Crowding and disease: Combined effects of larval rearing density and parasitism on survival and development of monarch butterflies *(Danaus plexippus)*

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Abstract

Monarch butterflies (*Danaus plexippus*) inhabit islands and continents worldwide and form migratory and nonmigratory populations that differ according to local population density and exposure to the protozoan parasite, *Ophryocystis elektroscirrha*. Specifically, monarchs in non-migratory populations develop at higher densities and have higher parasite loads, whereas monarchs in migratory populations develop at lower densities and have much lower rates of parasitism. **The goal of my study was to experimentally examine the effects of larval rearing density on the susceptibility and responses of monarch butterflies to infection with *O. elektroscirrha*.** I predicted that monarchs reared at high densities would be more susceptible to infection and would experience more severe negative effects of disease. Monarch larvae were inoculated with calibrated parasite doses and reared in replicate containers at low (single larva), medium (5 larvae), and high (10 larvae) densities. Effects of density and parasite treatments were evaluated by measuring larval and adult survival, development rates, pupal mass, adult wing size, body pigmentation, and quantitative parasite loads. Results showed that rearing density significantly affected morphometric variables and development times. Parasite infection influenced monarch development rates, final parasite loads and body pigmentation. There was an interactive effect of parasite infection and rearing density on pupal mass and total lifespan, and an interactive effect of density and sex on adult longevity. Thus, both rearing density and parasite infection significantly affected monarch development, survival, and final parasite loads.

Background

Monarch butterflies are found in three main populations in North America. A population east of the Rocky Mountains breeds at low densities (orange shaded region) and migrates annually to central Mexico. A population west of the Rocky Mountains breeds in the yellow shaded region and migrates a shorter distance to coastal CA. A population in S. FL breeds year-round at high densities and does not migrate.

All monarch populations examined to date are infected with the neogregarine protozoan parasite, *Ophryocystis elektroscirrha*. Parasite development is highly tied to the life cycle of the host. Parasites are spread from infected adults to their progeny when dormant parasite spores are scattered onto milkweed leaves and ingested by larval hosts. After eclosion, infected adult butterflies emerge covered with thousands of spores on the outsides of their bodies.

Parasite prevalence varies dramatically among wild monarch populations. Only 8% of the eastern migratory population is heavily infected, about 30% of the western migratory population is infected, and nearly 80% of the resident S. FL population is infected.

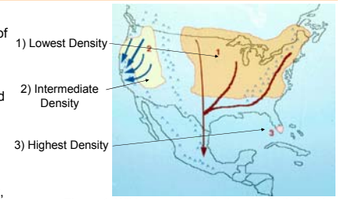


Figure 1. Wild monarch populations in North America

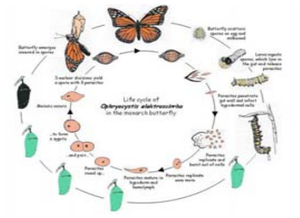
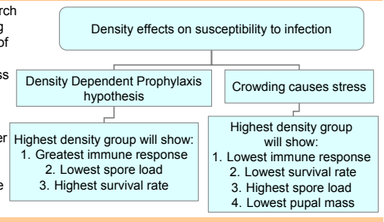


Figure 2. Lifecycle of *Ophryocystis elektroscirrha*

Predictions

Larval rearing density could affect monarch susceptibility to infection. Two competing hypotheses arise from previous studies of insect-parasite interactions. If monarchs reared at high densities experience stress due to crowding (right side of diagram), they could be more susceptible to parasites and experience more severe effects of disease. If high densities trigger monarchs to invest more in immune defenses (left side of diagram) then monarchs reared at high densities will be more resistant to infection.



Methods

Table 1. Total number of larvae used in each experimental group. Number of replicate counts shown in parenthesis.

	Low (1 larva)	Moderate density (5 larvae)	High density (10 larvae)
Control	40 larvae, 40 containers	60 larvae, 11 containers	110 larvae, 11 containers
Parasitized	40 larvae, 40 containers	60 larvae, 11 containers	110 larvae, 11 containers

Figure 3. Cages used to collect eggs (L), containers used to rear larvae (C), and dishes for inoculation (R)

Figure 4. Low (L), medium (C), and high (R) rearing densities.

Measured Data		
Sub-lethal Effects		Lethal Effects
•Development Time		•Larval Mortality
•Morphometric Measures		•Pupae Mortality
*Wing Density	*% Black on Wing	•Adult Survival
*Wing Area	*Pupal Mass	
*Infection Status	*Quantitative Spore Measure	

Developmental Times

- •Larval Development = Oviposition to Pupation
- •Adult Development=Pupation to Eclosion
- •Adult Lifespan= Eclosion to Death
- •Total Development= Oviposition to Eclosion
- •Total Lifespan= Oviposition to Death

Results

During the course of the experiment **167** larvae and pupae died. The cause and source of death are unknown, but one possibility is that baculovirus was the cause. The larvae and pupae that were afflicted turned dark black and rapidly deteriorated.

Larval and Pupal "Black Death"

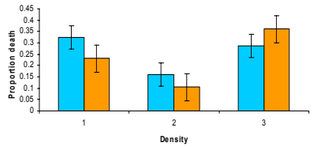


Figure 5. Number of deaths from the "black death" in each treatment and density group.

The "black death" affected the low and high density groups the most, while the medium group was the least affected (Figure 5). The unexplained deaths could mask any effect density may have had on the measured variables, by removing those individuals that are less fit. Figure 5 also shows that *O. elektroscirrha* did not affect susceptibility to the "black death."

Since not much is known about the "black death" larvae and pupae dying from this were removed from statistical analysis.

Key: Orange= Monarchs exposed to parasites and Blue= Monarch not exposed to parasites

Survival to adulthood

- Monarchs reared at the high density treatment had lowest survival to adulthood, and those in the moderate density treatment had the highest survival to adulthood (data not shown).
- Survival to adulthood was similar for monarchs exposed and not exposed to parasites.

Development Times

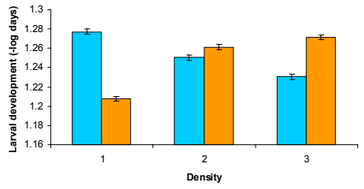


Figure 6. Time in days (log transformed) for larval development.

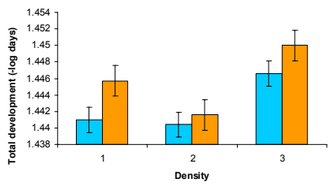


Figure 7. Time in days (log transformed) for total development.

- Larval development time, from oviposition to pupation, was longer for the parasite exposed monarchs in the medium and high densities, however it was longer for the control monarchs at low density (Figure 6).
- Total development time, from oviposition to adult eclosion, was longer for monarchs exposed to parasites relative to the control monarchs (Figure 7).
- Monarchs reared at the highest density had slower development times to adulthood. Monarchs reared at moderate densities developed the fastest overall (Figure 7).
- Males developed faster than females (results not shown).

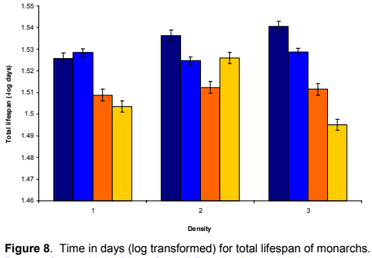


Figure 8. Time in days (log transformed) for total lifespan of monarchs. Purple= Control Male, Blue= Control Female, Orange= Infected Male, Yellow= Infected Female

Total lifespan

- Monarchs exposed to parasites had a significantly shorter lifespan than control treatment monarchs (Figure 8).
- Females exposed to parasites and reared at high densities had the shortest lifespan, and control males reared at high densities had the longest lifespan (Figure 8).

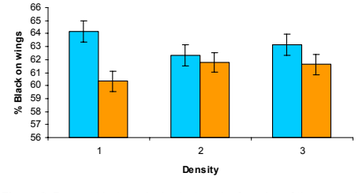
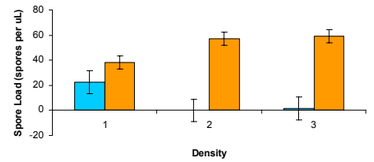


Figure 9. Percent black (melanization) on the forewing of the monarch.

Morphometric measures

- For monarchs exposed to parasites, pupal mass was greatest in the moderate density treatment and lowest in the high density treatment (data not shown).
- Control monarchs were darker than infected monarchs in both the low and high density treatment (Figure 9).
- For the % of the area of black pigmentation on forewings, females were darker than males, and monarchs exposed to parasites were lighter than control monarchs (data not shown).



Parasite loads

- Most monarchs exposed to parasites emerged with many parasites spores (Figure 10).; control treatment monarchs showed very low contamination.
- Spore loads on adult butterflies increased with increasing larval rearing density (Figure 10).

Figure 10.Mean number of spores per micro liter from monarchs in the infected treatment. Four control individuals that were infected with spores were removed from statistical analysis.

Table 2. Analysis of variance results based on model simplification procedures, where AICC was used to determine minimum adequate model. Values were averaged within each rearing container prior to analysis (*n=102 to 112* for all traits). X represents *P*<0.05 and ~ represents parameters that remained in the minimum adequate model but were not statistically significant.

Dependent Variables	Treatment	Density	Sex	Treatment*Density	Density*Sex	Treatment*Sex	Density*Treatment*Sex
Pupal mass	~	x	x	x			
Larval Development	x	x					
Adult Development		x	x				
Adult Lifespan	x						
Total Development	x	x	x				
Total Lifespan	x	~	~	x	x		
Spores per µl	x	~	~	~	~	~	~
Mean Wing Density	~	~	x	~			
% Black on Wing	~	~	x	~	~	~	~
Wing Area	~	x	x	~	~	~	~

Table 3. Correlations between dependent variables. Pearson's correlations between all the measured data two-tailed significance. *n=111 to 124* for all traits. **P*<0.05; ***P*<0.01

	Larval Development	Adult Development	Adult Lifespan	Total Development	Total Life span	Spore Load	Wing Density	% Black on Wing	Wing Area
Pupal Mass	**-0.1697***	**0.2392****	**0.1933***	0.0582	**0.2128***	-0.0774	**0.3866****	**-.04385****	**0.6337****
Larval Development		**-0.5569****	-0.0864	**0.5479****	0.1670	0.1183	0.0027	-0.0323	-0.1000
Adult Development			-0.0591	**0.3660****	0.1353	0.0320	**0.2596****	**-0.2965****	0.0586
Adult Lifespan				**-0.1876***	**0.8512****	**-0.7238****	-0.0679	0.0515	**0.4615****
Total Development					**0.3202****	0.1711	**0.2394****	**-0.3213****	-0.0156
Total Lifespan						**-0.6204****	0.0777	-0.1159	**0.3863****
Spore Load							-0.0450	-0.0571	-0.1631
Wing Density								**-0.8536****	0.1434
% Black on Wing									**-0.2316***

Conclusion

- Density significantly affects on all morphometric measurements (except wing color) and all development times.
- Treatment significantly affected larval and total development, adult and total lifespan and spore load.
- Sex had an effect on the all morphometric measures, adult development and total development.
- Treatment by density significantly affected pupal mass and total lifespan.
- Density by sex significantly affected total lifespan.

Our results do not conform to either the density dependent prophylaxis (DDP) or the crowding hypotheses. However, there are components that independently support both hypotheses specifically:

- Spore load fits well with the idea that crowding increases stress, because those in the medium and higher densities were found to have higher spore loads
- Survival rate for the uninfected and infected males fits the DDP hypothesis because total lifespan increased for the above groups

Significance

• Gain a better understanding of the relationship between infectious disease and immune defense to parasitic infection.

• Destruction of milkweed due to urban sprawl forcing monarch to find the same milkweed clusters, thereby increasing larval population densities.

• Global warming creates a temperature increase allowing milkweed to grow in different regions, thereby altering the geographical range and density of monarch populations.

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