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## THE EFFECTS OF AUDITORY PREDATION RISK CUES ON CATERPILLAR GROWTH, DEVELOPMENT, AND FITNESS

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THE EFFECTS OF AUDITORY PREDATION RISK  
CUES ON CATERPILLAR GROWTH, DEVELOPMENT,  
AND FITNESS

BY

ZACHARY A. LEE

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE  
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MASTER OF SCIENCE THESIS

OF

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## ABSTRACT

Predation risk is a major factor that impacts the growth and behavior of organisms. Being able to detect potential predators before contact offers a major competitive advantage, but these anti-predator behaviors often come with a significant energetic cost. Domestication, which can remove predation stress as a selective force, can lead to a reduction in these costly anti-predator behaviors in favor of increased growth and reproduction. Monarch (*Danaus plexippus*) and spongy moth (*Lymantria dispar dispar*) caterpillars both use auditory cues as a primary detection method for airborne predators. We exposed both species to a series of auditory cues in order to elicit anti-predator behavior and quantify the fitness costs of said behaviors in the absence of actual predation. In the case of *L. dispar*, we compared the reactions of domesticated and wild larvae to determine the effects of domestication on their response to predation cues. *D. plexippus* caterpillars displayed shorter time to pupation and lower pupal weight when exposed to predator cues, implying that they accelerate their development and pupate more quickly to escape the risk of predation. This accelerated development leads to the caterpillars pupating at a lower weight, potentially reducing their fecundity and overall ecological fitness. When exposed to auditory predator cues, wild-type *L. dispar* larvae suffered increased mortality, while domesticated caterpillars showed no such response. This suggests a loss of predation risk sensitivity following domestication, the first to be found in an insect species.

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## PREFACE

This manuscript was written to comply with the University of Rhode Island Graduate Manuscript Thesis Format for a masters degree in biological science. This thesis contains a total of two separate manuscripts both intended for separate submissions.

### **Manuscript 1**

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

Co-Authors: Alex K. Baranowski and Evan L. Preisser

Intended Journal: *Acta Oecologica*

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### **Manuscript 2**

Title: The costs of domestication: examining reduced response to predation risk in caterpillars

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**MANUSCRIPTS**

**Chapter 1**

**Auditory predator cues affect monarch (*Danaus plexippus*; Lepidoptera:**

**Nymphalidae) development time and pupal weight**

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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.

### **Key words**

Predation risk, anti-predator behavior, *Danaus* , auditory cues, non-consumptive effects, predator cues

## 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 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.

## **METHODS**

### **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 350ml clear plastic deli cups.

### **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 ( $187.5 \pm 1.5$  [SD] Hz), while caterpillars in the harmless sound treatment were exposed to a recording of harmless mosquito (*Aedes* sp.) buzzing ( $613.6 \pm 141.0$  [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 two-second intervals, repeating every six seconds, 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 decibels 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 (13m x 6 m) lab space, with 6m between each treatment group. This distance virtually eliminated between-treatment sound transmission; a BAFX 3370 decibel meter (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 21-23C and humidity ranged from 13-15%. Larvae were reared on *A. syriaca* and checked daily for survival and pupation each day. Fresh 15-20cm *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.

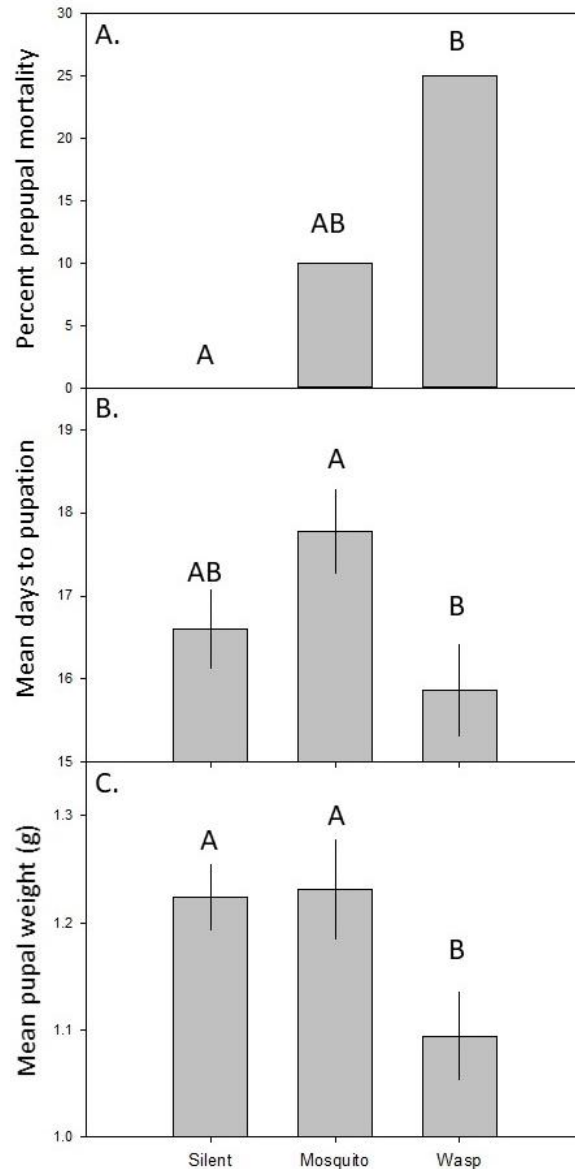


*Statistical analysis:* Differences in mortality between treatments were analyzed using a Chi-square analysis. Time to pupation and final pupal weight were analyzed using ANCOVA models with starting size and hatching date as covariates. All analyses were conducted using JMP 9.0.0 (SAS Institute, Cary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

## RESULTS

### 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



*Figure 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  $\pm$  SE. Capital letters denote significant treatment-level differences ( $P < 0.05$ ).*

only a small number of caterpillars died, all subsequent analyses were performed only on caterpillars that survived to pupation.

### **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.

### **Pupal weight**

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.

## **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 (Kemprij 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) (Kemprij 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 six seconds over a 12-hour 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 interspersions, 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 interspersions 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.

### **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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### **Credit author statement**

**Zachary Lee:** conceptualization, methodology, formal analysis, investigation, writing - original draft, writing - review & editing, visualization, project administration. **Alex Baranowski:** methodology, validation, investigation, writing - review & editing. **Evan Preisser:** conceptualization, formal analysis, resources, data curation, writing - review & editing, visualization, supervision, funding acquisition

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**Chapter 2**  
**The costs of domestication: examining reduced responses to predation risk in caterpillars**

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## **ABSTRACT**

Domestication can lead to significant changes in the growth and behavior of organisms. While the threat of predation is a strong selective force in the wild, this pressure is relaxed in captive rearing environments, resulting in a reduction in sensitivity to biotic stressors. Previous studies have noted this phenomenon in vertebrate species, but no work has been done on domestication-related losses of predation risk sensitivity in insects. We exposed both wild and domesticated *Lymantria dispar dispar* (Lepidoptera: Erebidæ) larvae to recorded predator cues to compare the effects of predation risk on the two stocks. Our results show that while wasp buzzing has a significant negative impact on larval survival in wild caterpillars, domesticated larvae show no such response. These results imply that domesticated *L. dispar* larvae have lost their sensitivity to predation risk and suggest that captive colony-reared insects may not always be analogues to their wild counterparts for behavioral studies.

### **Key Words**

*Lymantria dispar*, domestication, predation risk, auditory cues, non-consumptive effects

## **INTRODUCTION**

Domestication, which includes adaptation of a population to artificial rearing conditions, occurs when the conditions and selective pressures of artificial environments differ from those in natural habitats (Hoffmann and Ross 2018). This allows for factors such as food availability and environmental conditions to be kept at

optimal levels to maximize population growth or other valued traits. Such changes may also, however, alter resistance to starvation, temperature, desiccation, or other abiotic constraints (Hoffmann et al. 2001) and have been linked to shifts in environmental stress tolerance in captive-reared populations (Jones et al. 2021). Artificial and inadvertent selection are key drivers of these domestication-related phenotypic changes, but they can also result from inbreeding and genetic drift (Perez et al. 2021). The short generation times and high fecundity of many insect species means that these changes occur relatively quickly, making them useful for research exploring how domestication affects behavior and physiology (Liedo et al. 2007).

The changes in abiotic stress tolerance seen in captive-reared populations are often accompanied by decreased sensitivity to environmental cues (Price 1999). In laboratory settings, artificial selection for docility, crowding tolerance, and easy handling can yield animals at ease in conditions that free-living organisms would find intolerable (Blanchard et al. 1986, Stanley and Kulathinal 2016). Such altered response thresholds are particularly apparent in the response of captive-reared versus free-living populations to predation risk (Álvarez and Niecieza 2003, Solberg et al. 2020). In free-living organisms, the high fitness cost of a successful predator attack selects for prey capable of behavioral/physiological responses. These defensive responses can be costly: wild-caught dragonfly larvae exposed to visual predator cues experience increased mortality (McCauley et al. 2011). In contrast, the anthropogenic protection afforded domesticated organisms means that they are at little or no risk from predators and other natural enemies. As a result, energy spent on anti-predator behaviors in such environments is wasted (Swaney et al. 2015). A predator-free

environment thus selects for individuals that allocate energy to growth and reproduction at the expense of anti-predator behavior (Storsberg et al. 2018). Although domestication is generally thought to increase predator susceptibility (Solberg et al. 2020) and multiple studies have explored the effects of domestication on insects (Hoffmann and Ross 2018), we are unaware of any research exploring how it affects their responses to predation risk.

The spongy moth (*Lymantria dispar dispar* ('*Lymantria*'; Lepidoptera: Erebidae)), a generalist herbivore, is an ideal model system for exploring how domestication affects insect responses to predator cues. It was introduced into the United States in the 1890s, where it quickly became a devastating forest pest (Alalouni et al. 2013). Because of its substantial economic impacts, a laboratory colony collected in the invaded range has been maintained in captivity for research since 1967 (Grayson et al. 2015). *Lymantria* remains easily found in the forests of the northeastern United States, so both lab-reared and wild-type *Lymantria* strains are readily available. Research comparing the two strains found that domesticated individuals developed more quickly, underwent a shorter diapause, and had higher fecundity than did wild-collected individuals (Grayson et al. 2015). We classify the lab-reared *Lymantria* population as domesticated according to the five-step "domestication level" classification system (Lecocq 2019) that rates captive populations from one (wild population relocated to human-controlled environment) to five (selective breeding and/or bioengineering for specific traits). The lab-reared *Lymantria* population meets the criteria for level four (full human control of life cycle in artificial environments without external gene flow) of this classification system.

We report the results of research measuring the growth, development rate, and survival of wild-type and domesticated *Lymantria* larvae exposed to auditory predator cues (pre-recorded wasp buzzing), auditory non-predator cues (pre-recorded mosquito buzzing), or a no-sound control treatment. Predator and non-predator cues were played at the same volume and timing to control for the effect of sound *per se*. Exposing caterpillars to auditory predator cues is an effective way to elicit anti-predator behavior (Breviglieri and Romero 2019, Taylor and Yack 2019, Lee et al. 2021) and allows us to compare the levels of sensitivity to predation risk between the two stocks. We hypothesized that domesticated *Lymantria* larvae would exhibit reduced or nonexistent responses to predation risk relative to their wild counterparts.

## **METHODS**

### **Insect rearing**

In July 2020, wild *L. dispar* larvae were collected from various host plants at the University of Rhode Island East Farm Research Facility (Kingston RI USA). To guard against the possible spread of field-acquired pathogens, larvae were housed separately in 118mL polypropylene cups with airtight lids and fed sweetgum (*Liquidambar styraciflua*) leaves that had been sprayed with a 2% bleach (0.6% sodium hypochlorite) solution and allowed to air dry. Pupae were sexed and individual pairs of male-female pupae transferred into 473mL paper cups. Adults mated within these cups and females oviposited along the cup walls. Individual egg masses were transferred to separate 473mL clear polypropylene cup with airtight lids. A 3cm x 3cm moistened paper towel square was placed in the bottom of each cup to prevent desiccation. Egg masses were maintained at ambient room temperature until late

October, when they were transferred to a 7° C cooler. In October 2020, we obtained egg masses (USDA permit # P526P-18-01749) from a long-term lab colony maintained for research purposes by the Otis USDA APHIS lab (Buzzard Bay, MA) for 80 generations (Nadel et al. 2020); these egg masses were placed in the same 7° C cooler at the same date. In April 2021, all egg masses were removed from the cooler and placed in ambient temperatures to emerge. All emerging caterpillars were fed fresh crabapple foliage (*Malus* sp.) and kept in 950mL clear plastic cups (approx. 200 per cup) until the start of the experiment.

### **Experimental design**

The experiment was conducted using 240 third-instar caterpillars that had molted the previous day. The newly molted caterpillars from the two lineages (120 wild-type and 120 lab-type) were randomly assigned to one of three auditory risk treatments: buzzing of a predatory insect (*Mischocyttarus* sp.; caterpillar-hunting paper wasp), buzzing of a harmless insect (*Aedes* sp.; mosquito), and no-cue control. Our experiment thus crossed caterpillar lineage (wild-type, lab-type) with predation risk (wasp buzzing, mosquito buzzing, control) for a total of six treatments, with 40 caterpillars per treatment. Styrofoam coolers were used to reduce the risk of sound transmission between treatments. We used a BAFX 3370 dB m (BAFX Products LLC, Muskego WI) decibel meter to measure levels of transmission: sound transmission from one box to the next was measured at <2 dB, while sound treatments within the boxes were measured to provide an 18-20dB increase over ambient levels. Lighting within the boxes was provided by LED light strips that were turned on from 8AM to 8PM. Sound treatments were only played during the 12-hour lighted period.

The 40 caterpillars in each treatment were randomly split into eight five-caterpillar groups; each group was held individually in a 473 mL polypropylene cup. We then weighed each five-caterpillar set to determine initial larval weight per cup; this data was used as a covariate in our models (see below). Four plastic deli cups containing five caterpillars each were then placed in each of 12 Styrofoam coolers. In each cooler, two cups contained lab-type larvae and two contained wild-type larvae (Fig. 1). Each cooler contained a speaker (NiZHi TT-028, Shenzhen Powerunion Technology Co., Guangdong, China) playing the sound treatment: caterpillars in the harmless sound treatment were exposed to a recording of harmless mosquito (*Aedes* sp.) buzzing ( $613.6 \pm 141.0$  [SD] Hz), while the predator treatment groups were exposed to a recording of predatory wasp (*Mischocyttarus* sp.) buzzing ( $187.5 \pm 1.5$  [SD] Hz). The no-sound control group speakers played a loop of silence to control for the possible effects of the speaker (visual effects, heat, etc.). Both insect sound files (mosquito and wasp) were generously provided by Drs. C. Breviglieri and G. Romero (University of Campinas, Sao Paulo, Brazil). These files have been used in previous research assessing behavioral responses of caterpillars to auditory predation, and the wasp buzzing has been shown to elicit antipredator behavior in both *Hylesia nigricans* (Breviglieri and Romero, 2019) and *Danaus plexippus* (Lee et al. 2021). Wasp and mosquito sound files were set to run for 2-second intervals, repeating every 6 seconds, from 8AM to 8PM. The rationale behind this exposure frequency is detailed in the discussion; briefly, this exposure frequency is consistent with previous work and has successfully elicited anti-predator responses in caterpillars (Lee et al. 2021). Caterpillars were fed fresh crabapple (*Malus* sp.) foliage ad libitum, with daily checks



to replace any wilted foliage with new material. Caterpillars were checked daily for mortality and pupation. Survival to pupation as well as the time to pupation and weight of each pupa was recorded; the latter information was used to calculate mean time to pupation and mean pupal weight per cup. All pupae were also sexed; because male and female larvae are externally identical, we were unable to determine the gender of deceased larvae.

### **Statistical analysis**

Prepupal mortality was analyzed using GLM with a gaussian distribution, the main effects treatment, stock, and treatment\*stock, and initial larval weight as a covariate. Because we were interested primarily in whether or not they survived (rather than when they died) we analyzed mean survival per cup rather than changes in individual survival over time. Time to and weight at pupation were analyzed using the same main effects and both initial larval weight and % female at pupation as covariates. Response variables were normally distributed. Initial models included coolers as a blocking variable, but this was not found to be a significant covariate and was removed from subsequent models. All analyses were conducted using R (R Foundation for Statistical Computing, Vienna, Austria).

## **RESULTS**

### **Pre-pupal mortality (Fig. 2A)**

Both stock ( $X^2_{1\text{ df}} = 28.6$ ,  $p < 0.001$ ) and treatment ( $X^2_{2\text{ df}} = 13.2$ ,  $p = 0.001$ ) significantly affected prepupal mortality. Wild-type larvae suffered higher mortality than domesticated larvae ( $37 \pm 4.2\%$  [SE] versus  $13 \pm 2.8\%$ , respectively). Mortality in the wasp treatment ( $36 \pm 6.3\%$ ) was higher than mortality in the control treatment

( $15 \pm 4.3\%$ ; Tukey's HSD with  $\alpha = 0.05$ ); mortality in the mosquito treatment ( $25 \pm 4.0\%$ ) differed from neither the wasp nor control treatments. The significant effect of treatment was driven by a strong response of wild-type larvae to wasp buzzing; prepupal mortality in domesticated larvae was not affected by treatment (treatment\*stock interaction:  $X^2_{2 \text{ df}} = 6.09$ ,  $p = 0.048$ ). There was also a significant effect of initial larval weight ( $X^2_{1 \text{ df}} = 4.03$ ,  $p = 0.045$ ).

### **Time to pupation (Fig. 2B)**

Time to pupation varied significantly by larval stock ( $X^2_{1 \text{ df}} = 8.38$ ,  $p = 0.0038$ ), with domesticated larvae pupating 13% faster than wild larvae ( $30 \pm 0.8$  days vs  $34 \pm 0.9$  days, respectively). Neither treatment, the treatment\*stock interaction, nor the covariates affected time to pupation (all  $p > 0.05$ ).

### **Weight at pupation (Fig. 2C)**

Larval stock significantly affected pupal weight ( $X^2_{1 \text{ df}} = 20.7$ ,  $p < 0.0001$ ). Probably because of their longer time to pupation, wild-type larvae had higher pupal weights ( $81 \pm 4.2$  [SE] mg) than domesticated larvae ( $56 \pm 2.7$  mg). While there was no effect of treatment or the treatment\*stock interaction (both  $p > 0.05$ ), pupal weight was significantly affected by both initial larval weight ( $X^2_{1 \text{ df}} = 7.15$ ,  $p = 0.008$ ) and % female pupae per cup at pupation ( $X^2_{1 \text{ df}} = 36.9$ ,  $p < 0.001$ ).

## **DISCUSSION**

Auditory predator cues increased prepupal mortality in wild-type larvae but not their domesticated counterparts. The fact that auditory cues from a harmless insect did not evoke a similar response suggests that wild-type larvae were specifically reacting to the buzzing of a predator rather than to sound *per se*. The increase in prepupal

mortality, perhaps due to risk-induced feeding cessation, demonstrates that chronic predator stress can be fatal to lepidopteran larvae. The domesticated caterpillars, however, showed no difference in prepupal mortality between the sound treatments.

Neither wild-type nor domesticated caterpillars showed any treatment-level differences in time to pupation or pupal weight. We had anticipated that individuals would show a gradated stress response, with more affected individuals dying and less affected individuals exhibiting altered growth and development. Instead, it appears that the individual-level response to risk was bimodal – larvae either died or were unaffected. While unexpected, this result is consistent with previous work (McCauley et al. 2011) exploring the response of larval odonates to predator risk. In their experiments, exposure to caged predators increased prepupal mortality but affect neither larval nor adult body size of the surviving individuals. They suggested that this may have resulted from any negative effect of risk on the surviving larvae being compensated for by reduced competition and lower foraging costs (McCauley et al. 2011); a similar dynamic may occur in our system. The lack of an effect on surviving larvae may also be explained by the populations differing in their proportion of risk-averse versus risk-tolerant individuals, e.g., the shy-bold behavioral syndrome (Sih et al. 2012). While predation on wild populations can favor risk-averse individuals that respond strongly to predator cues, lab rearing selects for risk-tolerant individuals (see below). If our treatments affected risk-averse individuals but not their risk-tolerant counterparts, it would explain both the higher mortality in wild versus domesticated populations and the lack of a growth/development response in the surviving individuals.

Domesticated caterpillars pupated more quickly and at a lower weight than wild-type caterpillars. This decrease in development time is consistent with prior work on our lab-reared *Lymantria* population (Grayson et al. 2015) that found domesticated larvae pupated more quickly (but at a higher weight) than wild-type larvae when reared on red oak. The fact that wild-type larvae pupated at a higher weight in our work may stem from our use of a different wild population or reflect differences in diet or other rearing conditions (Grayson et al. 2015). Regardless, we hypothesize that the increased development rate of domesticated larvae is associated with decreased investment in anti-predator behavior; our experiment was originally inspired by noting behavioral differences between the wild and domesticated stocks.

This stark difference in sensitivity to predation risk suggests a domestication-related loss of anti-predator behavior. Reduction of activity following domestication has been seen in a number of species, including fruit flies, silkworms, and psyllids (Stanley and Kulathinal 2016, Jones et al. 2021, Zhang et al. 2021); in some cases, this change can be seen after as few as two generations in captivity (de Mestral and Herbinger 2013). This loss of sensitivity could be due to several factors. Mass rearing environments are highly stressful to wild insects, selecting for high levels of stress tolerance (Hoffmann and Ross 2018). As predators pose no risk in captivity, any energy put into predator detection/anti-predator behavior is wasted, potentially giving a fitness advantage to those who invest more energy into growth and reproduction (Swaney et al. 2015).

In a larger context, our findings caution against extrapolating the results of experiments using lab-reared insects onto wild populations. While lab-reared insects

may be genetically indistinguishable to their free-living kin, domestication-related changes in behavior and physiology may make them unreliable analogs to their wild counterparts. Specifically, the responses of domesticated insects to predator cues may tell us little about how these stressors affect free-living populations (a phenomena also noted in rats (Blanchard et al. 1994)). This has implications for studies on insect behavior and reactions to predation risk, where captive-reared insects are often used for convenience and availability (Hermann and Thaler 2014, Kempraj et al. 2020, Lund et al. 2020, Piovezan-Borges et al. 2020, Humphreys et al. 2021). In our case, the impact of auditory predator cues on wild-type caterpillars suggests some potential for the use of sound to reduce herbivory as part of an integrated pest management plan.

While our results highlight differences in risk sensitivity between domesticated and wild insects, there are a few caveats that need to be considered. We exposed caterpillars to auditory constantly over an extended period, with cues playing for two seconds every eight seconds for twelve hours a day. This exposure regime has been shown to successfully elicit responses in caterpillars (Lee et al. 2021) and was chosen to maximize the likelihood of observing an effect. Such chronic exposure is likely higher than what occurs in the wild, and future studies should explore whether lower exposure levels induce similar responses. Our study also focused on the effect of predator cues on mortality, growth, and development time; additional studies should explore risk-induced changes in behavior between wild-type and domesticated caterpillars. Other potential future studies involve field trials in more natural environments and investigations into the reactions of both stocks of caterpillar to live

predators. Caveats aside, we believe this study to be the first to demonstrate a loss of anti-predator behavior in insects because of domestication.

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## FIGURES

Figure 1: Arrangement of replicates within coolers. ‘W’: replicates (cups) containing wild-type *L. dispar* larvae; ‘D’: replicates containing domesticated *L. dispar* larvae. Each replicate contained five caterpillars. Speakers within each cooler produced the sound treatments.

Fig. 2. Pre-pupal mortality (A), time to pupation (B), and pupal weight (C) of domesticated and wild-type *L. dispar* larvae exposed to either no-cue control, mosquito buzzing, or wasp buzzing. Bars represent means  $\pm$  SE. Lowercase letters denote significant differences at  $\alpha = 0.05$  (Tukey’s HSD).

Figure 1.

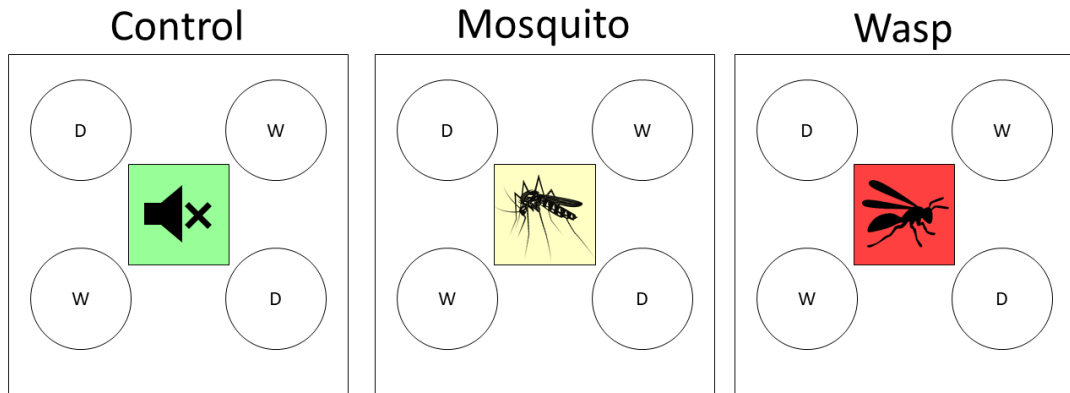


Figure 2.

