Caterpillar talk: Acoustically mediated territoriality in larval Lepidoptera

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We provide evidence for conspecific acoustic communication in caterpillars. Larvae of the common hook-tip moth, *Drepana arcuata* (Drepanoidea), defend silk nest sites from conspecifics by using ritualized acoustic displays. Sounds are produced by drumming the mandibles and scraping the mandibles and specialized anal "oars" against the leaf surface. Staged interactions between a resident and intruder resulted in escalated acoustic "duels" that were typically resolved within minutes, but sometimes extended for several hours. Resident caterpillars generally won territorial disputes, regardless of whether they had built the nest, but relatively large intruders occasionally displaced residents from their nests. All evidence is consistent with acoustic signaling serving a territorial function. As with many vertebrates, ritualized signaling appears to allow contestants to resolve contests without physical harm. Comparative evidence indicates that larval acoustic signaling may be widespread throughout the Lepidoptera, meriting consideration as a principal mode of communication for this important group of insects.

Caterpillars are key constituents of most terrestrial ecosystems and include some of the most serious pests of crops and forests. Their ecological and economic importance has promoted extensive research on how they interact with heterospecifics, including host-plants, predators, and parasitoids (1, 2). Conspecific communication among caterpillars has received little attention, however, despite many species living gregariously, where interactions involving group defense, aggregation, competition, and foraging may be crucial for survival (3, 4). The extent to which caterpillars communicate with one another, the modalities used, and the function of any communication all remain largely unexplored (3). Pheromones and tactile cues have been described for a few species (3, 5), and vision is unlikely to be an important modality for communication, because of the simplistic nature of the larval optical system (6). Acoustic signaling has not been investigated rigorously, despite its obvious potential for substrate-bound organisms like caterpillars.

Drepana arcuata Walker is locally common throughout deciduous woodlands of northeastern North America, feeding primarily on birch (*Betula*) and alder (*Alnus*) (7). Despite the common occurrence of this species, little is known of its larval behavior and life history. Sounds produced by modified anal structures had been noted long ago for *Drepana* (8, 9), but the functional significance of these sounds had not been investigated. On discovering that *D. arcuata* larvae produced a combination of three distinct sounds, we investigated the nature of these signals and the context in which they occurred, and tested the hypotheses that *D. arcuata* are territorial and that the acoustic signals serve in territoriality. If *Drepana* larvae are territorial, then: (*i*) residents should maintain exclusive use of nests, (*ii*) residents should defend nests against conspecifics, (*iii*) intruders should only rarely displace residents, and (*iv*) intruders experimentally given vacant nests should defend those nests against original owners (10, 11). If acoustic displays signal territory ownership, then displays will be: (*i*) restricted to the territory and (*ii*) given only in response to intruding conspecifics.

Materials and Methods

Animals. Adult *D. arcuata* were collected from the wild between July and September 1999 and May and September 2000 at the Queen's University Biology Station in eastern Ontario, Canada (45 37′N, 76 13′W). Females (>30) laid eggs on cuttings of paper birch (*Betula papyfera*), or on potted birch saplings maintained in outdoor enclosures. Of the *ca.* 600 larvae reared, 60 were used for unmanipulated natural history observations, with the remainder providing subjects for experiments. All larvae used in experiments were those occupying solitary nests, in their fourth and fifth larval instars.

Sound Recordings. Airborne sounds were recorded with a Sony DAT PCM-M1 at a sampling rate of 44.1 kHz, using a Sony ECM-MS957 microphone placed 1–2 cm from the back of the leaf. Substrate vibrations were recorded with a ceramic phonograph cartridge in contact with the leaf. The signals were amplified and recorded onto a Sony DAT. Temporal and spectral characteristics of signals were analyzed by using the CANARY BIOACOUSTICS RESEARCH PROGRAM (Cornell Laboratory of Ornithology, Ithaca, NY).

Behavioral Experiments. *Staged encounters.* Intruders were kept in a container with bare birch twigs for 15–20 min. Residents were observed for 5 min before introducing the intruder. Using a fine paintbrush, intruders were transferred from the holding container to the petiole of the leaf, where the petiole attaches to the branch. The interaction between the two individuals was monitored until 5 min after one contestant left (i.e., when one contestant "won" the encounter). When both the resident and the intruder stayed, observation continued until at least 30 min after the intruder began to construct its own nest. Weights of contestants (recorded following each trial) in the 53 encounters were similar overall (mean difference = −0.003 g; paired *t* test, *t* = −0.44, *P* = 0.66), but weight asymmetry was intentionally varied (SD ± 0.05, range = 0.131 to −0.146 g). Trials were videotaped with a Sony TR7000 Digital Handicam and a remote Sony audio microphone (ECM-MS907) placed 1–2 cm behind the leaf. For trials longer than 40 min, video recording was occasionally interrupted, and observations recorded by hand. Videotapes from the first 30 trials were analyzed to quantify signaling patterns in each consecutive 5-sec interval. Variation in signaling rates with distance between contestants was quantified for the first 15 trials in which the contestants' heads came in contact, using three stages: 20 sec following the intruder's head reaching the leaf, 20 sec following the intruder being midway on the leaf, and 20 sec following initial contact (see Fig. 3).

Squatter experiment. Established residents were removed and placed in holding containers. Squatters were held in containers for 15–20 min before being placed on the petioles of the leaves
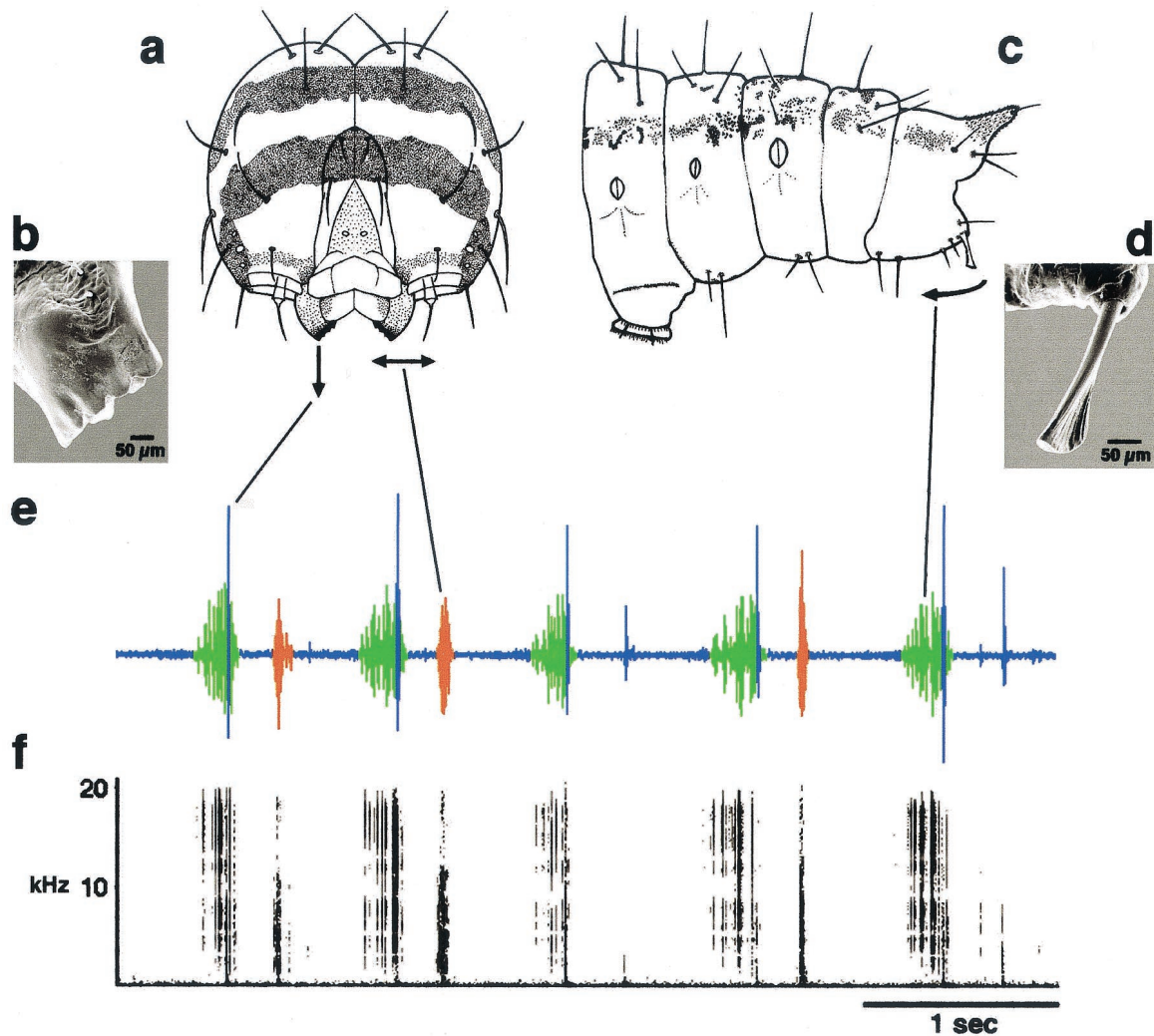
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Fig. 1. Acoustic signals and sound-producing structures of *D. arcuata* larvae. (*a* and *b*) "Mandibular drumming" involves repeatedly striking a leaf with the serrated edges of opened mandibles. "Mandibular Scraping" occurs when the mandibles are scraped laterally on the leaf surface. (*c*) A lateral view of abdominal segments 6–10, showing the specialized oars (*d*) that are dragged anteriorly across the leaf surface to produce a scratching sound (anal proleg swipe). (*e* and *f*) Oscillogram and spectrogram illustrating the three signal components (blue, mandibular drumming; green, anal proleg swipes; orange, mandibular scraping).

with vacant nests. Once squatters established residency by moving into nests (from 3–9 min), original residents were placed on the petioles of leaves with their respective nests and subsequent interactions videotaped.

Distance experiment. A small branch was stripped of all but one leaf with an occupied nest. Another larva of equal size was placed at the base of the branch, and the behavior of the two individuals was videotaped as the intruder approached the resident.

Results

Female moths oviposit in clusters [7.6 ± 5.8 eggs per leaf (mean ± SD); range 3–29; *n* = 35) on foliage. Hatchlings typically form communal silken nests within which they feed, expanding the nest as they grow. As the leaf is consumed, caterpillars in their third (≈7–12 days old) and subsequent instars establish solitary nests on fresh neighboring leaves. Leaf edges are tied with silk threads and a silken mat is laid over the leaf surface. After initial construction (1 h), larvae add to their nest and remove frass, leaving the nest only to feed at the leaf edge. Once the leaf and nest are consumed, larvae establish new

nests nearby, using and consuming several leaves and nests before pupating.

A resident larva confronted with an approaching conspecific typically stops feeding, backs into its nest, and begins signaling. The first of the three distinct signals used is a rasping sound (366.9 ± 145.4 ms; *n* = 112 from 30 individuals) produced by dragging a pair of chitinous oar-shaped structures across the leaf surface by using anteriorly directed abdominal contractions (Fig. 1). The second signal is staccato drumming (15.9 ± 12.5 ms; *n* = 180 from 30 individuals), produced by striking the leaf sharply with the serrated "incisors" of the open mandibles (Fig. 1 and Movie 1, which is published as supporting information on the PNAS web site, www.pnas.org). Signals one and two are often coordinated, with 1–8 mandibular strikes occurring during a single anal swipe (Movies 2 and 3, which are published as supporting information on the PNAS web site). A third, rasping sound (59.2 ± 17 ms; *n* = 49 from 22 individuals) is produced when the opened mandibles are swept across the leaf surface (Fig. 1 and Movies 1 and 2). The energy of the signals is spread over a broad bandwidth (Fig. 1). Larvae produce sounds audible to humans up to 4 m in still air, and mandibular scrapes are the most audible of the three signal
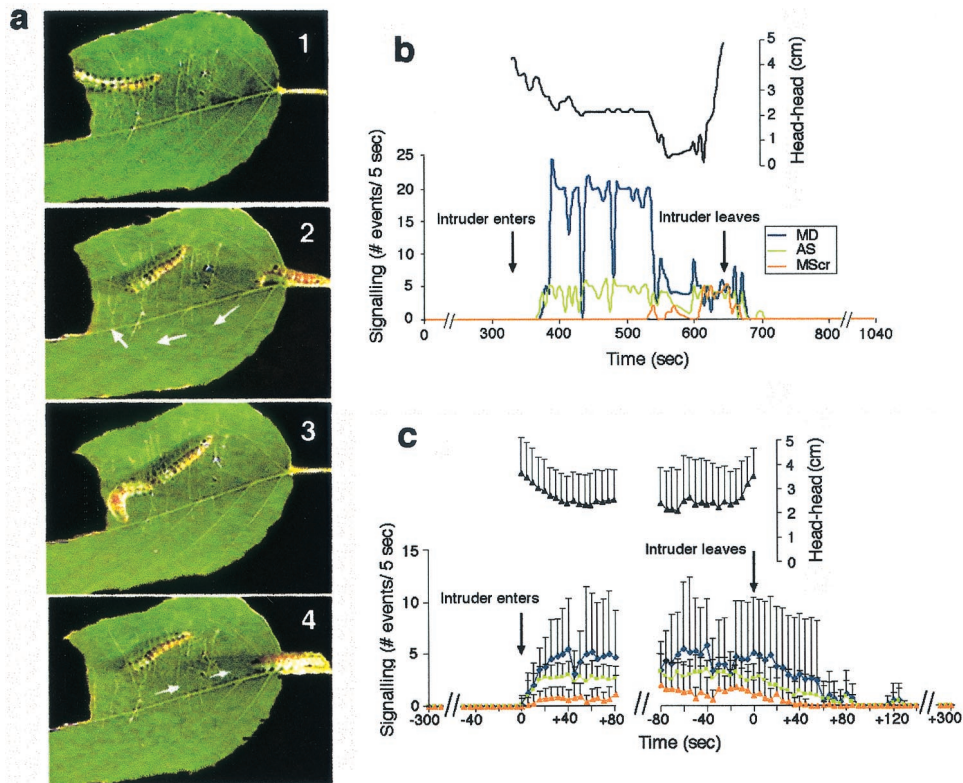
Fig. 2. Resident signaling during a single agonistic encounter (*a* and *b*), and over 24 encounters (*c*). (*a*) Video clips taken at different stages of the encounter depicted in *b*. Frame 1: The resident is feeding outside the nest before the trial. Frame 2: The intruder arrives and the resident backs into its nest and begins signaling. Arrows indicate the path taken by the intruder before chewing an opening through the silk and entering the nest. Frame 3: The intruder has entered the nest while the resident signals continuously. Frame 4: The intruder crawls through the nest and leaves the leaf. (*b*) Number of signals in consecutive 5-sec intervals throughout the encounter, including 5 min before the trial and 5 min following the intruder's departure. (*c*) Mean (+SD) signaling rate of resident larvae before the trials, for the first 80 sec of each trial and 80 sec following the departure of the intruders, and the mean (+1 SD) distance between the two heads at the beginning of each 5-sec interval. The duration of the central portion of contests varied among trials, but signaling rates remained relatively constant (see Table 1).

types. Vibration recordings of the signals revealed temporal and spectral patterns similar to audio recordings.

We staged 53 encounters where an intruder was placed on the petiole of a leaf with an occupied nest. In all trials the intruder walked onto the leaf and/or entered the nest. Residents won 86.8% of these trials, intruders won 7.5% of trials, and in three trials intruders built new nests on the same leaf as residents. Contests won by residents were significantly shorter than those won by intruders (mean $\pm$ SD $= 12.0 \pm 32.7$ vs. 412.1 ± 389.7 min; range $= 0.56–185$ vs. $28.28–900$ min; Mann–Whitney U test, $Z = -3.15$, $P = 0.002$). Intruders were significantly heavier than residents in trials ending in takeovers (mean difference $\pm$ SD $= 0.078 \pm 0.052$ vs. 0.0004 ± 0.040 g; t test, $t = -3.660$, $P = 0.0006$). Encounters lasted longer as intruders increased in size relative to residents ($r = 0.36$, $P = 0.01$). However, that pattern was not evident among those trials won by residents ($r = 0.09$, $P = 0.54$). Thus, relative size appeared to affect the likelihood that intruders attempted a takeover, and when that occurred, protracted contests resulted.

Residents were typically silent (Fig. 2 and Movie 2) until the introduction of an intruder (responses in 100% of trials). Residents always signaled first (mean latency $= 17.7 \pm 32.7$ sec SD; range 3–227). Other than trials involving takeovers, residents remained in their nests throughout an encounter (Fig. 2*a*). Residents escalated signaling as intruders approached (Figs. 2*c* and 3). Close approaches occasionally resulted in head butting, but biting or other physical interactions were not observed. Residents occasionally interrupted their otherwise continuous

signaling (Table 1) to rest or feed during longer encounters, and continued signaling for an average of 61.0 ± 54.5 sec SD; range 0–225 sec; $n = 46$) after the intruder departed (Fig. 2 *b* and *c*).

Intruders signaled in 38.7% of trials, creating acoustic duels (Movie 3) where interactions lasted longer than trials in which intruders did not signal (mean $= 36.3$ vs. 4.3 min; Mann–Whitney U test, $Z = -3.28$, $P = 0.001$). Intruders signaled at lower rates than residents, rarely performing mandibular scraping or head butting (Table 1). However, intruders frequently damaged residents' nests by biting through the silk strands.

To determine whether larvae would assume ownership of undefended nests, 10 residents were removed and intruders placed on the leaf petioles. In all trials intruders assumed residency, maintaining the nest and feeding. When original residents were replaced (10–20 min later) "squatters" always signaled first (mean $\pm$ SD latency $= 20.0 \pm 18.0$ sec, range $= 7–71$). Squatters won eight trials, with both larvae leaving the nest in the other two trials. Original residents signaled in half the trials and encounters were relatively short (mean $\pm$ SD $= 158.3 \pm 111.3$ sec).

Territorial interactions could be elicited in the absence of a nest, but not a leaf. In 10 trials where a larva was placed on a fresh leaf followed 2 min later by a second larva, the first individual signaled in response to the second individual in seven trials. In one trial the second individual signaled first, and neither individual signaled in the other two trials. When two larvae were placed on a fresh leaf simultaneously, at least one individual signaled in eight of ten trials, always in response to the approach
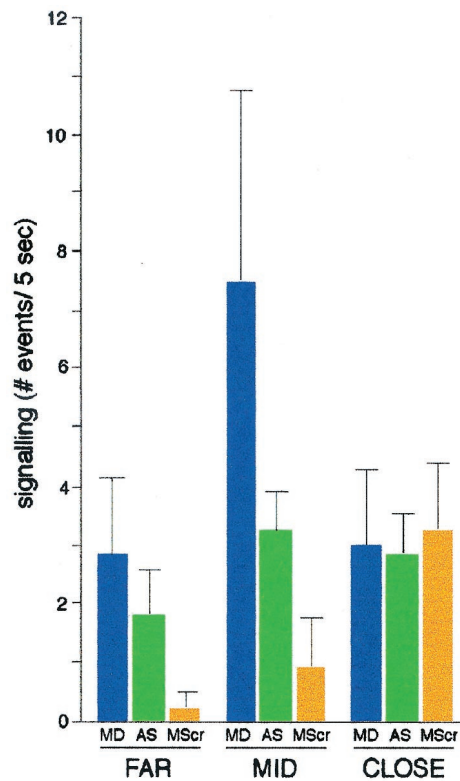
Fig. 3. Mean (+1 SD) signal rates by residents in 15 trials with direct contact, at three stages of the intruder's approach. FAR, the 20-sec period following arrival of the intruder on the leaf; MID, the 20-sec period midway between FAR and CLOSE; CLOSE, the 20-sec period following first contact. Mandible drumming (MD) and anal proleg swiping (AS) increased significantly from FAR to MID (ANOVA: $P = 2.3 \times 10^{-6}$ $F = 24.9$ and $P = 4 \times 10^{-7}$ $F = 29.2$, respectively). MD returned to FAR levels, and AP did not change from MID to CLOSE. Mandible scraping (MScr) increased significantly at each stage (ANOVA: $P = 0.0016$ $F = 10.4$ and $P = 3.7 \times 10^{-8}$ $F = 105.2$ for FAR to MID and MID to CLOSE, respectively).

of the other individual. Response latencies were long (mean = 290.3 sec) and signaling rates low compared with trials involving established residents with nests. Neither of two individuals placed on a birch twig without foliage signaled, even if they made direct contact ($n = 30$).

Circumstantial evidence suggests that the larvae do not use acoustic signals for purposes other than conspecific territoriality, such as startling predators or warning predators of chemical or physical defenses. Signaling was restricted almost exclusively to nest sites, was never followed by an escape response, and could not be elicited by a variety of disturbances, including wind, sudden visual movements or light intensity changes, loud sounds, or gentle shaking of the branches. Larvae occasionally responded to gentle disturbances of the nest leaf with a paintbrush (five of ten trials), but response durations were brief (18.4 ± 28.2

sec) and signaling rates low. Other than our previously stated observations that larvae are cryptic and possibly protected by their nest, we do not know how they defend themselves against predators. We have observed prepupal, fifth instar larvae being consumed by ants, and the larvae were eaten when fed to captive brown-headed cowbirds (*Molothrus ater*), indicating that larvae are palatable to predators. Larvae have no obvious spines or scales, nor do they employ physical defenses (e.g., biting) that might deter a predator. Other unidentified species of invertebrates [two spiders (Salticidae), five Lepidoptera larvae (four Geometridae, one Tortricidae), three ants, and two leaf-hoppers (Cicadellidae), all taken from birch] would occasionally elicit mild responses of short duration from a resident larva. Unlike trials with conspecific larvae, which were clearly "interested" in exploring the leaf and nest of the resident, it was difficult to get the various other heterospecifics to remain on the leaf long enough to get a fair test of how residents would respond, so results from these trials must be considered preliminary. The possibility that the larvae respond acoustically to heterospecifics cannot be ruled out and requires further testing with carefully chosen species.

Discussion

Our observations support the hypotheses that *D. arcuata* larvae are territorial and that territoriality is mediated through acoustic displays. Most contests were resolved quickly, residents generally won, and only a few minutes of residency established ownership. However, when intruders "decided" to compete for a territory, contests became longer and takeovers occurred. Size asymmetries between contestants had some effect on the duration and outcome of contests. Damaging of nests by intruders could represent attempts to reduce the value of nests, and thus the willingness of owners to defend them.

Territoriality by *D. arcuata* is consistent with nests being expensive to build and valuable to own. Many species of caterpillar build silk shelters, with reported benefits including improved microclimate and protection from predators, parasitoids, and weather (3, 12, 13). Costs of nest construction include time lost from feeding, plus energy investment in silk (12). Usurping an existing nest garners the benefits without the costs. Because *D. arcuata* do not travel far from their initial communal nest and/or hatching site, the potential for competition is high. Our numerous observations of unstaged acoustic contests in the lab and *in situ* suggest territorial interactions are common in nature. In two other experimentally demonstrated cases of conspecific competition in larval Lepidoptera (12, 14), territorial defense involves physical attacks that can result in injury or death. In comparison, *D. arcuata* behave more like many vertebrates, where ritualized signaling appears to allow contestants to assess one another and resolve most contests without fighting.

Our data support our hypothesis that acoustic displays signal territory ownership. Acoustic displays are restricted to a territory, and are performed almost exclusively by a resident or intruder in the context of a conspecific encounter. Ideally one would like to confirm this interpretation by assessing how intruders respond to a silent resident. We did find that when

Table 1. Mean encounter durations and signaling rates of residents and intruders

	Dur, min	Res MD/5s	Res AP/5s	Res MScr/5s	Int MD/5s	Int AP/5s	Int MScr/5s
Mean	13.31	5.80	2.55	0.74	0.07	0.04	.00
SD	37.3	6.96	1.53	1.49	0.60	0.34	.03
Med	4.17	4	3	0	0	0	.00
Range	1.8–187	0–30	0–6	0–10	0–8	0–4	0–1

AP, anal proleg swipes; Dur, encounter duration; Int, intruder; MD, mandibular drums; MScr, mandibular scrapes; Res, resident 24 trials; see Fig. 2c.

recently killed, intact residents were placed inside their nests, intruders readily moved in and took up residence along side the "silent" resident. Although this result is consistent with the hypothesis that acoustic signals are territorial displays, we cannot rule out the possibility that in using dead residents we may also have eliminated pheromones or visual stimuli that residents might use to deter intruders. The ideal experiment would be to use silenced, but otherwise functional larvae. However, given the nature of the sound production by these larvae, devising such an experiment will prove challenging.

D. arcuata larvae are insensitive to airborne sounds and appear to lack hearing organs, suggesting that they detect territorial signals by means of substrate-borne vibrations. Because larvae only signal when intruders are close (mean distance from resident = 2.39 ± 1.24 cm, range = 0.5–4.5 cm, $n = 10$), signals would appear to be much stronger than necessary (c.f. quiet signals between ants and lycaenid larvae; refs. 15 and 16). Therefore, these signals may also be directed at potential predators. Larval *D. arcuata* are visually camouflaged, remove frass from their nests, and clip petioles of leaves they have eaten—characteristics that suggest they are preyed on by birds (2). Birds are known to use acoustic cues to locate invertebrate prey (2, 17). Therefore, by signaling loudly, residents could put intruders (unprotected by a nest) at risk of avian predation, which would explain why contests are generally brief, and ownership respected.

Looking beyond our direct demonstration of conspecific acoustic communication in larval Lepidoptera, comparative evidence suggests that the phenomenon is widespread. Abdominal oar structures occur in at least eight other drepanid genera worldwide (18–20) and anal scraping has been noted for three other *Drepana* species (9). We have confirmed that *Drepana bilineata* also employs acoustic signaling in territorial disputes, and we have evidence that early instar, gregarious *D. arcuata* use vibrational signaling to interact with nestmates. There are numerous reports of larval sound production in other Lepidoptera. Predator defense is one suggested function of the sounds (9, 21), although experimental validation is presently lacking. Vibrational signals in other Lepidoptera facilitate heterospecific, mutualistic relationships with attendant ants (15, 16). Our study confirms that acoustic signaling in caterpillars can serve in conspecific communication, and evidence from other species of diverse lepidopteran taxa (22, 23) suggests this is not unique to drepanids. Signaling between early instar larvae may be particularly prevalent, because early instars often live in groups (3). Caterpillars that live gregariously must regularly face tradeoffs between individual and inclusive fitness. Exploring the role of acoustic communication in managing these tradeoffs may reveal previously "unheard of" complexity in larval social behavior.

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