Effects of Ambient Temperature on Monarch Larval Immunity

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Introduction

Changes in global climate have been shown to influence species interactions, including those between hosts and parasites. Host-parasite relationships can be affected by pathogen responses to warmer temperatures, and also by changes in immune defense for ectothermic host species.

Fig. 1: Adult monarch (*Danaus plexippus*)

Monarchs are migratory insects that experience a range of temperatures in natural populations, especially in eastern North America where their breeding range extends from southern Canada into the southern U.S., and where their annual migration takes them to overwintering sites in Central Mexico. They are also host to a debilitating parasite, *Ophryocystis elektroscirrha.* As such, monarchs are well-suited for studying the relationships between temperature and immune defense.

Phenoloxidase (PO) activity and hemocytes (blood cells) play important roles in the invertebrate immune system. Hemocytes encapsulate and phagocytize pathogens, while PO activity suppresses parasite growth via melanization. Thus, PO activity and hemocyte counts have been used as measures of immune defense against pathogens in multiple insect species.

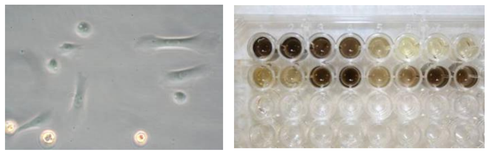


Fig. 2: Hemocytes from larval monarch showing 3 of the 4 major immune cell types (L). Samples showing individual variation in melanization (PO activity).

Experimental Questions

- How do measures of innate immune defense change with ambient temperature?
- Does temperature affect the severity of parasite infection?
- Do immune measures further depend on other host factors (genetic background or size)?

Hypotheses and Prior Work

- Past work shows conflicting evidence for the relationship between immunity and temperature in insects. Adamo et al. (2011) showed that warmer temperatures increased immune enzyme activity and bacterial resistance in the cricket *Gryllus texensis*. A study on mosquitoes showed that melanization and phagocytic cell activity peaked at intermediate temperatures and were lowest at higher temperatures (Murdock et al. 2012).
- Increased temperatures are known to speed up larval development in insect species (Zalucki 1982, Adamo et al. 2011), but can also increase rates of adult mortality, and may pose a trade-off between rate of development and immunity (Karl et al. 2011).
- Based on this information, we predicted that monarch larval immune defense might be highest at intermediate temperatures (29°C) and lowest at more extreme hot and cold conditions.

Materials and Methods

- Larvae were reared from captive adult monarchs and represented the outcrossed F3 offspring of wild adults captured at the Central Mexico overwintering sites in Feb 2013.
- At the 2nd instar, 100 larvae were placed into incubators set at one of the four daytime temperatures: 23°C, 26°C, 29°C, 32°C. Daylight hours were from 0600 to 2100, and nighttime temperature were 2°C below diurnal temperatures.
- 20 larvae per temperature treatment were uninfected, and 5 larvae per temperature treatment were inoculated with 10 spores each of the protozoan parasite, *Ophryocystis elektroscirrha* (OE).
- Larvae were reared singly in 32 oz. containers and fed cuttings of greenhouse-raised swamp milkweed (*Asclepias incarnata*)

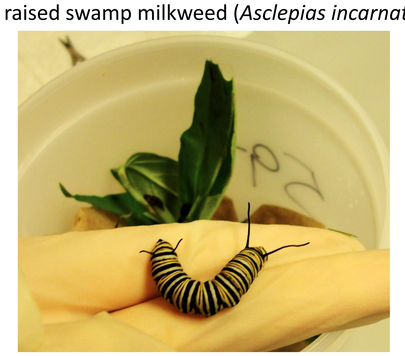


Fig 3: Monarch larva in single container (L) and incubator chamber (R)

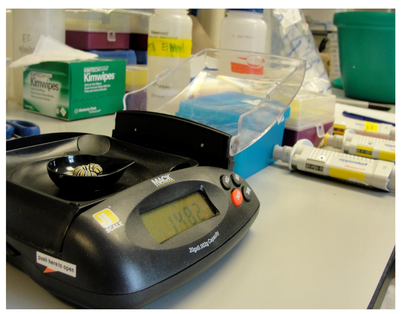


Fig. 4: Larvae were weighed (L) and hemolymph was collected from a clipped front tubercule (R)

- At the 5th instar, larvae were bled to obtain hemolymph for quantifying hemocyte concentrations and for PO assays.
- We used a Biotek EL-808 microplate reader to measure the maximum absorbance at 490 nm and slope at linear phase of the melanization process over a period of 3 hrs. Hemocytes were counted with phase microscopy at 400X magnification.
- For infected larvae, qualitative spore load and quantitative spore counts were recorded from adults to determine the severity of infection.
- Before bleeding, we recorded larva mass. We also recorded the number of days from hatching to pupation and eclosion. After eclosion, we recorded adult sex, mass, and OE infection status.

Results

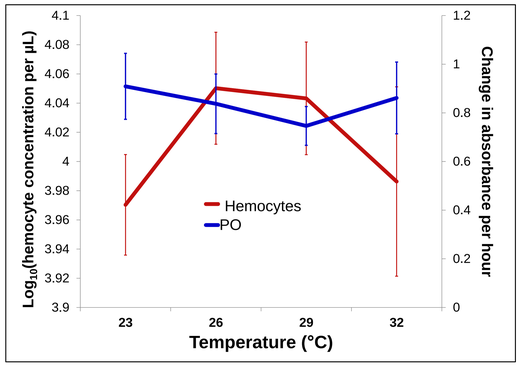
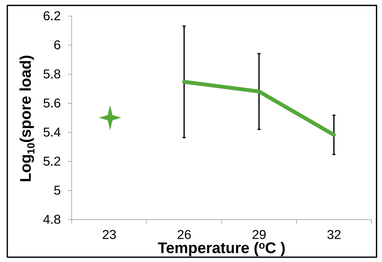


Fig. 5: Effect of temperature on mean (± SE) PO activity and hemocyte concentration.

Hemocyte concentration was highest at intermediate temperatures, though the trend was not significant. Conversely, PO activity tended to be highest at the coolest and warmest temperatures, though again the trend lacks statistical significance. At the individual level, there was no correlation between PO activity and hemocyte concentration (data not shown).

The final spore load in infected monarchs tended to decrease with temperature.



The three lineages of monarchs differed significantly in PO activity (p=0.031).

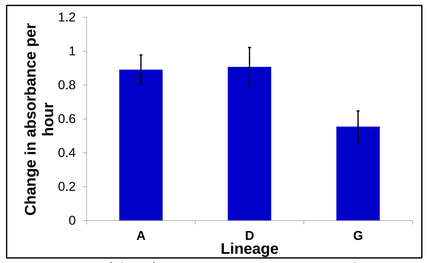


Fig. 6: Mean (± SE) OE spore load across temperatures. *Data pending for 23° incubator.

Fig. 7: Mean (± SE) PO activity across monarch lineages differing in genetic background.

- Neither PO or hemocyte concentration were affected by larval body size at bleeding.
- Development time from hatch to pupation decreased with temperature.
- Mortality rates were equal across temperatures and among lineages.

Conclusions

From our data, we conclude that:

- For monarch larvae, PO activity and the number of hemocytes are independent measures of immune defense.
- Neither PO activity or hemocyte concentration changed significantly across temperatures in the range we selected, although trends indicated that hemocyte concentration was highest, and PO activity was lowest, at intermediate temperatures.
- PO activity varied significantly among genetic lineages of monarchs.

In the future, we would like to repeat the study with a larger sample size, and include a wider range of temperature treatments.

Acknowledgements and References

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