

Invitation by vibration: recruitment to feeding shelters in social caterpillars

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Abstract

Sociality is widespread in caterpillars, but the communication mechanisms used for group formation and cohesion are poorly understood. Here, we present the first evidence that caterpillars produce complex vibratory signals to advertise food and shelter sites to conspecifics. We first tested the hypothesis that early instars of the masked birch caterpillar (*Drepana arcuata*) actively form groups. Larvae placed alone on different leaves of a birch twig began assembling within minutes and forming groups of 2–6 at a median time of 2 h. In Y-choice experiments, larvae joined arms occupied by conspecifics significantly more frequently than unoccupied arms. To test the hypothesis that group formation is vibration-mediated, signals were monitored in solitary residents of silk leaf shelters before and during natural recruitment events. Four distinct signal types were recorded: anal scraping, mandible drumming, mandible scraping, and buzz scraping. Anal scraping and buzz scraping were the most common in residents prior to being

approached, and these signals were strongly correlated to feeding and laying silk. Signaling occurred in 100% of residents, and higher signal rates resulted in significantly faster recruitment times. As a recruit approached a resident, complex signaling interactions occurred, which may communicate information about resource quality or location. We conclude that caterpillars, similar to other social animals, use acoustic communication to advertise resources. The vibratory signaling repertoire of these tiny caterpillars exhibits a complexity rivaling that of eusocial insects. Further investigations of vibroacoustic communication are essential to fully appreciate the intricacies of social interactions in caterpillars and other juvenile insects.

Significance statement

Group living provides many survival benefits to juvenile insects such as caterpillars, but little is known about the communication signals mediating social interactions such as group formation. Our study shows that caterpillars use vibration signals to “invite” conspecifics to social gatherings. Pinhead-sized early-instar caterpillars (*Drepana arcuata*) are capable of locating conspecifics on birch leaves to form small groups. But how do they accomplish this? We report that individual resident caterpillars established in a silk shelter produced complex vibrations by dragging their anal segments, scraping and drumming their mouthparts, and tremulating their bodies to advertise a feeding spot and shelter. These results provide the first evidence that caterpillars use vibratory signaling to form social groups, providing insight into the poorly understood role of vibratory communication in juvenile insects.

Keywords Group living · Communication · Vibration · Recruitment · Larvae · Sociality

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Introduction

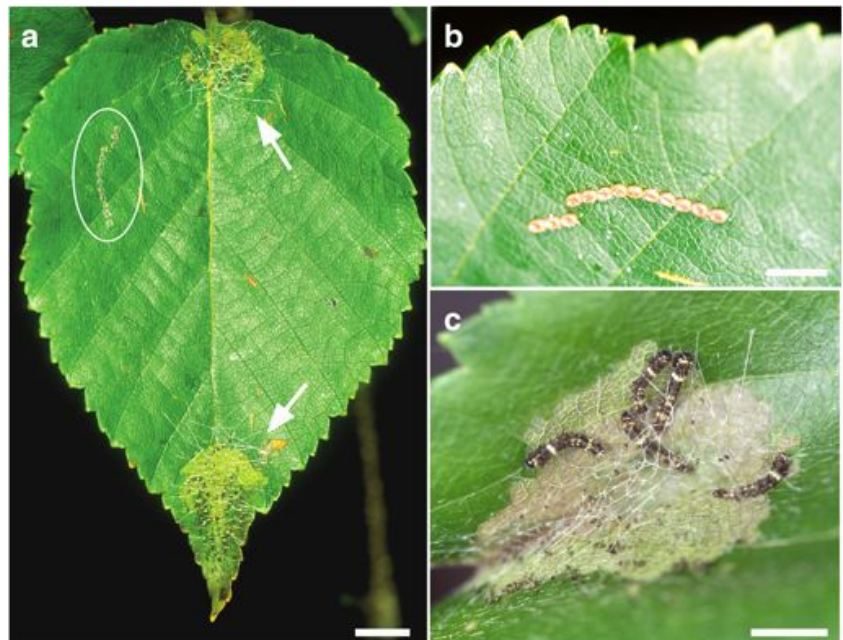
“Communication is the glue that binds individuals to one another” (Costa 2006). Group living is widespread throughout the class Insecta, occurring in more than 12 orders, and communication is essential for mediating the formation, cohesion, and organization of such groups (Costa 2006). While communication signals and mechanisms have been extensively studied in the eusocial insects such as bees, wasps, ants, and termites, much less is understood of these mechanisms in the other social insects, such as caterpillars (Costa 2006; Cocroft and Hamel 2010).

Many species of larval Lepidoptera across a diverse array of taxa form social groups at some stage of their development (Costa and Pierce 1997; Zalucki et al. 2002; Costa 2006). Groups can range in size, from two to several hundreds of individuals, and complexity, from patch-restricted foragers that share a feeding shelter to large groups that exhibit coordinated foraging expeditions and division of labor (Fitzgerald and Peterson 1988; Costa 2006; Dussutour et al. 2008). Benefits derived from group living in caterpillars include predator defense, feeding facilitation, and thermoregulation (Costa and Pierce 1997; Prokopy and Roitberg 2001; Costa 2006). While such benefits have been well documented, the communication mechanisms necessary for mediating social interactions, such as group formation and coordination of group activities, are poorly understood (Costa and Pierce 1997; Costa 2006). Group formation by neonates may be initially facilitated by adult females, as many species lay eggs in rows or clusters or multiple females may lay on the same host plant, ensuring that larvae are in close proximity to one another (Prokopy and Roitberg 2001). However, female egg-laying habits are usually not sufficient for larvae to form and maintain social groups that must respond to changing food and

environmental conditions (Fitzgerald and Peterson 1988; Costa and Pierce 1997; Zalucki et al. 2002). Most reported examples of social communication in caterpillars involve chemical markers, often as pheromone trails laid by processionary species, and in some species, tactile contact is used for maintaining processions (Costa and Pierce 1997). There is little evidence that visual communication signals are used (Costa and Pierce 1997; Prokopy and Roitberg 2001), probably owing to the simple eyes that larval insects possess (Warrant et al. 2003). Vibratory communication should be optimal for short-range communication in small plant-borne insects (Virant-Doberlet and Cokl 2004; Cocroft and Rodriguez 2005; Yack 2016), but at present, there is no direct evidence for vibratory-mediated group coordination in caterpillars. To expand our knowledge of the functions and mechanisms of social interactions in caterpillars, detailed observations of the insects on their host plants during social activities, in addition to experimental manipulations, are essential. This study is the first to explore the role of vibratory communication as a mechanism of social recruitment in caterpillars.

Early-instar larvae of the masked birch caterpillar, *Drepana arcuata* (Lepidoptera: Drepanidae), are good models to test hypotheses on vibratory-mediated group formation. The species occurs throughout northeastern North America (Rose and Lindquist 1997) and exhibits five larval instars that feed on birch (*Betula* spp.) and alder leaves (*Alnus* spp.) (Fig. 1). Late instars (third to fifth) live solitarily on individual leaves and use vibratory signals during territorial disputes over silk shelters (Yack et al. 2001; Scott et al. 2010; Guedes et al. 2012; Yack et al. 2013). Early instars (first and second) on the other hand have been observed to form small groups (Yack et al. 2001). Moreover, early instars have been noted to generate vibratory signals (Yack et al. 2001), although these signals

Fig. 1 Eggs and early-instar larvae of *Drepana arcuata*. **a** Birch leaf (*Betula papyrifera*) showing a row of 13 egg cases (inside oval) and 2 groups of early-instar larvae (indicated by arrows) comprising 15 individuals in total. Scale bar 5 mm. **b** Row of unhatched eggs. Scale bar 3 mm. **c** Group of five first-instar larvae inside a silk shelter. Scale bar 2 mm



have not been formally studied. Finally, because these insects reside in open leaf shelters, their behaviors and vibratory signals are amenable to being simultaneously monitored during social interactions with minimal disruption.

We first tested the hypothesis that early instars actively form groups. We predicted that (1) larvae placed on separate leaves of a birch twig will form groups; (2) larvae will not always establish shelters at the same location of a leaf; and (3) in Y-choice experiments, larvae will choose to join conspecifics over choices with no larvae present. Second, we tested the hypothesis that vibratory signals are associated with group formation. We predicted that (1) early instars generate vibratory signals; (2) solitary larvae established in shelters (residents) will signal; (3) vibratory signals generated by residents are associated with activities that would be attractive to potential recruits, such as feeding and shelter building; (4) vibratory signals are associated with recruitment events; (5) residents signal more than potential recruits; and (6) residents that signal at higher rates recruit conspecifics more quickly. Our results show that during group formation events, caterpillars engage in complex vibratory signaling patterns that are unprecedented for most social insects.

Methods

Study animals

Female *D. arcuata* (Lepidoptera: Drepanidae) moths were collected at ultraviolet lights at the Queen's University Biology Station (Chaffey's Lock, Ontario, Canada, 44.5788° N, 76.3195° W) and other regions near Ottawa, ON, Canada (45.4215° N, 75.6972° W) between May and September 2010–2015. Moths oviposited on cuttings of paper birch (*Betula papyrifera*) held in water-filled plastic vials and on paper bag clippings. Hatchlings were carefully transferred to fresh birch cuttings with a fine paintbrush and reared indoors (18–24 °C and 16L:18D) in glass jars (~22 cm long, 8 cm wide). Only the early instars (first and second) were used in experiments. Individuals used in experiments were of mixed parentage (i.e., larvae were not necessarily kin) as eggs from multiple females were mixed. Due to the nature of the experiments, which involved direct measurement of signals from focal individuals, blind scoring was not possible.

Group formation experiment

The first hypothesis predicts that larvae placed on separate leaves of a birch twig will move from their respective individual leaves to form groups. To test this, we conducted 30 “group formation” experiments. In each experiment, a birch twig (12–15 cm long) with five or six leaves (each 7–9 cm long and 4–6 cm wide) was positioned in a water-filled plastic vial (Fig. 2a). Using a fine

paintbrush, one larva was placed on each of the five leaves within a 1-min period from placement of the first larva. Larvae were left undisturbed in a plastic bin and monitored for the number and sizes of groups at the following time points: 0, 0.5, 1, 2, 3, 4, 5, and 18–24 h. All trials were performed in a greenhouse setting at a temperature of 22–26 °C during daylight hours (10:00–16:00), and no individuals were used more than once. We calculated the percentage of individuals in groups at each time interval over the 30 experiments. To follow the progression of group formation over time, a time-failure analysis (i.e., survival analysis) was performed using Kaplan-Meier estimators (PROC LIFETEST; SAS software, SAS Institute, Cary, NC, USA). Regression analysis was subsequently used to assess the prevalence of group size at the end of group formation, when the groups became stabilized, and its relationship to the number of groups formed (PROC GLM; SAS software, SAS Institute, Cary, NC, USA). We also predicted that larvae would not always form shelters at the same location on the leaf. We noted the locations of shelters at 48 h and analyzed the probability of shelter formation on any given portion of the leaf. This was done by dividing the leaf into four sections (tip, base, outer edge, and middle; Fig. 2b), calculating the percentage of shelters in each section, and performing a χ^2 test to determine if shelters in any one region occurred more than expected by chance (i.e., 25%).

Y-choice experiments

If early-instar larvae actively form groups, we predicted that they would preferentially choose to join conspecifics in a Y-choice experiment. Two different experiments were performed (Fig. 3). The first assessed whether a test larva would choose the arm of a Y occupied by conspecific larvae over an unoccupied arm. Fresh birch leaves were cut into a Y (3-cm-long, 2.5-mm-wide arms). The Y was clamped into a horizontal position using a paper clip at the base of the test arm and supported by reusable adhesive putty (Staples®). Three larvae were placed on one arm and the other arm was left unoccupied. A fourth larva (the test larva) was then placed within 2–5 s at the base of the Y. The test larva was considered to have “chosen” an arm when it reached the middle of either arm within 1 min of the beginning of the trial. Between trials, positions of the unoccupied and occupied arms were alternated to preclude any positional bias. The second Y-choice experiment assessed whether larvae would be attracted to an arm that had a potential chemical residue of previous occupants. The Y was cut and positioned as described above with the following modifications: Two custom-made lightweight foam barriers (1.5 cm long, 0.7 cm wide) with slits were placed at the base of each arm, and then three larvae were placed on one of the arms distal to the barrier. Larvae were allowed to walk over the arm and were continuously monitored so that they could be placed back on the arm if they attempted to wander over the barrier. The other arm was left unoccupied. After 30 min, the larvae and barrier were removed and the test larva placed at the

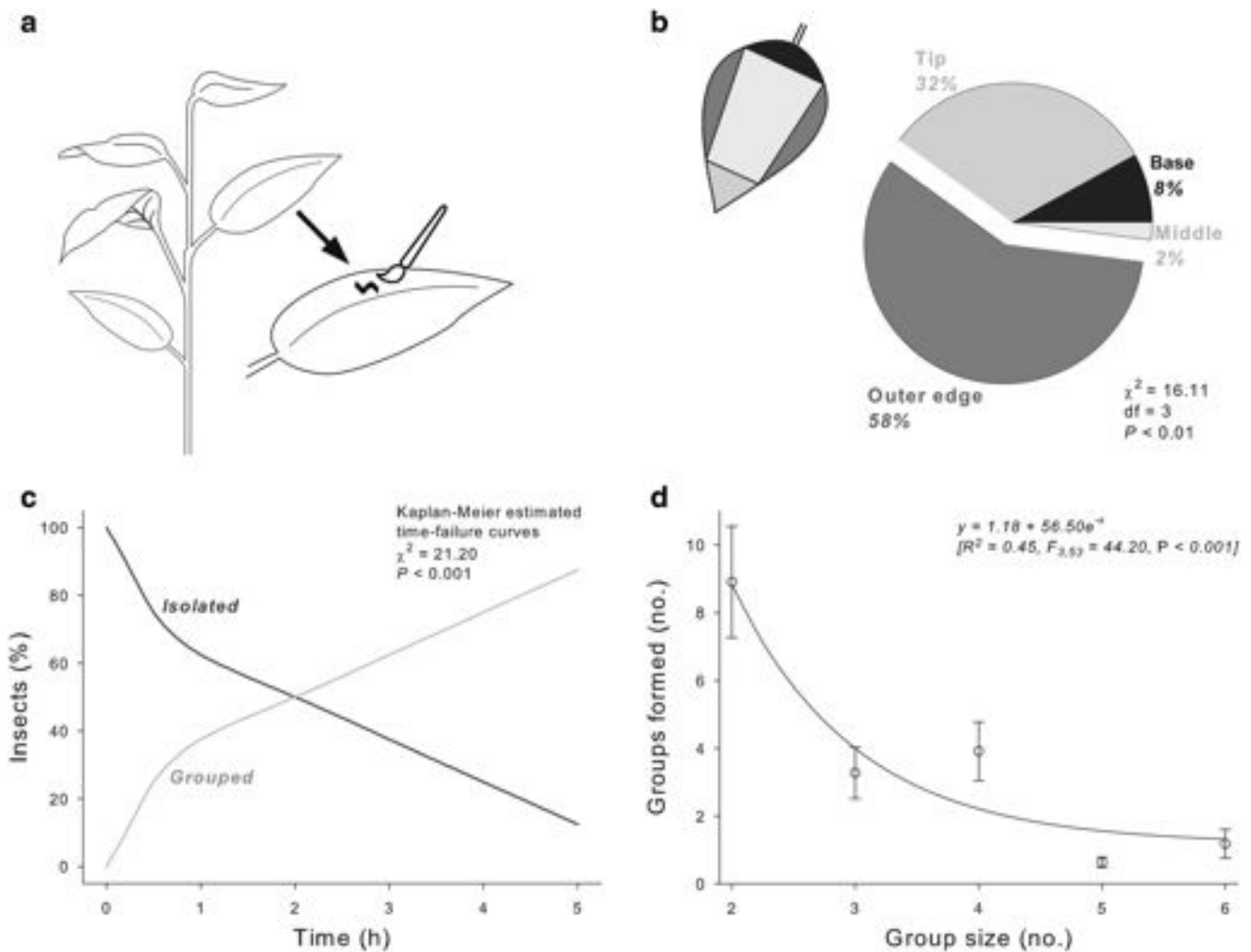


Fig. 2 Group formation experiment. **a** Experimental setup where 5–6 early-instar larvae were placed individually on separate leaves of a birch (*Betula papyrifera*) twig. Black arrow points to close up of a leaf showing placement of a larva. **b** Areas of leaf where larvae resided in leaf shelters at the end of the experiment. Shelters occurred on all parts of the leaf, but with a preference for the outer edges. **c** Percentage of larvae that formed

groups or remained solitary during the first 5 h of the observation period. It took a median time of 2 h for the larvae to form groups regardless of the group size as shown by time-failure analysis ($\chi^2 = 21.20$, $p < 0.001$). **d** Group sizes ranged from two to six, with prevalence of small group sizes (two larvae per group), negatively related to the number of groups formed

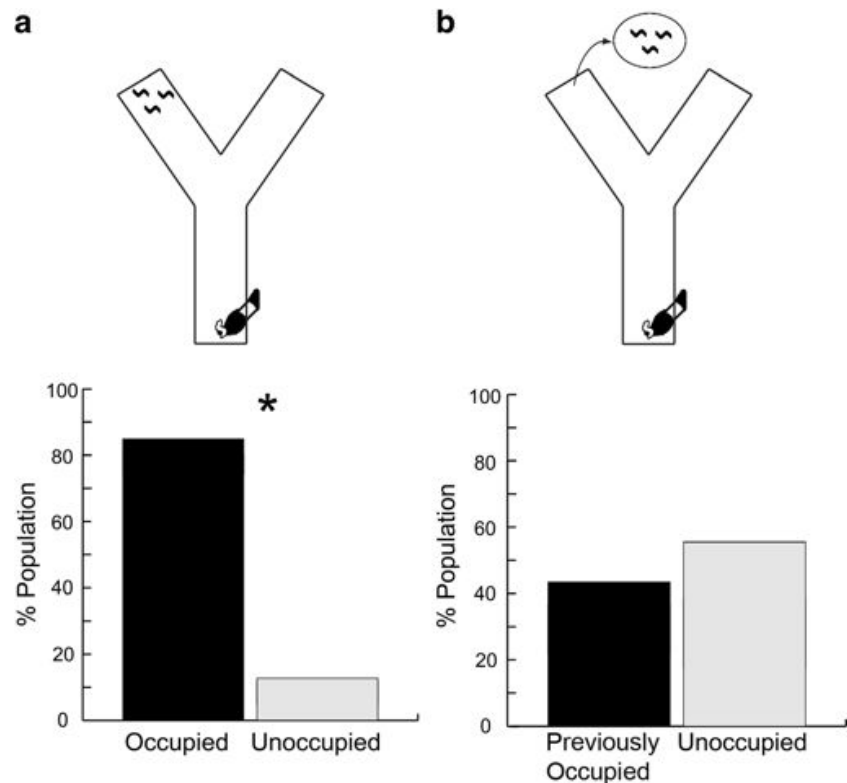
base of the Y, and its choice of arm recorded using the criterion outlined above. Thirty trials were completed for each experiment, using different larvae and a new Y made from a fresh leaf for each individual trial. All trials were performed at room temperature (21–24 °C). Statistical analysis was performed using two-tailed χ^2 test with GraphPad Prism software.

Vibration signal types and rates

Our first goals were to document if and how early instars produced vibration signals, measure signal types and rates for solitary shelter residents, and then determine how signaling is associated with other non-signaling behavioral activities.

Vibration signal types To identify the diversity of signal types produced by early instars, we reviewed video recordings of larvae that had settled in groups of 1–5 individuals. Larvae were placed randomly on birch twigs containing 3–5 leaves and left undisturbed over a 12-h period during which time they became established in shelters of different group sizes. Vibrations were recorded using a laser-Doppler vibrometer (PVD-100, Polytec Inc., Irvine, CA, USA) (velocity 20 mm/s; high-pass filter off; low-pass filter 20 kHz) by focusing the laser beam on a circle of reflective tape (2 mm diameter) attached to the upper leaf surface within 1–2 cm from a shelter. Trials were simultaneously videotaped using a camcorder (Sony hd-rxr520v Handycam, Sony Corp., Tokyo, Japan) with the output from the laser connected to the microphone port. Vibrations were also recorded as .wav files to a data

Fig. 3 Y-choice experiments testing preferences of test larvae for joining conspecifics. **a** Bar graph showing that 86.7% of test larvae chose the arm occupied by conspecifics, indicating a significant preference (indicated with an asterisk) for the Y arm occupied by conspecifics ($\chi^2 = 16.1, p < 0.0001$). **b** Bar graph showing that 43.3% of test larvae chose the previously occupied Y arm, indicating no significant preference over a previously unoccupied arm ($\chi^2 = 0.53, p < 0.465$)



recorder (Marantz PMD 671, Marantz Corp., Kanagawa, Japan) at a sampling rate of 48 kHz. Trials were performed in an acoustic chamber (C-14A MR, Eckel Industries Ltd., Cambridge, MA, USA). In total, 38 half-hour videos were examined, comprising nine replicates each for groups of two and four individuals and ten replicates each with solitary individuals and groups of five individuals.

Vibration signals of solitary residents If vibration signals function as recruitment signals, we predicted that solitary individuals established in a shelter would generate signals and that signaling would be associated with activities attractive to potential recruits, such as feeding or shelter construction. We recorded ten solitary residents using the video and laser recording methods described above. The individual was left undisturbed in its shelter for 5–10 min prior to recording. Time-sequenced files were created using JWatcher Video 1.0 (Blumstein et al. 2010). Video recordings of each trial were watched at reduced speed, and each signal event was time-marked over the 30-min period to calculate overall signal rates (i.e., rates of the different signals pooled) as well as rates of each individual signal type. The signal rate data were transformed to $\log_{10}(x + 1)$ and subjected to ANOVA and Fisher's LSD test (PROC GLM; SAS software, SAS Institute, Cary, NC, USA) to compare rates of different signal types. To assess if and how signals were associated with different behavioral states, rates of each signal type were calculated over the total time course of the four most common behaviors, feeding,

laying silk, walking, and resting, using JWatcher Video 1.0. A multivariate analysis of variance (MANOVA) and canonical variate analysis (CVA) of rates of each signal type were performed to test if signals would differ among the non-signaling behavioral states. Such results were subsequently subjected to complementary analyses of variance as well as Fisher's LSD test (at $p = 0.05$) (PROC GLM; SAS software, SAS Institute, Cary, NC, USA).

Vibratory signaling during group formation

Another set of experiments was conducted to determine how vibratory signaling was associated with group formation events. A fresh leaf (~10 cm long, ~6 cm wide) was set up for laser and video recordings as described above with the following modifications: Prior to placing larvae on a leaf, the reflective tape was attached to the upper surface of the leaf 1–2 cm from the leaf base. Five to eight larvae were then placed at random positions on the leaf and left undisturbed for 2.5–3 h or until at least one established group was formed. Fourteen experimental trials were conducted (involving 102 larvae in total). No larvae were reused between trials.

Video and laser recordings were scored for the time it took a resident to establish a shelter or feeding site on the leaf and the time to its first visitor or joiner (see below for definitions). The number and types of vibratory signals generated by caterpillars surrounding recruitment events were also recorded. The following definitions and scoring criteria were

established: A resident (hereafter referred to as “R”) is an individual that establishes itself on the leaf and is eventually visited or joined by another caterpillar. By “establishment,” we refer to shelter construction and/or commencement of feeding. A shelter consists of a silk mat on the leaf surface and one or more strands of silk joining edges of the leaf. A visitor (hereafter referred to as “V”) is an individual that comes within 0.5 cm of the R but then leaves, and a joiner (hereafter referred to as “J”) enters the shelter or feeding area and feeds and/or contributes to the shelter construction. A group was considered to be established when the R and J remained together for 30 min or longer. To score signaling surrounding recruitment events, we identified the time frame as the time R was established until 30 s after it was joined or visited. Signaling was scored as a conspecific approached the R through different zones. To do this, the leaf was divided into four 1-cm zones (D–A), with D being the farthest and A being the closest to the resident. A transparent acetate sheet was positioned over the computer monitor screen, and zones scaled based on leaf dimensions. The scoring session was initiated when the V or J entered zone D (farthest) and was ended 30 s after the V or J entered zone A (closest). The following measurements were obtained: the number and types of signals produced by the R as a V or J traversed through each zone, the number and types of signals produced by the V or J as it passed through each zone, and the time spent by V or J in each zone.

Data from this experiment were analyzed to address predictions 4–6 outlined in the introduction: Prediction 4 stated that vibratory signals are associated with recruitment events. To test this, we asked the following questions about the R signals: (1) Does an R signal prior to being approached by a V or J? This was addressed by noting the % of trials that resulted in R signaling at least once while being approached by V or J through any of the zones; (2) How do signal rates change as V or J approach? Changes in R overall signal rates (regardless of signal type) were measured as V or J approached across the four zones and compared using ANOVA and Tukey’s HSD test (at $p = 0.05$; PROC GLM; SAS software, SAS Institute, Cary, NC, USA); and (3) How do the types of R signals change as a V or J approaches? This was analyzed by comparing the proportion of signal types across zones using χ^2 contingency table (4×4 ; $p = 0.05$; PROC FREQ; SAS software, SAS Institute, Cary, NC, USA). We also asked questions about V and J signaling: (4) Do V or J signal? This was reported as the percentage of V + J (i.e., V and J combined) that signal at least once during the trials; (5) Do V + J signal rates change as they approach R? Rates were calculated as for the R signaling and results also analyzed using ANOVA and Tukey’s HSD test (at $p = 0.05$); and (6) Do the types of V + J signals differ between zones as they approach the resident? This was analyzed by comparing the proportion of signal types per zone using a χ^2 contingency

table (4×4 ; $p = 0.05$). Prediction 5 stated that R would signal more than V or J. To address this, signal rates of R and V + J were subjected to ANOVA and Tukey’s HSD test ($p < 0.05$; transformed to $\log_{10}(x + 1)$; PROC GLM; SAS software, SAS Institute, Cary, NC, USA). We also compared the signal types and proportions for R and J + V using a χ^2 contingency table (4×4 ; $p = 0.05$; PROC FREQ; SAS software, SAS Institute, Cary, NC, USA). Prediction 6 stated that higher R signal rates will result in faster V + J recruitment. To test this, we measured the total number of signals generated by R from the time it started feeding and/or laying silk until a V or J reached within 0.5 cm. A regression analysis was conducted by plotting R signal rates against the time taken by V + J to reach R (PROC GLM; SAS software, SAS Institute, Cary, NC, USA). Finally, we examined if there were differences between signaling rates of J and V, regardless of their distance to the resident using ANOVA (transformed to $\log_{10}(x + 1)$; PROC GLM; SAS software, SAS Institute, Cary, NC, USA).

Results

Group formation and locations

During group formation experiments, larvae began to establish groups within the first 30 min. The proportion of the population residing in a group steadily increased over time (at 1 h, 48%; 2 h, 62%; 3 h, 70%; 4 h, 76%; 5 h, 84%; and 24 h, 86%; $N = 30$ trials with 172 individuals). The median time taken for group formation was 2 h and stable group sizes were reached within 5 h (Fig. 2c). Group sizes ranged from two to six and were negatively related to the numbers of groups formed, with a high number of smaller (i.e., less than four larvae) groups (Fig. 2d). Larvae do not always form shelters on the same region of the leaf (Fig. 2b), but the probability of forming a shelter on any of the four regions is different than expected by chance ($p < 0.01$), with a higher prevalence of shelters occurring at the outer edges.

Y-choice experiments

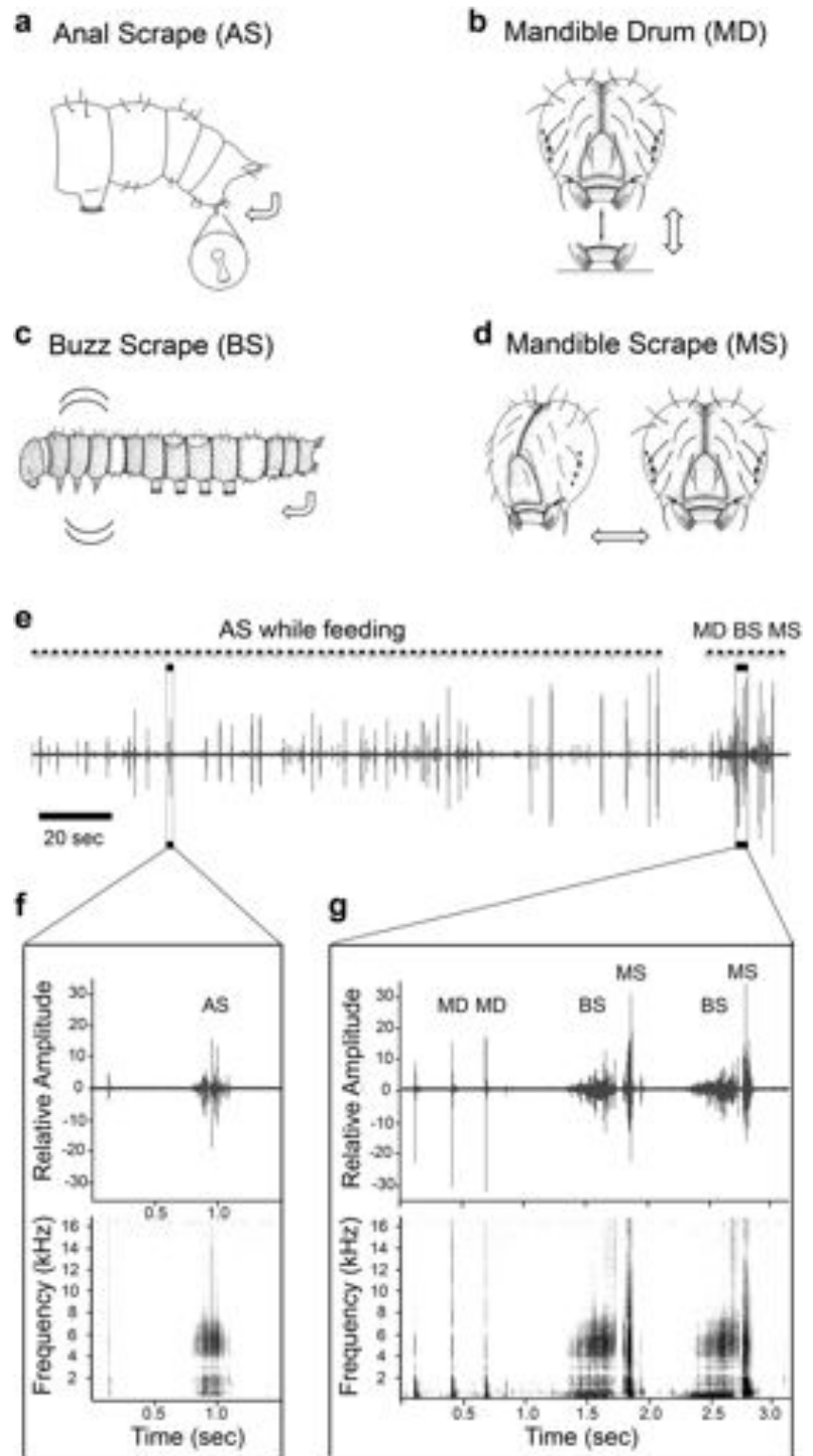
In the first Y-choice experiment (occupied vs. unoccupied), 26 of 30 test larvae chose the arm with larvae over the arm without larvae, showing a significant preference for occupied arms ($\chi^2 = 16.1$, $p < 0.0001$) (Fig. 3a). In the second experiment (previously occupied vs. unoccupied), 13 of 30 test larvae chose the previously occupied arm, not exhibiting a preference ($\chi^2 = 0.53$, $p < 0.47$) (Fig. 3b). These results indicate that early-instar larvae are attracted to conspecifics and not to putative chemical residues on the leaves.

Vibration signal types

Four distinct signal types were identified: anal scraping, mandible drumming, buzz scraping, and mandible scraping (Fig. 4; Supplementary materials: Sound S1, S2, Video S3,

Video S4). Anal scrapes (hereafter referred to as AS) are produced when the caterpillar contracts its terminal abdominal segments (A7–A10) anteriorly and scrapes a pair of thickened posterior proctor setae (PP1) located on the terminal abdominal segment across the leaf surface (Fig. 4a). Mandible drums

Fig. 4 Vibration signals. **a–d** Early-instar (I, II) *Drepana arcuata* produce four distinct types of vibrations using different body parts. **e** Laser vibrometer recording of a solitary resident in its shelter. The first part of the recording shows anal scraping (AS) while feeding; the second part shows the other three signal types (MD, MS, and BS) occurring as the resident is approached. **f–g** Waveforms and spectrograms showing the four signal types



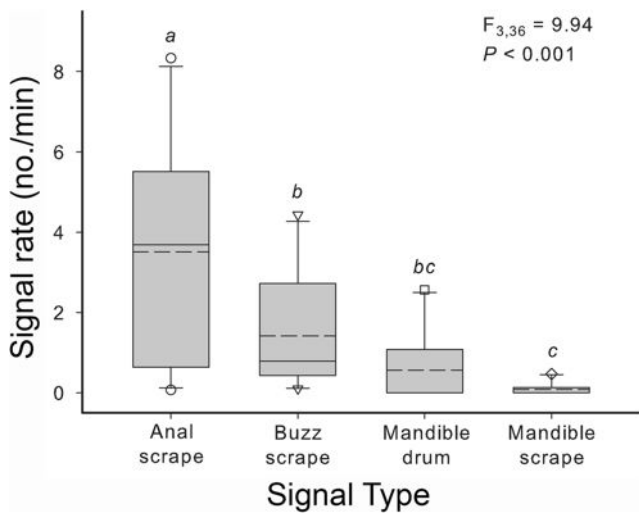


Fig. 5 Vibration signal types and rates for solitary early-instar *Drepana arcuata* larvae in shelters. Rates for each signal type are significantly different from each other (ANOVA and Fisher's LSD, $F_{3,36} = 9.94$, $p < 0.001$). Box plots indicate the range of data dispersion (lower and upper quartiles and extreme values), mean (dashed line), median (solid line), and outliers (symbols)

(hereafter referred to as MD) are produced by vertically striking the leaf surface with opened mandibles (Fig. 4b). A buzz scrape (hereafter referred to as BS) is produced when the caterpillar tremulates its body while anal scraping (Fig. 4c). Tremulation is a fast oscillation of the body, transmitting vibrations to the plant substrate (Hill 2008; Yack 2016). Mandible scrapes (hereafter referred to as MS) are produced by rapidly scraping opened mandibles laterally against the leaf surface (Fig. 4d).

Signaling rates and associated behavioral states of residents

Undisturbed residents generate 5.57 ± 3.69 signals/min (all signals combined; $N = 10$ individuals \times 30 min each). There were significant differences in the rates of different signal types ($F_{3,36} = 9.94$, $p < 0.001$; ANOVA and Fisher's LSD test at $p = 0.05$) (Fig. 5). Anal scrapes occurred at significantly higher rates than all other signal types, at 3.50 ± 0.90 signals/

Table 1 Vibratory signals in early-instar *D. arcuata* caterpillars during different behavioral states. Signal rates (#/min) \pm SD generated during four behavioral states—resting, walking, eating, and laying silk

	Buzz scrape	Anal scrape	Mandible scrape	Mandible drum
Resting	1.68 ± 3.79	0.09 ± 0.38	0.08 ± 0.29	0.77 ± 2.33
Walking	6.5 ± 3.83	0.29 ± 0.97	0.36 ± 0.87	1 ± 1.99
Eating	0	0	11.48 ± 7.55	0
Laying silk	0.29 ± 1.39	0	2.66 ± 4.08	0

min, followed by BS (1.42 ± 0.45), MD (0.56 ± 0.29), and MS (0.087 ± 0.051) (Fig. 5).

Signaling occurred during all four behavioral states—eating, laying silk, walking, and resting—although the types and rates of signals differed between these activities (Table 1; Fig. 6). Early-instar larvae feed by chewing on the leaf surface (i.e., skeletonization) and, while chewing, generate AS regularly at a rate of 11.5 ± 7.55 /min (Fig. 4e; Supplementary materials: Video S3). While constructing the silk shelter, AS was the most frequently occurring signal (2.66 ± 4.08 /min) (Supplementary materials: Video S3), with a small amount of BS, and no MD or MS. While adding silk to the shelter, the R's anal segment is in contact with the leaf and AS is performed while the upper part of the body is on the silk shelter (Supplementary materials: Video S3), whereas, in order to BS, the caterpillar briefly descends from the silk shelter, performs BS, and then resumes silk-laying activity. While walking, BS was the most common signal (6.5 ± 3.8 signals/min), with small amounts of the other three signal types. Caterpillars generated BS while walking within the shelter from one activity to the next. While resting, the most common signal was BS (1.68 ± 3.79 signals/min), with smaller amounts of the other three signal types (Fig. 6).

We also asked if the rates of each signal type—AS, BS, MD, and MS—differed across the four behavior states. MANOVA indicated overall significant differences in signal rates across behavioral states (Wilks' lambda = 0.0713, $F_{\text{appr.}} = 9.48$, $df_{\text{num};\text{den}} = 12;66$, $p < 0.001$). A CVA indicated that the signaling rate patterns of larva while either walking or resting are indistinguishable but differ from those when the larva is eating and laying silk, which also differ from each other (Table 2; Fig. 6a). Such differences were mainly due to AS and BS, which were the main contributors for the composition of the two significant CVA axes (i.e., provided higher canonical loads; Table 2); AS and BS were significantly different across each of the four behavioral states ($F_{3,30} > 12.95$, $p < 0.05$) with AS prevailing while the larvae are eating and BS prevailing when the larvae are walking (Fig. 6b, c).

Vibratory signaling associated with group formation

Of the 14 group formation experiments, four were excluded owing to poor quality of laser recordings due to changes in the leaf position over the course of the experiment. Of the 10 remaining experiments, data for the first 20 established R that were joined or visited were analyzed. For these 20 R, it took 41.17 ± 38.02 min from the beginning of the trial to become established on the leaf. Of these 20 R, 10 were joined and 10 were visited within the observation period. Mean times for V and J to arrive at R's shelter were 6 ± 7.04 min (median time of 3 min) and 25 ± 20.65 min (median time of 24 min), respectively.

Fig. 6 Relationships between behavioral states (i.e., not signaling activities) and vibratory signals in early-instar *Drepana arcuata* larvae. **a** Ordination diagram of the relationship among behaviors based on the rate of vibratory signals; vibration signal rate profiles during walking and resting are indistinguishable from each other but different from eating and laying silk, which are different from each other. **b, c** Rates of anal scrape and buzz scrape signals, respectively, during the four non-signaling behavioral states. Anal scrape and buzz scrape differ significantly during four different behaviors ($F_{3,30} > 12.95$, $p < 0.05$), with anal scrape occurring significantly more during eating, whereas buzz scrape occurring more during walking. *Box plots (b, c)* indicate the range of data dispersion (lower and upper quartiles and extreme values), mean (*dashed line*), median (*solid line*), and outliers (*symbols*)

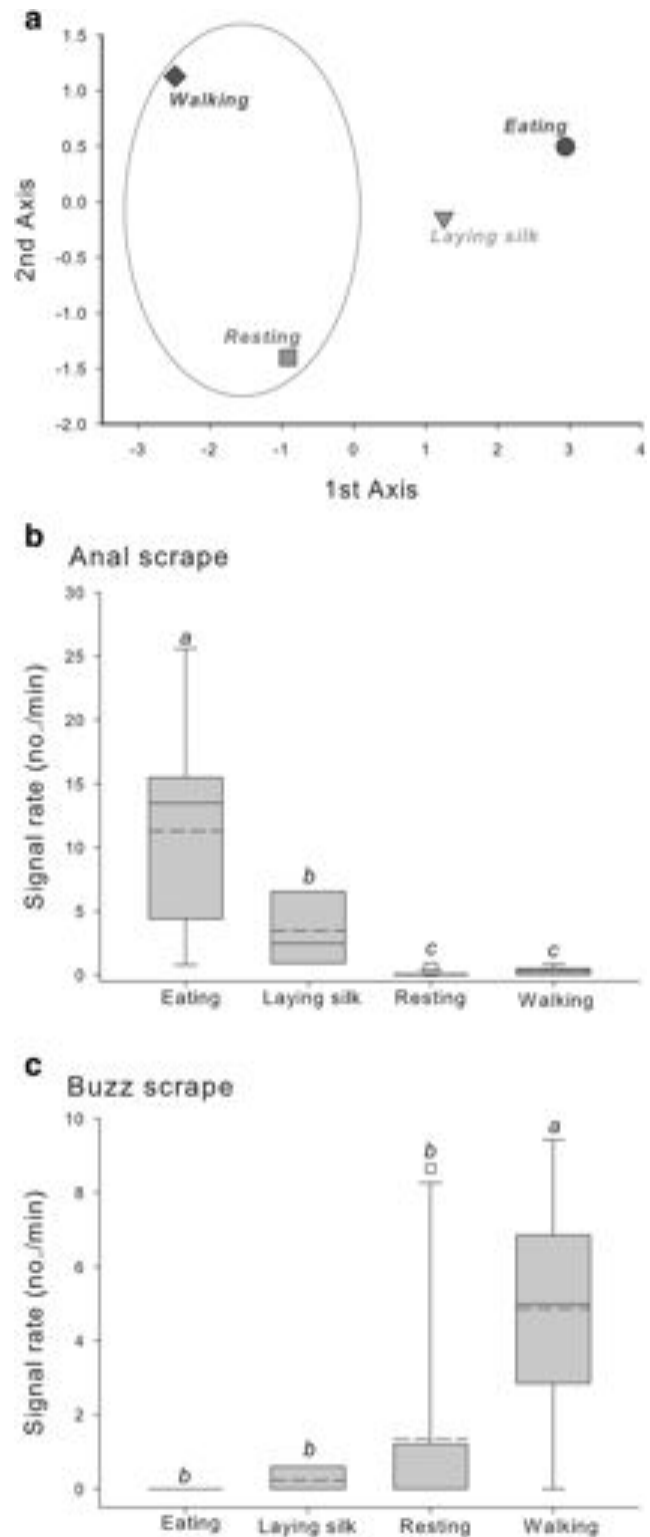
Resident signaling during recruitment events All 20 residents (100%) generated vibratory signals prior to being joined or visited (Fig. 4; Supplementary materials: Video S4). As the potential recruit approached from zone D (farthest from resident) to A (closest to resident), R signal rates increased significantly (ANOVA and Tukey's HSD test; $F_{3,76} = 6.89$, $p < 0.001$) (Fig. 7a). The proportion of signal types produced by the R also changed significantly as it was approached (χ^2 contingency test; $\chi^2 = 62.55$, $df = 9$, $p < 0.001$) with AS prevailing at farther distances (zone D) and BS prevailing at closer distances (zone A) (Fig. 7a).

Visitor and joiner signaling during recruitment events Recruits also signaled, but at lower rates and with different signal types than R (Fig. 7b). V + J signaled at least once in 60% of the 20 events, and signal rates varied significantly between zones (ANOVA and Tukey's HSD test; $F_{3,72} = 3.50$, $p = 0.02$), although much less so than observed in R signals (Fig. 7a, b). However, the proportion of signal types did not vary significantly from zones D to A ($\chi^2 = 4.07$, $df = 9$, $p = 0.91$) with BS prevailing throughout (Fig. 7b). Signaling rates were higher overall for residents (7.10 ± 1.40 signals/min) than V + J (2.51 ± 0.62 signals/min) ($F_{1,152} = 14.99$, $p < 0.001$), and this difference was even more pronounced at closer distances ($F_{3,152} = 7.19$, $p < 0.001$); significant differences prevail for each signal type and proportion, which vary with distance between R and recruits (V + J) ($\chi^2 = 85.47$, $df = 9$, $p < 0.001$) (Fig. 7a, b). Signal rates of J (3.22 ± 1.23 signals/min) were higher than those of V (1.57 ± 0.63 signals/min) ($F_{1,72} = 5.36$, $p = 0.02$). There was a significant negative relationship between the R's signal rate and the time taken by the V and J to reach within 0.5 cm of R ($R^2 = 0.35$, $F_{1,10} = 5.33$, $p = 0.04$) (Fig. 8).

Discussion

"...the largest gap in our knowledge of social Lepidoptera lies in the feature most essential to their sociality: communication." (Costa and Pierce 1997)

Social interactions in caterpillars, like for many of the "other social insects," can be complex, but a full understanding of



this complexity hinges upon our understanding of their communication systems (Costa and Pierce 1997; Cocroft and Hamel 2010; Costa 2006). To the best of our knowledge, there has been no evidence to date that vibrations play a role in coordinating social activities between conspecifics in larval

Table 2 Canonical loadings from canonical variate analysis (CVA) of the signal rates of early-instar *Drepana arcuata* associated with four behavioral states (eating, laying silk, resting, and walking) (MANOVA: Wilks' lambda = 0.0713, $F_{\text{appr.}} = 9.48$, $df_{\text{num;den}} = 12;66$, $p < 0.001$)

Signals	Canonical axes	
	1st	2nd
Anal scrape	0.76	0.61
Buzz scrape	-0.42	0.51
Mandible drum	-0.14	-0.15
Mandible scrape	-0.13	0.19
Proportion of variance explained	0.81	0.18
$F_{\text{appr.}}$	9.48	4.27
Degrees of freedom (num;den)	12;66	6;52
p	<0.001	0.001

Anal scraping and buzz scraping were the main contributors to the two significant CVA axes, highlighted in bold

Lepidoptera. Considering that there are several reports of larval Lepidoptera using vibrations for territoriality and mutualistic interactions with ants (see Travassos and Pierce 2000; Costa 2006; Scott et al. 2010; Yack 2016), the lack of evidence for vibratory-mediated sociality is surprising. This study provides the first evidence that caterpillars use vibrations to coordinate social activities and, specifically, to advertise food and shelter sites to conspecifics.

Early-instar caterpillars actively form groups

Our results support the hypothesis that early-instar *D. arcuata* actively form groups. During group formation experiments, larvae began assembling in shelters within the first 30 min, and by 5 h, 84% had formed groups. These tiny caterpillars (1–2 mm in length) travelled long distances (up to an estimated 420× their body lengths) before settling in a group. Also, while larvae prefer to form shelters on the edges of a leaf, most likely because leaf edges are more easily drawn together with silk, there was not one consistent location chosen, ruling out the likelihood that larvae simply aggregate at the same location based on physical cues. In Y-choice trials, test larvae chose to join an arm of a birch leaf with conspecifics over an unoccupied arm. Collectively, these results support the hypothesis that larvae actively seek out conspecifics to form groups. Adult female *D. arcuata* and congeners lay eggs in rows at various locations of the tree, including the upper and lower surfaces of leaves, petioles, and on twigs and branches, and, upon hatching, wander away from the egg cases before becoming established on a leaf (Bryner 1999; unpublished data JEY). Despite where eggs are laid, most neonate Lepidoptera larvae wander in search of a location to settle (Zalucki et al. 2002) and groups form and dissolve as food resources are depleted or as they are confronted with changing

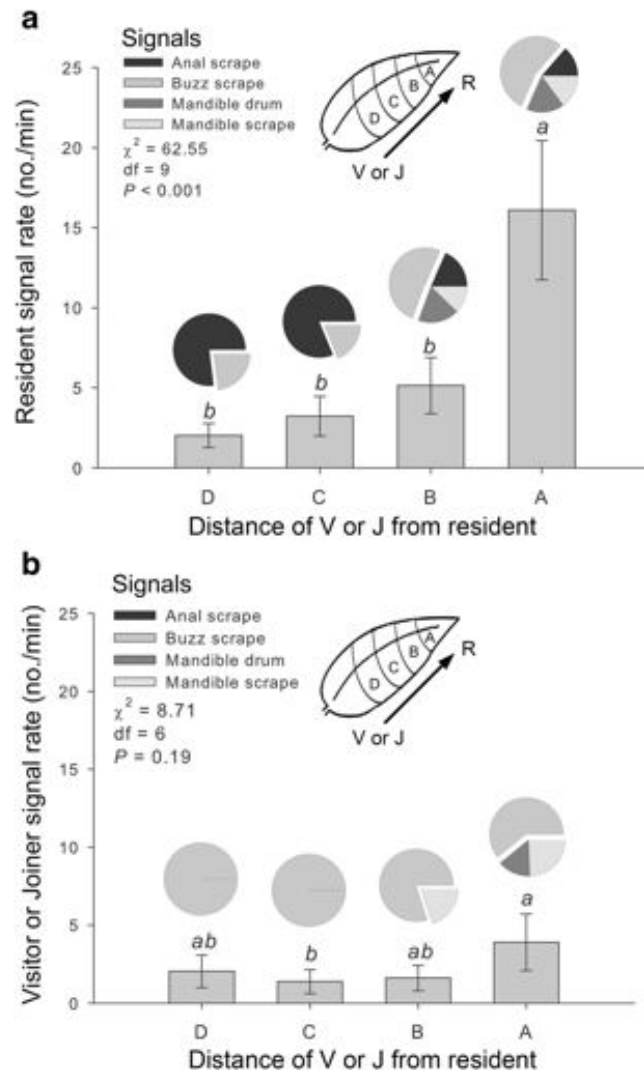


Fig. 7 Vibratory signaling during grouping events in early-instar *Drepana arcuata*. **a** Vibration signal rates of the resident (R) as it is approached by a potential recruit (ultimate visitor (V) or joiner (J)) as the latter passes through zones D to A (far to near the resident). Signal rates and proportion of signal types are significantly different across zones (ANOVA and Tukey's test, $F_{3,76} = 6.89$, $p < 0.001$; $\chi^2 = 62.55$, $df = 9$, $p < 0.001$). The leaf inset is a schematic representation of distance zones D to A with the R shown at the leaf tip. However, in trials, R may have been at any location on the leaf. **b** Vibration signal rates of the potential recruit as the latter approaches the resident. Signal rates vary significantly across zones (ANOVA and Tukey's test, $F_{3,72} = 3.50$, $p < 0.02$); however, there is no significant difference in the proportion of signal types across zones D to A ($\chi^2 = 8.71$, $df = 6$, $p < 0.19$)

environmental conditions. To establish social groups, communication mechanisms are required. We propose that for *D. arcuata*, vibratory communication signals play an important role in this process.

Vibrations advertise food and shelter sites

Our results indicate that vibratory signals function to advertise food and shelter sites to conspecifics, resulting in the

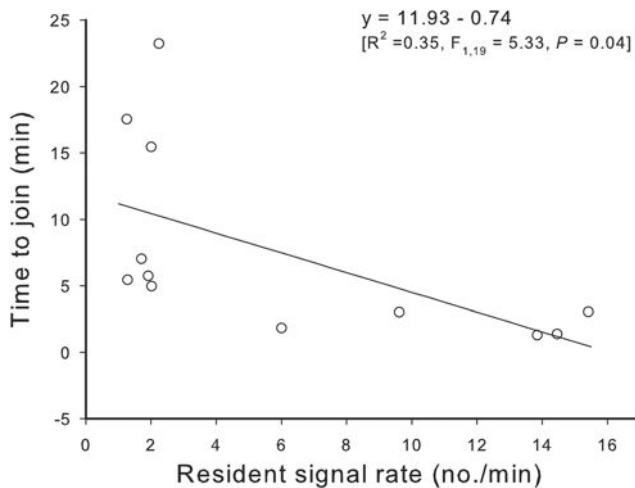


Fig. 8 Vibration signal rates relative to the duration and success of a recruitment event. Residents with overall higher signal rates recruit conspecifics faster than those with lower rates ($R^2 = 0.35$, $F_{1,10} = 5.33$, $p = 0.04$)

formation of small social groups. Acoustic signals are employed by many vertebrates to attract conspecifics to food sources (Bradbury and Vehrencamp 2011), but comparatively little is known about how insects use sounds or vibrations in this context. Vibratory recruitment to a food source is best known for the eusocial insects (e.g., dances of honeybees) (Hunt and Richard 2013), but in the “other social insects,” examples are limited to a few species of nomadic sawfly larvae and treehopper nymphs (Cocroft and Hamel 2010). Until now, there have been no reported examples of vibratory recruitment signals to food sources in caterpillars. Moreover, the vibratory communication system in *D. arcuata*, with four distinct signal types and changing signal dynamics during recruitment, rivals the complexity of food advertisement signals reported for eusocial insects (cf. Hunt and Richard 2013). To gain insight into this novel form of communication in caterpillars, we discuss our results in the context of other acoustic food advertisement signals in both vertebrates and invertebrates.

Several lines of evidence support the hypothesis that vibrations function to advertise food and shelter resources to conspecifics in early-instar *D. arcuata*: First, solitary Rs always generate signals prior to being approached; second, the R signals significantly more than does the recruit; third, the most frequent signals, AS and BS, are strongly associated with feeding and laying silk; and fourth, higher R signal rates resulted in faster recruitment times. Similar results are reported for advertisement calls in primates (e.g., Caine et al. 1995; Di Bitetti 2003, 2005; Gros-Louis 2004; Slocombe et al. 2010) and birds (e.g., Elgar 1986; Mahurin and Freeberg 2009; Suzuki 2012), where individuals that first locate a resource call to potential recruits and higher call rates result in more successful recruitment. In the eusocial insects, vibratory recruitment signals to food sources are mostly reported for

central-place foragers, where a scout advertises a remote food source by generating vibrations (see Hunt and Richard 2013; Hrnčir and Barth 2014). In the other insect societies (i.e., the non-eusocial insect societies), vibratory-mediated recruitment to food sources has been reported only for a few species of treehopper nymphs and sawfly larvae. In the treehopper *Calloconophora pinguis*, a nymph that locates a food source generates vibrations to recruit conspecifics to the feeding site and higher quality of food is communicated with higher signal rates (Cocroft 2005). In one species of sawfly, *Hemichroa crocea*, larvae produce vibrational signaling while feeding to attract conspecifics to a feeding site (Hograefe 1984). Similar to our observations in *D. arcuata*, sawfly larvae “scratch” their terminal abdominal segment on the leaf surface while feeding, and, as for the abovementioned treehopper nymphs, signal rates are proposed to relate to leaf quality. Based on these comparisons to previous studies, we propose that *D. arcuata* residents signal to advertise food and shelter resources to potential recruits and that variation in signal rates functions to advertise the quality of the shelter or food resource. Future studies should investigate the relationship between site quality, signal rates, and recruitment times by manipulating leaf conditions and conducting playback experiments.

Our results also showed variation in signaling patterns as R was approached by a potential recruit: First, R signal rates increased as a recruit approached the shelter; second, R signal types changed as the recruit came closer; and third, potential recruits also signaled, but at much lower rates than R and with different signal types. We have not been able to find examples of similar signaling patterns between founders of resources and recruits in other insects, emphasizing the need for more detailed studies such as those conducted with birds and primates (e.g., Clay et al. 2012; Szimpl et al. 2015). Variation in the types and rates of signals by participants during recruitment may serve a number of functions, including communicating levels of motivation, calling to additional recruits, species or kin recognition, resource localization, or resource quality.

Alternative hypotheses for group formation mechanisms

Our results support the hypothesis that vibratory signals mediate group formation, but it is important to recognize that locating a shelter and feeding site is likely a complex process involving a series of behaviors and different sensory modalities. While searching for a shelter site, a larva first typically wanders along the leaf edge, and upon approaching, a resident in a shelter may experience not only vibratory signals but also chemical (olfactory or gustatory), visual, or other forms of mechanical stimuli. We consider alternative or complementary mechanisms based on comparisons with other larval insects. Recruitment to food sources by means of chemical trails is the most commonly reported for caterpillars, but in all reported examples, these are central-place or nomadic foragers

that move together to new food sources or move back and forth from the food source to the shelter (Costa and Pierce 1997; Costa 2006). Because early-instar *D. arcuata* are patch-restricted foragers (i.e., remain in the same patch), chemical trails would not be likely because they feed within the shelter. It is possible that olfactory cues or pheromones deposited on the silk or in frass attached to the shelter are detected by potential recruits. Vision seems to be an unlikely mechanism involved in recruitment, as larvae possess fairly simple optical systems (Warrant et al. 2003). However, visual cues may be involved in the searching process, such as detecting the leaf edges during wandering (Gilbert 1994). We rule out the involvement of tactile cues in *D. arcuata* larvae because there was no physical contact observed between larvae during recruitment events. We conclude that vibratory signals play an important role as recruitment signals in *D. arcuata* but surmise that other signals or cues, such as pheromones in the silk shelter or frass, may also contribute to group formation.

Alternative hypotheses for vibratory signaling

Our results support the hypothesis that vibratory signals advertise food and shelter resources to conspecifics. However, alternative hypotheses explaining the functions of these signals should be considered. One hypothesis is that vibrations function as distress signals to recruit help (Cocroft 1996; Travassos and Pierce 2000). However, there is no evidence at present to support this hypothesis in *D. arcuata*. First, when disturbed by plucking at the shelter to simulate an invertebrate predator, larvae become silent rather than increase their signal rates, and there is no evidence of group antipredator defenses such as dropping, regurgitation, thrashing, or flicking (Matheson 2011; unpublished data JEY). Second, *D. arcuata* larvae are not tended by ants, and parental care is absent. Another hypothesis is that vibratory signals could be used to enhance feeding by a vibratome effect as observed in ants (Tautz et al. 1995), but this is not possible in *D. arcuata* as chewing does not coincide directly with AS or any of the other signals. Another hypothesis is that signals function as territorial signals. If so, it would be predicted that (1) R would generate signals only when approached by an intruding conspecific, and this is not the case, as R signals even in the absence of a conspecific, and (2) the approaching conspecific would not join and share the shelter with the resident larva, and we have shown that joining occurs following signaling of the resident. While our results do not support the hypothesis that signaling functions as a territorial defense signal by excluding a conspecific from the shelter, it remains possible that within the group, larvae maintain their own territories. However, this remains to be tested experimentally and would require analysis of established groups of two or more individuals, which was beyond the scope of the current study.

Conclusions

Caterpillar social groups are diverse and exhibit complex interactions between conspecifics for purposes of foraging, defense, and shelter construction (Costa and Pierce 1997). Yet, like for many of the “other social insects,” there is still a dearth of information on communication (Costa 2006). Scientists are only beginning to appreciate the importance of vibratory communication in insects, which is believed to be particularly important for close-range interactions in juvenile insects, such as caterpillars, which are substrate-bound (Cocroft and Rodriguez 2005; Yack 2016). Cocroft and Hamel (2010) proposed that vibratory recruitment to feeding sites is probably widespread in the “other insect societies,” but at present, there are few documented examples. Our study provides the first example in caterpillars. However, we anticipate that vibratory communication in early-instar *D. arcuata* functions beyond group recruitment. In the current study, we focused on the role of vibratory signals only during the initial stages of group formation. However, once groups are formed, complex vibratory interactions occur between individuals and these interactions are hypothesized to play roles in division of labor, shelter construction, taking turns to feed, spacing, and orientation (Matheson 2011). The importance of vibratory signals and cues remains poorly documented for most group-living insects, and future studies should combine detailed behavioral observations with experimental manipulations and playback studies to gain a full appreciation of the rich vibratory landscapes of these insects.

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References

- Blumstein DT, Daniel JC, Evans CS (2010) JWatcher V1.0. University of California, Los Angeles and Macquarie University, Sydney
- Bradbury JW, Vehrencamp SL (2011) Principles of animal communication, 2nd edn. Sinauer Associates, Sunderland
- Bryner R (1999) Drepanidae - Drépanidés. In Les Papillons et leur Biotopes: Espèces; Danger qui les menacent; Protection. Suisse et régions limitrophe, vol. 2 (ed. L. S. p. I. P. d. I. Nature), pp. 447–476 Switzerland: Pro Natura
- Caine NG, Addington RL, Windfelder TL (1995) Factors affecting the rates of food calls given by red-bellied tamarins. Anim Behav 50: 53–60
- Clay Z, Smith CL, Blumstein DT (2012) Food-associated vocalizations in mammals and birds: what do these calls really mean? Anim Behav 83:323–330
- Cocroft RB (1996) Insect vibrational defence signals. Nature 382:679–680

- Cocroft RB (2005) Vibrational communication facilitates cooperative foraging in a phloem-feeding insect. *P Roy Soc Lond B Bio* 272: 1023–1029
- Cocroft RB, Hamel JA (2010) Vibrational communication in the “other insect societies”: a diversity of ecology signals, and signal functions. In: O’Connell-Rodwell CE (ed) *The use of vibrations in communication: properties, mechanism and function across taxa*. Transworld Research Network, Kerala, India, pp 47–68
- Cocroft RB, Rodríguez RL (2005) The behavioral ecology of insect vibrational communication. *Bioscience* 55:323–334
- Costa JT (2006) *The other insect societies*. The Belknap Press of Harvard University Press, Cambridge
- Costa JT, Pierce NE (1997) Social evolution in the Lepidoptera: ecological context and communication in larval societies. In: Choe JC, Crespi BJ (eds) *The evolution of social behavior in insects and arachnids*. Cambridge University Press, Cambridge, pp 407–422
- Di Bitetti MS (2003) Food-associated calls of tufted capuchin monkeys (*Cebus apella nigrinus*) are functionally referential signals. *Behaviour* 140:565–592
- Di Bitetti MS (2005) Food-associated calls and audience effects in tufted capuchin monkeys, *Cebus apella nigrinus*. *Anim Behav* 69:911–919
- Dussutour A, Nicolis SC, Despland E, Simpson SJ (2008) Individual differences influence collective behaviour in social caterpillars. *Anim Behav* 76:5–16
- Elgar MA (1986) House sparrows establish flocks by giving chirrup calls if the resources are divisible. *Anim Behav* 34:169–174
- Fitzgerald TD, Peterson SC (1988) Cooperative foraging and communication in social caterpillars. *Bioscience* 38:20–25
- Gilbert C (1994) Form and function of stemmata in larvae of holometabolous insects. *Annu Rev Entomol* 39:323–349
- Gros-Louis J (2004) Responses of white-faced capuchins (*Cebus capucinus*) to naturalistic and experimentally presented food-associated calls. *J Comp Psychol* 118(4):396–402
- Guedes RNC, Matheson SM, Frei B, Smith ML, Yack JE (2012) Vibration detection and discrimination in the masked birch caterpillar (*Drepana arcuata*). *J Comp Physiol A* 198:325–335
- Hill PSM (2008) *Vibration communication in animals*. Harvard University Press, London
- Hograefe T (1984) Substrat-stridulation bei den koloniebildenden Blattwespenlarven von *Hemichroa crocea* (Geoff.) (Hymenoptera: Tenthredinidae). *Zool Anz* 213:234–241
- Hmcir M, Barth FG (2014) Vibrational communication in stingless bees (Meliponini): the challenge of interpreting the signals. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) *Studying vibrational communication*. Springer, New York, pp 349–374
- Hunt JH, Richard FJ (2013) Intracolony vibroacoustic communication in social insects. *Insect Soc* 60:403–417
- Mahurin EJ, Freeberg TM (2009) Chick-a-dee call variation in Carolina chickadees and recruiting flockmates to food. *Behav Ecol* 20:111–116
- Matheson SM (2011) *Vibratory mediated spacing in groups of insect larvae (Drepana arcuata, Lepidoptera; Scolytus multistriatus, Coleoptera)*. M.Sc. Dissertation, Carleton University
- Prokopy RJ, Roitberg BD (2001) Joining and avoidance behavior in nonsocial insects. *Annu Rev Entomol* 46:631–635
- Rose AH, Lindquist OH (1997) *Insects of eastern hardwood trees*. Canadian Forestry Service, Ottawa, Forestry Technical Report 29
- Scott JL, Kawahara AY, Skevington JH, Yen SH, Sami A, Smith ML, Yack JE (2010) The evolutionary origins of ritualized acoustic signals in caterpillars. *Nat Commun* 1:1–9
- Slocombe KE, Kaller T, Turman L, Townsend SW, Papworth S, Squibbs P, Zuberbühler K (2010) Production of food-associated calls in wild chimpanzees is dependent on the composition of the audience. *Behav Ecol Sociobiol* 64:1959–1966
- Suzuki TN (2012) Long-distance calling by the willow tit, *Poecile montanus*, facilitates formation of mixed-species foraging flocks. *Ethology* 118:10–16
- Szipl G, Boeckle M, Wascher CAF, Spreafico M (2015) With whom to dine? Ravens’ responses to food-associated calls depend on individual characteristics of the caller. *Anim Behav* 99:33–42
- Tautz J, Roces F, Hölldobler B (1995) Use of a sound-based vibratome by leaf-cutting ants. *Science* 267:84–87
- Travassos MA, Pierce NE (2000) Acoustics, context and function of vibrational signaling in a lycaenid butterfly-ant mutualism. *Anim Behav* 60:13–26
- Virant-Doberlet M, Čokl A (2004) Vibrational communication in insects. *Neotrop Entomol* 33:121–134
- Warrant EJ, Kelber A, Kristensen NP (2003) Eyes and vision. In: Kristensen NP (ed) *Handbook of zoology, Part 36, Lepidoptera, moths and butterflies, vol 2: morphology, physiology and development, vol IV*. Walter de Gruyter, Berlin/New York, pp 325–359
- Yack JE (2016) Vibrational signaling. In: Pollack GS, Mason AC, Fay RR, Popper AN (eds) *Springer handbook of auditory research: insect hearing*. Springer, New York, pp 99–123
- Yack JE, Gill S, Drummond-Main D, Sherratt TN (2013) Residency duration and shelter quality influence vibratory signaling displays in a territorial caterpillar. *Ethology* 120:354–364
- Yack JE, Smith ML, Weatherhead PJ (2001) Caterpillar talk: acoustically mediated territoriality in larval Lepidoptera. *P Natl Acad Sci USA* 98:11371–11375
- Zalucki MP, Clarke AR, Malcolm SB (2002) Ecology and behavior of first instar larval Lepidoptera. *Annu Rev Entomol* 47:361–393