Taylor & Francis
Taylor & Francis Group

Bioacoustics
The International Journal of Animal Sound and its Recording

ISSN: 0952-4622 (Print) 2165-0586 (Online) Journal homepage: www.tandfonline.com/journals/tbio20

Stridulation-like behaviour in the Red Wood ant (*Formica rufa*)

G. L. R. Barbier, M. Rollo, S. Hall & J. Klaminder

To cite this article: G. L. R. Barbier, M. Rollo, S. Hall & J. Klaminder (2025) Stridulation-like behaviour in the Red Wood ant (*Formica rufa*), Bioacoustics, 34:4, 468-480, DOI: 10.1080/09524622.2025.2500391

To link to this article: https://doi.org/10.1080/09524622.2025.2500391

BIOACOUSTICS
2025, VOL. 34, NO. 4, 468–480
https://doi.org/10.1080/09524622.2025.2500391

Taylor & Francis
Taylor & Francis Group

Stridulation-like behaviour in the Red Wood ant (*Formica rufa*)

G. L. R. Barbier[a], M. Rollo[a,c], S. Hall[b] and J. Klaminder[c]

[a]Department of Ecology and Environmental Science, Umeå University, Umeå, Sweden; [b]Division of Solid Mechanics, Lund University, Lund, Sweden; [c]Department of Forest Ecology and Management, SLU, Umeå, Sweden

ABSTRACT

Insects are known for communicating via sounds created by rubbing body parts, a behaviour referred to as stridulation. Red Wood ants (*Formica rufa* group) are not known to possess stridulatory organs and have historically been categorised as a non-stridulating species. In this exploratory study, we report that Red Wood ants generate stridulation-like sounds, being about 0.7 ± 0.2 seconds-long (mean ± standard deviation), rattling sounds that were repeatedly generated in our laboratory setting (about 0.6 productions h-1 individual-1). In addition, we assess Red Wood ant behavioural responses to playbacks of this stridulation-like sound. Our playback experiments show that the stridulation-like sound initiates a reduced locomotory speed among conspecifics, an effect not seen when the ants were exposed to silence. However, this response was not different from that generated by an artificial pure tone, making the use of this stridulation-like sound uncertain. We hypothesise that the stridulation-like sound is produced by rubbing the leg against a ridge structure located on the anterior margin of the pronotum, a structure that we describe using X-ray micro-tomography. Our exploratory study suggests that the recorded sound may be part of *Formica* ant communication that is hard to detect and easily missed in behavioural assays.

ARTICLE HISTORY
Received 25 November 2024
Accepted 22 April 2025

KEYWORDS
Biophony; behaviour; invertebrates; soil; bioacoustics; stridulation

Introduction

It is estimated that more than 195,000 insect species communicate via near-field airborne sounds and substrate-borne vibrations (Cocroft and Rodríguez 2005). For example, male stone flies (Plecoptera) create vibrations on surface waters to attract females and male crickets (Orthoptera) rub their legs to generate acoustic signals that attract reproductive partners (Nielsen and Dreisig 1970; Sandberg and Stewart 2006). The latter behaviour is called 'stridulation', which is when the friction between body parts is used to generate airborne sounds. By extracting the number of stridulating ant families from Golden and Hill (2016) and using the estimated number of species in each family from Schultheiss et al. (2022), we approximate that there are likely around 6000 ant species that stridulate.

CONTACT G. L. R. Barbier garance.barbier@umu.se
Supplemental data for this article can be accessed online at https://doi.org/10.1080/09524622.2025.2500391

This latter number corresponds to about 18% of all ant species. While ants are recognised for communicating both via near-field airborne sounds and substrate borne vibrations, it remains uncertain why some species stridulate while others do not (Cocroft and Rodríguez 2005).

Stridulation should not be confused with vibrational communication that occurs between ants through 'drumming', where ants hit the ground or the surrounding tunnel with their mandibles, legs or abdomen (Hölldobler 1999). For ants, communication via stridulation was first hypothesised to originate as a distress call from a buried ant, trapped by a collapsed tunnel, but literature reviews indicate that the stridulating species are mostly tree-living species (arboreal), making the burial signal hypothesis less likely (Markl 1973; Golden and Hill 2016). Other possible functions of stridulations in ants may be that the vibration functions as an alarm, a warning against potential attackers, an echolocation system, a signal used for recruitment of conspecifics, a signal to initiate exchange of regurgitated liquids between individuals (trophallaxis), a tool for leaf-cutting or an identification-sign among conspecifics (Hölldobler and Wilson 1990; Esperson 1994; Ware 1994; Roces and Hölldobler 1996; Chiu et al. 2011; Casacci et al. 2013; Schönrogge et al. 2017). On top of having species-specific uses, stridulation may show intra-specific plasticity, where an ant species can signal differently in a context of feeding versus entrapment (Masoni et al. 2021). Common to all ant stridulations is that they appear as short (i.e. less than a second), identical and consecutive pulses that are referred to as trains or rattles (Golden and Hill 2016). The organs in ants that have been recognised to produce stridulation (henceforth referred to as 'stridulatory organs') consist of a file (also known as *pars stridens*) and a scraper (Hernández et al. 2002; Hölldobler and Wilson 1990). In most ant groups studied by Golden and Hill (2016), the scraper is located in the petiole or post-petiole and rubs against striations on the first gaster segment. Grasso et al. (1998) note that there are two species (*Nothomyrmecia macrops* and *Streblognathus aethiopicus*) and one sub-family (Ectacominnae) that have stridulatory organs that differ from this scheme. In these exceptions, the file and the scraper are still present, but the location and number of these elements differ (Grasso et al. 1998). Organs that may serve as receivers of the stridulation vibrations include the chordotonal organs on the legs that are sensitive to substrate-borne vibrations, as well as the trichoid sensilla on the cuticle and the Johnston organs, both antennal stretch receptors that show sensitivity to airborne sounds (Hickling and Brown 2000; Hill 2009; Masoni et al. 2021). Notably, Roces and Tautz (2001) have argued that Johnston organs are insensitive and likely to only detect near-field airborne sounds, and ants' ability to detect airborne sounds is debated (Markl 1973; Roces and Tautz 2001; Golden and Hill 2016).

Red Wood ants (*Formica rufa* group) represent a keystone species in forests of Eurasia (Robinson et al. 2016; Stockan et al. 2016) and biophony from their mounds has been suggested as a future target for soil macrofauna studies (Sørensen et al. 2024). Red Wood ants belong to a subfamily (*Formicinae*) of ants that are considered non-stridulating as they lack known stridulation organs (Golden and Hill 2016). In this study, we report that *Formica rufa* ants seem to generate a stridulation-like sound and present audio files and spectrograms of their generated vibrations. We demonstrate that the recorded short rattles produced by the ants were not a by-product of other activities (generated by drumming, walking, grooming or feeding) by conducting simultaneously acoustic and

video recordings. We evaluate to what extent the rattles fitted that of a communication signal using criteria outlined previously by Scott-Phillips (2008), suggesting that this type of signal needs to (i) be repeated over time and (ii) generate behavioural responses among conspecifics. To evaluate these criteria, we recorded biophony of Red Wood ants in video-monitored laboratory experiments and conducted a playback experiment to test eventual ant behavioural responses to our recordings. In addition, we surveyed possible stridulation organs for Red Wood ants using *X*-ray microtomography (or micro-CT).

Material and methods

Acoustic detection of stridulation

We collected a few hundred *Formica rufa* ants from a mound located outside the Campus of Umeå University (63.817491°N, 20.309429°E) during the summer and fall of 2023. The ant colony was collected and transported along with the natural nest materials to avoid stressing the transported individuals. Ants were collected from the upper (<20 cm) part of the mound to obtain a mix of workers of various ages and castes. Once in the lab, the ants were kept at 60% (±10%) humidity and at 22 (±2)°C. The light cycle was fixed at 12 hours of light per day. The colony was placed in an open glass tank ($100 \times 45 \times 45$ cm^3) with their nest material and allowed to habituate for a week before we recorded them. Food was provided as a solution made by mixing 20 g of glucose with 100 mL of water, following the recipe proposed by Madsen and Offenberg (2020). This glucose solution was provided *ad libitum* in two small, ant-sized drink-troughs per terrarium.

We performed acoustic recordings of the worker ants using a small Plexiglas box ($10.4 \times 10.4 \times 8.6$ cm^3) as testing arena (the walls of the box were oiled with paraffin oil to prevent ants from escaping). Taking inspiration from Elias et al. (2003) for the transmission material, we glued a piece of 80 g m^{-2} printer paper (10.9×9.1 cm^2) across the box at approximately halfway up the sides of the box; see Figure 1. A piece of gold-plated copper wire (7.3 cm length, diameter 1 mm) was put in contact with the paper bottom on one end and with a contact microphone (3.0 m/6.3 mm contact microphone, Leaf Audio) on the other end. The microphone and the wire were clamped together on the wall of the box. The contact microphone was linked to a preamplifier (DM2 TNT by sE electronics), a connector (4 STAR A JF3 XM3 by Adam Hall) and a recorder (DR-40X by TASCAM). We used a sampling rate of 48 kHz, 24-bit for all recordings. The box was placed in a cabinet that, when covered by acoustic foam, became an anechoic chamber. The top of the chamber was pierced to let a camera (Point Grey Grasshopper) record the box from above (Figure 1(a)). We placed groups of 5 ants in the box for 15 minutes, and the procedure was repeated for 18 groups. The audio files were examined in search for a series of brief pulses (about 1 sec long) acoustically resembling stridulation described for other ant species (see Grasso et al. 2000 for an example). If stridulation-like sounds were detected, time-synchronised video and sound files were used to assess the vibro-acoustic generating behaviour. Acoustic parameters were analysed for each detected stridulation-like sound, including time (beginning, end, duration), frequency (lowest, highest, range), power (average, peak, delta) and energy using Raven Pro Interactive Sound Analysis Software (Yang and Center for Conservation Bioacoustics at the Cornell Lab of Ornithology 2024).

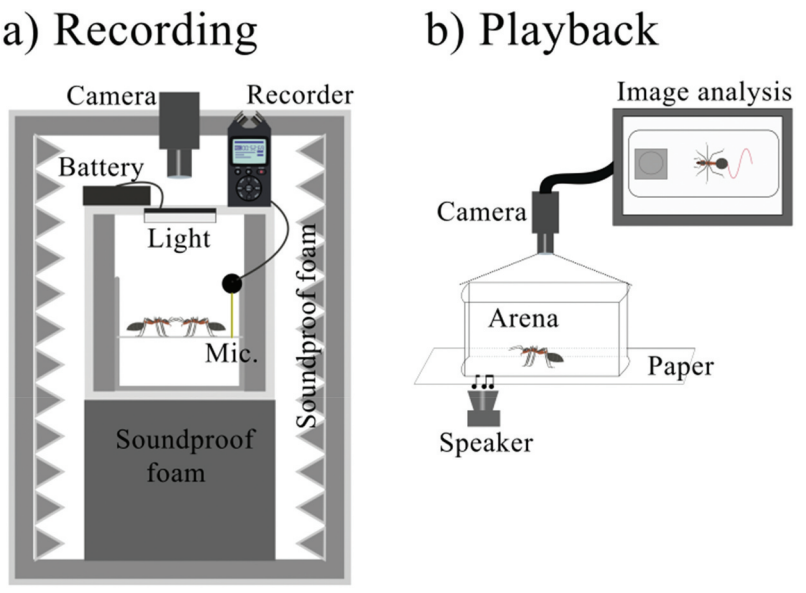

Figure 1. (a) A schematic representation of our recording protocol conducted inside a soundproof cabinet (the foam used for insulation is represented in grey). Ants are standing on a piece of paper glued to the sides of the arena. Also shown are the components used (camera, battery, light and recorder) and their approximate placing. (b) Illustration of the playback experiment where we show placement of the camera, speaker and the tested ant individual. Also shown (right upper corner) is an illustrated view of our behavioural arena seen from above. This latter view was what we used in the image analysis and tracking of ant behavioural responses.

Playback experiment

For each behavioural trial in our playback experiment, one ant was placed in an arena ($104 \times 210 \times 74\ cm^3$) as illustrated in Figure 1(b). The bottom of the arena was made of a white paper, replaced prior to each trial, overlying a speaker (Go Essential, JBL) playing the recorded stridulation-like sounds. We selected three different acoustic event recordings from our stridulation experiment and looped each sound to create a 1-minute file with 10 seconds of silence between the stridulation-like sounds. To ensure consistency, the playback sounds were recorded and processed in the same way as the experimental ones. The rationale for selecting sound-silent cycles where that this approach offered the best balance between two identified risks. First, playing only one stridulation-like sound increases the risk for the ant to miss the induced vibrations. Second, a long uninterrupted event would sound unnatural. In our playbacks, we used recordings that contained the least background noise. The three unique recorded stridulation-like sounds that we used in our experiments (available on Figshare https://doi.org/10.6084/m9.figshare.28704548.v1) were as follows: (i) Stridulation-1, a high-frequency sound (dominant frequency 6.2 kHz, peak power density −33.83 dB FS/Hz) with an indistinct envelope; ii) Stridulation-2, a broad-frequency sound (dominant frequency 4.4 kHz, peak power density −23.89 dB FS/Hz) with high power density and iii) Stridulation-3, a pulse-varied sound (dominant frequency 3.6 kHz, peak power density −23.03 dB FS/Hz). Prior to the behavioural trial, we performed a calibration process to ensure that the speaker accurately replicated the

intended vibrations. We made the played sounds closely match the natural amplitude of the original emissions by reducing their amplitude by 30 dB to compensate for the preamplifier's gain. We used a vibrometer (Polytec VibroGo-200 laser-Doppler vibrometer) to further verify that our playbacks were transmitted to the paper arena. To assess eventual different responses between our stridulation-like sounds and control for other sounds, we created an artificial pure-tone sound with Audacity 3.4.2 (Audacity Team 2023) consisting of 20 sounds of 1 second each at 300 Hz frequency, played at the same volume as the stridulation-like sounds. Single nestmate ant individuals, collected from the surface of the mound to assure they were all foragers, were released into the arena for each trial. A single ant's behaviour was tested per trial, but each trial consisted of two time-periods (before and after exposure to our playbacks) following a BACI-design. During the first minute of each trial, ant locomotory activity was filmed in the arena without any acoustic treatment (silence). During the second minute, ants were exposed to one of the five different treatments: a silent control ($n = 18$), artificial pure-tone sound ($n = 18$), Stridulation-1 on loop ($n = 18$), Stridulation-2 on loop ($n = 18$) and Stridulation-3 on loop ($n = 18$). This method allowed us to avoid order effects triggered when the stimuli were played one after the other. We opted for a minute-long loop to maintain equal time spent in silence and with the stimulus, simplifying comparisons between the first and second minute. We followed recommendations from Kroodsma et al. (2001) and did not create a composite of several stridulation-like sounds (each group was exposed to only one type of stridulation-like sound). The movies of the ants' behaviours were analysed using Animal TA (Chiara and Kim 2023). Behavioural traits measured were speed and meandering of the ants between the first and the second minute. The output from Animal TA is in arbitrary units and meandering is a value that represents how many turns are found in the trajectory of the animal.

Statistical methods

All the statistical analysis was done with R v4.4.0 (The R Core team 2024). The response variables measured in the playback experiment were the average speed of the ants and their meandering. For the statistical analysis, we used a general linear mixed model (GLMM) with a template model builder (TMB) with the glmmTMB function of the glmm TMB package (Brooks et al. 2017). Each response variable had its own model and was analysed the same way: treatment (five levels: silent control, artificial pure-tone sound, stridulation-1, stridulation-2, stridulation-3) and time (two levels, minute one before treatment and minute two after treatment) were considered as fixed factors. The interaction between those factors was included in the model formula. For both factors, we used the default Gaussian distribution as the distribution family.

We analysed each unique stridulation-like recording separately to avoid pseudo-replication that is otherwise common for these kinds of experiments (Kroodsma et al. 2001). Despite being tested once, each ant is represented in the data set by two recordings: one for the first minute and one for the second minute. For this reason, we had to code ant identity as a random factor. We used the simulateResiduals function of the DHARMa package to check for distribution and make sure there were no outlier residuals (Hartig 2022). The Anova function

was used to investigate the relationship between the factors (Fox and Weisberg 2019). Whenever the Anova showed a significant interaction between the factors, post-hoc pair-wise Tukey tests were performed using the lsmeans function of the lsmeans package (Lenth 2016).

Observations of stridulatory organs

Potential stridulation organs were screened with *X*-ray micro-computed tomography (micro-CT) using a ZEISS Xradia 520 Versa 3D X-ray Microscope, equipped with ZEISS Scout and Scan Control System software (version 16.1.14271.44713), at the 4D Imaging Lab at Lund University. The randomly selected worker specimens ($N = 3$) were inserted in 2 cm long MicroLumen high-performance polyimide tubes for the scanning. Each scan involved acquiring 1601 radiographic projections over 360-degree sample rotations with a 4× detector objective, exposure times of 0.5 or 1 second (depending on the sample and image resolution) and with camera binning of 2 × 2 pixels. The source voltage and power were set to 80 kV and 7 W, respectively, and no source filter was used. Scanning duration was in the order of 1 hour per scan. We captured the full specimen scans at lower spatial resolution with vertical stitching of 2–4 datasets along the specimens' length. We only obtained higher resolution scans over key regions of the specimens. We reconstructed the tomographic images with the Zeiss Reconstructor software to provide 3D images of about 1000 × 1000 × 1000 voxels (height × width × depth) with cubic voxel with side length ranging from 1.5 µm to 4.855 µm. We saved the 3D reconstructions in TIFF format slices. We used 3D Slicer v. 5.6.2 r32448 to postprocess the TIFF data, since it enables virtual examinations of the 3D surface models with volume rendering functions (Fedorov et al. 2012). To achieve desired volume renderings, adjustments were made to the colour space range, ensuring the exterior surface of specimens remained visible while maintaining the highest available quality.

Results

Acoustic detection of stridulation

We detected repeated signals ($N = 18$) characteristic for ant stridulation. Here, a single stridulation-like event typically consisted of a 0.7 ± 0.2 second-long (mean ± standard deviation) long train of pulses (Figure 2). In our recordings, the mean frequency and amplitude varied somewhat depending on acoustic settings, where the samples with least interference from other ant biophony indicate mean frequencies of 5 ± 1 kHz (mean ± standard deviation), while the strength of the signals typically ranged between −33 and −22 dB FS/Hz. We rarely observed several trains of pulses happening in a row. The frequency (number of signals divided normalised to the number of individuals in the trial and time) at which we detected stridulation-like sounds in our recordings was approximately of 0.6 signals h^{-1} $individual^{-1}$. Our video monitoring revealed that these sounds were not produced by drumming or any rubbing against artificial surfaces. We did not observe any aggressive response, like mandible opening or dashing, at the time of the stridulation-like recordings.

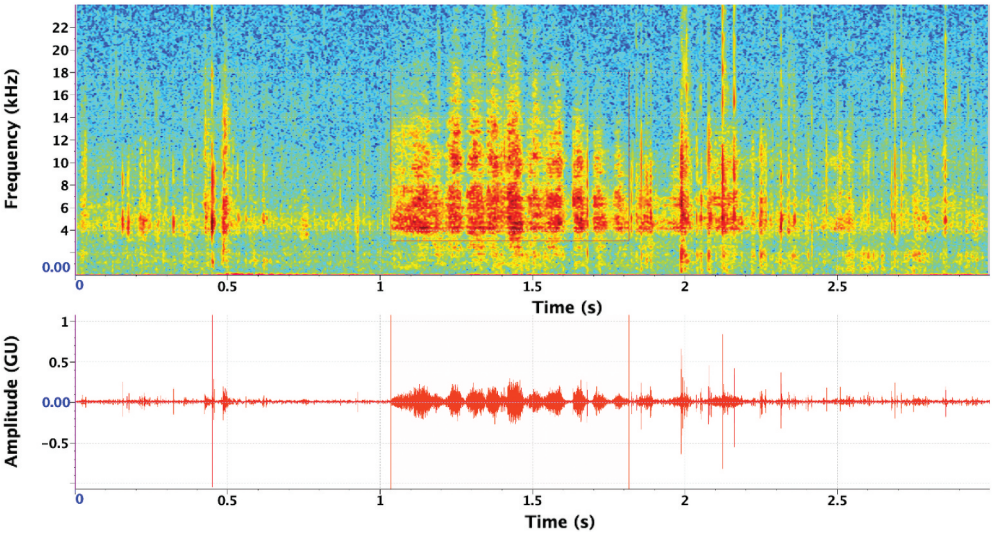

Figure 2. Spectrogram (upper panel) and oscillogram (lower panel) of a recorded stridulation-like sound. The frequency is in kiloHertz and the amplitude in arbitrary units. Spectrogram settings: window type Hann, 512 points, 3 dB filter bandwidth 135 Hz, 87.5% overlap, grid spacing 93.8 Hz.

Playback behavioural trial

In our playback experiment, ants in the silent treatment showed a higher average speed during the second minute of recording, which contrasted with trends seen for the other treatments that included exposure to sounds (artificial 300 Hz pure tones or stridulation-like sounds), where the average speed was reduced during the second minute (Figure 3(a)). The latter effect was strong enough to generate an overall reduction in speed over time (Table 1). We found a significant interaction effect with time (Treatment × Time) for our stridulation treatments, where our BACI design suggested that ants exposed to our playbacks, in contrast to the silent control, reduced their speed during the second minute. Here, our post-hoc analysis revealed that this effect was driven mainly by a 30% reduction in speed for ants exposed to Stridulation-1. Even though reduction in average speed was noted also for the other two recorded stridulation-like sounds and the pure tone (see Supplementary Material Table S1 for the full results), the effects were not significant in these treatments. We found no main effect of Time (minute 1 vs minute 2) or Treatment on the meandering behaviour of the studied ants (Figure 3(b), Table 1). While the meandering increased on average during exposure to our stridulation-like sounds playbacks as well as for the pure tone, we found no significant Time × Treatment interaction effects.

Observations of stridulatory organs

We found a ridge on the pronotum (first thorax segment) (Figure 4(a)) of all three examined Red Wood ants that was hollow and had a rough external structure (Figure 4(b)). The ridge in question was a few millimetres long and ran along the anterior margin of the ant's pronotum. This ridge was within the reach of the ant's front legs, but

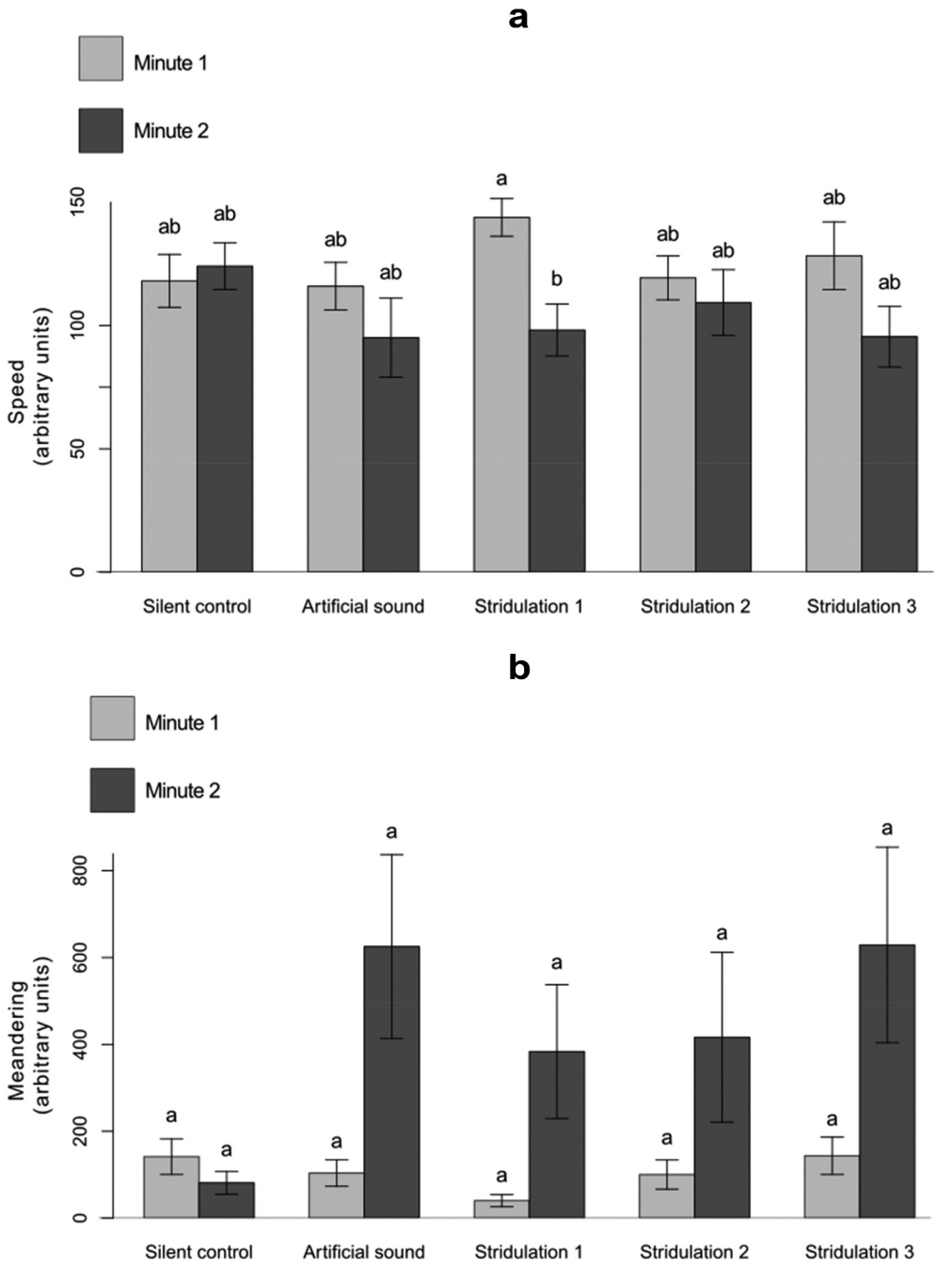

Figure 3. Average speed (panel a) and average meandering (panel b) for the ants in our playback experiment. Averages are calculated for the first minute prior to treatments (light grey) and for one minute during our sound treatments (dark grey). Measured responses are grouped as a function of our different treatments (silence, an artificial pure-tone sound and our three different stridulation-like sounds). Note that we refer to the stridulation-like sounds as 'stridulation' in the figure for simplicity. Similar letters on top of the bars indicate no statistical difference ($p > 0.05$) in our post-hoc analysis. Error bars indicate the standard error.

Table 1. Summary statistics for our general linear mixed effect model outlining effects of our treatment and time (first minute vs second minute) on two behavioural traits (speed and meandering).

	Variable	Chi Squared	Degrees of Freedom	P-Value
Speed	Treatment	2.1924	4	0.7004
	Time	13.065	1	0.0003
	Treatment × Time	9.8434	4	0.0431
Meandering	Treatment	1.8647	4	0.7606
	Time	0.2008	1	0.6541
	Treatment × Time	6.6949	4	0.1529

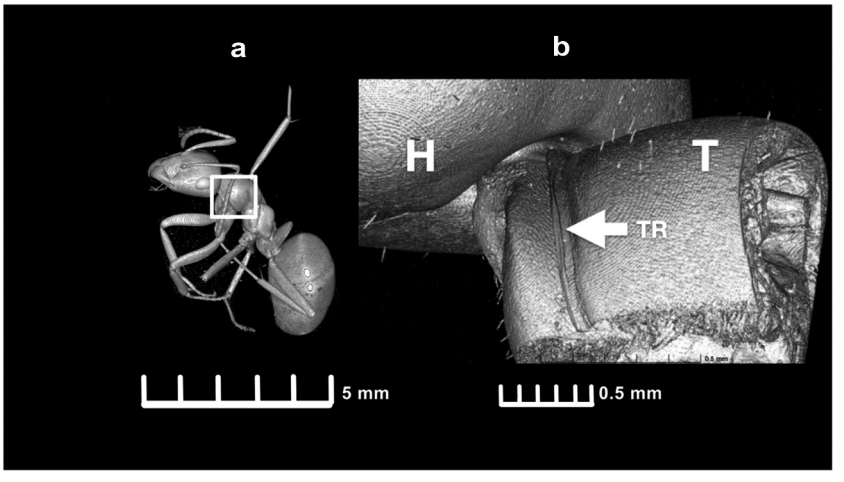

Figure 4. 3D renderings of a micro-CT image of a specimen of the *Formica rufa* group. Shaded surface display volume renderings extracted from 3D scans: full view (a) zoom on the highlighted region thorax ridge side view (b) showing the extension of the thorax ridge (TR) legend: left and right-side views: h – head, *t* – thorax, tr – thorax ridge.

we did not observe any evident structures on the legs that could definitively function as scrapers rubbing against the identified ridge. We suspect sclerotised edges could scrape against the ridge. On the other hand, the micro-CT scan (Figure 4(a)) did not reveal any stridulatory structures on the abdomen or the petiole that could act as a file in the stridulation process (Figure S1, Supplementary Material).

Discussion

Red Wood ants (*Formica rufa* group) produce stridulation-like sounds that are not generated through drumming or interactions with artificial substrates. To classify as a communication signal, Scott-Phillips (2008) suggests that a recorded signal needs to both be repeated over time as well as being able to trigger a behavioural response among conspecifics. Evidently, the 0.7-second-long train of pulses that we recorded was repeated over time, making our stridulation-like sounds fulfil the first criteria. It is, however, less self-evident whether our recordings fulfilled the second criteria. Indeed, the playbacks of our stridulation-like sounds caused a reduced speed among conspecifics in comparison to ants active in silence that increased their speed over time. However, the induced

behaviour response among conspecifics was not significantly different from that generated by a pure tone sound (300 Hz), even though our statistics indicate that only Stridulation-1 had an interaction effect with time. Given the similarities in trends generated for a pure tone and our stridulation-like sound, both for speed and meandering, we cannot exclude that the effect we measured was not specific to the sounds of our playbacks – but rather a response to sound in general. Therefore, we henceforth in the text continue to refer to the recorded vibrations as stridulation-like sound, rather than communication signals or stridulation.

Nevertheless, it is intriguing that *Formica* (belonging to the Formicinae family) generates such a stridulation-like sound, given that these ants are currently categorised as non-stridulatory (Golden and Hill 2016). We know from the phylogenetic results of Golden and Hill (2016) that stridulation has likely evolved several times independently within different sub-families of ants, but our exploratory study indicates that Formicinae do indeed generate vibrations that resemble that produced by other stridulating ant species. It was first hypothesised that stridulation sounds were used in cases of accidental burial of ants (Markl 1973). Nonetheless, recent phylogenetic studies have shown that most genera that possess a stridulatory organ are arboreal and formally rejected the burial alarm hypothesis (Golden and Hill 2016). In this context, our study species is particularly interesting as the *Formica rufa* family nest buried under twigs, soil and needles but spends also considerable time in trees (Risch et al. 2016). However, the sound that we recorded is unlikely signalling burial as our recordings were conducted in hard-bottomed arenas without soil.

While most ant species use organs in the abdomen for stridulation (Grasso et al. 1998), we have yet to find such a structure in *Formica* as it was not seen in our analysis of Red Wood ant individuals. However, the simplicity of the stridulation-like sound itself also indicates a simpler source than the petiole-generated sound observed for other species. Here, our recorded sound showed no frequency modulation, nor any seriation (meaning there is no substructure in the train of pulses). A simple sound suggests that the organ in charge of generating it has likely a simple structure. We propose that the thorax ridge that we have uncovered acts as a potential file against which the ants' hairy legs act like a scraper (it is possible that tibial spurs or other hardened cuticular features, such as hooks or sclerotised edges on the joints, could also fulfil this role). Unfortunately, the resolution on our recorded videos was too low to fully capture the trajectory of ant leg motions, making it difficult to fully validate that the legs acted like a scraper; the films only allow us to exclude drumming and other locomotory actions. Noteworthy, we did not hear the stridulation as frequently as we saw ant-grooming behaviour, making it unlikely that the recorded vibrations were unintentionally generated during self-grooming.

Note that our BACI-design revealed that our playback treatments prevented the increase in speed seen during the second minute for ants in our silent control. We can only speculate about why playbacks with stridulation-like and pure tone artificial pure-tone sound reduced speed among conspecifics as this variable is tied to many independent behaviours. The fact that ant conspecifics slow down to stay in the area for longer when sensing a (vibro)acoustic stimulus (airborne or substrate-borne) is characteristic of attraction, as flight responses are typically viewed as a stress response in *Formica* (Wallis 1963). However, there is another aversive response in ants: aggression. We cannot

exclude that the reduced speed was part of an initiated aggressive behaviour as Red Wood ants spray acid from their gaster and do so standing still (Johansson and Gibb 2016). Nonetheless, we did not smell any acid or observe any mandible opening that would be typical for an aggressive response; there is still the possibility that the ants got into a spraying position without projecting acid.

We noticed that, despite the trend of speed decreasing for all the sound samples, only one of our stridulation-like playbacks had a significant effect in our post hoc test: Stridulation 1. We hesitate to interpret this result as direct evidence for a unique response to the latter sound, as the result may also be an artefact of too low statistical power in the Stridulation 2 and Stridulation 3 treatments that showed comparable average reductions in speed. Nevertheless, the fact that our studied ants were responsive to the playbacks, either via near-field airborne sounds or via substrate-borne vibrations, supports previously developed theory, suggesting that vibro-acoustic communication in ants – if existing – is likely for short-ranged use only (Roces and Hölldobler 1996; Jackson and Ratnieks 2006). As high frequencies get absorbed by the substrate quickly, they cannot travel far: our high-pitched ant sounds must be limited to short-range use (Caldwell 2014).

Conclusion

Red wood ants generate stridulation-like sounds. These sounds are both faint for human ears and rare, which are factors that likely explain why previous studies have missed them. Our model for generating the stridulation-like sound suggests that the vibrations are generated by rubbing legs against a ridge on the pronotum, but this model should be considered tentative given the descriptive nature of our study. However, it is clear that Red Wood ants are able to generate sounds that are perceived by conspecifics. Although it is not possible from our results to fully resolve the purpose of this stridulation-like sound, it seems evident that this vibration-type is something conspecifics pay attention to. Our results highlight the need for studies assessing potential vibro-acoustic signalling in *Formica rufa* and its potential use across different contexts.

Acknowledgements

We would like to thank Jérémy Monsimet for his advice on ant keeping, Saúl Rodríguez-Martínez for his help with the statistical methods and two anonymous reviewers for their comments that made this manuscript clearer and more precise.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by FORMAS (Dnr 2021- 01192) and the Swedish Research Council (2021-05445).

References

Audacity Team. 2023. Audacity (Version 3.3.0) [software]. https://www.audacityteam.org.

Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Maechler M, Bolker BM. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J. 9(2):378–400. doi: 10.32614/RJ-2017-066.

Caldwell MS. 2014. Interactions between airborne sound and substrate vibration in animal communication. In: Cocroft RB, Gogala M, Hill PSM, Wessel A, editors. Studying vibrational communication. Berlin, Heidelberg: Springer Berlin Heidelberg; p. 65–92.

Casacci LP, Thomas JA, Sala M, Treanor D, Bonelli S, Balletto E, Schönrogge K. 2013. Ant pupae employ acoustics to communicate social status in their colony's hierarchy. Curr Biol. 23 (4):323–327. doi: 10.1016/j.cub.2013.01.010.

Chiara V, Kim SY. 2023. AnimalTA a highly flexible and easy-to-use program for tracking and analysing animal movement in different environments. Methods Ecol Evol. 14(7):1699–1707. doi: 10.1111/2041-210X.14115.

Chiu YK, Mankin RW, Lin CC. 2011. Context-dependent stridulatory responses of leptogenys kitteli (Hymenoptera: Formicidae) to social, prey, and disturbance stimuli. Ann Entomol Soc Am. 104(5):1012–1020. doi: 10.1603/AN11027.

Cocroft RB, Rodríguez RL. 2005. The behavioral ecology of insect vibrational communication. Bioscience. 55(4):323–334. doi: 10.1641/0006-3568(2005)055[0323:TBEOIV]2.0.CO;2.

Elias DO, Mason AC, Maddison WP, Hoy RR. 2003. Seismic signals in a courting male jumping spider (Araneae: Salticidae). J Exp Biol. 206(22):4029–4039. doi: 10.1242/jeb.00634.

Esperson JR. 1994. Do ants use ultrasound for personal communication (Hymenoptera: Formicidae)? Aust J Entomol. 33(3):213–215. doi: 10.1111/j.1440-6055.1994.tb01220.x.

Fedorov A, Beichel R, Kalpathy-Cramer J, Finet J, Fillion-Robin J-C, Pujol S, Bauer C, Jennings D, Fennessy FM, Sonka M, et al. 2012. 3D slicer as an image computing platform for the quantitative imaging network. Magnetic Reson Imag. 30(9):1323–1341. doi: 10.1016/j.mri.2012.05.001. PMID: 22770690. PMCID: PMC3466397

Fox J, Weisberg S. 2019. An R companion to applied regression. 3rd ed. Thousand Oaks (CA): Sage. https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Golden TMJ, Hill PSM. 2016. The evolution of stridulatory communication in ants, revisited. Insectes Sociaux. 63(2):309–319. doi: 10.1007/s00040-016-0470-6.

Grasso DA, Mori A, Le Mou F, Giovannotti M, Fanfani A. 1998. The stridulatory organ of four messor ant species (Hymenoptera, Formicidae). Italian J Zool. 65(2):167–174. doi: 10.1080/11250009809386741.

Grasso DA, Priano M, Pavan G, Mori A, Le Moli F. 2000. Stridulation in four species of messor ants (Hymenoptera, Formicidae). Italian J Zool. 67(3):281–283. doi: 10.1080/11250000009356325.

Hartig F. 2022. Dharma: residual diagnostics for hierarchical (Multi-Level/Mixed) regression models. R package version 0.4.6. https://CRAN.R-project.org/package=DHARMa .

Hernández JM, Martínez MD, Ruíz E. 2002. Descripción del órgano estridulador en Messor barbarus (Linnaeus, 1767) (Hymenoptera. Formicidae). An de Biología. 24:167–174.

Hickling R, Brown RL. 2000. Analysis of acoustic communication by ants. J Acoustical Soc Am. 108(4):1920–1929. doi: 10.1121/1.1290515.

Hill PS. 2009. How do animals use substrate-borne vibrations as an information source? Naturwissenschaften. 96(12):1355–1371. doi: 10.1007/s00114-009-0588-8.

Hölldobler B. 1999. Multimodal signals in ant communication. J Comp Physiol. 184(2):129–141. doi: 10.1007/s003590050313.

Hölldobler B, Wilson EO. 1990. The ants. Berlin & Heidelberg: Springer.

Jackson DE, Ratnieks FL. 2006. Communication in ants. Curr Biol. 16(15):R570–R574. doi: 10.1016/j.cub.2006.07.015.

Johansson T, Gibb H. 2016. Interspecific competition and coexistence between wood ants. In: Stockan J, and Robinson E, editors. Wood ant ecology and conservation. Ecology, biodiversity and conservation. Cambridge: Cambridge University Press; p. 123–144.

Kroodsma DE, Byers BE, Goodale E, Johnston S, Liu WC. 2001. Pseudoreplication in playback experiments, revisited a decade later. Anim Behaviour. 61(5):1029–1033. doi: 10.1006/anbe.2000.1676.

Lenth RV. 2016. Least-squares means: the R package lsmeans. J Stat Softw. 69(1):1–33. doi: 10.18637/jss.v069.i01.

Madsen NE, Offenberg J. 2020. Seasonal changes in sugar and amino acid preference in Red Wood ants of the formica rufa group. Sociobiology. 67(2):144–152. doi: 10.13102/sociobiology.v67i2.3760.

Markl H. The evolution of stridulatory communication in ants. In: *International Union for the Study of Social Insects (7th), Proceedings*. London, 1973:258–265.

Masoni A, Frizzi F, Nieri R, Casacci LP, Mazzoni V, Turillazzi S, Santini G. 2021. Ants modulate stridulatory signals depending on the behavioural context. Sci Rep. 11(1):5933. doi: 10.1038/s41598-021-84925-z.

Nielsen ET, Dreisig H. 1970. The behavior of stridulation in orthoptera ensifera. Behaviour. 37(3–4):205–251. doi: 10.1163/156853970X00358.

R Core Team. 2024. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Risch AC, Ellis S, Wiswell H. 2016. Where and why? Wood ant population ecology. In: Stockan J, and Robinson E, editors. Wood ant ecology and conservation. Ecology, biodiversity and conservation. Cambridge: Cambridge University Press; p. 81–105.

Robinson EJH, Stockan JA, Iason GR. 2016. Wood ants and their interaction with other organisms. In: Stockan JA, Robinson EJH, editors. Wood ant ecology and conservation. Cambridge (UK): Cambridge University Press; p. 123–144.

Roces F, Hölldobler B. 1996. Use of stridulation in foraging leaf-cutting ants: mechanical support during cutting or short-range recruitment signal? Behav Ecol Sociobiol. 39(5):293–299. doi: 10.1007/s002650050292.

Roces F, Tautz J. 2001. Ants are deaf. J Acoustical Soc Am. 109(6):3080–3082. doi: 10.1121/1.1370085.

Sandberg JB, Stewart KW. 2006. Continued studies of vibrational communication (drumming) of North American Plecoptera. Illiesia. 2(1):1.

Schönrogge K, Barbero F, Casacci LP, Settele J, Thomas JA. 2017. Acoustic communication within ant societies and its mimicry by mutualistic and socially parasitic myrmecophiles. Anim Behav. 134:249–256. doi: 10.1016/j.anbehav.2016.10.031.

Schultheiss P, Nooten SS, Wang R, Wong MK, Brassard F, Guénard B. 2022. The abundance, biomass, and distribution of ants on Earth. Proc Natl Acad Sci USA. 119(40):e2201550119. doi: 10.1073/pnas.2201550119.

Scott-Phillips TC. 2008. Defining biological communication. J Evol Biol. 21(2):387–395. doi: 10.1111/j.1420-9101.2007.01497.x.

Sørensen LV, Rodríguez-Martínez S, Rollo M, Klaminder J. 2024. Continuous measurement of red wood ant (Formica rufa) outdoor behaviour using passive acoustic monitoring. Eur J Soil Biol. 123:103687. doi: 10.1016/j.ejsobi.2024.103687.

Stockan JA, Robinson EJH, Trager JC, Yao I, Seifert B. 2016. Introducing wood ants: evolution, phylogeny, identification and distribution. In: Stockan JA, Robinson EJH, editors. Wood ant ecology and conservation. Cambridge (UK): Cambridge University Press; p. 1–36.

Wallis DI. 1963. A comparison of the response to aggressive behaviour in two species of ants, formica fusca and formica sanguinea. Anim Behaviour. 11(1):164–171. doi: 10.1016/0003-3472(63)90025-3.

Ware AB. 1994. Factors eliciting stridulation by the ponerine ant streblognathus aethiopicus Smith (Hymenoptera: Formicidae). Afr Entomol. 2(1):31–36.

Yang KL, Center for Conservation Bioacoustics at the Cornell Lab of Ornithology. 2024. Raven Pro: interactive sound analysis software (Version 1.6.5) [Computer software]. Ithaca, NY: The Cornell Lab of Ornithology; https://www.ravensoundsoftware.com/.