Title: A Burrowing Spider, *Latouchia typica* (Araneae: Ctenizidae), Uses Vibrational Cues as a Trigger for Predatory Behavior

Running title: Cues triggering predation of burrowing spider

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Abstract. Spiders are one of the most dominant predators in terrestrial ecosystems. Although cues triggering predatory behavior in web-building and wandering spiders are well investigated, studies concerning burrowing species, the most ancestral group of spiders, are relatively limited. To clarify critical cues affecting the predatory behavior in burrowing species, we conducted vibration-reducing experiments using the trapdoor spider, *Latouchia typica* (Araneae: Ctenizidae), and nymphs of the speckled cockroach, *Nauphoeta cinerea* (Blattodea: Blaberidae), as prey. The spider achieved a high success rate of prey capture even in blindfolded conditions but reducing vibration with a rubber mat significantly decreased its predation success rate. In addition, the presence or absence of the blindfold did not affect the predation rates under the reducing vibration condition. These results indicate that substrate vibrations emitted from prey are critically important to trigger the predatory behavior in *L. typica*, but visual and chemical stimuli are not used even in the case when vibration cues are unavailable. This is the first report experimentally demonstrating the critical cues for predation in trapdoor spiders with vibration-reducing experiments.

Keywords: trapdoor spider, visual stimulus, chemical stimulus, vibration reduction

Introduction

Spiders are one of the most dominant predators in terrestrial ecosystems (Michalko et al., 2019; Sugiura, 2020; Valdez, 2020). They use a variety of cues in predatory behaviors, including vibration (Klärner & Barth, 1982), visual (Harland & Jackson, 2000), and chemical cues (Persons & Rypstra, 2000). Substrate-mediated vibrations are particularly important in web-building spiders (Barth, 2002; Wu & Elias, 2014) and recent studies focus on the physical properties of spider silk and how the vibration is transmitted when a prey animal makes contact with the spiderweb (e.g. Vibert et al., 2016; Mortimer et al., 2018). Jumping spiders (Salticidae) have a high visual dependency and use vibration stimuli not only in predatory but also in defense behavior (Shamble et al., 2016). In contrast, the wolf spider, *Hogna helluo* (Lycosidae), searches for prey by relying on chemicals emitted from the prey (Persons & Rypstra, 2000). Girard et al. (2011) confirmed by laboratory experiments that the jumping spider, *Maratus volans*, used a combination of visual and chemical stimuli during courtship. Therefore, a combination of some stimuli may be also important in predatory behaviors.

Burrowing species are the most ancestral group in spiders (Wheeler et al., 2017) and they produce sophisticated and concealed burrows to ambush their prey. As such, the burrowing spiders should have fine sensory organs to accurately detect the approach of prey to the burrow. Understanding the cues used for predatory behavior of burrowing spiders is important for elucidating the evolutionary processes of species-specific predation patterns and signal use in spiders. However, few studies have focused on the cues that trigger the predatory behavior of burrowing species to date.

Ctenizidae, known as trapdoor spiders, use the burrowing strategy and form a door at the entrance of the burrow (Buchli, 1969). Burrows produced by Ctenizidae are well concealed and difficult to find in the field, therefore, few studies have been conducted to detect the cues related to their predatory behaviors. Buchli (1969) observed the behavior of the ctenizid, *Nemesia caementaria,* in the field, suggesting that vibration cues transmitted from prey through the soil have an important role in its predatory behavior. However, the study is fundamentally based on observations and the involvement of other cues including visual and chemical cues has not yet been intensively explored. To confirm the cue(s) used by Ctenizidae for their predation, experimental studies manipulating candidate cues are needed.

In this study, we intend to clarify the critical cues triggering the predatory behavior of trapdoor spiders. *Latouchia typica* (Araneae: Ctenizidae) is distributed in Honshu, Shikoku, and Kyushu, Japan, with adult females appearing year-round and males from September to October (Shinkai & Takano, 1984; Nakamura, 2018). This species is found in lowland to low-mountain forests, urban parks, green spaces and gardens, and makes nests on slopes of the forest floor or along forest roads, in park plantings and stone walls, and beside building foundations (Ono & Ogata, 2018). In our census sites, its burrows are frequently found in the precincts of temples and shrines or in the stone walls of castles. The inside of the burrow is lined with thread. When small insects such as dung beetles and other prey pass near the burrow, the spider vigorously flings out its forelegs to catch and drag the prey into the burrow. During the breeding season in September and October, males of *L. typica* wander out of their burrows and search for the females' burrows to mate. Females lay eggs the next summer.

This species constructs its burrow with a trapdoor hinged on one side with silk and ambushes prey holding on to the underside of the door by its tarsal claws (Shinkai & Takano, 1987) (Movie S1). Some species of Liphistiidae, another burrowing group producing morphologically similar burrows to Ctenizidae, extend the signal thread radially from the burrow, to perceive the presence of prey outside the burrow via vibration cues (Sedgwick & Schwendinger, 1990). However, such structures were not observed around the burrows produced by *L. typica* (S. Nakamura, personal observations). Furthermore, another trapdoor spider, *Conothele fragaria* (Araneae: Ctenizidae), waits for prey by keeping the trapdoor half-opened (Nakahira, 1961), suggesting that visual information may also be critical for predatory behavior in these species.

We hypothesized that *L. typica* uses visual or vibrational cues as a trigger for predatory behavior. We conducted blindfolded and vibration-reducing experiments and examined the predation rate of *L. typica*.

Materials and Methods

Laboratory rearing of *L. typica*

All *L. typica* individuals were collected in Hirado City, Nagasaki Prefecture, Kyushu between October and November in 2018 and 2019. Because body length of adult females is known to be 12-20 mm (Ono & Ogata, 2018), *L. typica* females with body lengths greater than 10 mm, which were regarded as adults or subadults, were used in this study.

Latouchia typica individuals were individually maintained in acrylic containers (Fig. 1; 20 cm long, 1.5cm wide, and 13 cm high) filled with sterilized soil from the collection site, kept at 20 °C under a 12L:12D photoperiodic condition. One side of the acrylic container was processed to become removable, which allowed the spider to be picked out from the burrow for manipulation. A nymph of the speckled cockroach, *N. cinerea* (Blattodea: Blaberidae), was supplied weekly as food. The spiders were held approximately for one month in the laboratory before using in experiments.

Effects of visual cues on the predatory behavior

Females of *L. typica* forming burrows were picked out from the side of the acrylic container and anesthetized with ice for 10 minutes. Then the eyes (treatment, $N = 8$) or prosoma (control, $N = 7$) were coated with paint (Visible Implant Elastomer Tags, Northwest Marine Technology, Inc., Shaw Island, WA), which is a harmless dye frequently used in assessing animal behavior (Tazunoki et al. 2021) before reintroduction to the burrow (Fig. 1). The light-blocking rate of the paint was 93.5% (measured using Illuminance UV Recorder, TR-74Ui, T&D Corporation, Matsumoto). A nymph of *N. cinerea* was placed on the soil of each acrylic container four days after the treatment. The body length and head width of the *N. cinerea* nymphs were 10-15 mm and 5-8 mm, respectively. The behaviors of spiders and cockroaches were then recorded for two hours with a video camera (HDR-CX670, SONY, Tokyo) at the illuminance of 57.8 ± 0.17 lux (mean ± SD), to examine how many times the cockroaches have crossed the burrows and whether or not the spider successfully captured the cockroach within the two hours (=

predation success or failure). The experiment was conducted during the dark period of the rearing condition. Each *L. typica* individual was used once in the experiment.

Effects of vibrational cues on the predatory behavior

Prior to the experiment, a pilot study was conducted to verify whether a rubber mat (2 mm thick) had a vibration-reducing effect. An experimental apparatus was prepared as follows: an aluminum film (CHUWIT, China) was stretched on a plastic cup (12 cm diameter and 5.5 cm deep). Another cup, from which the bottom had been removed, was placed, upside down, on the first cup (Fig. 2a) to prevent the cockroaches from moving out of the film. Talcum powder (Wako Pure Chemical Industries, Ltd., Osaka) was applied at the inner side of the bottomless cup to prevent the cockroaches from climbing the side. Reflective tape (1 × 1 mm) was attached to the film to enhance a laser reflection. The experimental apparatus was placed on a vibration isolation table (UMX-0605, Nippon Boushin Industry Co., Ltd., Numazu). Then vibrations transmitted to the film were recorded using a Laser Doppler Vibrometer (LV-1710, ONO SOKKI CO, LTD., Yokohama; LDV, hereafter) in the situations when nothing was placed on the film (control), when a cockroach was placed on the film, and when a cockroach was placed on the rubber mat placed on the film (Fig. 2a). The distance between the reflective tape and sensor head of the vibrometer was approximately 210 mm, which is the optimal distance of the device. Output signals from the vibrometer were sent to a computer (Windows 10 Pro 1909, Dynabook Inc.) using data acquisition hardware (DS-0320 version 3.0.4.388, ONO SOKKI CO, LTD.) and monitored in real-time using Oscope2 software (2.10.2.14, ONO SOKKI CO, LTD.) for vibration recording. Vibration frequencies were obtained by converting voltages on the data acquisition hardware. In each measurement, the vibration waveform and frequency were recorded for 20 seconds and the video footage was also recorded by a video camera (HDR-CX670, SONY) to synchronize the behavior of the cockroach and recorded vibrations. Five replicates were performed for each treatment.

The vibration from the cockroach placed on the film had some peaks of strong vibration velocities over a wide range, especially in the low-frequency range below 1000 Hz (Fig. 2b). When the cockroach was placed on the rubber mat placed on the film, the vibration was reduced and became almost similar to that of the control group (Fig. 2c, d). As the effectiveness of the rubber mat was confirmed in the pilot experiment, the mat was used in the experiment to manipulate the effects of vibrational cues on the predatory behavior of *L. typica*. The rubber mat was installed on the soil of the container in which the spider formed a burrow. Rubber mats were cut approximately 20 cm long and 1.5 cm wide to fit the size of the acrylic container and placed on the soil surface. The center of the rubber mat was cut out 2-3mm longer and 1-2mm wider than the trapdoor to match the location and size of each spider burrow used in the experiment to minimize the effect of the rubber mat on movement of the trapdoor. In the control, the rubber mat was set along the sidewall to expose the soil surface and thereby remove its effect on vibrational signals from the cockroach. We did not remove the rubber mat in the control to ensure that the odor of the rubber mat does not affect the predatory behavior of *L. typica*. After three days acclimation to the installation of the rubber mat, a nymph of *N. cinerea* was placed into the container. Then, the behaviors of the spider and cockroach were recorded for two hours with the video camera (Fig. 1). Whether or not the spider successfully preyed on the cockroach within two hours (=predation success or failure) was recorded. Because all attacks from *L. typica* were successful in capturing the prey throughout the experiments, "predation success" means that the spider displayed predatory behavior during the 2-hour experimental period.

The same individuals used for the visual cues experiment were used also in this vibrational cues experiment to confirm whether the blindfold treatment affects the predatory behavior of *L. typica* under different vibration conditions. The vibrational cues experiment was conducted approximately one month after the visual cues experiment and each *L. typica* individual was used once also in this experiment.

Statistical analysis

The effects of blindfolding and vibration isolation on the predation success of *L. typica* were analyzed using a generalized linear model (GLMM) with a binomial distribution and a logit link function. In the model, blindfolding and vibration isolation treatments were included as the fixed effects and spider individuals as random effects. The number of crossings by the cockroaches were analyzed between treatments using Welch's t-test. All statistical analyses were performed using R ver. 4.0.3 (R Core Team 2020).

Results

Effects of visual and vibrational cues on predatory behavior

The effect of vibration isolation significantly affected the predation success of *L. typica* (GLMM, blindfold treatment: $\chi^2 = 70.22$, Df = 1, $p < 0.001$), but the effect of the blindfold treatment ($\chi^2 = 0.0093$, Df = 1, $p = 0.923$) as well as the interaction between the blindfold and vibration isolation treatments ($\chi^2 = 0.0183$, Df = 1, $p = 0.892$) were not significant.

In the absence of the rubber mat, predation success rates of *L. typica* were nearly 70%, regardless of the presence or absence of the blindfold treatment (Fig. 3a). Then, cockroaches were captured by spiders within four crossings of the burrows (1.5 ± 0.3 times in the blindfold treatment and 1.5 ± 0.2 times in the control group; mean ± SE). The number of crossings by the cockroaches over the burrow was not significantly different between the presence (7.5 ± 4.3 times) and absence (3.9 ± 2.6 times) of the blindfold (Welch's t-test, $t = -0.79$, Df = 11.17, $p = 0.45$).

When the rubber mat was installed on the soil, the predation success rate was lower than the control (Fig. 3b). Furthermore, in the vibration-reducing condition, cockroaches crossed the burrows for 13.0 ± 2.4 (mean ± SE) times during the experimental period. In contrast, in the control condition, cockroaches were captured by *L. typica* at the latest in the second crossing of the burrow (Movie S2).

Discussion

In our experiment, the predation success rate of *L. typica* did not decrease with the blindfold treatment, so the spider does not to rely on visual information during its predatory behavior. In contrast, the predation rate decreased under the vibration isolation condition. Therefore, vibration sensory stimuli acted as the critical cue triggering the predatory behavior of *L. typica.* Since no predation occurred when prey crossed over the burrow in the experimental area with rubber mats, the spiders in the burrow presumably detect the vibrations of approaching prey prior to capture. In addition, the presence or absence of the blindfold did not affect the predation rate in the vibration reducing experiment, and spiders did not capture prey in the absence of vibration even if the cockroach crossed the burrows many times. This suggests that even when vibration stimuli are unavailable, the spider does not use visual information from prey during predation. Furthermore, *L. typica* might not use chemical cues because they failed to capture the prey in the reducing vibration experiment, in which chemical cues were presumably available.

Vibrations are known to be a critical factor in the capture of prey for many other spiders (Barth, 1982). For example, the vibration of prey transmitted to the web triggers the predatory behavior in some web-building spiders (Klärner et al., 1982; Landolfa & Barth, 1996). Similar to web-builders, vibration cues are probably the most efficient stimulus for *L. typica* and other subterranean burrowing spiders to perceive prey. As far as we know, this is the first report demonstrating the critical cues for predation of burrowing spiders by experimental manipulation. Our present techniques are applicable for other burrowing species and will contribute to further understanding the cues used by them in various situations.

Web-building spiders are generally known to respond to vibration frequencies between 100 and 1000 Hz but not below 50 Hz, and are considered to have adapted to the wing-flapping frequency of flying insects (500-1000 Hz) (Masters, 1984; Landolfa & Barth, 1996; Mortimer, 2019). In our study, the cockroaches placed on the film generated vibrations in the frequency range below 1000 Hz (Fig. 2), which would have been transmitted to the burrow where *L. typica* recognizes and captures the prey (Fig. 2). As vibrations, especially those above 100Hz, are easily attenuated at the soil surface (Hill & Shadley, 1997, 2001), those transmitted in the actual foraging environment of *L. typica* might be different from ones measured on the film. However, it is possible that *L. typica* uses similar vibrations to those used by other spiders, because frequencies transferred from the prey were within similar ranges. As another possibility, *L. typica* might detect signal patterns characteristic of walking of prey (i.e., specific patterns, frequencies, amplitudes generated over time, etc.) to determine whether or not it exhibits the capture behavior. The tangle-web spider, *Enoplognatha ovata* (Theridiidae), consumes adults of a leafhopper, *Aphrodes makarovi* (Hemiptera: Cicadellidae) by eavesdropping on substrate vibrations emitted as sexual communication cues by the leafhopper (Virant-Doberlet et al., 2011, 2019). A web-building spider, *Achaearanea* sp. distinguished vibrations generated from leaves and prey insects impacting the web (Wignall & Taylor, 2011). As both cues had similar high amplitudes at impact, the presence of subsequent vibrations (e.g., pulling a leg, moving the head or thorax, etc.) was suggested to be an important factor in discrimination between prey and non-prey. Further playback experiments using manipulated vibration frequencies are needed to clarify the critical vibration stimuli that trigger the predation behavior of *L. typica*. In addition, the construction of an experimental system that enables us to observe the behavior of spiders in their burrows will be also useful in clarifying the details of the foraging system that uses vibration as a signal, because it would allow us to correlate the spider's behavior with the vibrations emitted by the prey approaching the burrow.

In conclusion, we conducted vibration-reducing experiments and demonstrated for the first time that predatory behavior of a trapdoor spider depends heavily on vibration sensory stimuli emitted from its prey.

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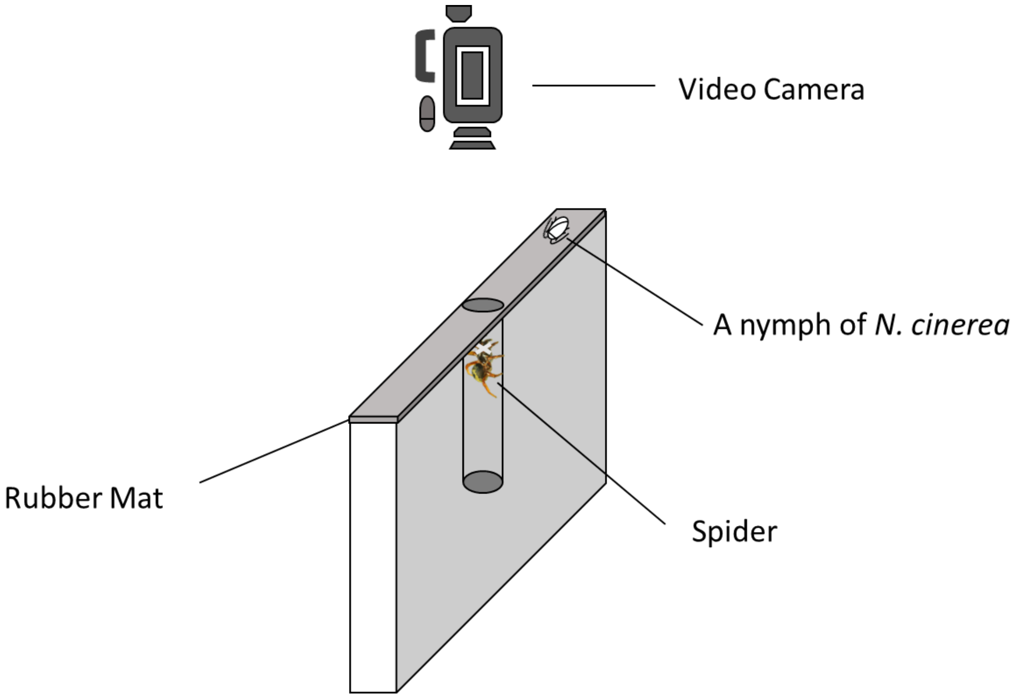
Figure captions

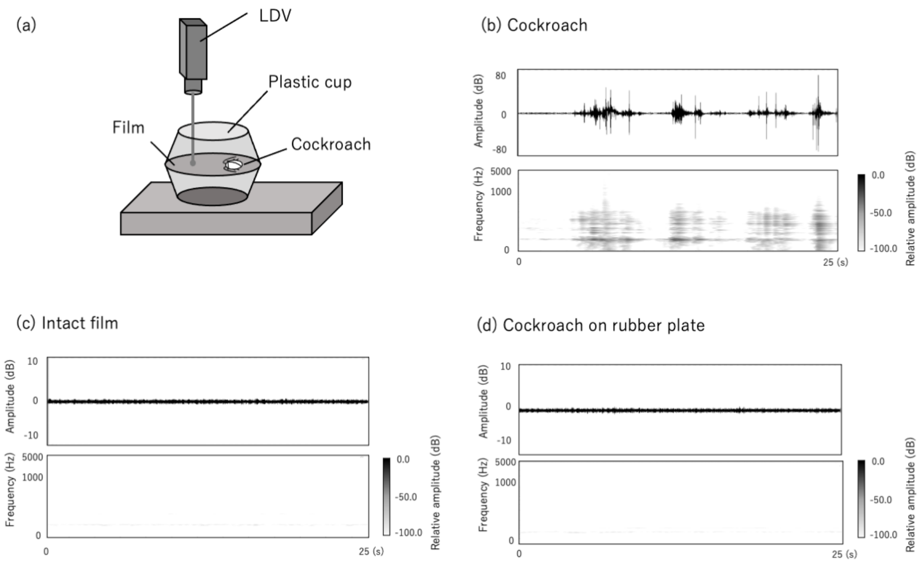
Figure 1. A schematic diagram of the experimental apparatus used for evaluating the vibration isolation effect of the rubber mat. During the evaluation of visual blocking, the rubber mat was not installed.

Figure 2. (a) Schematic diagram of the experimental apparatus used for measuring the vibration emitted from prey (see the text for a detailed explanation); and results of vibration measurements for (b) a nymph of *N. cinerea* walking on the film; (c) intact film; and (d) a nymph of *N. cinerea* walking on a 2 mm thick rubber mat placed on the film.

Figure 3. Predation rates of *L. typica* in blindfold and non-blindfold conditions, and with and without the rubber mat. Figure 3a shows the predation rates of *L. typica* in the presence and absence of paint on the eyes (no significant difference between the control and blindfold treatment; GLMM: exact test, $p = 0.923$). Figure 3b shows the predation rate with and without the rubber mat. The predation rate when the rubber mat was installed on the soil was significantly lower than the control (GLMM: vibration isolation treatment: $p < 0.001$).

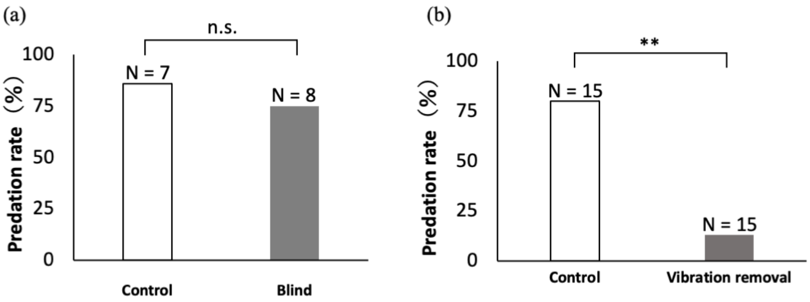


(Figure 1)



(a)
LDV
Plastic cup
Film
Cockroach
(b) Cockroach
Amplitude (dB)
80
0
-80
Frequency (Hz)
5000
1000
0
0
25 (s)
Relative amplitude (dB)
0.0
-50.0
-100.0
(c) Intact film
Amplitude (dB)
10
0
-10
Frequency (Hz)
5000
1000
0
0
25 (s)
Relative amplitude (dB)
0.0
-50.0
-100.0
(d) Cockroach on rubber plate
Amplitude (dB)
10
0
-10
Frequency (Hz)
5000
1000
0
0
25 (s)
Relative amplitude (dB)
0.0
-50.0
-100.0

(Figure 2)



(a)
n.s.
Predation rate（%）
100
75
50
25
0
N = 7
N = 8
Control
Blind
(b)
**
Predation rate（%）
100
75
50
25
0
N = 15
N = 15
Control
Vibration removal

(Figure 3)