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Hanging by a thread: Post-attack defense of caterpillars

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A B S T R A C T

Caterpillars use a diverse range of anti-predator defenses, including camouflage, making and hiding in shelters, mimicry, regurgitating, rolling, and biting. Some caterpillars also drop to the ground and hang themselves by a silk thread. This hanging behavior has been described for a long time but has surprisingly been overlooked in entomological research. In this study, we aimed to identify (1) the taxonomic distribution of the species showing the hanging behavior and (2) the type of sensory stimulus that induces the hanging behavior. We first located caterpillars in the forest and stimulated each caterpillar with three different types of sensory stimuli sequentially: visual approaching, wind-blowing, and poking. For those who responded to none of the stimuli, we further delivered harder tactile stimuli (pinching and detaching from the plants by grabbing) and observed whether they performed the hanging behavior. Among the surveyed 88 species, 46 species (comprising 11 different families) were confirmed to perform the hanging behavior. Most species responded to neither visual nor wind stimulus, but about half of the tested individuals responded to one of the tactile stimuli. Our results suggest that hanging by a silk thread is widespread across a range of lepidopteran groups, and they use this behavior as a post-attack defense.

Introduction

Lepidopteran larvae (commonly called caterpillars) are ecologically important animals in the terrestrial ecosystem because they are the main prey of many invertebrate/vertebrate predators (Hawkins et al., 1997). From caterpillars' perspective, predators exert strong selective pressures on them, which in turn has resulted in the evolution of various defensive strategies (Evans and Schmidt, 1990). Caterpillars' most common anti-predator defense is presumably camouflage, but other forms of defenses such as aposematism, mimicry, making shelters, regurgitating, spines, dropping, and whistling have also been reported (Awan, 1985; Bura et al., 2011; Greeney and Jones, 2003; Gross, 1993). One largely under-appreciated anti-predator behavior is dropping from the host plant and hanging by a silk thread.

While walking on a forest trail, one can easily find a caterpillar in the air, hanging by a thread. Some caterpillars falling from the tree canopy are able to hang by a thread produced from the spinneret on their head (Brackenbury, 1996; Craig, 1997). The benefit of hanging by a thread (over just dropping to the ground) is clear: this makes them easier to return to the host plant safely after the falling (Sugiura and Yamazaki, 2006). Then why do they fall in the first place? One reason might be that it helps them escape from imminent predatory threats. In *Semiothisa aemulataria*, substrate-borne vibrations by approaching parasitic wasps trigger the performance of hanging behavior (Castellanos and Barbosa, 2006). The study by Sugiura and Yamazaki (2006) reported 13 lepidopteran species performing the hanging behavior of which some caterpillars performed the hanging behavior in response to the disturbance generated by beating the twigs. While these studies paved the initial way for exploring the hanging behavior, how widespread it is and the sensory cues that elicit such hanging behavior are largely unknown. In this study, we aimed to (1) extend our knowledge on the taxonomic distribution of caterpillars performing the hanging behavior and (2) identify sensory cues that induce caterpillars' hanging behavior.

Methods

Field experiment on the caterpillars' response to different sensory stimuli

We conducted field experiments once or twice a week from May to September 2020 at Mt. Seungdal, Muan, South Korea (N34°54′, E126°27′). During the experiment, we walked along trails and located caterpillars by either i) visual search or ii) beating plants with a stick while holding a white sheet below. When a resting caterpillar was located visually without any disturbance, one experimenter (YK)

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stimulated it with three different sensory stimulus types that mimic a predator's approach/attack in the following order: visual approaching, wind blowing, and poking (see below for the stimulus description). We chose these stimulations because these stimuli are commonly associated with avian predators' approaches or attacks (Hanlon et al., 2018). Although other stimuli (such as chemical or vibrational stimuli) can be also crucial for predator detection (Castellanos and Barbosa, 2006; Greeney et al., 2012), these stimuli were impractical to be adopted for large-scale inter-specific comparison studies because they are often predator specific or difficult to be mimicked in the field conditions. Also, we did not randomize the testing order of each stimulus because the hanging behavior uses stored silk; once the hanging behavior was performed, there may be a chance that the individual cannot perform it again unless it recharges the silk. Thus, we started with the least likely stimulus to induce the hanging behavior. Preliminary testings on several individuals suggested that visual approaching and wind blowing hardly induced the hanging behavior, thus we used these two stimuli first, then proceeded to the tactile stimuli subsequently. For each stimulus type, we stimulated the caterpillar three times. If the caterpillar did not respond to one stimulus type, then we delivered the next type of stimulus. When the caterpillar responded to a stimulus and was hanged by a thread, we waited until it climbed up back to the plant and re-settled (i.e. became immobile at least for a minute on the plant) and delivered the next stimulus.

For visual approaching, the experimenter approached his palm towards the caterpillar's head at a roughly 60 cm/sec speed, starting from c.a. 40 cm distance. The approach stopped when the distance between palm and caterpillar was around 10 cm. For wind blowing, the experimenter blew the wind at a distance of 30 cm from the caterpillar. For poking, we used the tip of a tweezer to poke the dorsal center of the caterpillar's abdomen. While we had no prior hypotheses on which body parts caterpillars would respond, we tried to minimize the variation in the body location that the stimulus was given. We also tried to maintain a similar intensity of the poking stimulus in that poking always terminated when the tweezer pushed the abdomen surface around the half-width of the caterpillar body. If a caterpillar did not respond to any of the three stimuli, we further pinched it using a tweezer to examine whether caterpillars respond to a stronger tactile stimulus. Thus, pinch stimulus was given to those who did not respond to poke stimulus. We pinched their abdomen but did not pull them out from the host plant. Once all testings were done, the caterpillar was collected and brought to the laboratory for species identification. For those who did not respond to any of the given stimuli, we conducted a final tactile stimulation to reduce the false-negative error probability (i.e. to reduce the error that those who did not show the hanging behavior during the field testing actually could perform it); we put them on a plant substrate, waited until it settled on the plant, and grabbed them out from the host plant using a tweezer so that their body was detached to the resting substrate. Then, we recorded whether they showed the hanging behavior or not.

Species identification

We used caterpillars' morphology when there was a clear morphological cue for species identification using field guides. Otherwise, we raised them until they became adults and identified them based on adult morphology. Some unidentified caterpillars died before or during metamorphosis. In this case, we used DNA barcoding as a tool for species identification. We extracted the genomic DNA from the caterpillars' bodies using either TaKaRa MiniBEST Universal Genomic DNA Extraction Kit Ver.5.0 (Takara Korea Biomedical Inc., Seoul, South Korea) or DNeasy Blood & Tissue Kit (Qiagen, Germany), according to the manufacturer's instructions. We used universal DNA primers (LCO1490 and HCO2198) to amplify the mitochondrial cytochrome *c* oxidase subunit I (COI) genes (710-bp). Then, polymerase chain reaction (PCR) was conducted using AccuPower Taq PCR PreMix (Bioneer, Daejeon, South Korea) in the following conditions to amplify the extracted gDNA: pre-denaturation for 1 min at 95 °C followed by 30 cycles of 30 sec at 95 °C, 30 sec at 65 °C, 1 min at 72 °C, and a subsequent final extension of 5 min at 72 °C. We performed electrophoresis using Top Green Nucleic Acid Gel Stain (LED; Genomic Base, South Korea) in 1X TAE Buffer on 1% agarose gel to confirm the success of DNA amplification. PCR product was purified using TaKaRa MiniBEST Agarose Gel DNA Extraction Kit (Takara Korea Biomedical Inc., Seoul, South Korea) and sequenced in both forward and reverse directions. When there existed ambiguous bases, we manually edited the sequences by applying a bi-directional sequence using MEGA X (Kumar et al., 2018). We translated the sequenced bases into a protein sequence and compared them with publicly available sequence databases (BLAST; https://blast.ncbi.nlm.nih.gov) for species identification.

Results

In total, we tested 156 individuals of 88 species. Among those, 45% of individuals showed the hanging behavior (70 individuals of 42 species). The frequency of responded caterpillars differed among stimulus types (Chi-squared test; $\chi^2_2 = 88.41$, $P < 0.001$). Caterpillars hardly responded to neither visual approaching (only one individual responded) nor wind blowing (three individuals of three species) stimulus (Fig. 1). Instead, the hanging behavior was induced mostly by tactile stimuli. In response to the poking stimulus, 18 individuals (15 species) performed the hanging behavior. In response to the pinching stimulus, we found further 28 individuals (further 15 species) performing the hanging behavior. Lastly, during the additional lab testing by grabbing and detaching from the plant, we found 23 more individuals (12 more species) performing the hanging behavior (Table 1).

The family-level summary showed that the hanging behavior was observed in 11 families: Crambidae, Drepanidae, Erebidae, Geometridae, Gracillariidae, Noctuidae, Nolidae, Psychidae, Pyralidae, Tortricidae, Zygaenidae (Table 1). The most common five families in our survey (Crambidae, Erebidae, Geometridae, Noctuidae, and Tortricidae) occupied about 73% of the surveyed species, of which more than half of the species (except Noctuidae) showed the hanging behavior (Table 1). However, this does not exclude the possibility that species in other families have not evolved the hanging behavior because our results are not free from the risk of false-negative probability (See Discussion for details). We also note here that the performance of hanging behavior was highly context-dependent: we found a high degree of intra-specific variation in both (1) the performance of hanging behavior, and (2) the type of sensory stimulus that each individual responded. For example, in *Uproctis piperita*, only 11 out of 18 tested individuals performed the hanging behavior of which six responded to poking, seven responded to pinching, and one responded to further grabbing stimulus).

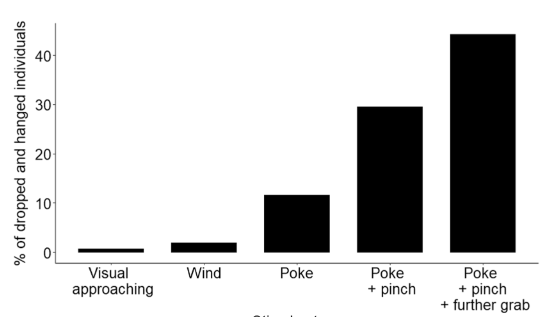

Fig. 1. The percentage of individuals that hung by a silk thread against each sensory stimulus. Both pinch and further grabbing stimuli were delievered only to those did not responded to any of the previously tested tactile stimulations (see Methods). Thus, the percentages in pinch and further grab include those that already responded to previous tactile stimulations and indicated with "+" sign. "Poke + pinch + further grab" group shows the overall percentage of individuals who responded to any of the tactile stimuli.

Table 1
Family-level summary of the species that showed the hanging behavior at least once during the field testing. Pinch stimulus was given to those who did not respond to poke stimulus. We delivered further grabbing stimulus to those who did not respond to any of the stimuli to reduce the error that those who did not show the hanging behavior during the field testing actually could perform the hanging behavior.

Family	Surveyed species	Tested individuals	Number of species that performed hanging behavior	Number of individuals responded to each sensory stimulus				
				Visual	Wind	Poke	Pinch	Further grab
Brahmaeidae	1	1	0	0	0	0	0	0
Crambidae	8	28	8	1	1	4	13	16
Drepanidae	2	2	1	0	0	1	1	1
Erebidae	11	38	8	0	0	6	14	19
Geometridae	24	29	15	0	1	3	8	18
Gracillariidae	1	1	1	0	0	0	0	1
Limacodidae	6	7	0	0	0	0	0	0
Noctuidae	13	17	3	0	1	0	2	2
Nolidae	2	2	1	0	0	0	0	1
Notodontidae	2	7	0	0	0	0	0	0
Papilionidae	1	1	0	0	0	0	0	0
Pieridae	1	2	0	0	0	0	0	0
Psychidae	1	1	1	0	0	0	1	1
Pyralidae	1	1	1	0	0	0	0	1
Sphingidae	3	4	0	0	0	0	0	0
Tortricidae	8	13	6	0	0	4	6	8
Zygaenidae	3	8	1	0	0	0	1	5
Total	88	162	46	1	3	18	46	73

Discussion

Our results demonstrate that the hanging behavior has evolved in a wide range of lepidopteran taxa than previously appreciated (Sugiura and Yamazaki, 2006). Our field survey does not bear the risk of false-positive (classifying a species as performing the hanging behavior when it actually cannot) but has the probability of false-negative error (i.e. erroneously classifying a species as not showing the hanging behavior when it actually can). Therefore we predict that the hanging behavior should be more widespread than our results show. Caterpillars are frequently exposed to the risk of falling from the host plant either incidentally (such as by strong wind blows) or voluntarily (e.g. under imminent predatory threats) (Castellanos and Barbosa, 2006; Yamazaki, 2011). In either case, dropping to the ground is costly because the caterpillars have to spend substantial time and energy to re-settle back to the host plant, during which they are under the risk of being exposed to predators dwelling/feeding on both ground and trees (Kenne and Dejean, 1999). Hanging by a thread reduces or eliminates such risk and enables the caterpillar to safely re-settle back in the host plant (Castellanos and Barbosa, 2011; Soares et al., 2009). While the energetic cost of using silk threads has not been demonstrated yet, it is likely not to outweigh the potential cost of falling to the ground given that the caterpillars could easily climb back to the host plant and feed more leaves to compensate for the energy use. Even some caterpillars re-consume the used silk threads (Shaik et al., 2017). This adaptive benefit could drive the widespread evolution of hanging behavior in caterpillars.

In our field survey, caterpillars particularly responded to tactile stimuli over visual or wind stimuli. While caterpillars have photoreceptors that may enable them to sense visual looming (Ichikawa and Tateda, 1982), they have highly coarse mosaic vision (Gilbert, 1994); thus it may be practically difficult for them to distinguish between the looming generated by a predator approach and environmental noise such as leaves trembling in the wind. The same holds for wind stimulus: the wind generated by a predator approach (e.g. birds) should be difficult to be distinguished by natural wind blowing so that the caterpillars' sensory capacities are unlikely to discriminate between those predator cues and environmental noise. These explain why they hardly responded to either sensory stimulus. However, tactile stimuli strongly elicited the hanging response. This suggests that caterpillars might use hanging behavior as a post-attack defense against predators. We consider that this post-attack performance of hanging should be an effective way to avoid predation by predatory arthropods (Castellanos et al., 2011; Greeney et al., 2012; Soares et al., 2009). For example, ants are one of the main predators of caterpillars that mostly subdue their prey after tactile contacts. While some caterpillars have evolved specialized anti-predator strategies to fight against ants (Dettner and Liepert, 1994), hanging by a thread could play a general avoiding response of caterpillars in response to ant attacks.

Among those species that multiple individuals were tested, we found substantial variation in the performance of hanging behavior. This may be related to the external context under which our stimulus was given. However, another factor that may be possibly associated with the hanging behavior is the caterpillars' size (or larval stage). It has been speculated that smaller (or earlier instar) caterpillars may engage in hanging behavior more than larger ones, presumably because smaller ones are easier to be held by a silk thread and are less affected by gravity during climbing back. While our data do not have sufficient statistical power to formally test this idea, the observed trend in two species, *Artaxa subflava* and *Kidokuga piperita* (the two species with the largest sample size), aligns well with the hypothesis. In *K. piperita*, the proportion of individuals who performed the hanging behavior decreased as their larval stage increased (100 % in early ($N = 6$), 67 % in mid (4/6), and 20% in the last stage (1/5)). We found a similar trend in *A. subflava* as well (33.3% in mid (2/6) and 0% in the last stage (0/4)). These suggest that the larval stage, or size of the larvae, may be one factor that explains the intra-specific variation in the performance of hanging behavior.

In summary, our field survey and experiments demonstrated that the hanging behavior has evolved in a wide range of lepidopteran groups. Tactile stimuli are the primary type of cues that caterpillars respond to perform the hanging behavior. However, we note here that other sensory stimulations (such as vibratory or chemical cues) could also induce the hanging behavior in caterpillars (Castellanos and Barbosa, 2006; Humphreys and Ruxton, 2019). Many caterpillars have a good sensory capacity to detect either (or both) vibrations and chemical cues; thus, if they can sense a predator approach through either sensory channel, it can also elicit an escape response, most likely in the form of dropping and hanging by a thread. This remains to be tested.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aspen.2022.101893.

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