Mimicry Complexes and Host Plant Species of Seed Bugs on Randall's Island

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Introduction

- This project investigates the relationships between Batesian and Müllerian mimicry in three species from the family Lygaeidae, the seed bugs, sampled in the Native Plant Garden of Randall's Island.
- The species studied include *Lygaeus turcicus*, the false milkweed bug, *Lygaeus kalmii*, the small milkweed bug, and *Oncopeltus fasciatus*, the large milkweed bug.
- Batesian mimicry is a system where a nonpoisonous organism (the mimic) takes on the phenotypic and behavioral signaling of a poisonous or unpalatable one (the model) in order to gain protection by taking advantage of a predator's learned avoidance of the model.
- In Müllerian mimicry, poisonous organisms evolve to have similar physical characteristics, so predators that have previously learned to avoid one avoid the other (Hlaváček et. al 2022).
- In the wild, large milkweed bugs only eat milkweed, which is toxic. Small milkweed bugs eat milkweed too, but they can also consume other plants and even some insects (Conservation Commission of Missouri 2024).
- False milkweed bugs eat the seeds from the family Asteraceae (Ozark Bill, 2015).
- Milkweed sap contains cardenolides, a steroid that inhibits cell functions. Large and small milkweed bugs have a mutation that prevents the cardenolides from working. False milkweed bugs do not have this mutation.
- Due to their aposematism, *O. fasciatus* and *L. kalmii* are examples of Müllerian mimics. On the other hand, Batesian mimicry operates between *L. kalmii* and *L. turcicus* because the latter is not poisonous.
- We explored the relationship between host plant and bug species, and the dynamics of Batesian and Müllerian mimicry in Native Plant Garden.
- We hypothesize that the population of *L. kalmii* will be larger than that of *L. turcicus* because Batesian model systems function best when the model has a greater population than the mimic (Pfennig and Mullen, 2010).
- Because each milkweed bug has a different diet, we expect to find a different number of each species on each host plant. We predict that *L. kalmii* and *O. fasciatus* will be found primarily on milkweed plants, while *L. turcicus* will be found on other, non-poisonous plants in the family Asteraceae.
- This is because the Müllerian mimics can only gain their protection from this poisonous plant, and the Batesian mimic cannot eat it.

Materials and Methods

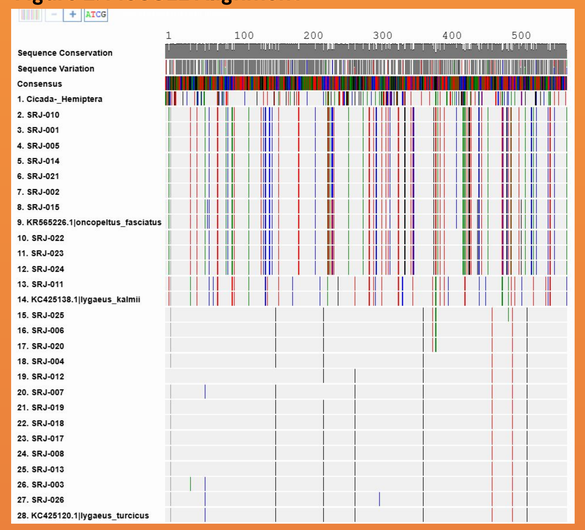
1. Thirty **samples were collected** along the designated path in all three sampling locations, one every ten paces.
2. The **suspected species of the insect was recorded** when possible using key phenotypic differences.
3. **DNA was successfully isolated** from twenty-four specimens using the silica method.
4. **DNA from the twenty-four specimens was amplified** using Ready to Go PCR Beads and the COI Ant primer.
5. **PCR products were analyzed** using gel electrophoresis, and twenty-four samples yielded bands.
6. These samples were then sent out for **sequencing**.
7. The sequence samples were uploaded to **DNA Subway**, trimmed, and analyzed using the Nucleotide Basic Local Alignment Search Tool (BLASTN) and MUSCLE.

Table 1: BLAST Data

Sample Number	Species	Alignment length	Bit score	Mismatches
SRJ-001	*Oncopeltus fasciatus*	644	1158	1
SRJ-002	*Oncopeltus fasciatus*	648	1169	0
SRJ-003	*Lygaeus turcicus*	626	1125	1
SRJ-004	*Lygaeus turcicus*	622	1113	2
SRJ-005	*Oncopeltus fasciatus*	654	1176	1
SRJ-006	*Lygaeus turcicus*	627	1113	4
SRJ-007	*Lygaeus turcicus*	634	1140	1
SRJ-008	*Lygaeus turcicus*	622	1118	1
SRJ-010	*Oncopeltus fasciatus*	644	1158	1
SRJ-011	*Lygaeus kalmii*	649	1167	1
SRJ-012	*Lygaeus turcicus*	622	1105	2
SRJ-013	*Lygaeus turcicus*	625	1123	1
SRJ-014	*Oncopeltus fasciatus*	651	1170	1
SRJ-015	*Oncopeltus fasciatus*	653	1178	0
SRJ-017	*Lygaeus turcicus*	625	1123	1
SRJ-018	*Lygaeus turcicus*	627	1127	1
SRJ-019	*Lygaeus turcicus*	623	1120	1
SRJ-020	*Lygaeus turcicus*	626	1112	4
SRJ-021	*Oncopeltus fasciatus*	649	1171	0
SRJ-022	*Oncopeltus fasciatus*	649	1158	3
SRJ-023	*Oncopeltus fasciatus*	647	1154	3
SRJ-024	*Oncopeltus fasciatus*	652	1163	3
SRJ-025	*Lygaeus turcicus*	625	1105	5
SRJ-026	*Lygaeus turcicus*	625	1123	1

The table displays the results of the DNA sequencing, assigning a species, alignment length, bit score, and number of mismatches to each sample that was processed.

Figure 2: MUSCLE Alignment



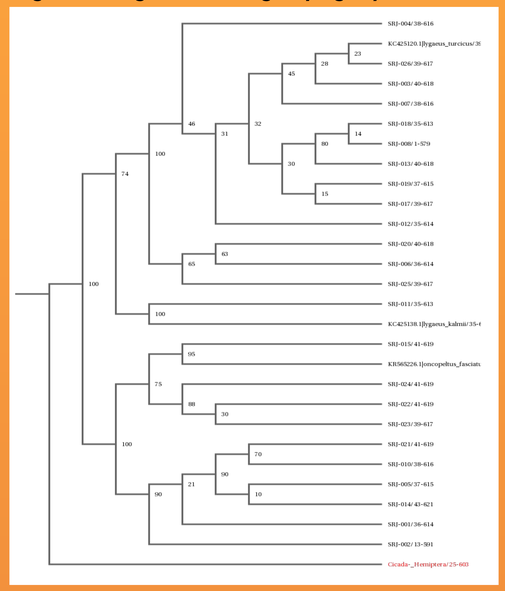
MUSCLE Alignment for 24 sequenced samples, including three reference sequences (one for each identified species) and a cicada outgroup.

Lygaeus turcicus

Oncopeltus fasciatus

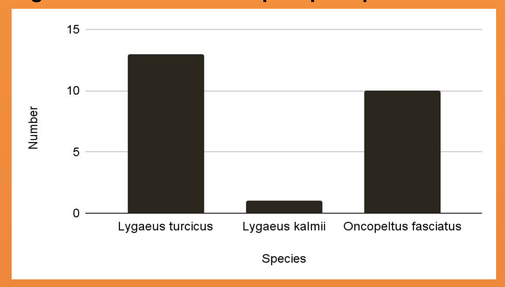
Lygaeus kalmii

Figure 1: Neighbor Joining Phylogeny



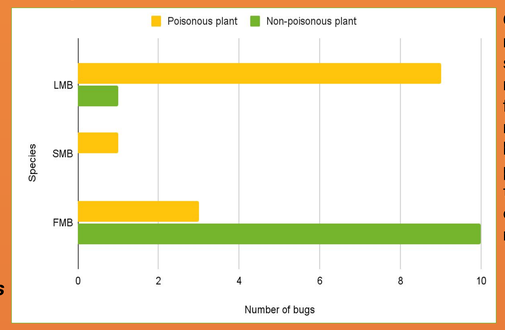
Neighbor joining phylogenetic tree for 24 sequenced samples, plus one reference sequence for each of three identified species. The cicada sequence is the outgroup.

Figure 3: Number of Samples per Species



Total number of *O. fasciatus, L. turcicus,* and *L. kalmii* samples collected.

Figure 3: Number of Bugs on Poisonous vs. Non-poisonous Plants



Graph showing the number of large, small, and false milkweed bugs found on the non-poisonous heliopsis or other plants compared to the number of each found on the milkweed plant.

Figure 4: Gel Electrophoresis

Gel analysis of each sample compared to a ladder, with sequences around 700 base pairs

Discussion

- The neighbor joining phylogeny shows three distinct monophyletic clades of seed bugs. As expected, *L. turcicus* and *L. kalmii* are monophyletic, sharing a common lygaean ancestor. Meanwhile, *O. fasciatus* is less closely related to the other two species, as it is not in the same genus.
- The MUSCLE alignment reveals that *L. turcicus* is most similar to the consensus sequence. *O. fasciatus* differs the most from the consensus, mostly in different places than *L. turcicus*, and *L. kalmii* tends to vary from the consensus in the same places as *O. fasciatus*.
- The hypotheses about host plant species was correct, with 100% of the *L. kalmii* samples and 90% of the *O. fasciatus* samples having been collected from milkweed. 77% of the *L. turcicus* samples were found on non-milkweed plants.
- However, our population predictions were incorrect. The ratio between false and large milkweed bugs was 13:10, and only one *L. kalmii* specimen was collected.
- Since the model's population was so small, can this even be called Batesian mimicry? The mimic would gain no benefits from phenotypic similarities with the model, and the predation on the mimic would only further reduce the population of the model. This raises the question: what kind of mimicry is operating in this habitat, if the basic requirements of Batesian mimicry are not being met?
- Even though *L. kalmii* has a negligible population at the sampling site, the coevolution of the Batesian system transfers the traits and benefits of Müllerian mimicry from the Müllerian mimic to the Batesian one. In other words, *L. turcicus* benefits from sharing the aposematic coloring with *O. fasciatus* as a result of its mimicry of *L. kalmii*.
- Hlaváček et. al (2022) described this system as a Batesian-Müllerian mimicry ring.
- Among seed bugs it appears to be quite effective, as demonstrated by the large populations of each insect observed in the native plant garden of Randall's island.
- Due to the aposematic coloring shared by *O. fasciatus* and *L. kalmii*, and the visual characteristics mimicked by *L. turcicus*, common predators of seed bugs, like birds, avoid them.
- However, these mimicry dynamics have only been observed on one day, in one habitat on Randalls Island. To gain further support for this hypothesized relationship, we would have to sample multiple locations on multiple different days to see if the pattern remained consistent.

References

DNA Learning Center. "Using DNA Barcodes to Identify and Classify Living Things." Cold Spring Harbor Laboratory. October, 2018.

Duncan, Bill. "False Milkweed Bug." *Ozark Bill*, December 30, 2015. https://ozarkbill.com/2015/12/30/false-milkweed-bug/

Additional references on the back.

Acknowledgements

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