Auditory predator cues affect monarch (*Danaus plexippus*; Lepidoptera: Nymphalidae) development time and pupal weight

Zachary A. Lee, Alex K. Baranowski, Evan L. Preisser [*]

Department of Biological Sciences, The University of Rhode Island, Kingston, RI, 02881, USA

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ABSTRACT

Predation risk is a key factor that impacts the growth and behavior of organisms. The ability to detect and react to potential predators provides a major competitive advantage, but the energetic costs associated with anti-predator behaviors can be severe. Monarch (*Danaus plexippus*) (Lepidoptera: Nymphalidae) caterpillars detect airborne predators through auditory predator cues, identifying the sound of a potential threat and exhibiting anti-predator behavior accordingly. Previous work on this species has examined only short-term behavioral changes in response to predation risk. We exposed monarch caterpillars to recorded predator cues in order to provoke anti-predator behaviors over an extended period of time in an effort to determine the long-term fitness costs associated with these behaviors. Our results show that exposure to wasp buzzing reduces development time and final pupal weight. These results imply that the stress of predation risk causes monarch caterpillars to accelerate their development, pupating more quickly in order to avoid the threat of predation. This shorter developmental time leads to the caterpillars pupating at a suboptimal weight, potentially reducing their future fecundity and lowering their ecological fitness as a whole.

1. Introduction

Predator-prey interactions play a major role in shaping the behavior and population dynamics of species within ecosystems. While prey mortality is the most visible outcome of predator-prey interactions, even the threat of predation can significantly affect organisms (Preisser et al., 2005). Prey assess risk by detecting and responding to predator cues; these cues, even in the absence of the predator itself, can alter prey behavior, habitat use, and morphology (Culshaw-Maurer et al., 2020; Hawlena et al., 2010; Weiss, 2018; Zaguri and Hawlena, 2019). In songbirds, for instance, auditory predator cues have been shown to reduce fecundity by up to 40% (Zanette et al., 2011). This reduction stems from individuals putting time and energy into antipredator responses, such as increased vigilance and avoidance behavior, rather than reproduction (Thaler et al., 2012; Zanette et al., 2011). Trade-offs between antipredator responses and growth/fitness are advantageous to the individual, since dead organisms cannot reproduce; when summed across a population, however, the cumulative impact of such risk responses can equal or exceed that of direct predator mortality (Preisser et al., 2005).

Insects rely on a variety of visual, auditory, and chemical cues to detect and avoid predators (Coss, 2019). Of particular interest are auditory cues, which can often be detected long before an individual can see a predator in terrestrial environments (Breviglieri and Romero, 2019); many insect species detect and respond to such cues (Goerlitz et al., 2020). Because they are generally slow, caterpillars are particularly vulnerable to predators and thus highly reliant on auditory cues (Breviglieri and Romero, 2019). They use these cues to detect wasps and other airborne predators, picking up sounds via structures known as filiform hairs (Breviglieri and Romero, 2019; Tautz and Markl, 1978). These hairs pick up on near-field sounds (particle displacement) in the air, allowing them to detect and react to incoming flying predators. The detection of auditory cues in some species is sensitive enough to identify the type of aerial predator (birds vs wasps) and exhibit defensive behaviors proportional to the threat posed. (Breviglieri and Romero, 2019). Caterpillar reactions to auditory risk cues include freezing in place, body contractions, squirming, and rearing (lifting the forelegs off the host plant) (Haverkamp and Smid, 2020; Tautz and Markl, 1978). Caterpillars also exhibit avoidance behavior, moving towards the main stem of the host plant in order to seek cover from predatory wasps (Stamp and Bowers, 1988). Since the outer leaves of plants provide the highest quality food, movement towards the main stem has a

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detrimental effect on caterpillar health and growth (Stamp, 1997). Conversely, some species of caterpillars respond to predation threat by increasing feeding, gaining weight faster to outgrow vulnerable early life stages (Lund et al., 2020). The high energetic cost of antipredator behavior alone (decreasing or stopping foraging to avoid predators) can be enough to increase mortality even without any actual predation events (Baranowski and Preisser, 2018).

Monarch (*Danaus plexippus*) caterpillars detect auditory cues through filiform hairs on the upper thoracic segment, the removal of which renders the caterpillar unable to detect and react to auditory stimuli (Taylor and Yack, 2019). The caterpillars respond to low-frequency sounds (100–900 Hz) by flicking their anterior segments, contracting their bodies, or freezing in place (Taylor and Yack, 2019). Similar behavioral responses have been noted in response to sounds such as insect buzzing, human voices, and aircraft and road noises (Rothschild and Bergstrom, 1997). Monarch sensitivity to auditory cues reflects the fact that aerial predators and parasitoids (such as predatory wasps and parasitoid tachinid flies) take a high toll on monarch caterpillars (Oberhauser et al., 2017). While this fact suggests that monarch caterpillars should be willing to engage in energetically costly antipredator behavior, these potential growth and developmental costs have not been quantified.

We measured monarch caterpillar growth, development, and survival when exposed to auditory predator cues (wasp buzzing), auditory non-predator cues (mosquito buzzing), and in a no-cue control. Auditory cues from the predatory and non-predatory insect were played at the same volume to control for the effect of sound per se. By exploring whether the short-term behavioral responses observed by other researchers (Cinel and Taylor, 2019; Rothschild and Bergstrom, 1997) incur long-term costs, our work begins to assess the potential ecological consequences of predation risk for monarchs. We hypothesized that exposure to auditory predator cues would hamper the caterpillars' development and long-term fitness.

2. Methods

2.1. Insect rearing

Adult monarch butterflies (*D. plexippus*) were collected in South Kingstown, RI in spring 2020 and hand-paired to produce eggs. Eggs from multiple females were combined and the offspring reared together on common milkweed (*Asclepias syriaca*) collected from a nearby field. Prior to being fed to larvae, *A. syriaca* leaves were sprayed with 2% bleach solution and allowed to air-dry to reduce disease risk. Both larvae and adults were reared in a lab at the University of Rhode Island's East Farm research facility (Kingston, RI) under ambient lighting and temperature regimes. Once the offspring of wild-caught individuals had pupated and emerged as adults, they were again hand-paired and the above process repeated. Eggs from multiple females were again mixed; larvae emerging on the same day were reared together in groups of 20 or fewer in 950 ml plastic deli cups. Larvae entering the third instar were each weighed and placed in individual 350 ml clear plastic deli cups.

2.2. Experimental design

The experiment started when 60 caterpillars (selected for similar third-instar weights and hatch dates) were again weighed and randomly assigned to one of three treatment groups: predatory insect sounds, harmless insect sounds, and no sound (no-cue control). Caterpillars in the predator treatment were exposed to a recording of predatory wasp (*Mischocyttarus* sp.) buzzing (187.5 ± 1.5 [SD] Hz), while caterpillars in the harmless sound treatment were exposed to a recording of harmless mosquito (*Aedes* sp.) buzzing (613.6 ± 141.0 [SD] Hz). Both the wasp and mosquito sound files were provided by Drs. C. Breviglieri and G. Romero (University of Campinas, Sao Paulo, Brazil), who had used them in research assessing behavioral responses to sound in *Hylesia nigricans*

caterpillars (Breviglieri and Romero, 2019). Wasp and mosquito sound files were set to run for 2-s intervals, repeating every 6 s, from 10AM to 10PM. Treatment continued daily from the start of the experiment until pupation. There were 20 caterpillars in each of the three treatment groups.

The 20 caterpillar-containing deli cups in each of the three treatment groups were grouped together and surrounded by eight speakers (NiZHi TT-028, Shenzhen Powerunion Technology Co., Guangdong, China). All speakers were turned on and playing either a sound loop (the predator and harmless treatments) or no sound. Speaker volume was regulated between treatments so that both the wasp and mosquito groups were exposed to an increase in 18–20 dB over ambient levels. While we would have preferred to have interspersed individual replicates from the three treatments, pilot experiments using an interspersed design found an unacceptably high level of between-treatment sound transmission. Even surrounding an individual cup and speaker with commercial-grade acoustic foam (Foamily Inc., Los Angeles CA) was ineffective at stopping the lower-frequency wasp buzzing from affecting larvae in other treatments, and covid-19 restrictions prevented us from using multiple separate rooms for the experiment. The experiment thus took place in a single large (13 m × 6 m) lab space, with 6 m between each treatment group. This distance virtually eliminated between-treatment sound transmission; a BAFX 3370 dB m (Bafx Products LLC, Muskego WI) found that neighboring treatment groups experienced a <2 dB change in sound levels. Each treatment was rotated to a new spot in the room daily in order to expose all treatments to the same environmental conditions and control for any minor differences in microclimate within the lab. Laboratory temperatures ranged from 21 to 23C and humidity ranged from 13-15%.Larvae were reared on *A. syriaca* and checked daily for survival and pupation each day. Fresh 15–20 cm *A. syriaca* leaves were added daily, ensuring that the larvae always had plentiful host plant material available. We ensured that leaves were not yellow or brown or otherwise senescent. Uneaten leaf material was removed after 3–4 days. Once larvae pupated, they were weighed, sexed, and the pupation date was recorded.

2.3. Statistical analysis

Differences in mortality between treatments were analyzed using a Chi-square analysis. Time to pupation and final pupal weight were analyzed using ANCOVA models with starting size and hatching date as covariates. All analyses were conducted using JMP 9.0.0 (SAS Institute, Cary NC.)

3. Results

3.1. Mortality

Prepupal mortality differed between treatments (Chi-square analysis, $X^2 = 7.731$, p = 0.021; Fig. 1A). While no caterpillars died in the no-cue treatment, 2/20 died in the mosquito treatment and 5/20 caterpillars died in the wasp treatment. The difference in survival rates between the wasp treatment and the no-cue treatment was significant, while the mosquito treatment was not significantly different from the other two treatments. Because only a small number of caterpillars died, all subsequent analyses were performed only on caterpillars that survived to pupation.

3.2. Time to pupation

There was a significant effect of treatment on time to pupation (ANCOVA test, $F_{2,50} = 3.32$, p = 0.044; Fig. 1B). Individuals in the wasp treatment group pupated an average of nearly two days earlier than those in the mosquito treatment group. The no-cue treatment did not differ significantly from either the mosquito or wasp treatment group.
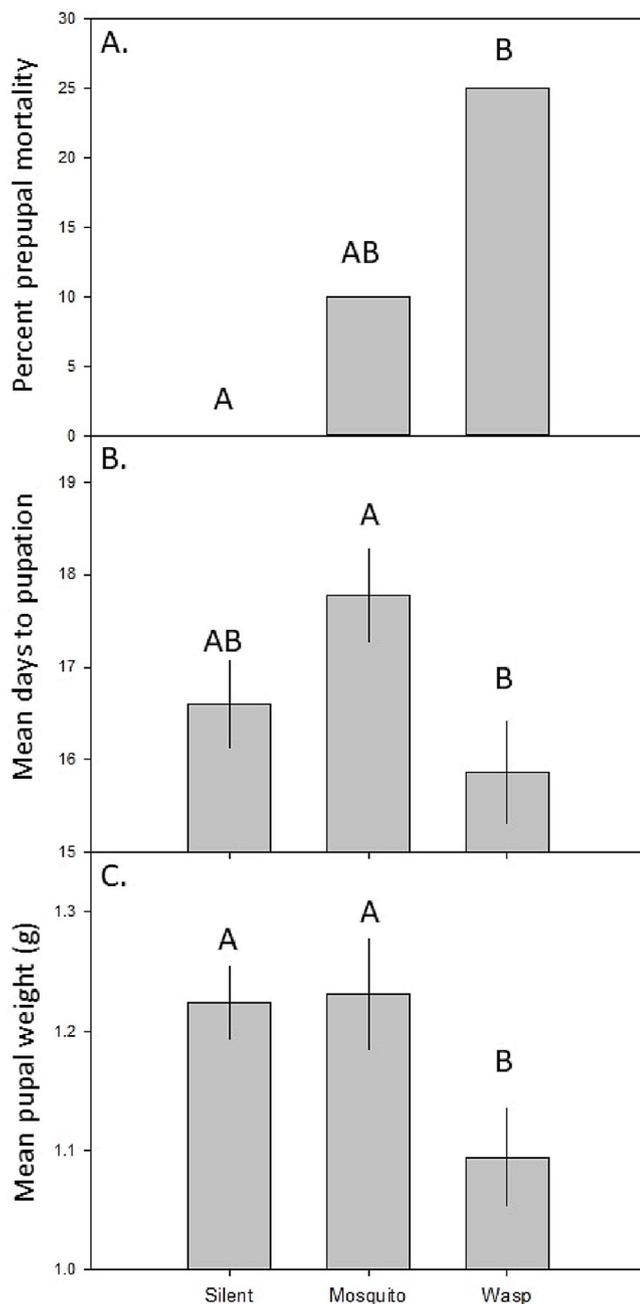
Fig. 1. Mortality (A), time to pupation (B), and final pupal weight (C) of monarch larvae exposed to either wasp buzzing, mosquito buzzing, or no cues. Bars represent means±SE. Capital letters denote significant treatment-level differences (P < 0.05).

3.3. Weight at pupation

Pupae in the wasp treatment were significantly lighter than pupae in the mosquito or no-cue treatments (ANCOVA test, $F_{6,46} = 3.69$, p = 0.033; Fig. 1C). The no-cue and mosquito treatments did not differ significantly from each other.

4. Discussion

Auditory predator cues affected monarch survival, growth and development. This was not a function of sound per se, since auditory cues from harmless insects played at the same volume did not evoke a similar response. This suggests that the caterpillars recognize the sound

of a predator, rather than simply reacting to sound. Prepupal mortality was higher in the wasp treatment than the no-cue control, suggesting that chronic predator stress can even be fatal. The reduced time to, and lighter weight at, pupation reveals that predation risk can induce monarch caterpillars to accelerate development, presumably in order to escape the vulnerable larval life stage. While *D. plexippus* caterpillars are at high risk of wasp predation, these predators pose little threat to pupae (Rayor, 2004). Accelerated development in response to predator cues has been noted in mayflies (Peckarsky et al., 2001), spiders (Li and Jackson, 2005), and grasshoppers (Danner and Joern, 2003). While presumably effective at reducing individual risk, the cost of these and other anti-predator responses can include increased energy consumption and reductions in foraging effort and energy intake (Kempraj et al., 2020; Phuge et al., 2020; Preisser et al., 2005). Fecundity can also be affected, both as a result of physiological constraints (reduced feeding resulting in less energy for producing offspring) or behavioral changes (individuals not mating when exposed to predation risk) (Kempraj et al., 2020).

Insect fecundity is directly linked with female body size, with heavier females producing more eggs across a wide range of species (Honěk, 1993). Predator-induced reductions in larval growth have been found to affect adult body size in a range of insect species (Jourdan et al., 2015). Female monarchs rely on nutrients ingested during the larval stage for egg production, and pupal weight correlates with adult female body size (Oberhauser, 2004b). Because egg production in monarch scales with female body size at eclosion (Oberhauser, 2004a), our data thus suggests that auditory predator cues have the potential to reduce adult fecundity by causing larvae to pupate at a smaller size.

While our results point to a role for predation risk in altering monarch demography, there are several caveats that need to be considered. Since risk-related costs to growth and development had not previously been assessed in monarch caterpillars, our experiment was designed to test for them by eliciting the strongest possible response. Caterpillars were exposed to auditory cues constantly over an extended period, with the cues playing for two of every 6 s over a 12-h period each day for roughly 2–3 weeks until pupation. Such chronic exposure to predation risk likely exceeds that experienced by monarch larvae in the wild; now that responses have been shown, follow-up work should investigate whether more acute exposure to predator cues produces similar responses. Despite multiple attempts to sound-insulate our replicates enough to allow for proper interspersion, we were also unable to block short-range (~1 m) transmission of the low-frequency wasp buzzing. Because covid restrictions prevented us from solving this problem by placing individual replicates in different lab spaces, we were forced to group replicates together by treatment and rotate the groups daily within the lab to control for microclimatic variation. Additional work should be conducted in larger spaces to allow for the spatial interspersion of treatments necessary to fully guard against pseudoreplication.

These caveats notwithstanding, our study appears to be the first to confirm that auditory predator cues can, by themselves, affect lepidopteran fitness. Previous research on auditory cues has focused on short-term behavioral responses (Breviglieri and Romero, 2019; Tautz and Markl, 1978; Tautz and Rostás, 2008); our study builds on this work by exploring the long-term effects of those responses. In addition to being a first step towards understanding the ecological effects of predation risk on monarch populations, our findings may also have implications for lepidopteran management and ecology. Because *D. plexippus* has been observed responding to car and aircraft noises (Rothschild and Bergstrom, 1997), populations located near roads or other high-noise environment may engage in anti-predator behavior sufficient to reduce adult size and thus fecundity. If so, noise levels may prove a useful factor to consider when identifying promising environments for monarch habitat restoration efforts.

Credit author statement

Zachary A. Lee: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Project administration. Alex K. Baranowski Methodology, Validation, Investigation, Writing – review & editing. Evan L. Preisser: Conceptualization, Formal analysis, Resources, Data curation, Writing – review & editing, Visualization, Supervision, Funding acquisition.

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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