Anna Shattuck. The effect of infection on thermoregulatory behavior of Monarch caterpillars (*Danaus plexippus)*

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Monarch butterfly (*Danaus plexippus*) populations in the United States are in decline for several reasons, one of which being infection caused by *Ophryocystis elektroscirrha* (OE), a protozoan parasite. Ectothermic organisms have been shown to respond to parasitism by displaying behavioral fever where an infected individual acutely changes its thermal preference, usually for high temperatures, to favor immune response and promote survival. No experiment has yet tested thermal preferences of monarch caterpillars or if thermal preference varies based on infection status. I hypothesized that monarch caterpillars parasitized with OE will favor warmer temperatures to mitigate infection outcomes and increase survival. To test this, I placed caterpillars in a photothermal gradient and measured body temperature using a thermal imaging camera over a set time interval. Preliminary results suggest fever is being displayed by monarch caterpillars in response to OE infection. This work elucidates the thermal preferences of monarch caterpillars.

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1.0 Introduction:

Monarch butterflies, well-known for their transcontinental migration between Canada/United States and Mexico, are commonly parasitized by *Ophryocystis elektroscirrha* (OE), a protozoan parasite. Parasitism, along with habitat destruction and host plant loss, has resulted in widespread monarch population declines for several decades (Pocius et al. 2022). Due to its parasitic lifestyle, OE must be in a host to grow and reproduce; however, it can be maintained in the environment for extended periods of time as dormant spores and is resistant to various extreme conditions. Monarchs become infected with OE as caterpillars when they consume the infectious spores. When a caterpillar becomes infected, it cannot spread OE and does not display overt signs of OE infection until adulthood.

OE can cause both lethal and sub-lethal effects in monarch butterflies. Probability of survival decreases with increasing parasite load in monarchs, (De Roode et al. 2006). Heavy OE infections can cause wing and proboscis deformity after pupation in adult monarchs (Lester & Bulgarella 2021). Infected butterflies do not recover and remain covered in spores for the entirety of their lifespan and so OE is considered a threat to the survival of monarch populations across the United States (Pocius et al. 2022).

Previous research has shown that OE is a thermally sensitive parasite with warmer temperatures reducing the viability of transmission stages (Sánchez et al. 2021). Furthermore, unpublished data has shown that when monarch caterpillars inoculated with OE are reared at high constant temperatures (34 C), no individual showed signs of infection as an adult (presented by Izzy Ragonese, 2021). While there was a decrease in

longevity of the monarchs reared at 34 C, all inoculated individuals were able to clear infection completely.

Organisms can respond to infection in several ways. A common adaptive response displayed across taxa is fever which can be generated through physiological metabolic processes or changes in organismal behavior (Clancy et al. 2018; De Roode and Lefèvre 2012). Ectotherms cannot use physiological processes to generate fever and instead must seek warmer temperatures in their environment to increase their body temperature to mitigate the costs of infection or clear infection. This is called behavioral fever and has been documented across various ectothermic taxa including reptiles, amphibians, and insects (Clancy et al. 2018; De Roode and Lefèvre 2012). In insects, behavioral fever has been displayed in desert locusts and ranchman's tiger moth caterpillars (Clancy et al. 2018; Karban 1998). While behavioral fever is costly for uninfected individuals to display, it can result in various benefits for infected individuals including suppression of parasite development in hosts due to suboptimal temperatures for pathogen growth, improved immune system function, and decreased mortality (De Roode and Lefèvre 2012; Clancy et al. 2018; Elliot, Blanford, and Thomas 2002).

With high temperatures proving to be suboptimal for OE parasitism, infected monarch caterpillars may benefit from generating fever responses to parasitism to either clear or modulate the more serious effects of infection. While there have been a myriad of experiments exploring the effects of rearing monarchs under various temperature regimes or exploring how temperature plays a role in monarch ecology and development, no experiment has tested thermal preferences of monarch caterpillars or if thermal preference varies based on infection status. Understanding the role of temperature in

infection outcome is critical in understanding how monarchs might response to climate change. This thesis will elucidate thermal preferences of monarch caterpillars and explore how thermoregulatory behavior, specifically behavioral fever, might allow for infected monarch caterpillars to survive with a reduced parasite burden into adulthood such that that they can spread OE through breeding and environmental contact and contribute to the high infection prevalence in the Gulf South. I hypothesized that monarch caterpillars infected with OE will favor warmer temperatures on average to mitigate OE infection outcomes.

2.0 Methods:

Trials for Experiment 1 were conducted during the Fall 2022 semester. These trials were preliminary, and the results informed the design of Experiment 2. Experiment 2 implemented a similar overall design as Experiment 1 by testing thermoregulatory responses of infected and uninfected caterpillars in a photothermal gradient; however, there were important changes made to create an optimal environment for producing a fever response. In Experiment 2, I tested caterpillars under higher infection doses and during the morning as both conditions have been shown in other systems to produce the strongest fever responses (Clancy et al. 2018).

2.1 Monarch Rearing:

Monarch caterpillars in both experiments were reared from hatch to pupation on *Asclepias curassavica*. *A. curassavica* plants were obtained from Joyful Butterfly (Blackstock, SC 29014), repotted, and left to sit for 2 weeks prior to being fed to any caterpillars. Plant leaves were soaked in a 2% bleach solution and then rinsed with tap

water before being fed to caterpillars to kill any pathogens and OE spores. Clean plant tissue was refrigerated in water and replaced as needed due to wilting or consumption.

Monarch caterpillars were all kept in the same Conviron A1000 reach-in growth chamber on a 12.5-hour day and 11.5-hour night cycle (Table 1). Chamber temperature varied between 19.5 - 28.5 C (Table 1). Chamber humidity was maintained at 50-90% (Table 1). Experiment 1 followed regular day:night cycling that matched the day:night period outside of the chamber. Experiment 2 used day:night cycling that was opposite to the day:night conditions outside of the chamber (i.e. morning in the chamber was timed to be in the evening outside the chamber). Throughout early instars, caterpillars were kept in petri dishes with a damp paper towel square, *A. curassavica* leaf cutting, and changed to a new petri dish every other day. Once large enough (3-4 instar), caterpillars were moved into 32 oz. clear deli containers and given new milkweed and paper towel bedding every day until pupation. After pupation, adults were moved to be stored in glassine envelopes in a refrigerator at 12 C.

Hour	Temperature (C)	Humidity (%)	Light Level
7:00	28.5	90	1
16:00	25	90	1
18:00	23	90	1
19:30	19.5	90	0

Table 1:Conviron growth chamber rearing conditions for monarch caterpillars and pupae during Experiment 1.

Hour	Temperature (C)	Humidity (%)	Light Level
4:30	25	90	1
6:30	23	90	1
7:00	19.5	90	0
19:30	28.5	90	1

Table 2: Conviron growth chamber rearing conditions for monarch caterpillars and pupae during Experiment 2.

2.2 Host and Parasite Sources:

Both wild-caught and lab-bred monarch caterpillars were used in Experiment 1. Only wild-caught monarchs were used in Experiment 2. Wild-caught individuals were collected from gardens around New Orleans as eggs or 1st instar larvae and reared in laboratory conditions (Section 2.1). All wild-caught eggs collected were bleached (2%) for 2 minutes then rinsed with tap water to remove any presence of wild OE spores that might cause unintentional infection. Lab-bred caterpillars were produced by mating New Orleans wild-caught individuals from separate family lines. I only mated uninfected wild-caught individuals to reduce the chance of accidental infection with OE spores.

OE parasite spores from lineage E10 were used to inoculate caterpillars during early instars. E10 spores are a known lineage originating from St. Paul, Minnesota. These spores have been maintained in lab by continuously infecting caterpillars with the newest spores from the known lineage.

2.3 Host Inoculation and Infection Measurement:

When caterpillars reared from eggs reached the late second to early third instar, individuals were randomly assigned an infection or control treatment. To infect caterpillars in Experiment 1, I placed 10-20 OE spores on a hole-punch-sized milkweed leaf that was later fed to each caterpillar in the infection treatment. Inoculations using 10-20 spores cause a sublethal infection that does not usually result in significant fitness costs such as wing deformity or premature mortality. Caterpillars in the control treatment were fed a bleached hole-punch-sized leaf. Caterpillars were only given more milkweed once they had fully consumed the inoculated or bleached milkweed leaf cutting. In Experiment 2, I inoculated individuals with 70-100 spores (Thierry et al. 2010; Altizer &

Oberhauser 1999). A higher inoculation dose generates a heavier infection burden that might result in an increased need to produce an adaptive response (like fever) to mitigate the effects of infection.

Adult butterflies were measured for the presence of OE spores using the tape method (Project Monarch Health). For this, tape was pressed to the underside of the adult butterfly's abdomen. The tape was peeled off the abdomen, pulling off any spores and abdominal scales it contacted. The tape was then placed on a white background and visually assessed under a dissecting microscope for the presence or absence of spores.

2.4 Photothermal Gradient Construction:

Thermal gradients range in their construction when exploring the thermal preferences of various animals and have been utilized by researchers for decades (Barbour & Racine 1967; Uvarov 2009). Photothermal gradients are widely used throughout thermal preference studies and are constructed using a lamp that heats and lights one end of the gradient (Mathies and Andrews 2003; Bowker et al. 2013). A drawback to using a photothermal gradient is the addition of a light source which cannot be present if the study species is phototaxic (Dillon et al. 2009).

To test for the thermal preference of monarch caterpillars in Experiment 1, I constructed a photothermal gradient using 6.5 cm diameter cylindrical clear plastic tubes cut in half to make each gradient lane (58 cm). Mesh netting was glued to each tube's surface and over the open size of the tube to create a fully enclosed lane, so the caterpillars could not crawl out. Gradient lanes were placed in a growth chamber set to 16 C. On one side of the chamber, three heat lamps with 150-watt bulbs were aligned to generate a gradient of surface temperatures ranging from 16 – 40 C. These temperatures

were chosen to create conditions at either end of the gradient that are intolerable for caterpillars.

In Experiment 2, I constructed a new photothermal gradient using cardboard wrapped in mesh. The new design had smaller lanes (45 x 3 x 3 cm) which allowed for more caterpillars to be tested at once, increasing possible sample size. Gradient lanes were placed in a growth chamber set to 16 C. On one side of the chamber, three heat lamps with 150-watt bulbs were aligned to generate a gradient with surface temperatures ranging from 17 – 38 C.

Each lane in Experiment 2 was labeled with a number and reserved for a specific infection status to prevent any accidental OE contact. Caterpillars were randomly placed in lanes corresponding to their infection status, and lane number was recorded for each trial to account for any differences that might occur between lanes. Uninfected and infected lanes were alternated in the growth chamber, so one type of lane was not concentrated on one side of the growth chamber.

2.5 Thermal Preference Trials:

The trial period began when the caterpillars were large enough (usually >150 mg) to not slip through the holes in the mesh of the gradient lanes. Before being placed into the gradient, caterpillars were given fresh milkweed and placed in another growth chamber for 30 minutes. The chamber was set to 28.5 C which I considered to be the pre-trial acclimation temperature. After acclimation, a single caterpillar was put into each gradient lane at a chosen gradient surface temperature of 30 C (surface temperature of the gradient was measured by using thermal imager). Immediately upon placement into the gradient, a thermal image of each caterpillar was taken to determine the starting

temperature of each trial (0:00). In Experiment 2, I also marked the placement position of each caterpillar with a pencil. Each caterpillar remained in the gradient for 30 minutes in Experiment 1, and its body surface temperature was recorded using a thermal camera at 0:10, 0:20, and 0:30 (OMEGA TI-426 Thermal Imager).

In Experiment 2, each caterpillar remained in the gradient for 50 minutes (20 min longer than Experiment 1 to remove the effects of placement temperature on thermal preference), and its body surface temperature was recorded using a thermal camera at 0:30, 0:40, and 0:50 (OMEGA TI-426 Thermal Imager). The final position of the caterpillar (at 0:50) was marked with pencil and the distance between the placement position mark and final position mark was measured and recorded (value given negative sign if the final position was closer to the cold end of the gradient than the placement position). The caterpillars had no contact with the pencil marks as they were made outside each gradient lane and erased between trials.

After each trial period was finished, each caterpillar was removed from the gradient and weighed. All trials for Experiment 1 were conducted during the late afternoon to early evening of the preset day:night cycle. All trials for Experiment 2 were conducted in the morning. The same trial process was conducted for each caterpillar once a day until they pupated. Adult butterflies were then sexed and tested for infection status.

2.6 Thermal Image Analysis:

Thermal Images taken throughout the trial period were uploaded to the PC software OMEGA TI Analyzer to obtain relevant values for caterpillar surface body temperature. Once uploaded to OMEGA TI Analyzer, I visually identified the caterpillar. In Experiment 1, I drew a line following the center of the caterpillar's body from its head

to last abdominal segment. The maximum and minimum thermal pixel values along the line were averaged to determine the caterpillar's average body temperature which was then recorded in a separate datasheet. For Experiment 2, I chose 3 points along the center of the caterpillar's body (concentrated in 1-6 segments of abdomen). The three temperature values were then averaged to determine the caterpillar's average body temperature which was then recorded in a separate datasheet. A copy of each annotated image was then saved if needed for future reference (Figure 1).

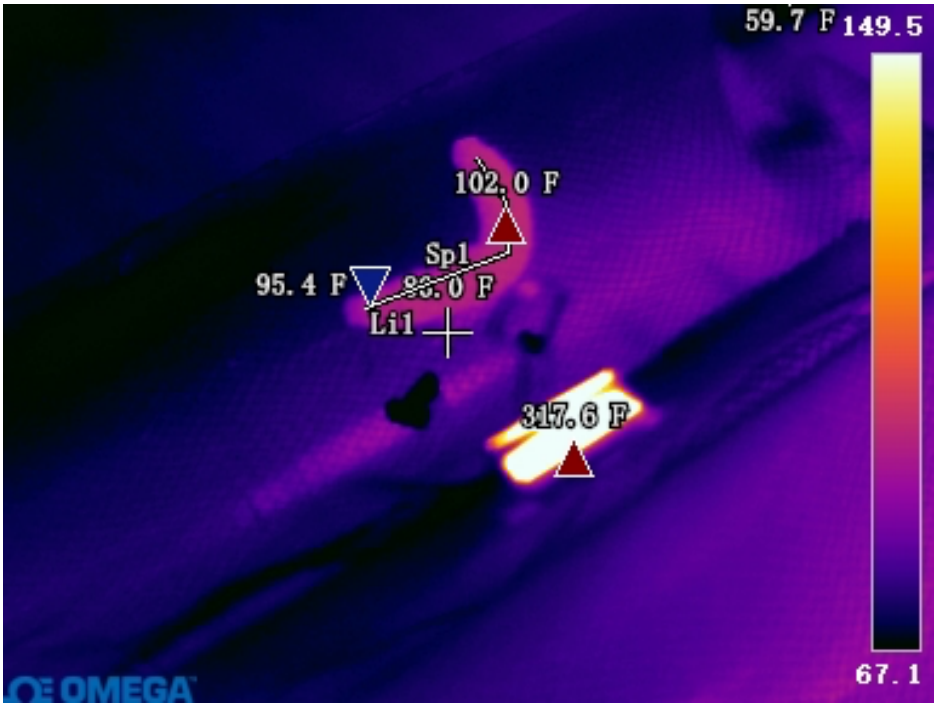

Figure 1: Thermal image of monarch caterpillar in OMEGA TI Analyzer software. White line drawn along the caterpillar's body, generating high (102.0 F) and low (95.4 F) temperature values that I average to determine average body temperature of the caterpillar (method used in Experiment 1).

2.7 Statistical Analysis:

Data was analyzed in RStudio (2022.07.2+576) using R package nlme. Using linear mixed-effects models, I tested for the fixed effects of weight, infection status, and starting temperature on thermal preferences of monarch caterpillars (Table 2). I also added random effects of individual, family line, and origin (wild-caught or lab-bred) for

Experiment 1 and individual, family line, and gradient lane for Experiment 2. I ran a post-hoc analysis after each model to analyze the difference between more than two levels of data (i.e., Exposed, Infected, Uninfected).

3.0 Results for Experiment 1:

In Experiment 1, I tested thermal preferences of 38 caterpillars (24 males and 14 females): 16 of which were wild-caught as eggs (n=14) or 1st instar caterpillars (n=2) with the rest (n=22) were lab-bred. Of the 18 caterpillars inoculated with 10-20 OE spores, 11 displayed signs of infection as adults when tested for the presence of OE spores. There were no other visible signs of infection (wing/other physical deformity, darkening pupa, diminutive size, etc.). The remaining 7 inoculated but uninfected caterpillars were categorized as "Exposed." No caterpillars that were not given the OE parasite inoculation became infected with OE.

3.1 Infection Status:

After 30 minutes in gradient conditions, infection status had a partially significant effect on thermal preference (Figure 2, Table 3a/b). There was no difference between thermal preferences of infected and uninfected individuals or infected and exposed individuals; however, there was a significant difference between the thermal preferences of uninfected and exposed individuals (Figure 2, Table 3a/b).

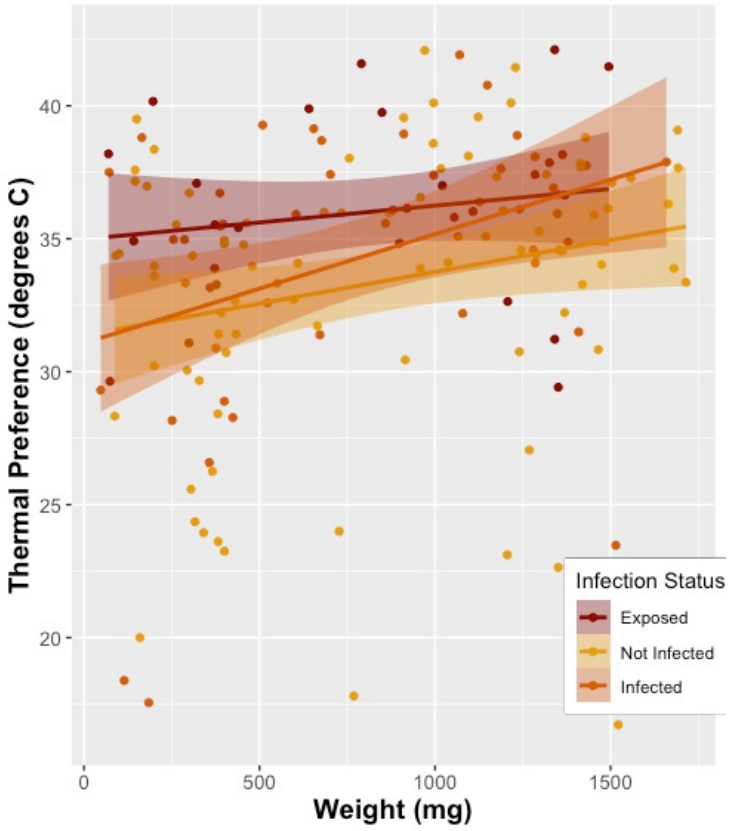

Figure 2: Thermal preference of exposed, infected, and uninfected caterpillars through development. Thermal preferences are based off measured temperatures of caterpillars after 30 minutes in the gradient. Exposed (dark red), infected (dark orange), and uninfected (light orange) caterpillars appear to display a preference for increasing temperatures throughout development as they get heavier.

3.2 Weight:

Weight was a significant predictor of thermal preferences of uninfected and infected caterpillars (Figure 2, Table 3a). As caterpillar's weight increased, it preferred warmer temperatures on average.

	Value	Std. Error	DF	t-value	p-value
(Intercept)	34.332	1.426	151	24.068	0.000
Weight	**0.003**	**0.001**	**151**	**3.539**	**0.001**
Infection Status (N)	**-2.556**	**1.047**	**151**	**-2.441**	**0.0158**
Infection Status (Y)	-1.518	1.141	151	-1.331	0.185
Sex (M)	-1.121	0.864	151	-1.298	0.196

Table 3a: Results of linear mixed-effects model exploring the effects of weight and infection status on preferred temperature of caterpillars after 30 minutes in gradient conditions.

Contrast	Estimate	SE	df	t.ratio	p.value
E - N	**2.56**	**1.047**	**151**	**2.441**	**0.042**
E - Y	1.52	1.141	151	1.331	0.380
N - Y	-1.04	0.896	151	-1.158	0.480

Table 3b: Results of post-hoc analysis of model in Table 3a exploring the differences between infection statuses with regard to thermal preference after 30 minutes in the gradient. E stands for exposed caterpillars. Y stands for infected caterpillars. N stands for uninfected caterpillars.

3.3 Starting Temperature:

Starting temperature (Time0) was defined as the temperature of each caterpillar immediately after they were placed in the gradient. Starting temperature had a significant effect on caterpillar thermal preferences measured at 10 minutes (Table 4) and 20 minutes (Table 5) after placement in the gradient. At 30 minutes, the effect of starting temperature on thermal preference became insignificant.

	Value	Std. Error	DF	t-value	p-value
(Intercept)	3.063	7.479	103	0.410	0.683
Weight	0.001	0.001	103	1.847	0.068
Infection Status (Y)	2.028	0.079	23	2.821	0.01
Time0	1.022	0.258	103	3.962	0.0001

Table 4: Results of linear mixed-effects model exploring the effects of weight and infection status on preferred temperature of caterpillars at 10 minutes in gradient conditions.

	Value	Std. Error	DF	t-value	p-value
(Intercept)	4.794	8.387	103	0.572	0.569
Weight	0.003	0.001	103	3.724	0.0003
Infection Status (Y)	0.665	0.930	23	0.715	0.482
Time0	0.925	0.290	103	3.193	0.002

Table 5: Results of linear mixed-effects model exploring the effects of weight and infection status on preferred temperature of caterpillars at 20 minutes in gradient conditions.

4.0 Results from Experiment 2:

Experiment 2 involved 24 caterpillars. There were 11 males (Infected = 5, Uninfected = 6) and 13 females (Infected = 5, Uninfected = 8). All 10 caterpillars inoculated with 70-100 spores that survived to adulthood displayed signs of infection as

adults when tested for the presence of OE spores. There were also other visible signs of infection (wing/other physical deformity, darkening pupa, diminutive size, etc.) that were not seen in Experiment 1. No caterpillars that were not given the OE parasite inoculation became infected with OE except for 3 caterpillars that were caught as first instars in the wild. These individuals were likely exposed to an unknown amount of OE spores prior to entering the lab and therefore were excluded from analysis. The other 4 caterpillars that were uninfected but caught as first instars were also removed because I could not determine if they were exposed to OE and cleared infection or were never exposed to OE in the wild.

4.1 Infection Status

Infection status had a positive but non-significant effect on the average thermal preferences of monarch caterpillars tested in Experiment 2 (Fig. 3, Table 6). Starting temperature in Experiment 2 had no significant effect on the thermal preference of caterpillars at 30, 40, and 50 minutes in gradient conditions (Table 7).

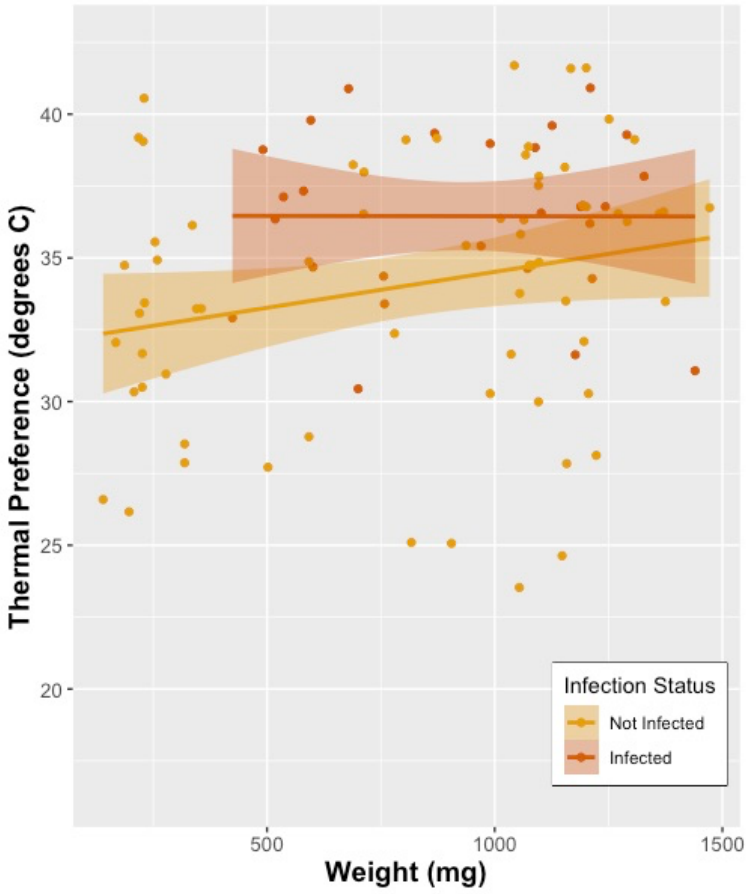

Figure 3: Thermal preference of infected and uninfected caterpillars through development. Thermal preferences are based off measured temperatures of caterpillars at 30, 40, and 50 minutes in the gradient.

	Value	Std. Error	DF	t-value	p-value
(Intercept)	33.614	1.151	63	29.200	0.000
Weight	0.001	0.001	63	1.205	0.233
Infection	2.073	1.119	21	1.853	0.078
Sex (Male)	-0.779	1.071	21	-0.727	0.475

Table 6: Results of linear mixed-effects model exploring the effects of weight, infection status, and sex on averaged preferred temperature of caterpillars at 30, 40, and 50 minutes in a temperature gradient. Infection status did not have a significant effect on thermal preferences of caterpillars.

	Value	Std. Error	DF	t-value	p-value
(Intercept)	21.534	11.676	57	1.844	0.070
Time0	0.422	0.375	11	1.125	0.285

Table 7: Results of linear mixed-effects model exploring the effects of starting temperature on preferred temperature of caterpillars at 30, 40, and 50 minutes averaged in gradient conditions. Starting temperature (Time0) has no effect on thermal preference.

4.2 Weight

Weight is not a significant predictor of thermal preference of monarch caterpillars in Experiment 2 (Table 6).

5.0 Discussion:

While there have been many experiments exploring the effects of temperature on monarch growth, development, and ecology, no experiment has tested thermal preferences of monarch caterpillars or if thermal preference varies based on infection status. The purpose of these trials was to assess whether behavioral fever was being displayed by monarch caterpillars in response to OE infection. I hypothesized that monarch caterpillars infected with OE would favor warmer temperatures on average to mitigate OE infection outcomes. In Experiment 2, when inoculation dose was high and individuals were tested in the morning, there was not a significant effect of infection status on thermal preferences of monarch caterpillars.

In Experiment 1, there was a significant difference in thermal preferences of exposed and uninfected caterpillars. Exposed caterpillars were caterpillars that were inoculated with a low dose (10-20 OE spores) but showed no sign of infection as adults. Uninfected caterpillars were never inoculated with OE spores. Because exposed caterpillars preferred warmer temperatures than uninfected individuals, this could suggest that exposed caterpillars were displaying fever and able to successfully clear infection as a result. When caterpillars ingest OE spores, the spores lyse in their gut and release individual parasites. These parasites then penetrate the gut wall of the caterpillar and infect hypodermal cells. During pupation, the parasites replicate and burst out of

hypoderm cells. The increased thermal preferences displayed in exposed caterpillars could be in response to the parasitic stage of OE where the parasite lyses in the gut, penetrates the gut wall, and infects hypodermal cells. Disrupting the early proliferation of OE could result in unsuccessful infection of caterpillars.

With limited evidence of behavioral fever in Experiment 1, I decided to make changes to the overall method design before starting my second round of experiments. Caterpillars in Experiment 1 were placed in the thermal gradient for 30 minutes with thermal images taken every 10 min. From this design, I found that starting temperature for each caterpillar (from thermal images taken at time 0:00) in the gradient had a significant effect on thermal preference up until 30 minutes into the gradient which was when the last thermal image was taken, and each caterpillar was removed from the gradient. Therefore, because the effect of starting temperature only became insignificant at 0:30, the thermal preferences in Figure 2 only reflect preferences at 30 minutes instead of an average across each trial period. Basing thermal preference off one point in time in a gradient study is less accurate than averaging thermal preferences measured across multiple times in a gradient to account for movement of the caterpillars. To respond to this issue of method design found in Experiment 1, I extended trial period in Experiment 2 to run for 20 extra minutes to include a 30-minute period of acclimation within the gradient. Extending the trial period resulted in no effects of starting time on thermal preferences of caterpillars imaged at 30, 40, and 50 minutes in the gradient.

I also improved the laboratory conditions to better promote fever in Experiment 2. Fever is an energetically costly and risky behavior to display in response to infection as an individual raises their body temperature above their optimal temperatures (De Roode

& Lefèvre 2012; Boorstein & Ewald 1987). Therefore, it would make sense that fever would only be displayed if an organism is significantly threatened by infection. In Experiment 1, caterpillars were inoculated with 10-20 OE spores, which corresponds to a sublethal dose of infection that typically does not impact fitness of adults (Lefèvre et al. 2010). If sublethal infection elicited some fever response, a heavier infection load might lead to a more drastic fever response as there would be higher negative fitness impacts of heavy infection.

To increase infection burden and create more optimal conditions for fever to be displayed, I increased inoculation dose in caterpillars to 70-100 spores in Experiment 2 (Lefèvre et al. 2010). Furthermore, individuals were tested in the morning as time of day has been shown in other systems to produce the strongest fever responses (Clancy et al. 2018). The reasoning behind highest fever responses being displayed in the morning is that this is the first period of the day in which an ectotherm can thermoregulate and therefore the first time of the day an individual can respond with behavioral fever to infection that might have increased in load overnight.

In Experiment 2, there is a positive but non-significant effect of infection status on thermal preferences of monarch caterpillars ($p = 0.078$) with infected individuals seeming to prefer warmer temperatures than uninfected individuals. This experiment has a small sample size, so the lack of a significant result may be due to that. I am planning to test more individuals inoculated with a high (70-100 spores) and low (10-20 spores) infection dose to add to this dataset.

Unexpectedly, weight, in Experiment 1, was seen to be a significant predictor of thermal preference with heavier caterpillars preferring warmer temperatures on average.

Weight was not seen to be a significant predictor of thermal preference in Experiment 2; however, there was a non-significant positive trend in uninfected caterpillars. Small sample size in Experiment 2 could have resulted in the non-significant effect of weight in caterpillar thermal preference.

Many studies have shown effects of temperature on development time in monarch caterpillars (York & Oberhauser 2002). Long periods of heat stress increase development time and mortality in monarch caterpillars (York & Oberhauser 2002). This effect is most pronounced in first and third instar caterpillars and least pronounced in fifth instar caterpillars (York & Oberhauser 2002). In contrast, short-term daily exposure to heat stress has no differential effects on mortality and decreases development time in all monarch caterpillar instars. While monarch caterpillars experience decreased development time throughout all instars when exposed to short pulses of heat stress, fifth instar caterpillars may be the most tolerant to heat stress. Therefore, heavier caterpillars may be selecting for warmer gradient temperatures to decrease development time when they are least susceptible to heat stress. Decreasing development time may benefit monarchs by decreasing their exposure to parasites and predators during juvenile life stages.

Another possible explanation for the relationship seen between weight and thermal preference could be that it is an artifact of increased melanism of caterpillars in later instars. Various studies have shown that melanism in monarch caterpillars is linked to temperature with darker individuals developing at colder temperatures and darker individuals achieving body temperatures that were 4-6 C warmer than lighter individuals (James 1986; Davis et al. 2005; Solensky & Larkin 2003). However, it is unlikely that

melanism alone can explain the relationship between weight and thermal preference as darker individuals would be able to move to lower temperatures than lighter individuals to achieve the same body temperature if there was no difference in temperature preferences.

During development, monarch caterpillars grow rapidly, increasing through several orders of magnitude of body size in the span of two weeks. Changes in weight and body size occur simultaneously in monarch caterpillars and result in changes to how they interact with their thermal environment. Using thermal imaging, Woods (2013) showed that increases in body size throughout the development of tobacco hornworm larvae also correspond to increases of 3-7 C on average in larval body temperature. This could mean that the positive relationship seen between weight and thermal preference is the result of changes to average body temperatures throughout ontogeny. Essentially, larger, heavier caterpillars could just be warmer on average.

Thermal imaging is a powerful tool to use to attempt to measure body temperature of smaller individuals where probing might not be possible, but it has limitations. Body surface temperature can vary widely from actual internal body temperature. In this thesis, surface body temperature was compared between caterpillars as a proxy to compare thermal preference; however, based on literature values, body surface temperature is likely not a good measure of internal body temperature for monarch caterpillars as it is likely several degrees higher than body temperature. Monarch caterpillars experience optimal performance at 29 C (Zalucki 1982; Malcolm et al. 1987). Temperatures above 31 C have been shown to significantly increase mortality in monarch caterpillars (Zalucki 1982; Malcolm et al. 1987). The average surface body temperatures that were measured

from caterpillars in these trials was 34.13 C with the highest temperature measured at 45.36 degrees C, well above the maximum thermal limits of monarch caterpillars.

In summary, there was evidence of behavioral fever displayed in monarch caterpillars exposed to OE in Experiment 1 which might have resulted in some individuals being successful in clearing a sublethal infection. Unexpectedly in Experiment 1, weight was a significant predictor of thermal preference with heavier caterpillars preferring warmer temperatures on average. The positive relationship between weight and temperature could be a result of the benefits of increasing development rate or be a result of ontogenic changes in body size that correspond to changes in average body temperature. In Experiment 2, there was an absence of fever being displayed which could be the result of small sample size. There also was not a significant effect of weight on thermal preference. While there have been many experiments exploring the effects of temperature on monarch ecology, no experiment has tested thermal preferences of monarch caterpillars or if thermal preference varies based on infection status. Understanding the role of temperature in infection outcome is critical in understanding how monarchs might response to climate change.

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