



Is disturbance stridulation in the passalid beetle *Odontotaenius disjunctus* a form of social communication?

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Abstract

Among insects, stridulation (the rubbing of body parts to generate sound) is an important component of many essential behaviors including courtship, predator deterrence, and social recruitment. The subsocial passalid beetle *Odontotaenius disjunctus* boasts the largest acoustic repertoire of any beetle and performs a distinct type of stridulation when disturbed by predators. We investigated whether disturbance stridulation in this species functions as a form of social communication. Because *O. disjunctus* live in small colonies with high relatedness, we hypothesized that disturbance stridulation may serve as a warning to kin, potentially benefiting inclusive fitness during a predation event. To test this, we placed individuals from the same colony in an arena and exposed them to disturbance stridulations, observing behavioral diversity, whether each individual moved away or toward the sound, and time spent locomoting. According to information theory, behavioral diversity is predicted to decrease after communication has occurred. Adult beetles were significantly less likely to locomote and exhibited significantly less behavioral diversity when hearing disturbance stridulation than when they were in silence. There was no significant directionality to the movement of beetles toward or away from the sound source, whether in the treatment or negative control groups. Together, this suggests that these beetles respond to stridulation sounds with a “freeze” response rather than “fight” or “flight”. However, more work is needed to elucidate whether defense stridulation specifically communicates different information from other types of stridulation. These results encourage future research into the adaptive value of insect stridulation in social contexts.

Keywords Acoustic communication · Bess beetle · Defensive stridulation · Information theory · Passalidae

Introduction

Acoustic and vibratory communication are poorly studied compared to other modes of communication within insects, such as visual and chemical (Ali and Morgan 1990; Low et al. 2021; Richard and Hunt 2013; Schönrogge et al. 2017; Wilson 1965). Sound production is energetically expensive and affects fitness (Schuster 1983), placing strong selection pressure on vibroacoustic adaptations in certain species. Vibroacoustic communication can refer to vibrations that are both airborne (commonly referred to as sound) or

substrate-borne (commonly referred to as vibration). Saproxylic species, those that depend on dead wood for survival, tend to rely more heavily on vibroacoustic communication than visual or olfactory communication because visual and olfactory signals are not propagated as well in dark, confined environments (Bedoya et al. 2021). For instance, termites rely heavily on vibratory-based communication for a variety of purposes including, but not limited to, conspecific alarm, recruitment to nestmates, reproductive signaling, and warnings to predators (Evans et al. 2005, 2009; Hager and Kirchner 2013; Pailler et al. 2021).

One hypothesized driver of the evolution of communication complexity is the evolution of sociality (Freeberg et al. 2012), though support for this hypothesis in insects is mixed, with relationships between these two factors in species with intermediate levels of sociality playing a key role in shaping our understanding (Nehring and Steiger 2018). Alarm communication is one such behavior that predates eusociality in

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the shared lineage of termites and cockroaches (Order: Blattodea) and was present in less social, more basal termite species (Sillam-Dussès et al. 2023). These basal species share similarities in their presociality and communication with the societies of other presocial arthropods. For instance, the horned passalus beetle, *Odontotaenius disjunctus*, is also a saproxylic species that produces sound via stridulation. Individuals of this species tend to live as mated pairs inside a single log of decaying wood, but often live as a cooperative family unit of adults and their adult offspring (Schuster and Schuster 1985) until the adult offspring disperse to look for new nests (Jackson et al. 2009). Passalid beetles are considered to be subsocial because they coexist with overlapping generations and perform cooperative brood care (Schuster and Schuster 1985) but lack reproductive division of labor (Wilson and Hölldobler 2005). Similar to termites, passalid beetles are thought to have evolved sociality as an adaptive strategy enabling adults to pass on wood-digesting gut symbionts to offspring (King and Fashing 2007; Ulyshen 2018). Therefore, parental care of these beetles is essential to offspring survival.

In addition to these social traits, *O. disjunctus* has also evolved one of the largest acoustic repertoires of any arthropod with adults exhibiting 5 unique sound types (distinguished by discrete pulses of sound and groups of pulses, termed bars; as described by Schuster 1983) across 11 different behavioral contexts for a total of 14 known acoustic signals (Schuster 1983). Underneath the hard outer wings or elytra, the adults repeatedly rub the ridges on their abdomen (plectrum) against the soft inner wing (file or pars stridens) to produce a “squeaking” sound (Reyes-Castillo and Jarman 1983; Schuster 1975). The larvae also stridulate by scraping their metathoracic legs against a file on the mesothoracic coxae (Schuster 1983). These signals can broadly be categorized into the contexts of courtship, aggression, solitary behaviors (occasional instances when sound is produced in the absence of others, such as when feeding, walking, or generally inactive), and disturbance. Disturbance stridulation, or defensive stridulation, is defined as any stridulatory sound produced in response to predator attacks or threats (Low et al. 2021).

Due to their broad acoustic repertoire and the tendency to live in cooperative groups, it has been hypothesized that disturbance stridulation in *O. disjunctus* has adaptive value as a form of social communication. However, disturbance stridulations may function in several non-mutually exclusive ways, as suggested by Low et al. (2021): (1) as a startle behavior, or deimatic behavior, with the function of startling and deterring predators (Drinkwater et al. 2022; Masters, 1979); (2) as a form of acoustic aposematism advertising toxicity; (3) to interfere with predator senses to avoid detection; (4) as a form of mimicry; or (5) to warn other conspecifics of danger. The hypothesis that disturbance stridulation causes

a startle response in predators has been tested using hand-reared crows (Buchler et al. 1981) and deer mice (Gotch 1997), though these studies reported conflicting results and have been critiqued because vertebrates are not the most common predators of *O. disjunctus* (Ulyshen 2018). These studies reported no interference with predator senses or adverse effects after beetles were consumed (Buchler et al. 1981; Gotch 1997), however, studies of more realistic predatory species are needed. Schuster (1975) suggested Batesian mimicry as a possibility because disturbance stridulation is found across many insect taxa, some of which accompany stridulation with bites or stings. However, this may be unlikely because these sounds differ vastly among and within the many arthropod species that produce them (Low et al. 2021).

In this study, we present a test of the hypothesis that disturbance stridulation functions as a form of conspecific social warning communication in *O. disjunctus*. This social warning hypothesis was partially tested previously by Buchler et al. (1981), who reported no evidence that adult beetles were attracted or repelled by a stridulating beetle held against a box containing a colony, or when a recording of disturbance stridulation was played on one side of a Y-maze. Here, we present a follow-up investigation of this topic. We tested whether disturbance stridulation functions as a form of social communication using information theoretic predictions. Information theory posits that communication occurs (information has been transferred) if the diversity of behaviors of the receiver is reduced after the signal is sent (McCowan et al. 1999). Using behavioral categories described in published ethograms for this species (Ebot-Ojong et al. 2019; Wicknick and Miskelly 2009), we compared behavioral diversity of adult beetles before and during exposure to disturbance stridulation sounds while in naturalistic behavioral arenas with other nestmates.

We then conducted two independent explorations of how locomotion is affected by disturbance stridulation, checking not only for evidence of rescue (“fight”) responses (moving towards the stimulus) or “flight” responses (moving away from the stimulus), but also evaluating proportion of time spent engaging in non-directional locomotory behavior to test whether these beetles “freeze” in response to disturbance stridulation which may be adaptive as a method of predator avoidance in habitats where the prey are less detectable (Cooper and Sherbrooke 2010; Takada et al. 2018).

Methods

Field collection

We collected subject *O. disjunctus* colonies from Lake Thoreau Environmental Center in Hattiesburg, MS (31.349,

-89.418) intermittently from 23 February 2023 to 2 August 2023. This region is primarily considered to be a longleaf pine ecosystem (McFarland et al. 2020). We located colonies by searching for entrance tunnels and beetles underneath fallen decayed logs, specifically larger fallen logs with intermediate stages of decomposition without heart rot or evidence of recent flooding (Ulyshen 2018). All beetles found in the same log were considered to be part of the same colony, or social group, which is typical in this species (Schuster and Schuster 1985) and so were housed together. Groups of beetles collected more than 100 m apart were assumed to be separate colonies because previous work indicated the average dispersal distance for colonization of new logs to be 11.6 m (Jackson et al. 2009).

Colonies found with fewer than 3 adult individuals were excluded from adult assays. Colonies with fewer than 3 larvae were excluded from larval assays. Any individuals collected with visible injuries were not used in the study. A total of 38 beetles from 10 collected colonies were used for adult behavioral assays, and 32 larvae from 5 colonies were used for larval behavioral assays. Along with each colony, we collected nest material such as wood debris, frass, and dirt from their respective logs while avoiding any other insects that might be in the nesting material. All colonies were transferred to the laboratory within four hours. Gloves were worn during the collection process and any time the beetles or larvae were handled to prevent transfer of chemical signals between colonies and to prevent potential additional stress from direct contact with human hands.

Husbandry

Each colony was kept separately in a ventilated plastic rearing bin with a volume of 5.6 L and dimensions of 34.2×20.6×11.7 cm. Each rearing container had a mostly opaque lid and sides to minimize bright light exposure. Holes in the lid and sides allowed for air exchange and allowed a small amount of dappled light to enter. Rearing containers were housed in a 3900 Series ThermoScientific® Environmental Chamber under a 12hL:12hD light cycle at 26 °C and 60% to 65% relative humidity (RH). These conditions were based on RH and temperature readings recorded via three Hygrochron iButtons (Maxim Integrated, San Jose, CA) placed underneath the logs where colonies were found during the first week of collections. We misted each bin with water at least twice a week and provided new wood when needed to allow feeding *ad libitum*. Beetles in all colonies were allowed to acclimate to these conditions for one week before assays began, and they were returned to the areas in which they were originally found the week after assays were complete. All subjects were given individually identifiable paint markings on the pronotum using nontoxic Sharpie oil-based paint pens, which have been shown in other studies

to be non-disruptive to insect behavior (Baudier et al. 2019; Holbrook et al. 2011). Experimental trials began a week after paint marking the beetles.

Disturbance stridulation behavioral assays

We performed assays to assess individual behavioral responses to pre-recorded disturbance stridulation sounds. All adult beetles or all larvae (up to 6 larvae at a time, i.e. per batch) within a single colony were moved into an experimental arena consisting of an untreated pine wood box (33×33×4.5 cm) with 1 cm-thick sides to mimic sound passing through their natural nesting substrate. The arena also had a clear 2.3 mm-thick acrylic plastic lid. We used a marker to divide the lid equally into two sides to more easily observe beetle movement (Fig. 1). The arena was lined with a substrate of soft, moistened, untreated pine shavings of about 1 cm in depth to minimize discomfort for the beetles, to allow for burrowing behavior, and to allow us to observe them against a light-colored background. These pine shavings are not an exact mimic of their natural habitat, but the substrate approximates the large amount of chewed and fragmented wood that fills the galleries of their logs and create piles of excavated material underneath the logs (Ulyshen 2018). A small amount of pine shavings was also mixed into the material in one half of their non-experimental enclosures to assist in acclimation to the arena substrate.

During the trials, we placed the experimental enclosure in a dark and quiet room illuminated by a red light to mimic naturally dark conditions underneath or within a log. We placed a small Bluetooth speaker (Brand: onn. Rugged; dimensions of sound producing area in contact with the side of the box: 12×6.5 cm) such that the speaker would play directly into the wood (Fig. 1). Adults and larvae were observed in separate trials. After being placed in the center of the arena we let the subjects acclimate for 5 min underneath a transparent plastic cup. Once the cup was lifted and the plastic lid placed back over the box, the trial began. During each trial, we scan-sampled the beetles every 30 s for 5 min; the first time point being taken after the first 30 s. For adult beetle trials, at each time point we recorded the location, locomotive state, and behavior exhibited using an ethogram (Table 1) with the following *a priori* categories: avoidance, defensive, social, and other/neutral behavior (modified from Wicknick and Miskelly (2009) and Ebot-Ojong et al. (2019)). For trials with larvae, only the location was recorded, as no larval ethograms have been published for this species.

Each colony underwent an initial control trial consisting of an identical set-up but with no sound playing from the speaker. After establishing each colony's neutral state, they each underwent one experimental trial. The beetles remained in the arena under the plastic cup between the control and

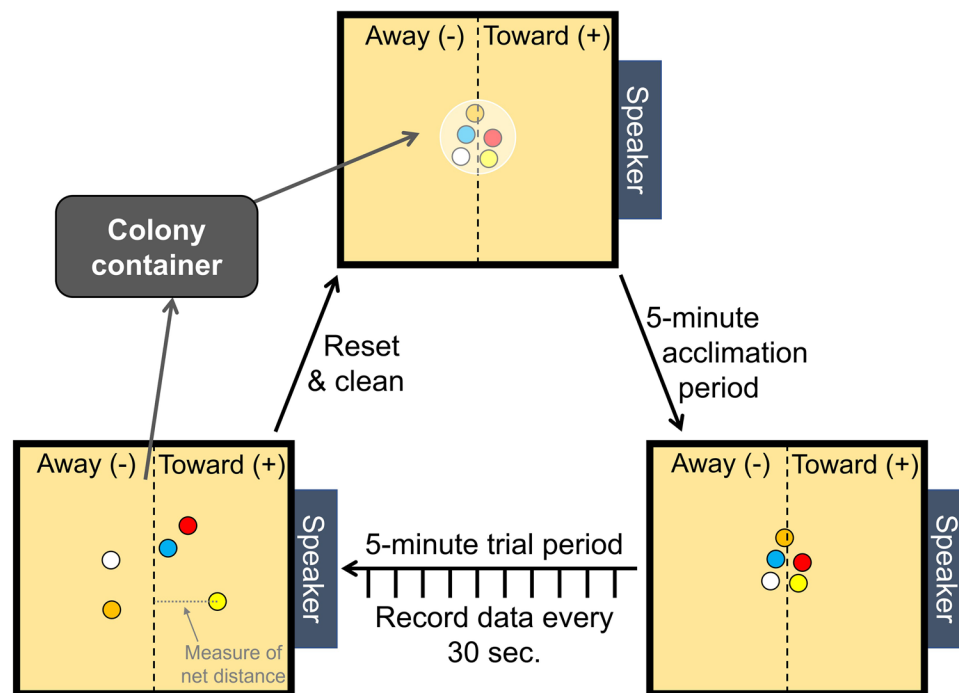


Fig. 1 Experimental procedure of disturbance stridulation trials. Each of the yellow boxes represents a top-down view of the wooden trial enclosure. The five colored circles represent different individual beetles. Beginning at the top of the diagram, all beetles are taken from their colony container and placed within a cup, represented by the translucent circle, to acclimate before the trial so they would have a similar starting position. For each colony, the first trial was silent for

the negative control, and the second trial involved the speaker playing the stridulation. For 5 min, data were recorded every 30 s. The final location of each beetle was recorded and used to measure the net distance traveled. Following both control and experimental trials, the beetles were placed back in their colony's container, and the arena was cleaned before the next trials

Table 1 Ethogram behaviors assessed during adult trials. At each sampling time point, one locomotive state and one behavior from one of the other categories was recorded. Modified from previous research (Ebot-Ojong et al. 2019; Wicknick and Miskelly 2009)

Locomotive states	
Stationary	Remaining in one place
Locomoting	Travelling from one location to another
Behavioral Categories	
<i>Avoidance</i>	
Moving away from sound	Traveling away from sound source
Bury	Moving underneath substrate
Grouping together	Touching or clustering of 3 or more individuals
<i>Defensive</i>	
Moving to Sound	Traveling toward sound source
Opening mandibles	Movement of mandibles together and apart repeatedly or maintaining mandibles in an open position (occasionally accompanied by a rear-up behavior)
<i>Social</i>	
Touching	Beetle is physically contacting another but not above or below another beetle
Climbing on each other	One beetle is physically on top of another beetle
<i>Other/Neutral</i>	
Antennal movement	Movement or vibration of antennae
Grooming/antennal grooming	Using legs to clean own antennae or other body parts
Other	Any other behavior not specified above

experimental trials but were still given another 5-min acclimation period. For the experimental trials, we played a loop of a 30 s pre-recorded *O. disjunctus* disturbance stridulation (Bibbs et al. 2011). The playback was standardized across all trials to be between 8,000–10,000 Hz and 40–60 Db using an android cellphone app, Spectroid, on a Samsung Galaxy S21. This was the average natural volume of stridulation reported in previous studies (Buchler et al. 1981; Reyes-Castillo and Jarman 1983; Schuster 1975, 1983). Each colony underwent only one control and one experimental trial to prevent acclimation or sensitization to the acoustic stimulus. All trials were also recorded using a Panasonic HC-VX981 4 K camera for later analysis of distance traveled. Once each colony completed their trials, the arena box was emptied of pine shavings and wiped with a damp cloth to reduce the probability of transferred chemical signals across colonies.

Statistical analysis

To test the hypothesis that disturbance stridulation is a form of social communication using the predictions of information theory, we compared behavioral diversity between the negative control and disturbance stridulation sound treatments. Information theory predicts that when information is exchanged (when communication occurs), there will be a reduction in the diversity of behaviors exhibited by the receiver of the information. The Shannon entropy test was originally developed for this purpose before being later used as a diversity index (McCowan et al. 1999; Shannon 1948). Behavioral diversity of each beetle in each trial was estimated using the Shannon Diversity Index. We compared Shannon Diversity between control and sound treatment groups using a generalized linear mixed effect model (GLMM) with an inverse Gaussian distribution and the individual beetle identification (ID) and colony as a random

variable. Inverse Gaussian was used because it had the lowest AIC score compared with other statistical families assessed. Mixed effect models were fitted using R package “lme4”. The *p* values of all models were obtained from a subsequent Type II Wald chi-square test from the “ANOVA” function in the R package “car”.

To explore in more detail the effects of disturbance stridulation on locomotory behaviors, we also compared net distance traveled and the proportion of time spent locomoting versus being stationary between disturbance stridulation treatments and negative control. Net distance traveled was estimated as the perpendicular distance from the starting centerline to an individual’s end location (Fig. 1). This was estimated for both adults and larvae using ImageJ (Schroeder et al. 2021). A linear mixed effect model (LMM) was used to compare net distance traveled by each adult beetle between the negative control (no-sound) and sound treatment with the individual beetle ID and colony as random variables (Table 2). This model, and each subsequent model, was validated using the R package “DHARMa” to ensure that we satisfied assumptions of normality and homoscedasticity of residuals. The model for assessing whether larvae net distance differed across sound treatments was initially heteroscedastic when only using individual ID and colony as random variables. We added the batch number as a random variable which resolved the heteroscedasticity such that we could use a GLMM (Table 2).

The overall proportion of time spent locomoting was defined as the proportion of time points where each beetle was locomoting out of $n = 10$ timepoints. The proportion data was arcsine transformed, and a GLMM with the inverse Gaussian distribution was used to compare negative control and sound treatment groups with individual ID and colony as random variables. Inverse Gaussian was used because it had the lowest AIC score compared with

Table 2 Summary of statistical models

Statistical Model	Statistical Family	Response Variable		Fixed Predictor Variable(s)	Random Variable(s)
Generalized linear mixed effect model (GLMM)	Inverse Gaussian	Shannon Diversity of adult behaviors	~	Sound treatment	+ (1 Individual ID) + (1 Colony ID)
Linear mixed effect model (LMM)	Gaussian	Adult net distance traveled	~	Sound treatment	+ (1 Individual ID) + (1 Colony ID)
Generalized linear mixed effect model (GLMM)	Inverse Gaussian	Larvae net distance traveled	~	Sound treatment	+ (1 Individual ID) + (1 Colony ID) + (1 Batch ID)
Generalized linear mixed effect model (GLMM)	Inverse Gaussian	Arcsine(proportion of time adults spent locomoting)	~	Sound treatment	+ (1 Individual ID) + (1 Colony ID)
Generalized linear mixed effect model (GLMM)	Binomial	Locomotion status	~	Sound treatment* Time	+ (1 Individual ID) + (1 Colony ID)

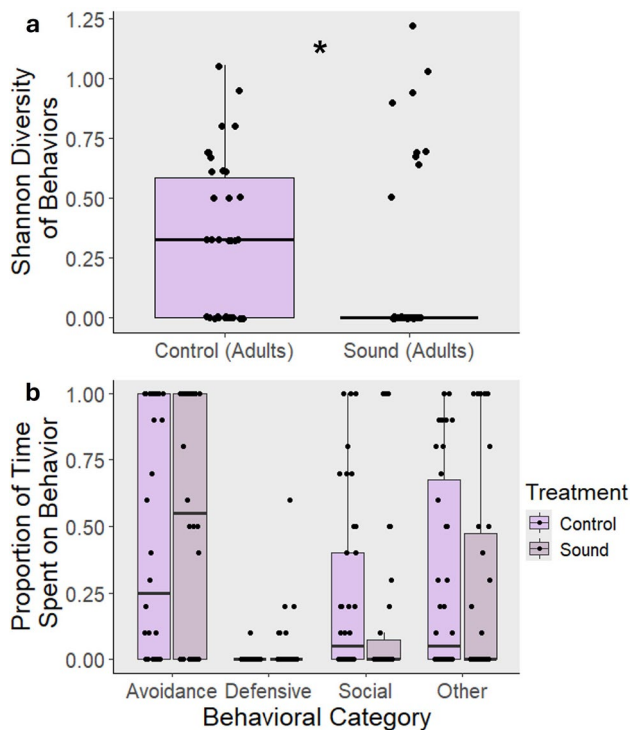


Fig. 2 Change in diversity of behaviors during disturbance stridulation trials. **a** The diversity of behaviors exhibited in each behavioral category (avoidance, defensive, social, or other) as defined in our ethogram was calculated using the Shannon Diversity Index for each beetle. The control exhibited significantly higher diversity ($n=38$, $\chi^2=4.00$, $df=1$, $p=0.045$). The asterisk (*) indicates $p<0.05$. **b** The proportion of time that each beetle spent performing each behavior during its control and treatment (sound) trial is also shown

other statistical families assessed. We also used a binomial GLMM with locomotion status (either stationary or locomoting) as the response variable to assess whether there was a difference between treatment groups at each time point during the trials (Table 2). All statistical analyses were performed using R (version 4.4.2).

Results

There was a significant decrease in the diversity of behaviors exhibited by adult beetles after exposure to disturbance stridulation sound ($\chi^2=4.00$, $df=1$, $p=0.045$; Fig. 2a).

There was no significant difference in net distance traveled by adult beetles in the negative control ($n=26$) versus the sound treatment ($n=31$) ($\chi^2=0.44$, $df=1$, $p=0.507$; Fig. 3a). The number of adults differed from control to experimental treatment because some beetles dug under the substrate during the trials, obscuring their markings and exact final position. There was no significant

difference in net distance traveled by larvae in the negative control ($n=32$) versus the sound treatment ($n=32$) ($\chi^2=0.11$, $df=1$, $p=0.735$; Fig. 3b).

The proportion of time spent locomoting was significantly lower when disturbance stridulation was being played than in the negative control ($\chi^2=10.53$, $df=1$, $p=0.001$; Fig. 4). When accounting for locomotion at each time point, we found that the beetles in the treatment group still exhibited significantly lower locomotion ($\chi^2=15.08$, $df=1$, $p<0.001$). There was no significant effect of time ($\chi^2=3.56$, $df=1$, $p=0.059$) or the interaction of treatment with time ($\chi^2=2.24$, $df=1$, $p=0.134$).

Discussion

This study contributed to the body of work investigating the adaptive significance of *O. disjunctus* disturbance stridulation. To test whether disturbance stridulation was a form of social communication, we applied principles of information theory which predict a decrease in the diversity of behaviors following exchange of information (communication) (McCowan et al. 1999). We report a significant reduction in the diversity of behaviors exhibited by adult beetles after they experienced disturbance stridulation stimuli. This supports the hypothesis that defense stridulation serves as a form of social communication, although further study is needed to assess whether other forms of stridulation may also cause the same response. The bimodality of diversity observed in Fig. 2a is due to beetles that only exhibited one behavior throughout the trials. This can be more easily seen in Fig. 2b which shows the proportion of time that each beetle spent performing behaviors belonging to each respective behavioral category used in this study. The treatment group exposed to the disturbance stridulation sound showed more individuals exhibiting no diversity in behavior, meaning only one behavior was performed.

Next, we investigated whether disturbance stridulation functions as a warning that could elicit a fight, flight, or freeze response among kin when in the presence of a predator. There was no difference in net distance moved toward or away from the auditory stimulus for adults or larvae when disturbance stridulation was and was not playing, indicating that the stridulation does not recruit aid or cause retreat among colony members. These results were consistent with descriptive observations reported by Buchler et al. (1981).

We did observe a significant decrease in the proportion of time beetles spent moving around the arena when disturbance stridulation was played as compared to negative controls. This suggests that *O. disjunctus* “freezes” or reduces movement in response to hearing disturbance stridulation. We did not test in this study whether this species also freezes in response to non-disturbance types of stridulation,

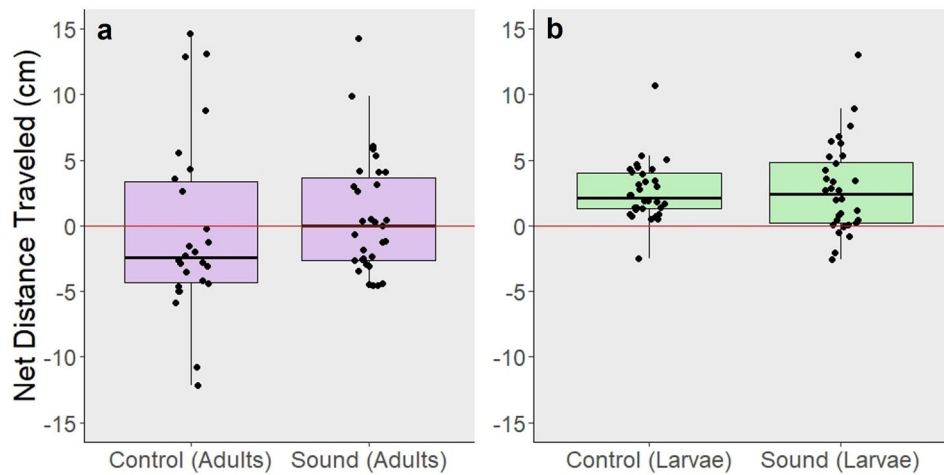


Fig. 3 Distance traveled during disturbance stridulation trials. Each data point represents the net distance traveled by each adult (**a**; control: $n=26$; sound: $n=31$) or larval (**b**; control: $n=32$; sound: $n=32$) beetle between the start and the end of the trial. Positive numbers indicate that the beetle moved toward the sound source while negative

numbers indicate that the beetle moved away from the sound source. The horizontal red line is drawn at 0 cm traveled to indicate no net movement from the starting position. Box plots show inner and outer quartiles with median values indicated by a bold horizontal line and overlaid data points plotted with jitter

a possibility that would lead us to question whether the adaptive value of freezing in response to disturbance stridulation might be something other than predator avoidance.

For many species, freezing is an adaptive response to predators to avoid visual or sound-based detection, particularly in habitats in which the prey are less detectable (Cooper and Sherbrooke, 2010; Takada et al. 2018). In *Hodotermes* termites, workers also exhibit brief freeze responses to disturbance (Sillam-Dussès et al. 2023). In *O. disjunctus*,

disturbance stridulation may improve the ability of nestmates, which are typically kin, to avoid detection when predators are present. This may be an especially effective strategy for species such as this one that live in wood and have small colony sizes, because a predator's cost of breaking apart an entire log is high, which makes prolonged search for a small number of behaviorally cryptic individuals maladaptive under optimal foraging theory (MacArthur and Pianka 1966). *Odontotaenius disjunctus* has been shown to display freeze behavior in response to startling noise, vibration, and light stressors, with females freezing for longer than males (Davis et al. 2023). However, comparisons to non-disturbance stridulation responses would be useful to test whether the adaptive value of freezing in response to disturbance stridulation is associated with predator avoidance specifically, or whether this observed freeze response may serve some unrelated function (e.g. reducing noise interference or improving signal processing during communication).

Future tests of disturbance stridulation as a form of communication in a more naturalistic setting (a log with galleries dug by beetles) may also be worthwhile. Additionally, it is not known whether beetles react differently to stridulation sounds of nestmates or beetles from the same source population. There are likely two distinct clades of *O. disjunctus* within the Southeastern United States (Garrick et al. 2019). The stridulation recording that we used from Bibbs et al. (2011) was presumed to be an individual from Florida. If and how disturbance stridulation differs between populations in southern Mississippi and Florida is unknown. There is some evidence that suggests this species may be able to determine nestmate from non-nestmate (Gotch 1997; King and Fashing 2007; Wicknick and Miskelly 2009), but the

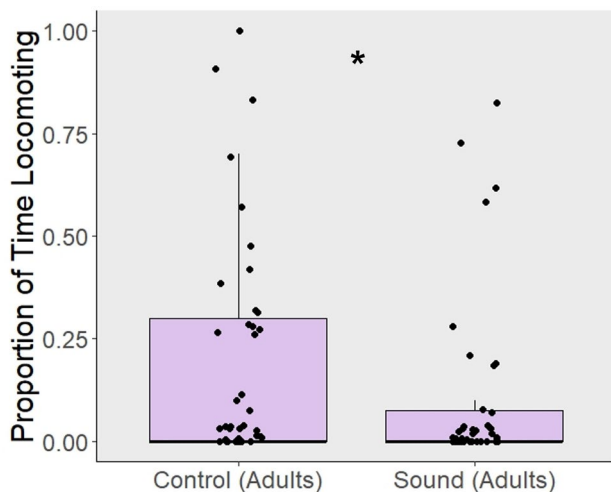


Fig. 4 The proportion of time beetles spent locomoting around the arena. The proportion of time locomoting was calculated as the proportion of time points that each beetle was actively moving and not stationary throughout the trial. The adults moved significantly less during the stridulation trial ($n=38$, $\chi^2=10.53$, $df=1$, $p=0.001$). The asterisk (*) indicates $p < 0.05$

mechanism of recognition is unknown and may very well be chemically based as it is for many insects (Basu et al. 2021). Whether *O. disjunctus* can recognize vibroacoustic communication differences between nestmates and non-nestmates and whether that would affect their behavioral response is unknown.

These beetles may also communicate defensively using multi-modal signaling, for instance stridulation at the same time as expressing pheromones to communicate chemically. Insects often use alarm pheromones, and they can cause a variety of responses depending on density of the population as well as the amount of pheromone released (Basu et al. 2021). Chemical signals can also be used in conjunction with vibrational signals to communicate alarm. For instance, when predated upon, many termites use both alarm pheromones and drumming behaviors to produce vibrations to communicate (Hager and Kirchner 2013; Sillam-Dussès et al. 2023). In a naturalistic setting, more multi-modal information would be available to *O. disjunctus* colonies during a predation event. Whether and to what extent this context affects behavioral responses to disturbance stridulation is unknown.

Future work is also needed to test the remaining hypotheses regarding the adaptive value of disturbance stridulation. Considering both predator and prey response behaviors to stridulation is important to gaining a better understanding of whether disturbance stridulation can cause a startle response and deter predators (Low et al. 2021). In *O. disjunctus*, this has not been effectively tested using realistic predators (Buchler et al. 1981; Gotch 1997) since vertebrate predators are relatively rare for these beetles (Ulyshen 2018). Understanding the adaptive value of disturbance stridulation in this species expands our understanding of subsocial communication, cooperation, and defense. More generally, this work adds to the growing body of information on two of the less frequently studied modalities of communication in insects: acoustic and vibrational (Low et al. 2021).

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00040-025-01024-2>.

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Author contributions KMR conceived and designed the project. KMR, ZCM, HS, KYRL, ANJ, GC, AN, and KMB contributed further to study design, data collection, analysis, data visualization, and drafting of the manuscript. CHZ and KMB provided support and supervision during field and lab work. All authors contributed to revising the manuscript and have approved the final submitted version.

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Data availability Data used in this study are available in the online supplementary information.

Declarations

Competing interests The authors declare no competing interests.

Ethics approval Insects are not currently subject to IACUC ethical regulations; therefore, no ethical approval was required for this study. However, we have adhered to the ethical guidelines of the Insect Welfare Research Society in our experimental design (Fischer et al. 2024). Beetles were housed according to conditions reported by previous research (Buchler et al. 1981; King and Fashing, 2007; Reyes-Castillo and Jarman, 1983) and according to our habitat measurements taken in the field. Despite our best efforts to minimize negative welfare impacts, early during the first collection period, two colonies totaling nine adult beetles died before any trials occurred, possibly from desiccation. After increasing the frequency of water misting, no more deaths occurred. Aside from this isolated incident, beetles potentially experienced stress during trials from the stridulation playback and when being handled such as during the initial collection, paint-marking, and between trials. Following the experimental trials, all beetles were returned to the locations where they were initially collected.

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