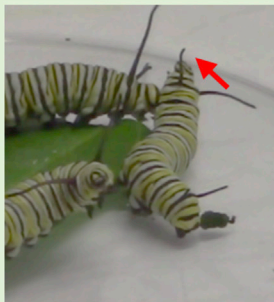


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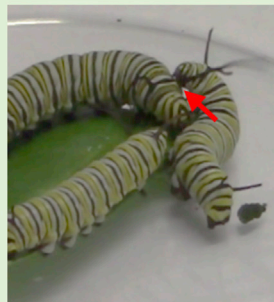
Aggression Is Induced by Resource Limitation in the Monarch Caterpillar

Monarch Caterpillar Aggressive Behavior

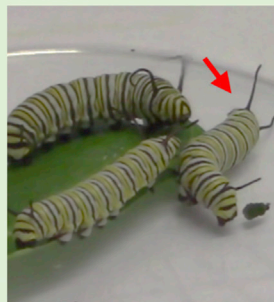
Pre-Attack



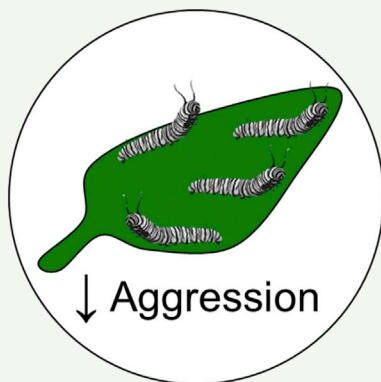
Attack



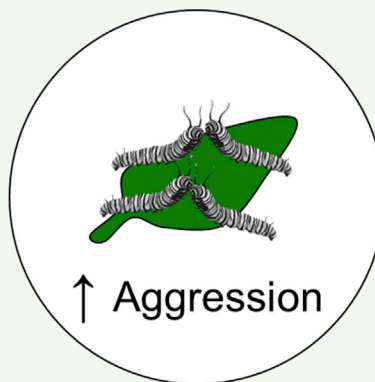
Post-Attack



High Food Density



Low Food Density



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HIGHLIGHTS

Monarch caterpillars
display stereotyped
aggressive behavior

Aggression is triggered by
limited food availability

Aggression peaks during
the late stages of
caterpillar development

Article

Aggression Is Induced
by Resource Limitation
in the Monarch CaterpillarJoseph Collie,¹ Odelvys Granela,¹ Elizabeth B. Brown,^{1,*} and Alex C. Keene^{1,2,*}

SUMMARY

Food represents a limiting resource for the growth and developmental progression of many animal species. As a consequence, competition over food, space, or other resources can trigger territoriality and aggressive behavior. In the monarch butterfly, *Danaus plexippus*, caterpillars feed predominantly on milkweed, raising the possibility that access to milkweed is critical for growth and survival. Here, we characterize the role of food availability on aggression in monarch caterpillars and find that monarch caterpillars display stereotyped aggressive lunges that increase during development, peaking during the fourth and fifth instar stages. The number of lunges toward a conspecific caterpillar was significantly increased under conditions of low food availability, suggesting resource defense may trigger aggression. These findings establish monarch caterpillars as a model for investigating interactions between resource availability and aggressive behavior under ecologically relevant conditions and set the stage for future investigations into the neuroethology of aggression in this system.

INTRODUCTION

Resource competition is a primary driver of behavioral evolution that shapes interactions within conspecifics and between species (Peiman and Robinson, 2010; Brumm and Ritschard, 2011). Animals often compete for diverse resources throughout their lifetimes including territory, mates, and food acquisition. Aggression and territoriality are present from invertebrates through primates, and many of the molecular and neural mechanisms governing aggression are conserved across phyla (Thomas et al., 2015). Food availability represents a critical resource that is particularly important in early life, as insufficient food consumption can potentially affect developmental progression and brain development (Ormerod et al., 2017; Brown et al., 2019). Further, even short periods of food restriction can induce mortality during early life when animals often have lower energy stores or are undergoing developmentally specified rapid growth (Dawirs, 1984; Tu and Tatar, 2003). Therefore, while resource competition and aggression are often studied in adult animals, these behaviors are also likely to be adaptive early in life.

Aggressive behavior represents a heterogeneous behavior that consists of offensive aggression, often to acquire resources, and defensive aggression, to protect against predation (Takahashi and Miczek, 2013; Ormerod et al., 2017; Wranghama, 2017). Aggression has been widely documented in eusocial and solitary insects where its function is used to defend territories, establish social hierarchies, and compete for food. For example, multiple species of butterflies have been studied for their territoriality, where males defend sunspots that attract potential mates (Davies, 1977; Wickman and Wiklund, 1983; Stutt and Willmer, 1998; Bergman et al., 2010). Under laboratory conditions, the fruit fly, *Drosophila melanogaster*, has become a leading model for studying the molecular mechanisms underlying aggression, which can be experimentally induced in the laboratory by pairing male flies in an arena with food (Chen et al., 2002). The standardization of this system, combined with powerful genetics, has allowed for dissection of genes and neural circuits regulating aggressive behavior (Kravitz and Fernandez, 2015; Anderson, 2016; Hoopfer, 2016). Together, intraspecies aggression can be studied in diverse insect species under natural and laboratory conditions, providing the opportunity to investigate how environmental and social factors contribute to this behavior.

Food availability in early life may be particularly important in insect larvae that consume food voraciously throughout most larval stages (Edgar, 2006; Tennessen and Thummel, 2011). Nutritional restriction during

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larval stages has been shown to delay larval development, as well as reduce adult body size, reproductive performance, and lifespan (Beadle et al., 1938; Dmitriew and Rowe, 2011; Runagall-Mcnaull et al., 2015; Poças et al., 2020). Food consumption in larvae is essential to accommodate the growth necessary to achieve metamorphosis. Larvae from many species are social and often compete for resources (Cody and Diamond, 1975; Kaplan and Denno, 2007). For example, acoustic territoriality in Lepidoptera caterpillars (Yack et al., 2001) and cannibalism in *Drosophila* larvae, beetles, and other insects (Dickinson, 1992; Vijendravarma et al., 2013; Orrock et al., 2017; Huang et al., 2018) suggest the presence of aggression in early developmental stages. The social interactions and competition for resources raise the possibility that limited food availability triggers aggression during the early stages of insect development.

The monarch butterfly, *Danaus plexippus*, is a model for investigating the mechanisms underlying migration, navigation, and circadian function (Reppert et al., 2010; Zhan et al., 2011; Tomioka and Matsumoto, 2015). Monarch caterpillars predominantly feed on milkweed and often strip entire plants bare of leaves over a two-week period. In many locations, milkweed is only available for part of the year, placing a significant constraint on monarch development (Yang and Cenzer, 2019). These ethological constraints suggest behavioral repertoires may be present in monarch caterpillars to acquire and defend resources.

To examine whether caterpillars display aggressive behavior, we established a group aggression assay and quantified the presence of aggressive lunges under a number of conditions, as well as the effect of attacks on target conspecifics. Here, we describe aggressive behavior in monarch caterpillars, which occurs during later stages of caterpillar development when resource competition may increase in the wild. Further, limiting food availability promotes aggression, suggesting that this represents a form of resource defense. These findings suggest monarch caterpillars may be used as a model to investigate how resource limitation contributes to regulation of aggressive behavior and subsequent effects on the animals' brain development and function.

RESULTS

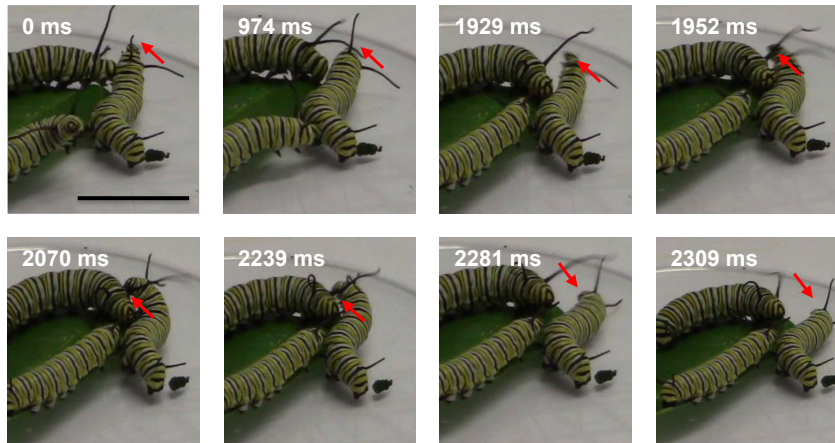
To measure aggression in monarch caterpillars, we sought to develop a quantifiable assay for aggression. We first observed caterpillars at the fourth and fifth instar stage, the final stages prior to pupation. In both stages, we noticed a stereotyped aggressive behavior sequence consisting of the aggressive caterpillar orienting toward the conspecific and performing a quick head snap that made physical contact with the target (Figures 1A and 1B and Video S1). This tended to elicit cessation of feeding and a movement away from jointly occupied space by the target caterpillar. We reasoned that these movements constitute aggression.

To determine the relationship between aggression and developmental stage, we quantified the number of attacks across multiple instars. Attacks were not observed in third instar caterpillars (data not shown). A comparison between fourth and fifth instar caterpillars revealed significantly more aggressive lunges between fifth instar caterpillars than between fourth instar caterpillars (Figure 2). Together, these findings suggest aggression increases in frequency over the later stages of larval development. We reasoned that the relatively larger size of older caterpillars may increase competition for food resources, thereby promoting aggression.

To directly quantify the relationship between food availability and aggression, we generated three conditions that differed in food availability (Figure 2A). Although the total number of attacks was lower among fourth instar caterpillars, there were significantly more attacks among fourth instar caterpillars when food availability was low, with a mean of 18.00 ± 3.17 , compared to when food availability was either intermediate or high, with means of 8.33 ± 2.01 and 4.81 ± 1.19 , respectively (Figure 2B). The number of attacks among fifth instar caterpillars also correlated with food availability (Figure 2C). We again observed a significantly greater number of aggressive attacks when food availability was low, with a mean of 54.75 ± 7.63 , compared to when food availability was either intermediate (28.89 ± 5.55) or high (13.32 ± 1.83). The number of aggressive attacks on intermediate food availability was also greater relative to high food availability. Together, these findings support the notion that low food availability triggers aggression in monarch caterpillars.

To identify when aggression occurs and its impact on the target conspecific, we generated an ethogram of four caterpillars from a single group, defining behaviors (e.g. foraging, feeding, resting) at the time of each

A 4th instar



B 5th instar

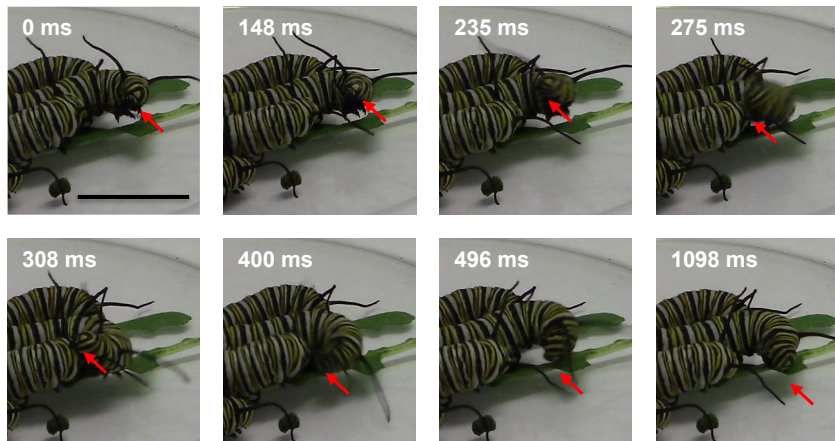


Figure 1. Characterization of Aggressive Behavior in fourth and fifth Instar Monarch Caterpillars

Example time lapse of a stereotypical aggressive encounter, characterized by a lateral lunge, between two (A) fourth instar or (B) fifth instar conspecifics. Red arrows indicate a change in caterpillar head movement as it progresses from recognition, initiation, contact, and then cessation of a single attack. The timestamp on each image denotes the duration of the encounter, in milliseconds. Scale bar represents 2cm.

aggressive encounter and their location (e.g. on leaf or off leaf). In nearly all cases, the attacked caterpillar was feeding at the time of the aggressive encounter, suggesting attacks may disrupt feeding and serve to protect a food source (Figure 3). Similarly, the attacking caterpillar tended to be foraging at the time of the attack, suggesting aggression usually occurs when animals are searching for food, rather than actively consuming food. In addition, we cannot rule out the possibility that crowding plays a role in inducing aggression, as this has been shown to contribute to aggressive behavior in many other insect species (Wang and Anderson, 2010; Stevenson and Rillich, 2016). Therefore, this qualitative analysis supports the notion that aggression is associated with food competition, but other factors are likely involved as well.

To further investigate the defining features of these aggressive attacks, we quantified multiple parameters during each aggressive encounter and its impact on the targeted caterpillar. In both fourth and fifth instars, nearly all aggressive attacks occurred on the leaf (Figures 4A and 4B). Aggressive attacks were significantly more likely to occur when the target caterpillar was actively feeding and never occurred when a caterpillar was resting (Figures 4C and 4D). The number of aggressive attacks that occurred during foraging was intermediate between those that occurred during feeding and during resting. Immediately following an attack, the attacking caterpillar almost always remained on the leaf and is consistent for both fourth and fifth instars (Figures 4E and 4F). In fourth instars, the target caterpillar left the leaf a significantly greater

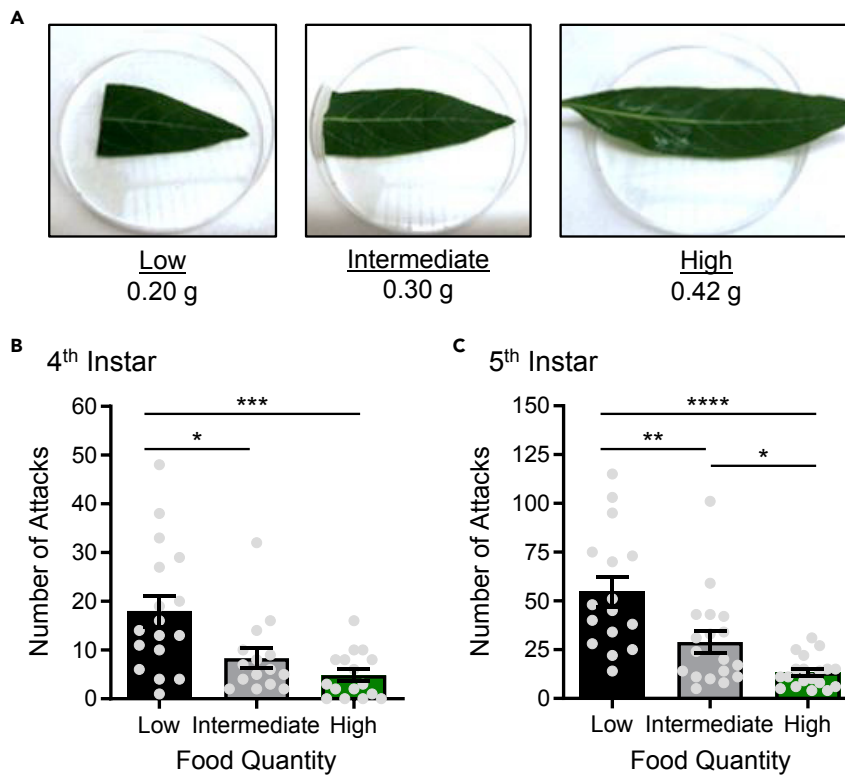


Figure 2. Aggressive Behavior Increases in Both fourth and fifth Instar Caterpillars with Decreasing Food Density

(A) *Asclepias curassavica* of low, intermediate, or high density was provided to fourth and fifth instar caterpillars and aggressive behavior was observed.

(B) There was a significant effect of food quantity on the number of aggressive attacks in fourth instar caterpillars ($F_{2, 45} = 8.84$; $p < 0.0006$). *Post hoc* analyses revealed a significant increase in the number of aggressive attacks on low food density relative to both intermediate ($p < 0.0109$) and high food densities ($p < 0.0006$), while no significant difference between intermediate and high food densities was observed ($p < 0.3002$).

(C) There was a significant effect of food quantity on the number of aggressive attacks in fifth instar caterpillars ($F_{2, 50} = 15.34$; $p < 0.0001$). *Post hoc* analyses revealed a significant increase in the number of aggressive attacks on low food density relative to both intermediate ($p < 0.0027$) and high food densities ($p < 0.0001$). The number of attacks were also significantly higher on intermediate food density in comparison to high food density ($p < 0.0375$). Data are represented as mean $\pm$ SEM. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$.

amount of time than it remained (Figure 4E), while results trended in this direction for fifth instar caterpillars (Figure 4F). Therefore, aggression increases the likelihood that the attacking caterpillar will remain on the food source, while the attacked caterpillar will be displaced.

Lastly, we sought to investigate whether sensory input contributes to aggressive behavior. Visually-mediated social behavior has been recently identified in *Drosophila* larvae (Dombrovski et al., 2019), suggesting that simple eyes may convey complex social information. Monarch caterpillars contain six bilateral ocelli allowing for vision (Khan Perveen, 2017). To determine whether visual input is required for aggression, we compared the number of aggressive attacks in fifth instar caterpillars on low food density recorded under standard light conditions or in complete darkness (Figure 5A). No differences in aggression were identified between the groups (Figure 5B), suggesting light input is dispensable for caterpillar aggression. We also investigated the contributions of the tentacles, large mechanosensory appendages that exhibit rapid growth during the fourth and fifth instar stages when aggression increases (Oberhauser and Kuda, 1997). To investigate the contributions of these structures, we compared aggression in caterpillars with bilateral ablation of the tentacles with intact controls (Figure 5C). There was no difference between the groups, suggesting that functioning tentacles are dispensable for the elicitation of aggression (Figure 4D). These findings suggest that alternative sensory modalities such as pheromonal, olfactory, or tactile cues that are independent of the tentacles initiate aggression.

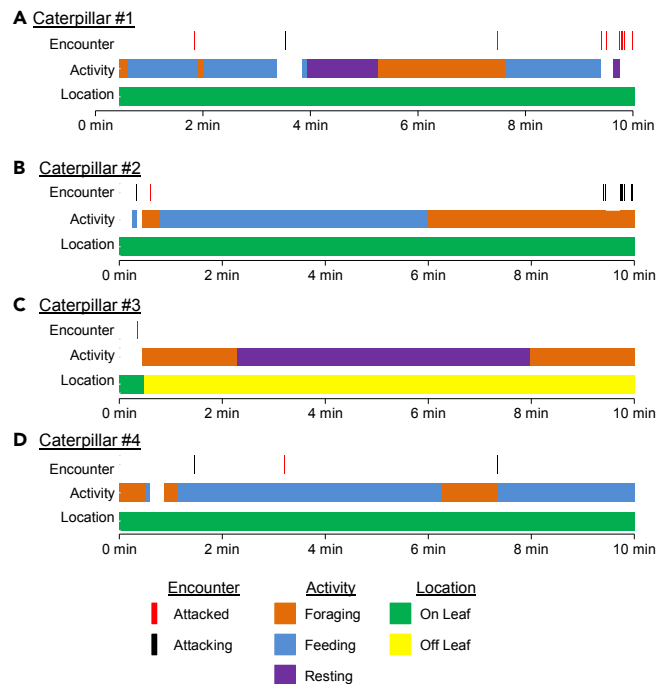


Figure 3. Ethogram of Four Representative fifth Instar Caterpillars on Intermediate Food Density during a 10-Min Video

For each caterpillar, the time at which an aggressive encounter occurs was recorded, as well as its activity and location, shown as fractions of time. Aggressive encounters were classified as either attacking (black) or being attacked (red). Activities were categorized into foraging (orange), feeding (blue), or resting (purple). The location of each caterpillar was also recorded (on the leaf: green; off the leaf: yellow).

DISCUSSION

Here, we describe aggressive behavior in a caterpillar that consists of physical contact presumably to defend a food source that is critical for development. The physical contact is more likely to occur on food and frequently results in displacement suggesting it may be related to resource acquisition. It is important to note that we observed a high degree of variability between individuals, raising the possibility that interindividual differences in exploration, social behavior, or boldness underlie response differences. Territoriality has been described in other caterpillar species (Kemp, 2000; Yack et al., 2001; Bowen et al., 2008), suggesting aggression may be widespread. Cannibalism is widespread in *Lepidoptera* larvae (Semlitsch and West, 1988; Dial and Adler, 1990; Zago-Braga and Zucoloto, 2004; Tang et al., 2016; Zhou et al., 2016), raising the possibility that aggression is associated with conspecific competition. This extends beyond *Lepidoptera* to other insect larvae, as crowded rearing conditions promote cannibalism in *Drosophila* larvae (Vijendravarma et al., 2013), suggesting a relationship between resource availability and this aggression-linked behavior. In our study, we exclusively examined behavioral lunges, a feature most commonly associated with aggressive behavior (Dankert et al., 2009). Examining acoustic communication and potential cannibalism in monarch caterpillars may inform additional behavioral components associated with resource competition.

Numerous sources of resource competition trigger aggression, including limited availability of territory, mates, and food. Our findings suggest limited food availability triggers aggression in monarch caterpillars. In contrast, in social species like the honeybee, *Apis mellifera*, rather than an increase in aggression, nutritional stress during larval development promotes cooperation, in which the queen increases colony investment and decreases reproduction (Walton et al., 2018). However, the effect of nutritional state on aggression is likely widespread throughout the animal kingdom as food scarcity induces aggression in amphibians, lizards, and birds (Moore and Marler, 1987; Drummond and Chavelas, 1989; Ducey and Heuer, 1991). In facultatively sublethal bird species, where aggression commonly results in death of the competitor, the “food amount hypothesis” contends that sibling aggression will vary inversely with food quantity (Mock et al., 1987; Drummond, 2001a). Although less is known about the proximate causes of aggression in

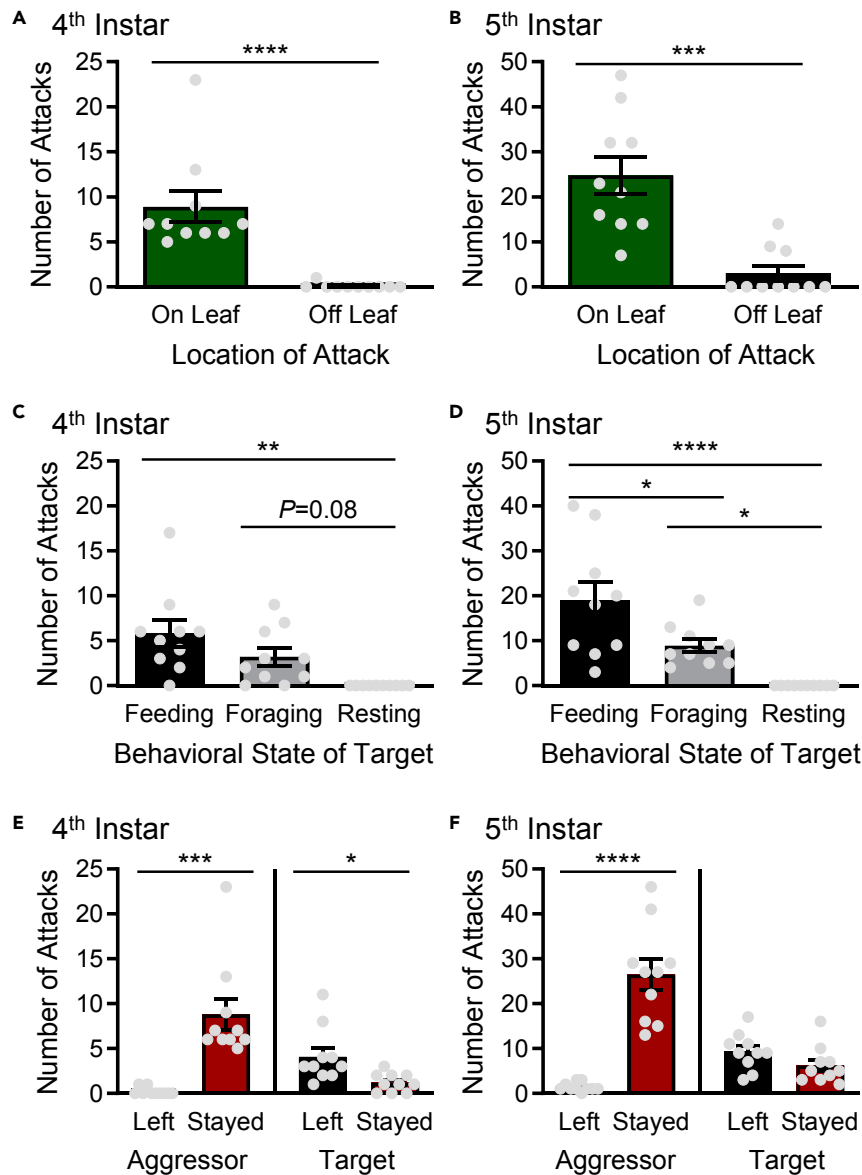


Figure 4. Aggressive Behaviors Tend to Occur on the Leaf during Feeding

In fourth (A) and fifth (B) instar caterpillars, there were significantly more aggressive attacks on the leaf than off the leaf (fourth instar: $t_{18} = 5.102$; $p < 0.0001$; fifth instar: $t_{18} = 4.876$; $p < 0.0001$).

(C) There was a significant effect of behavioral state on the number of aggressive attacks in fourth instar caterpillars ($F_{2,27} = 8.047$; $p < 0.0018$). *Post hoc* analyses revealed that significantly more aggressive attacks occur during feeding relative to resting ($p < 0.0012$). No significant difference between feeding and foraging ($p < 0.1903$) or between foraging and resting was observed ($p < 0.0876$).

(D) There was a significant effect of behavioral state on the number of aggressive attacks in fifth instar caterpillars ($F_{2,27} = 14.98$; $p < 0.0001$). *Post hoc* analyses revealed that significantly more aggressive attacks occur during feeding relative to both foraging ($p < 0.0191$) and resting ($p < 0.0001$). The number of aggressive attacks is also significantly higher during foraging in comparison to during rest ($p < 0.0418$).

(E) At the fourth instar stage, the aggressive caterpillar is significantly more likely to stay on the leaf ($t_{18} = 4.936$; $p < 0.0001$), while the attacked caterpillar is significantly more likely to leave the leaf ($t_{18} = 2.830$; $p < 0.0111$).

(F) At the fifth instar stage, the aggressive caterpillar is significantly more likely to stay on the leaf ($t_{18} = 7.325$; $p < 0.0001$), but the attacked caterpillar is equally as likely to leave the leaf as they are to stay ($t_{18} = 1.671$; $p < 0.1120$). Data are represented as mean $\pm$ SEM. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$.

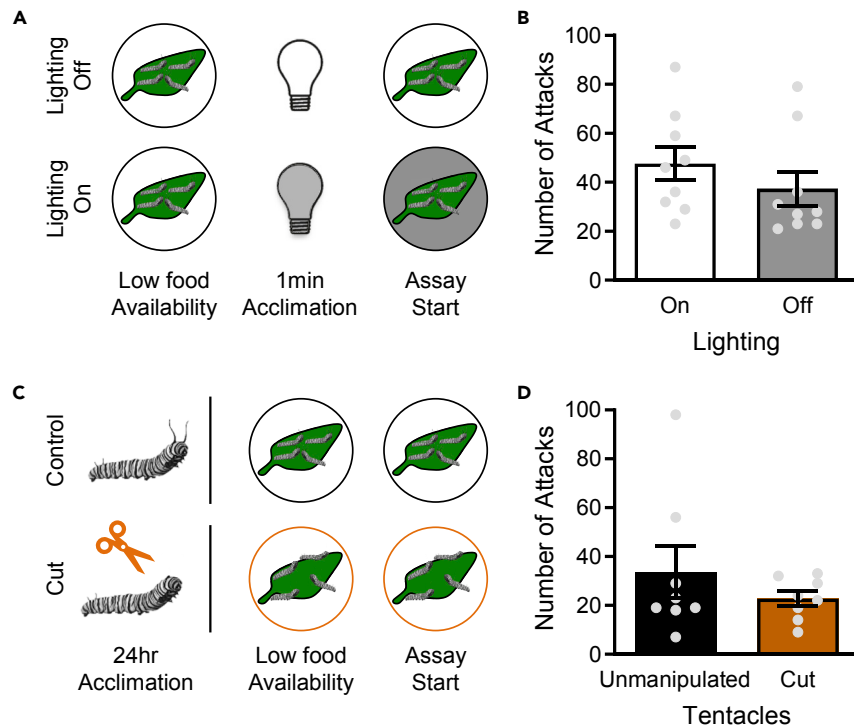


Figure 5. Lighting and Tentacle Laceration Have No Effect on Aggressive Behavior

(A) In the experimental treatment, lights were turned off 1 min prior to testing. The effect of lighting on aggressive behavior was then measured in arenas with low food availability.

(B) Caterpillars were equally as aggressive when the lights are off as they are in lighted conditions ($t_{16} = 1.054$, $p < 0.3076$).

(C) In the experimental treatment, tentacles from each caterpillar were removed 24 hr prior to testing. The effect of tentacle laceration was then measured in arenas with low food availability.

(D) Caterpillars were equally as aggressive without tentacles as they are with intact tentacles ($t_{14} = 0.9964$, $p < 0.3360$). Data are represented as mean $\pm$ SEM.

nonsiblicidal species (Drummond, 2001b), in the meerkat, *Suricata suricatta*, the severity of aggressive competition scales with food availability (Hodge et al., 2009). Similarly, in numerous mammalian models, moderate reductions in food availability promote aggression. For example in Siberian hamsters, *Phodopus sungorus*, food restriction promotes aggression during winter-like photoperiods, a signal of impending limited resources (Hodge et al., 2009; Bailey et al., 2017). Food restriction in utero is also associated with aggression. In baboons, *Papio hamadryas*, intrauterine growth restriction is linked to higher rates of aggressive display behaviors such as aggressive grunts, canine displays, and eyelid flashes (Huber et al., 2015). In *Drosophila*, which is widely used to study aggression in the laboratory, conspecific aggression is triggered by placing a small amount of high calorie food in the center of an arena (Chen et al., 2002; Lim et al., 2014). Therefore, we hypothesize that aggression induced by limited food availability in monarch caterpillars are likely present in many different species throughout the animal kingdom.

While we systematically tested the relationship between food availability and aggression, we cannot exclude other possibilities. For example, it is possible that low amounts of food brings caterpillars into closer proximity, thereby resulting in aggression. In this case, it is proximity, rather than food availability *per se* that induces aggression. The conditions, all of which included different amounts of food, were chosen to mimic conditions in the wild. The complete lack of food resulted in roaming behavior, where caterpillars are highly active. This likely represents a foraging state, and few aggressive events were observed (data not shown). Manipulating feeding state prior to testing may provide additional insight into the relationship between feeding state and aggression.

In most species studied, aggression is dependent on multiple sensory inputs, providing the opportunity to identify specified sensory stimuli that induce aggression (Chen and Hong, 2018). For example, in flies,

dedicated pheromone sensing neurons and their cognate receptors are required for aggression (Wang and Anderson, 2010). Further, disruption of the visual system also disrupts aggressive behavior (Hoyer et al., 2008; Ramin et al., 2014). Conversely, we find that aggressive behavior is similar under light and dark conditions, suggesting it occurs independently of visual function. Further, ablation of the tentacles does not impact aggression, suggesting mechanosensory stimuli from these organs are not required. It is possible that other sensory modalities such as acoustic communication, contribute to the regulation of aggression. For example, in the warty birch caterpillar, *Drepana bilineata*, acoustic cues are used to defend silk leaf mats and shelters from conspecific larvae (Bowen et al., 2008). Further, caterpillars of the silk moth family *Bombycoidea* display a wide array of defense sounds in response to simulated inter-species aggression (Bura et al., 2016). Future work examining the contributions of olfactory or alternative mechanosensory pathways may help identify neural mechanisms that trigger aggression.

Monarch caterpillars are specialists that feed nearly exclusively on the milkweed (Seiber et al., 1986; Malcolm et al., 1989). It is likely that food resources are scarcer for specialists than generalists or during life stages where animals are dietary specialists, raising the possibility that conspecific aggression is elevated in this species. Food competition may be more prevalent for monarch caterpillars that largely defoliate host plants, generating resource scarcity, particularly in the later stages of larval growth when we observed aggression (Kaplan and Denno, 2007). In addition, it is possible that conspecific aggression originated as a mechanism of inter-species defense. Over 100 different species are known to consume neonate monarch caterpillars (Hermann et al., 2019). Investigating the conditions that trigger aggression in the wild, and in additional *Lepidoptera* caterpillars, will allow for comparative analysis that may provide insight into the ecological factors that promote aggression.

Beyond the study of aggression in caterpillars, monarchs present an emerging model for studying the molecular mechanisms underlying behavior. Monarch caterpillars are widely studied for their migratory behavior that spans generations along the animals range from Southern Mexico through Canada (Brower, 1996; Reppert et al., 2010). Monarch butterflies detect polarized light and use a time-compensated sun compass for migratory navigation (Froy et al., 2003; Reppert et al., 2004). The genome of monarch butterflies has been sequenced, and the recent implementation of gene editing provides the opportunity to investigate the genetic mechanisms underlying behavior (Zhan et al., 2011; Merlin et al., 2013; Markert et al., 2016). Our findings that monarch caterpillars exhibited aggressive behavior under conditions of limited food availability provide the opportunity to examine the role of genes identified in other leading models of aggression in an ecologically relevant system.

Limitations of the Study

While our work identifies aggressive behavior in monarch caterpillars, a number of limitations to this work will guide future investigation. For example, our studies are limited to a laboratory setting and it will be critical to quantify the behavior in nature. To this point, it is possible that the behavior is regulated by the circadian clock and by seasonality. These aspects can be investigated by manipulating the circadian cycle under laboratory conditions or measuring the behavior in nature. Finally, our findings reveal that limiting food availability increases aggressive behavior, but this limitation may be a consequence of the reduction in food bringing animals into closer proximity. This can be directly addressed by systematically manipulating the nutrients within the available food or testing animals in different sized arenas.

Resource Availability

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Alex Keene (Keenea@fau.edu).

Material Availability

This study did not generate new unique reagents.

Data and Code Availability

This study did not generate any new code. All original and processed data will be made available upon request.

METHODS

All methods can be found in the accompanying [Transparent Methods](#) supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.isci.2020.101791>.

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AUTHOR CONTRIBUTIONS

Conceptualization, J.C., O.G., E.B.B., and A.C.K.; Methodology, J.C., O.G., E.B.B., and A.C.K.; Investigation, J.C., O.G., and E.B.B.; Writing –Original Draft, J.C., E.B.B., and A.C.K.; Writing –Review & Editing, E.B.B. and A.C.K.; Funding Acquisition, A.C.K.; Resources, A.C.K.; Supervision, E.B.B. and A.C.K.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Supplemental Information

**Aggression Is Induced
by Resource Limitation
in the Monarch Caterpillar**

Joseph Collie, Odelvys Granela, Elizabeth B. Brown, and Alex C. Keene

Transparent Methods

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Alex C. Keene (keenea@fau.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The published article includes all datasets generated or analyzed during this study.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Monarch caterpillars were field-collected between May and August of 2018 and 2019, on Florida Atlantic University's campus in Jupiter, Florida (26.887596, -80.115436). Caterpillars were collected during the 4th and 5th instar stage. Caterpillars were stored in 16 oz. plastic containers (DELI-36P-16OZ; Comfy Package, Brooklyn, New York), with four caterpillars per container to reflect the density generally observed at the time of collection and generate natural interactions. Caterpillars were fed *A. curassavica* milkweed leaves *ad libitum*; fresh leaves being replaced daily. Plastic containers containing caterpillars were housed in a climate-controlled room at a constant temperature of 25 °C and humidity of 55%, with a 12:12 light/dark cycle. All caterpillars were acclimated to these conditions for at least 1 hour prior to testing. Caterpillars were kept for no longer than three days following collection, and upon completion of behavioral analysis, were released back to the location of their capture.

METHOD DETAILS

Aggression assays

Aggression was measured in arenas containing four caterpillars of the same instar stage. To synchronize feeding, caterpillars were food restricted in an empty container for 1 hr prior to the initiation of each assay. Caterpillars were then transferred to an arena containing fresh milkweed leaves and allowed to acclimate for 1 min prior to testing. Aggressive encounters were recorded using a personal camcorder (Vixia HF R800; Canon, Tokyo, Japan) under standard light conditions over a 10 min period. All data were collected between ZT 5-9:30, where ZT0 represents the time of lights on.

Aggression was determined by manually quantifying the number of aggressive attacks, or lunges, that occurred during each video. Attacks were defined by rapid head movements that make contact with another caterpillar. We partitioned the attack sequence into four actions: (1) recognition, (2) initiation, (3) contact, and (4) cessation. First, the aggressive caterpillar recognizes the recipient caterpillar as a competitor. An aggressive encounter is then initiated upon drawback of the head, followed by a rapid, lateral lunge in the direction of the recipient caterpillar. Next, physical contact between the aggressive caterpillar and recipient caterpillar occurs. Lastly, cessation of an aggressive encounter occurs when the aggressive caterpillar retreats its head from the recipient caterpillar. Incidental contact would occur periodically by two or more caterpillars during food search behavior. An aggressive attack was differentiated by incidental contact by the quick head drawback and lunge movement. The total number of aggressive lunges for each caterpillar was manually counted over the 10-minute period and presented as the mean of all four caterpillars for a single behavioral assay.

Single animal analysis

Aggressive encounters and related behaviors from individual caterpillars in a representative 10-min video were characterized using the Behavioral Observation Research Interactive Software

(BORIS), which allows for single animal analysis through selective coding sequences and focal subject targeting (Friard and Gamba, 2016). Each of four caterpillars were selected as the core subject with the use of BORIS's parameter controls. With this setting, behavioral coding was used to identify single points (e.g. aggressive encounters) and timed occurrences for each caterpillar (e.g. activity and location metrics).

Food availability manipulations

We chose amounts of food to reflect variations in food availability that were within the range of naturally occurring conditions, based on our observations of caterpillars at the collection site. At the start of testing, 4th or 5th instar caterpillars were transferred to a petri dish containing low food availability (~ 0.20 g), intermediate food availability (~ 0.30 g) or high food availability (~ 0.42 g) of *A. curassavica*. Caterpillars were allowed to acclimate for a period of 1 minute and then the number of aggressive attacks were quantified, as described above.

Lighting manipulations

To assess the contribution of light on the number of aggressive encounters, a digital camcorder with a built-in infrared system was used (DCR-SR42; Sony, Tokyo, Japan). These experiments were performed as described above, using 5th instar caterpillars. Caterpillars were first introduced to low food availability with the lights on. Once the lights were turned off, caterpillars were allowed to acclimate for a period of 1 minute, then the number of aggressive attacks were quantified. As a control, similar manipulations were performed, but the lights were never turned off.

Tentacle ablation

To examine the effect of sensory input on the number of aggressive encounters, the tentacles of 5th instar caterpillars were removed using steel scissors. Tentacles were cut 2–3 mm from the base of each tentacle. Caterpillars were given 24hrs to recover, and then number of aggressive

attacks were quantified. Lacerated caterpillars were examined under the same experimental conditions as control caterpillars.

QUANTIFICATION AND STATISTICAL ANALYSES

All measurements are presented as bar graphs showing mean $\pm$ standard error. A t-test was used for comparisons between two treatments, while a one-way analysis of variance (ANOVA) was used for comparisons between more than two treatments. All post hoc analyses were performed using the Holm-Sidak multiple comparisons test. Statistical analyses and data presentation were performed using InStat software (GraphPad Software 8.0; San Diego, CA).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Bacterial and Virus Strains		
Biological Samples		
Chemicals, Peptides, and Recombinant Proteins		
Critical Commercial Assays		
Deposited Data		
Experimental Models: Cell Lines		
Experimental Models: Organisms/Strains		
Monarch Caterpillars	Jupiter, FL	N/A
<i>A. curassavica</i>	Jupiter, FL	N/A
Oligonucleotides		
Recombinant DNA		
Software and Algorithms		
BORIS	Friard and Gamba, 2016	N/A
Prism	Graphpad software	V8.0
Other		
Camcorder	Canon	Vixia HF R800
Infrared Camcorder	Sony	DCR-SR42
Plastic Containers	Comfy Package	DELI-36P-16OZ;

Excel Files

Supplemental Table 1. All raw data from Figure 2.

Supplemental Table 2. All raw data from Figure 4.

Supplemental Table 3. All raw data from Figure 5.

Multimedia Files

Supplemental Movie 1. Aggressive behavior is measured in 5th instar monarch caterpillars,
Related to Figure 1.