 1991; Travassos & Pierce, 2000). However, in *D. bilineata* or any Drepanoidea, there is no evidence for secretions or associations with ants. A third hypothesis is that signals function in conspecific recruitment, as seen in some gregarious sawfly larvae (Fletcher, 2007). However, this function in *D. bilineata* is unlikely because the larvae are not gregarious at any stage in their development.

Why defend a leaf?

If leaves are abundant, as might be assumed for a caterpillar feeding on birch or alder trees, why invest time and energy in leaf defence? It is suggested that the amount of energy a caterpillar invests in leaf defence is related to how much time and energy the caterpillar invests in building a shelter, and the probability of encountering another individual seeking to take over that shelter. In larval insects, silk leaf shelters are expensive to build and valuable to own because they provide an improved microclimate, as well as

protection from predators, parasitoids and weather (Berenbaum *et al.*, 1993; Cappuccino, 1993; Costa & Pierce, 1997). Taking over an existing nest from a resident caterpillar would reap the benefits without the costs of building a shelter. In some larval species that invest in silk shelters, territorial defence involves physically aggressive acts that sometimes lead to serious injury or death (Weyh & Maschwitz, 1982; Okuda, 1989; Berenbaum *et al.*, 1993; Poirier & Borden, 1995; I. Hasenfuss, personal communication). It is proposed that, like other animal territorial systems, vibrational signalling in caterpillars has evolved to avoid the costs of physical aggression. The relationship between signalling and the defence of costly shelters is noted for three other caterpillars to date, including the cherry leaf roller *Caloptilia serotinella* (Fletcher *et al.*, 2006), the jumping caterpillar *Sparganothis pilleriana* (Russ, 1969), and the masked birch caterpillar *Drepana arcuata* (Yack *et al.*, 2001). Signalling in these species also appears to be highly ritualized and escalated injurious fighting has not been reported.

Comparisons between the sympatric congeners *D. arcuata* and *D. bilineata* also reveal a possible relationship between silk investment and leaf defence. Overall, *D. arcuata* invests more in leaf defence, by signalling sooner and at significantly higher rates than *D. bilineata*. Also, *D. arcuata* produces a third signal (i.e. mandible scraping) that is lacking in *D. bilineata*. *Drepana arcuata* not only signals more than *D. bilineata*, but also 'wins' more contests. On average, *D. bilineata* were more 'tolerant' of each other when they occupied different ends of a larger leaf, and this resulted in more ties. It is proposed that these differences in signalling effort are related to differences in nest-building behaviour. *Drepana arcuata* invests significantly more time in nest building than does *D. bilineata*. In addition to laying a silk mat, *D. arcuata* can spend up to 2 h constructing a leaf shelter. *Drepana bilineata*, on the other hand, do not build leaf shelters, but simply lay a silk mat on the leaf, spending an average of 9 min doing so in the first hour of occupying a new leaf.

Aside from differences in silk investment, the two species differ in their social structure during early developmental instars. *Drepana arcuata* lives gregariously in cooperatively constructed shelters during their first two instars before moving to and defending multiple solitary leaves during their final developmental stages. The chances of encountering a wandering sibling therefore are expected to be higher in *D. arcuata* than in *D. bilineata* because the latter disperses earlier than does *D. arcuata*.

The present study contributes to the understanding of vibrational communication in larval holometabolous insects. Experimental evidence supports the hypothesis that signalling in *D. bilineata* functions to advertise 'ownership' of a resident's leaf. It is suggested that investment in signalling is linked to the amount of time and energy invested in building silken leaf shelters. Drepanoidea caterpillars offer a unique opportunity for studying the evolution of vibrational signalling in larval insects. Further comparisons of behavioural and morphological observations combined with phylogenetic mapping of Drepanoidea larvae behaviours are currently

underway, and should shed light on the proximate and ultimate pathways that lead to ritualized signalling in this interesting group of insects.

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