*Sophia Experiments II*

Iron Tolerance in Blood-Feeding vs. Obligate Non-Biting Populations

of the Pitcher-Plant Mosquito, *Wyeomyia smithii*

Plain English Objective: To determine concentrations of iron that are toxic to mosquitoes for potential use in sugar-baited traps to attract and kill adult mosquitoes.

Specific Objective: To determine adult survivorship and fecundity of *Wyeomyia smithii* when their carbohydrate source (raisins) are spiked with increasing concentrations of ferrous iron (FeSO4).

Who cares? Mosquitoes have been called the Earth’s most dangerous animal due to their ablity to transmit some of the most heinous diseases of humans, livestock, and wildlife. Adult mosquitoes are attracted to sugar-baits that can be laced with toxic pesticides. Iron is a much more-environmentally friendly molecule than toxic pesticides. If successful, my studies can lead to iron-containing sugar baits that may reduce mosquito populations by killing or debilitating males and/or females while having minimal impact on the environment.

**Abstract**

Only adult female mosquitoes consume vertebrate blood (bite), thereby obtaining nutrients for egg production. At the same time, the blood meal contains toxic levels of iron, which may be rapidly excreted by blood-engorged females. Males do not bite, but if provided blood in liquid form, some species have been shown to be highly susceptible to iron toxicity. Both adult male and female mosquitoes rely on carbohydrates for adult nutrition and are attracted to sugar-baited traps. For laboratory populations of the pitcher-plant mosquito, *Wyeomyia smithii*, carbohydrate is provided in the form of pesticide-free (organic) raisons. Raisins (carbohydrate source) saturated with increasing levels of iron in the form of ferrous sulfate (FeSO4) will be used to determine potential iron toxicity in adult *W. smithii* and whether toxic thresholds vary between males and females and between biting and obligate non-biting populations.

**Background**

When blood is consumed by mosquitoes, the hemoglobin in the red blood cells is broken down, releasing first toxic levels of heme and ultimately, potentially toxic levels of heme-bound iron. The iron is rapidly excreted, females retaining only about 13% for their own metabolism and 13% for provisioning their eggs. In blood-feeding females, the anterior of the mid-gut (proventriculus) secretes a net-like peritrophic matrix that thickens after a blood meal and binds heme, a reactive oxygen species that interferes with normal cell oxidation-reduction reactions and damages DNA. Heme is a complex ring compound with an iron atom at its core. Upon degradation of the heme, potentially toxic levels of iron are released. Larvae and male *Aedes aegypti* have a less well developed peritrophic matrix and males that do not bite but are fed liquid blood are more susceptible to iron toxicity than females (XXX). Herein, the experiments with *Ae. aegypti* are replicated with the pitcher-plant mosquito, *Wyeomyia smithii*, with the added ability to determine whether iron tolerance is greater in blood-feeding that obligate non-biting populations.

*Wyeomyia smithii* completes its entire pre-adult life cycle only within the water-filled leaves of the purple pitcher plant, Sarracenia purpurea from the Gulf of Mexico to northern Canada. All populations of *W. smithii* are fully inter-fertile and produce one batch of eggs without biting. Southern populations require a blood meal in order to produce a second and any subsequent batches of eggs. Northern populations are obligate non-biters and produce multiple batches of eggs without ever seeking or taking a blood meal. At high concentrations, iron is toxic to larval *W. smithii*, with southern (biting) populations being more iron tolerant than northern (non-biting) populations, but there is no difference in iron tolerance between male and female larvae (Rasmussen, 2019). Herein, I extend these prior studies to ask whether adults, which are the potentially blood-consuming stage of the life cycle, also vary in iron tolerance among populations and, unlike larvae, vary between non-biting males and blood-females.

**Materials and Methods**

1. Obtain a crate each of two southern biting (LI, WI) and northern non-biting (ML, KC) and rear them to the F2 generation to minimize latent effects of long-term storage in diapause.
2. Set up 12 adult cages, three for each population, labeled XX0, XX1, XX2, where XX stands for the population acronym.
3. Make up ferrous sulfate solutions in 3L aliquots and store in refrigerator
4. 0 FeSO4 = 3L of distilled water = 0mg/l iron (control)
5. 6 Nature Made 325mg tables (= 65mg Iron) in 3L distilled water = 130mg/L iron
6. 300ml of above in 2.7L distilled water = 13mg/L iron
7. Rear 250 F2 from each population for each treatment (XX0, XX1, XX2) to adults, placing pupae in the respective pupal dish, along with a cut leaf, in each adult cage.
8. Soak 5 well-dried raisins in each solution the FMW before a MWF check of the cages
9. Starting on the first adult eclosion, record the date of adult eclosion and sex of all eclosing adults.
10. Add the 5 pre-soaked raisins to the top of each cage and change every MWF
11. Remove dead adults and record their date of death and sex
12. Remove, count, and record number of eggs; save eggs on water
13. After 5 days, count and record number of hatch
14. Continue until all larvae have metamorphosed, all adults are dead, and last eggs have hatched.
15. For each treatment and each population, calculate
16. Mean date of eclosion for males and females
17. Mean date of death for males and females
18. Mean adult longevity = B-A for males and females
19. Perform nested ANOVA

S of V df

[Fe] 2

B-nB(Fe) 3

Sex(B-nB) 6

Residual 12

Total 23

1. For each treatment and each population calculate:
2. Eggs per eclosed female
3. Hatch per eclosed female
4. Arcsin % of eggs hatching
5. Then perform nested ANOVA for A-C: [Fe], Biting vs non-Biting (Fe)

S of V df

[Fe] 2

B-nB(Fe) 3

Residual 6

Total 11

**Implications**

1. No significant effect of iron concentration on survivorship means adults are more tolerant of iron than larvae
2. No significant effect of sex on survivorship means that females are not more iron tolerant than males
3. No significant effect of iron concentration on Biting vs. non-Biting populations means that populations with the potential to blood feed are not more iron tolerant than populations that never bite – implies evolutionary (genetic) inertia of iron tolerance
4. No significant effect of iron concentration on fecundity (eggs per eclosed female), offpring production (hatch per eclosed female), or embryonic viability (% hatch) indicates that reproductive potential is not affected by environmental iron,
5. If 1-4 are true, iron added to sugar-baited traps has little potential for mosquito population control.
6. If any, some, or all of 1-4 are not true, then iron added to sugar-baited traps has potential for mosquito population control, depending on the specific magnitude of effects in 1-4,

See new relevant article: Ferrero et al. 2017. Assessment of the effects of orally administered ferrous sulfate on Oncopeltus fasciatus (Heteroptera: Lygaeidae). *Environ. Sci. Pollut. Res*. **24**:8551-8561.