Impact of Climate Change on the Pupal Parasitoid Wasp, *Pteromalus cassotis*, and its Interactions with Monarch Butterflies

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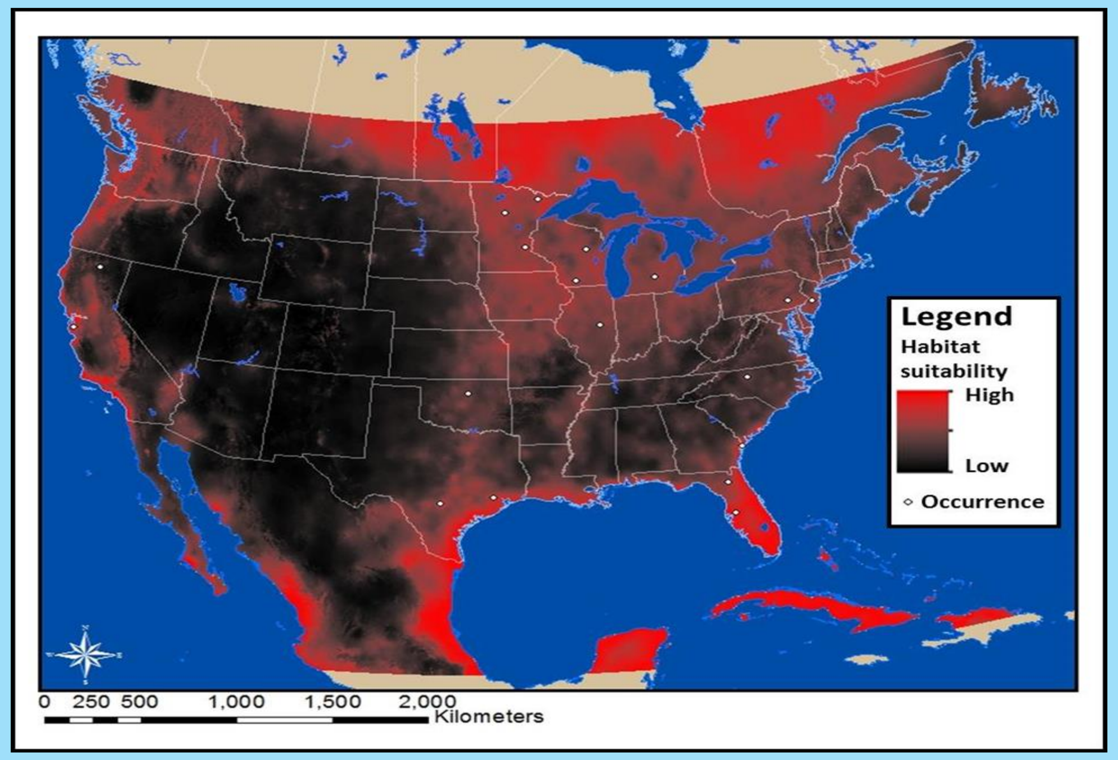
Introduction

Monarchs (*Danaus plexippus*) are a well known and well studied species of North American butterfly, but their population has declined significantly over the past two decades[1]. Monarch population dynamics may be influenced by several factors, including natural enemies. One such natural enemy is the recently rediscovered specialist parasitoid wasp of monarchs called *Pteromalus cassotis*[2], which attacks and kills monarchs by depositing eggs into the pupa, consuming the monarch pupal tissues as the eggs develop. These wasps can be an important factor in monarch mortality, causing up to 60% of monarch deaths in field experiments[3].

A female *Pteromalus cassotis* wasp laying its eggs inside of a newly pupated monarch. Taken by Carl Stenoien.

It is hard to study the interactions between *P. cassotis* and monarchs in the field due to the small size of the wasps and host camouflage. It can be even harder to measure how outside factors such as climate change will influence these dynamics. One way to measure the effects of climate change is through modeling current host and parasite distributions and determining suitable climatic conditions for both species. This suitable region where a species actually occurs is known as the realized niche[4]. The realized niche can then be used to track possible changes in their range with climate change estimates. *P. cassotis* distribution has been modeled and found that the wasp occurred all across the U.S., but prime habitat was located on the Gulf Coast, Florida, and Southern Canada near the U.S. Midwest and Northeast[5].

In order to go beyond the realized niche, I designed my experiment to determine the potential limits of temperature on *P. cassotis*. Building on previous research, I examined the potential for *P. cassotis* to parasitize monarchs at a range of different temperatures towards the extremes of what the wasp might experience, either presently, or in the future with a changing climate.



Adapted from McCoshum et al. (2016). Models habitat suitability for the parasitoid wasp, *P. cassotis*, across North America.

Methods

Wasps were exposed to three different temperatures. Incubators were kept at daytime temperatures of 18°C, 25°C, and 32°C, with nighttime temperatures 5°C lower at 13°C, 20°C, and 27°C, respectively. These temperatures were chosen to reflect the full range of temperatures (represent extremes on either side) *P. cassotis* wasps would be exposed to in the field.

A trial consisted of a minimum of three wasps, which were taken from a parent colony, with wasps divided evenly into each of the three incubators. Wasps were exposed to assigned temperatures 2-5 days before being used to start a trial. Trials in each temperature consisted of one monarch pupa and one wasp. They were paired together for 3 days, with multiple daily observations made to see if the wasp was on or off the pupae (as the wasp typically stays on the pupa from 1 hour to 2 days while ovipositing). After the wasps were taken out, the pupae were weighed, tied to the lid of a tub, and were left in the incubator until, wasps emerged, an adult butterfly emerged, or the pupa was determined to be dead (if nothing emerged after a month and a half). If wasps emerged, they were frozen, counted under a microscope, and their sex determined. All pupae were then dissected to find the number of adult unemerged wasps, as well as larvae and pupa (immature wasps). Data analyses were conducted using excel. Significance calculated using t.test function.

Results

Figure 1: Picture of parasitized monarch pupa dissection. All pupae (both with and without emerged wasps) were dissected under a microscope. Parasitized pupae (wasps present inside) were found for all three temperature treatments. Taken by Carl Stenoien.

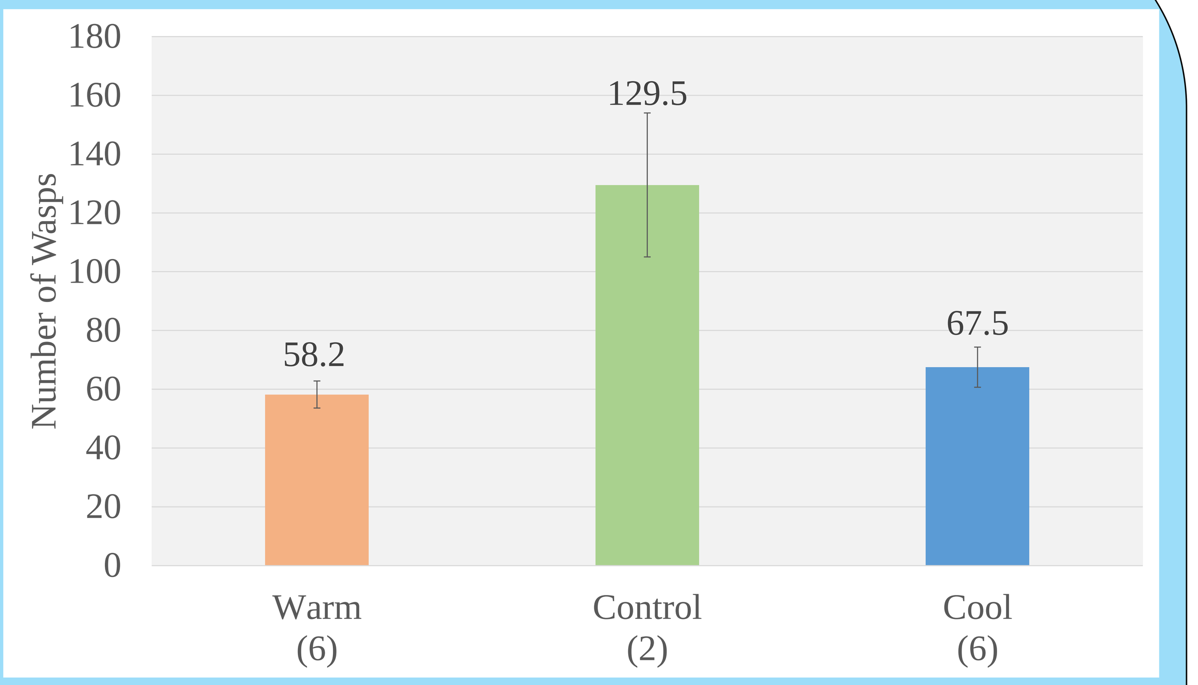


Figure 2: Average number of wasps found per pupa, ± SE. All life stages were counted, both inside and outside of the monarch pupa, with totals above error bars. Sample size for each temperature treatment is in parentheses. For cool, 5 of the trials were dissected before wasps could emerge.

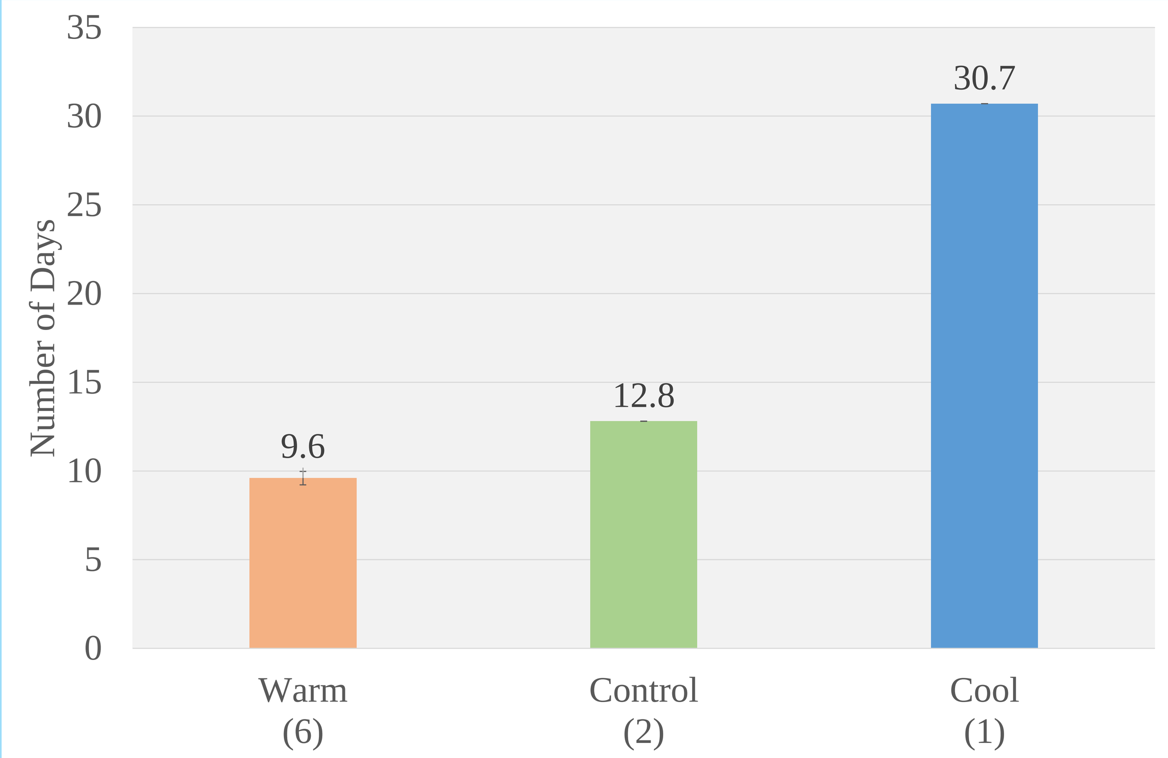


Figure 3: Average number of days it took for wasps to emerge from pupa after trial end date, ± SE. Sample sizes are below treatment in parentheses. The control treatment had no deviation between data points and the cool treatment had a sample size of one, hence there was no standard error estimate for either.

Discussion

The parasitoid wasp *P. cassotis*, was able to successfully parasitize monarch pupae (Figure 1) when exposed to all three temperatures, 32°C, 25°C, and 18°C, albeit with varying levels of success. This corresponds with the extreme temperatures of previous research on *P. cassotis* habitat suitability[5]. It also indicates that if temperatures were to shift to these more extreme temperatures with climate change, *P. cassotis* would be able to continue to parasitize monarchs.

The average number of days it took for wasps to emerge from the pupa for the warm, control, and cool treatments were 9.612 ± 0.612 days, 12.785 days, and 30.729 (only one data point) days (Figure 3). The results show a positive relationship with temperature and number of days to emergence. The control is relatively consistent with previous data showing an average of 15.831 ± 0.438 days (Carl Stenoien, unpublished data). The extended development time is unsurprising, since wasps are ectotherms and hence their rate of growth depends on the temperature of their external environment.

The average number of total wasps per pupa (±SE) were 58.2 ± 4.7 wasps, 129.5 ± 24.5 wasps, and 67.5 ± 6.8 wasps for the warm, control, and cool treatment temperatures, respectively (Figure 2). The control treatment was significantly higher than both the warm and cool treatment. The range of temperatures, although limited, seem to show the optimal temperature for offspring at 25°C. However, we can't tell whether this is due to behavioral changes (e.g., changes in the number of eggs a female wasp lays) or physiological changes (e.g., the temperature affects survival of the wasp offspring). Surprisingly, the control treatment has a higher average number of wasps than the UMN monarch lab's average of 80.4 ± 7.3 wasps (Carl Stenoien, unpublished data). Data have been collected over several years using the same lineage of wasps which were grown in room temperature, approximately the same as our 25°C control temperature. However, with such a small sample size, this difference may be reduced with further data collection.

Over the course of this experiment, the control incubator overheated twice, one time killing off both pupae and wasps, leading to smaller sample sizes than anticipated. Additionally, due to time constraints, some of the cool treatment pupa had to be dissected before they could emerge naturally. While they were not included for the time to emergence data, they were included for the average total wasps data. Since sample sizes were small, I plan on conducting additional research to confirm my findings.

As expected, it took longer for the parasitoid offspring to emerge in cooler temperatures than warmer ones. This means that as average temperatures rise, we can expect the growth rate of *P. cassotis* populations to increase, as the waiting period between generations decrease. This could potentially mean a higher parasitoid induced mortality rate for monarchs. However, my results show that the optimal temperature for the highest parasitism success rate is around 25°C. This is slightly unexpected as warmer temperatures are essential for insect growth. This means that as average temperatures rise, we can expect *P. cassotis* wasps to produce less offspring per pupa. But it is unclear how this will effect their population. They are still successful at the extreme temperatures, killing the monarch. If mortality is high for adult wasps in the field, a slightly higher mortality might not significantly affect their lifecycle.

More data was collected on each trial, but due to small sample size, did not allow us to come to any conclusions. They can however be used to direct future research, there is the possibility that warmer temperatures lead to higher male to female sex ratios or cause a lower proportion of the offspring to reach maturation.

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