
Figure 1: larval, pupal and adult stages

Influence of temperature on susceptibility and response of monarch butterflies to a protozoan parasite

Hugo Valin[1], Elizabeth Friedle[2], Sonia Altizer[2]

[1] Ecole polytechnique, France; [2] Department of Environmental Studies, Emory University

Abstract

In North America, three main populations of monarch butterflies (*Danaus plexippus*) are infected by the neogregarine protozoan parasite *Ophryocystis elektroscirrha* but show very different prevalence. To test whether climatic variations could explain those differences, we reared larvae exposed to cold, moderate and hot temperatures and increasing inoculation dose of the parasite. We observed that hot temperatures led to a significant decrease of parasite load for every level of infection. At the same time, larvae from the cold treatment were less harmed by the infection with higher survival to eclosion and fewer deformities. These results suggest that temperature is an important variable in dynamic of this host-pathogen system but its effect alone is not sufficient to explain the differences in prevalence among wild populations of monarchs.

Background

Monarch butterflies are spread world-wide and all populations examined to date harbor the parasite *O. elektroscirrha*. The development of this parasite is highly related to the metamorphic cycle of the host, which goes through a larval, pupal and adult stage like all Lepidoptera (cf. figure 1 on the left-top corner of this poster).

This parasite is carried by the adults as dormant spores on their abdomen and wings. Adults spread their spores to other adults but only larvae can be infected when they ingest some. Ingested spores are activated and replicate in the larvae hypoderm during larval and pupal stage. At eclosion, the adult is covered by hundreds of thousands spores on its abdomen and wings.

Infection by this parasite increases larval mortality and adult fitness, as they can be deformed after eclosion and have decreased life expectancy and wingspan.


Figure 2: Infected pupa and infected monarch failing to emerge

Monarch butterflies can be found in three major areas in North America. Each of them present a different prevalence of the parasite:

- The eastern population breeds in a temperate climate and migrates long distances to overwinter in central Mexico. Only 8% of the population is heavily infected
- The western population also migrates a shorter distance and 30% of adults are infected.
- The South Florida population does not migrate; monarchs live in tropical conditions and 80% of them are heavily infected by this parasite.

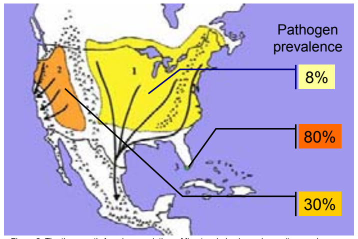

Figure 3: The three north America populations: Migratory behavior and parasite prevalence

Questions

What factors might explain these differences in prevalence among wild populations?

Three hypothesis to explore:

(1) ***Role of migratory behavior***: as the butterflies who migrate the farthest are less infected, selection during migration could reduce parasite spread.

(2) ***Role of genetic diversity:*** parasite strains from South Florida could be more virulent and monarchs from migratory populations more resistant to parasite infection.

(3) ***Role of climate:*** temperature and other local environmental factor could benefit to the infection in South Florida

Objective of this study:

To test the effect of temperature on the monarch-parasite interaction.

Methods

Design: Monarchs larvae were distributed into 3 temperature treatments and 4 inoculation dose treatments.

Total: 369 larvae	**Cold 21°C**	**Moderate 26°C**	**Hot 31°C**
Control (0 spore)	34 larvae	31 larvae	36 larvae
Low dose (50 spores)	30 larvae	31 larvae	28 larvae
Medium dose (500 spores)	31 larvae	31 larvae	29 larvae
High dose (1000 spores)	29 larvae	31 larvae	28 larvae

Larvae were obtained by crossing South-Florida freshly collected males with eastern and South-Florida female monarchs, after the spore checking showed they were uninfected. After inoculation with calibrated doses of a South Florida strain of *O. elektroscirrha*, larvae were reared in individual containers in growth chambers programmed with a day-night cycle (light and temperature fluctuation) and fed daily.

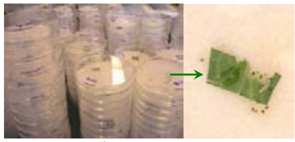
Figure 3: inoculation of 2nd instar larvae with the parasite: a calibrated dose of spore was deposited on a 1 cm^2 piece of leaf.

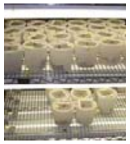
Figure 4: After inoculation, larvae were reared in individual containers

Data analyzed: we measured the following variables:

- Development time from inoculation to pupation and to eclosion.
- Infection level and spore load (using a standardize method to remove scales and spore from monarch abdomens and count them by image processing with Adobe Photoshop®).
- Levels of deformity: Any sign of wing deformity or failure to emerge by themselves was recorded among adults
- Adult lifespan and mass: after emergence, adults were removed from the treatment. Their mass was recorded 24 hours after eclosion and their lifespan (in days) was also reported.

All the data results were tested by Analysis of Variance (continuous variable) or Logistic Regression (binary variables) tests to evaluate the significance of the hypotheses.


Figure 5: Spores of *O. elektroscirrha* seen with light microscope (100X). Counting these spores using image analysis enabled us to quantify the severity of infection.

Results

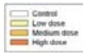

Figure 6, 7 and 8: The legend for color is 1 for control, 2 for low dose, 3 for medium dose and 4 for high dose. Error bars indicate the standard error of mean. Parenthesis indicate the sample size.

[a] Sample size uncompleted as all the data are not collected yet.

* Significant difference with the other temperature treatments.

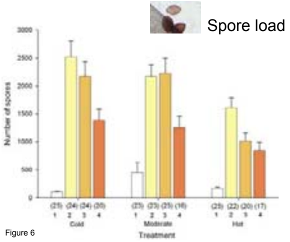

Figure 6

- Spore load decreased with increasing inoculation doses. This effect was due to difficulty in removing scales and spores from highly infected adults, which had damaged abdomens.
- Spore load decreased with increasing temperature. This significant result (P<0.001) was observed throughout all inoculation treatments.

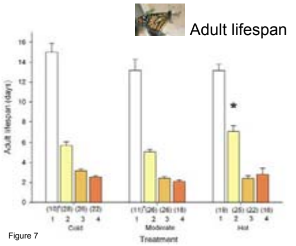

Figure 7

- Adult lifespan decreased with increasing inoculation doses. In treatments 3 and 4, adults did not survive more than 2 or 3 days.
- In the low dose treatment, hot temperature favored survival of monarchs after eclosion (P<0.001 – shown by the star on figure 7). But no global effect of temperature was observed in other treatments.

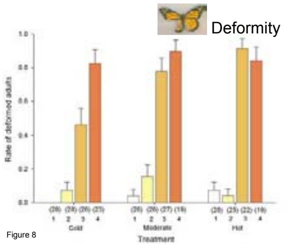

Figure 8

- Wing deformity increased with greater inoculation doses.
- For the medium dose treatment, low temperature limited levels of deformity. But this moderating effect was not observed in the other dose treatments.

Summarize of all the results

Dependent variable	Effect of infection increase	Effect of temperature increase	Interaction effect
Spore load	Increase ?	Decrease	Independent effects
Deformity	Increase	-	Interaction
Adult lifespan	Decrease	-	Interaction
Adult mass	Decrease	-	-
Development rate	Decrease	Decrease	Independent effects

Other results:

- Adult mass showed a decrease with increasing infection but no effects of temperature.
- Results showed a very high proportion of infected adults in comparison with previous experiments, suggesting the parasite from South Florida may be more virulent.
- The effect of temperature on development time of insects is well-known and a faster development was observed in high temperatures, as expected. But infection also slowed development times.

Effect of temperature: what we did not know before…

- Hot temperatures caused a 30% reduction on spore loads.
- Monarch deformities when inoculated with moderate doses were lower in the coldest treatments.
- Monarchs reared in hot temperatures survived longer to a low dose infection

Conclusions

This experiment showed that temperature has important effects on the development of this parasite.

HOWEVER

- The results show that high temperature lead to a decrease of the infection level: geographic differences in prevalence cannot be explained by this single effect. **Hypothesis (3) is not the right explanation**.
- Monarch fitness is strongly affected by the parasite, **reinforcing the relevancy of hypothesis (1).**
- The high virulence of the parasite from South Florida supports the idea that **genetic differences may explain some differences in the wild (hypothesis (2)).**

The mechanism of this system is more complex than expected, with a probable trade-off between several effects. Further investigations and modeling will be useful to be able to explain the current and future dynamic of this host-parasite system and foresee the exact consequences of global warming.

Acknowledgements

We thank Andrew Davis for his critical review of this work, advice, suggestions and assistance. We also thank Nick Vitone and Zack Baumann for their helpful laboratory assistance. We thank Les Real from the Biology department for the loan of the greenhouse facilities. We thank the SURE program of Emory University and the Howard Hughes Medical Institute for their financial support. We thank the Ecole polytechnique for offering to Hugo Valin the opportunity to take part in this project.

ÉCOLE POLYTECHNIQUE

EMORY