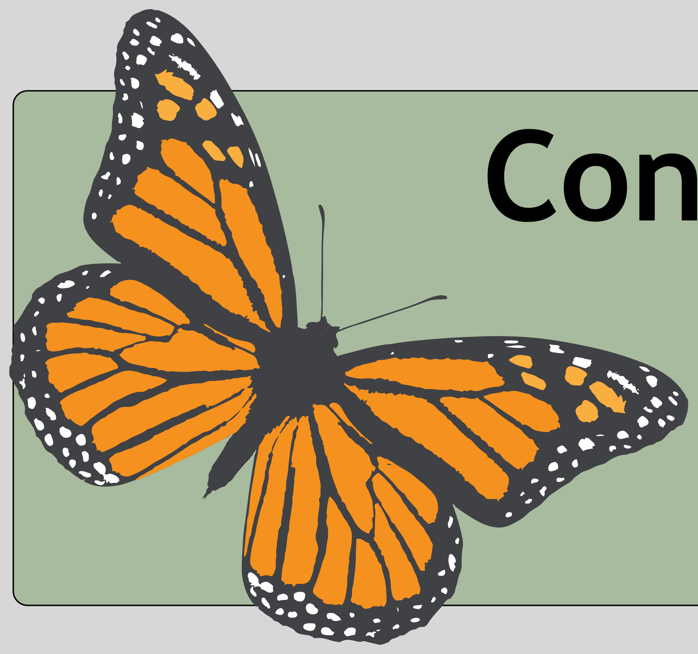

Contribution of bottom-up forces in the evolution of toxin resistance in milkweed butterflies

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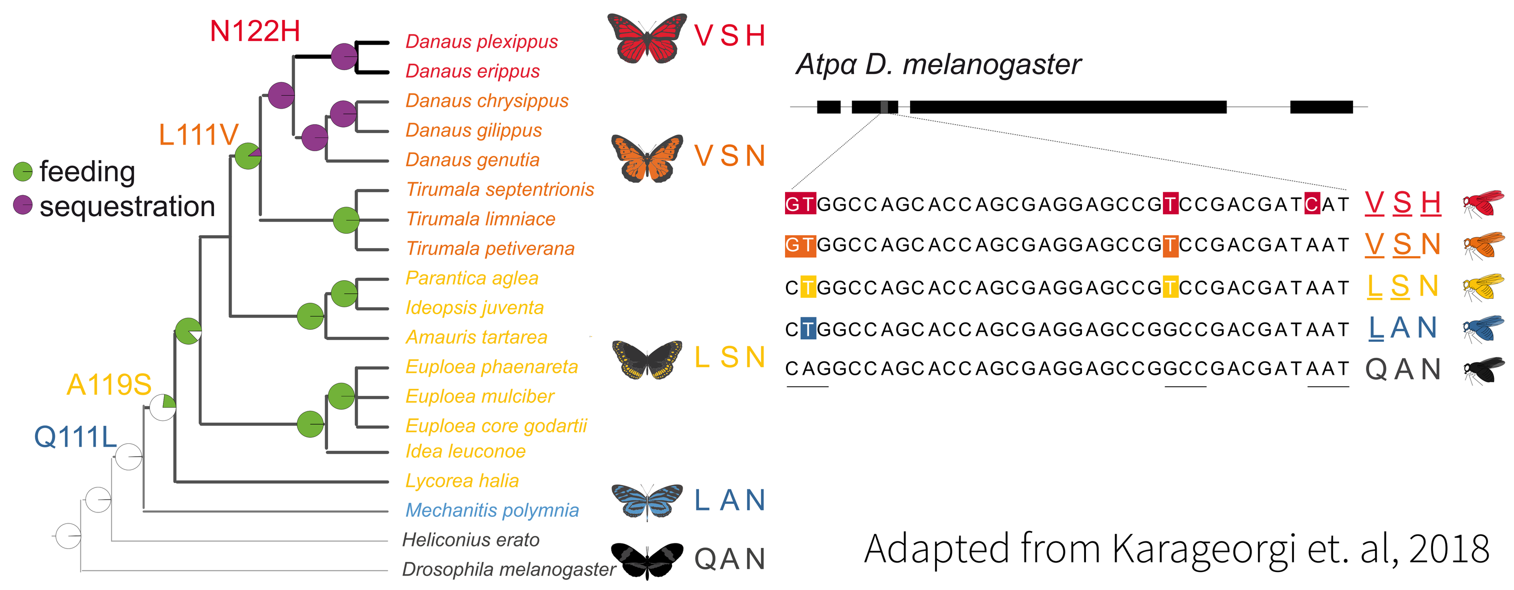
Abstract

A central question in evolutionary ecology is whether herbivore host plant specificity is driven by chemical coevolutionary arms races between plants and herbivores or by predators of the herbivores (Ehrlich and Raven, 1964; Bernays and Graham, 1988). Milkweed butterflies breed on a range of toxic milkweed host plants; some of them, including the monarch butterfly, also sequester toxic cardenolides present in milkweeds to avoid predation. In a stepwise manner, milkweed butterflies have evolved resistance substitutions in their sodium pump, the target of the cardenolides (Petschenka et al., 2012). Previously, it was shown that cardenolide resistance in the monarch butterfly is mainly linked to sequestration, and not the need to cope with toxic milkweeds (Petschenka and Agrawal, 2015). In the present study, we aim to characterize whether milkweed butterflies evolved resistance substitutions in their sodium pump primarily to feed on milkweeds (bottom-up selective force) or to sequester the cardenolides and avoid predators (top-down selective force). To address this question, we use CRISPR-engineered Drosophila flies, previously generated in our lab, that carry mutations in their sodium pump that confer different levels of cardenolide resistance "mimicking" the milkweed specialists (Karageorgi et al., 2019) (Figure 1). To test the hypothesis that the resistance mutations evolved for feeding on milkweed, we plan to perform feeding experiments with the CRISPR-engineered Drosophila flies and compare their performance (survival/growth) in media containing milkweed tissue with different cardenolide levels. To test the hypothesis that the resistance mutations evolved for sequestration, we plan to perform cardenolide (ouabain) injections in the hemolymph of CRISPR-engineered *Drosophila* flies and compare their survival. Our study will illuminate the role of bottom-up and top-down forces in the evolution of host specificity in milkweed butterflies.

In the present poster, we present the series of experiments we will conduct to characterize the bottom-up hypothesis.

Figure 1



Adapted from Karageorgi et. al, 2018

Question

• Do resistance substitutions in the sodium pump aid milkweed butterflies primarily in feeding on toxic milkweeds?

Strategy

• Compare performance (survival/growth) among *Drosophila* lines with different sets of resistance mutations (milkweed butterfly mimics) on media containing foliar milkweed tissue with different cardenolide makeups.

Methodology

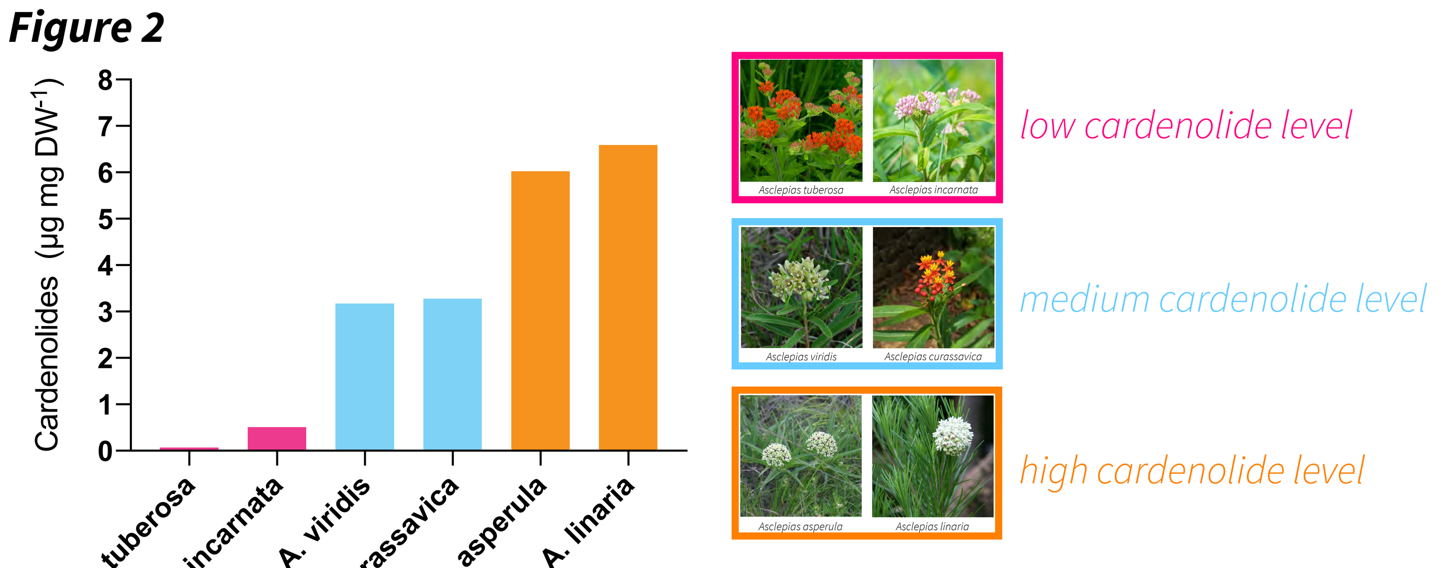
• Cultivate milkweed species that differ in their toxicity levels based on their cardenolide profiles (Figure 2).

• Use HPLC to analyze milkweed cardenolide profiles (in collaboration with Professor Agrawal).

• Perform feeding assays in which dried foliar milkweed tissue from each species is individually mixed with fly media (as in Karageorgi *et. al*, 2019).

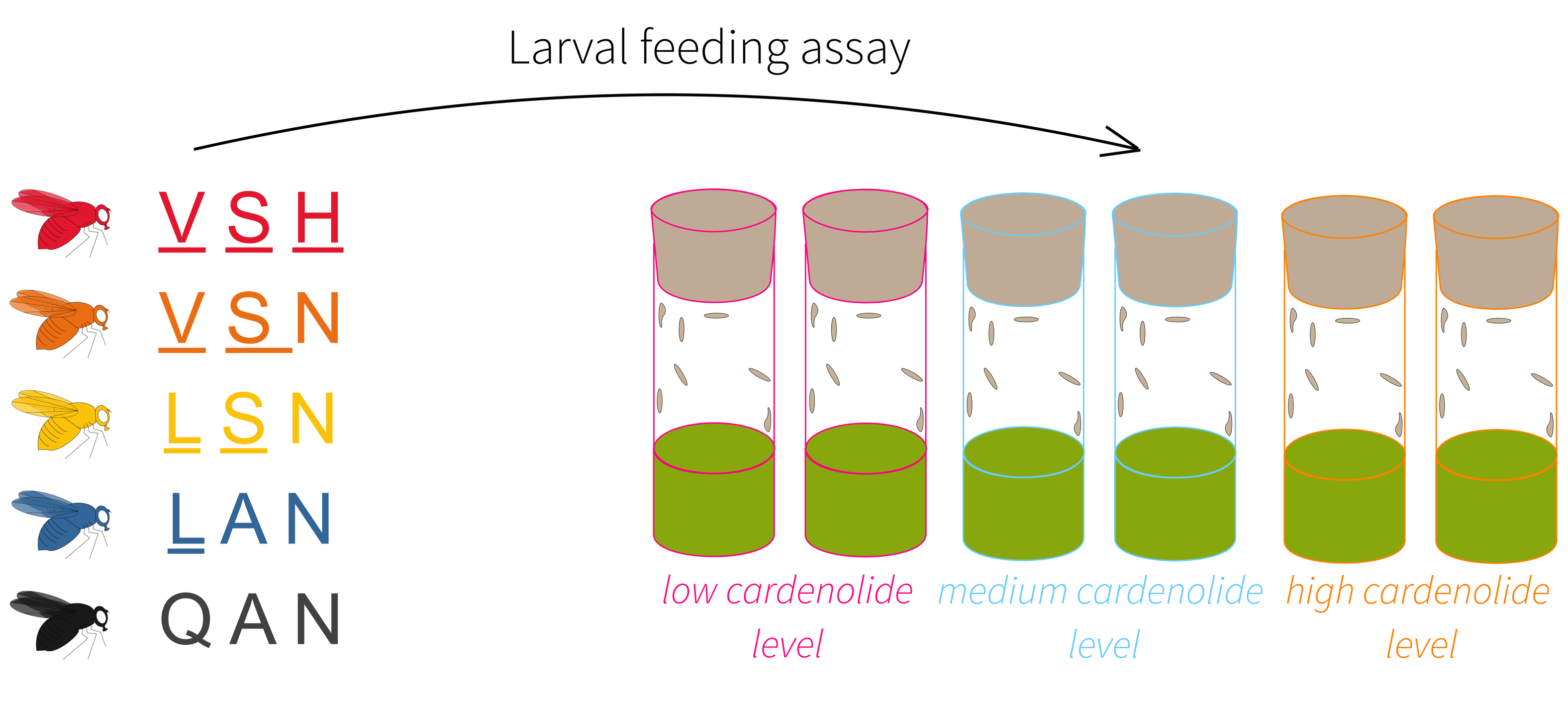
• Compare survival rates of *Drosophila* lines (QAN, LAN, LSN, VSN, VSH).

Figure 2



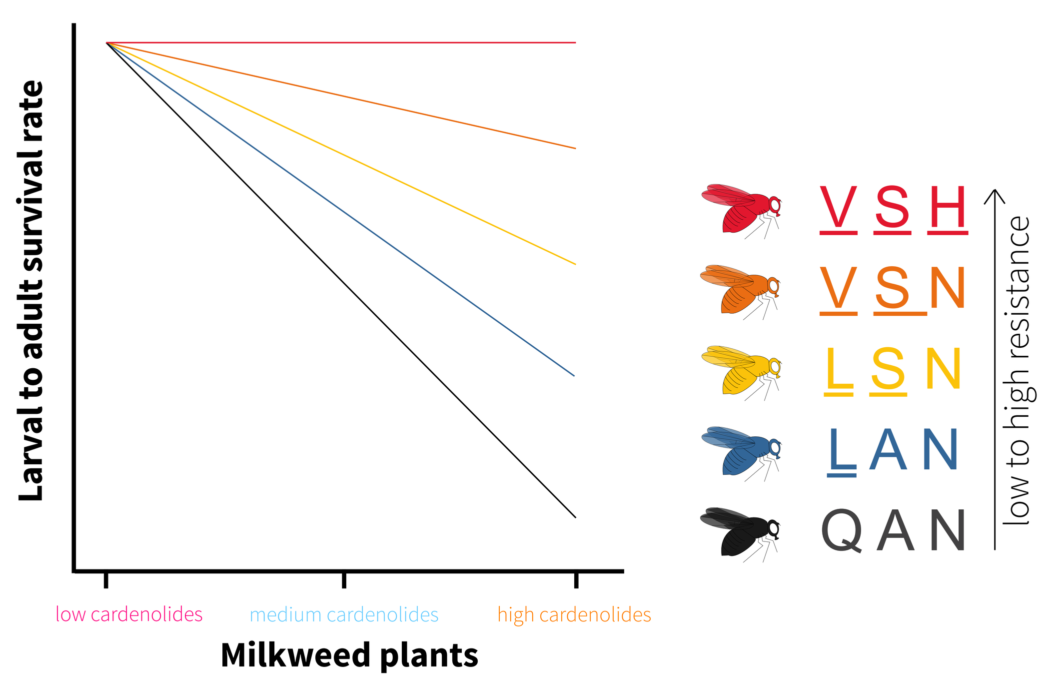
Adapted from Rasmann, 2011.

Expected Results



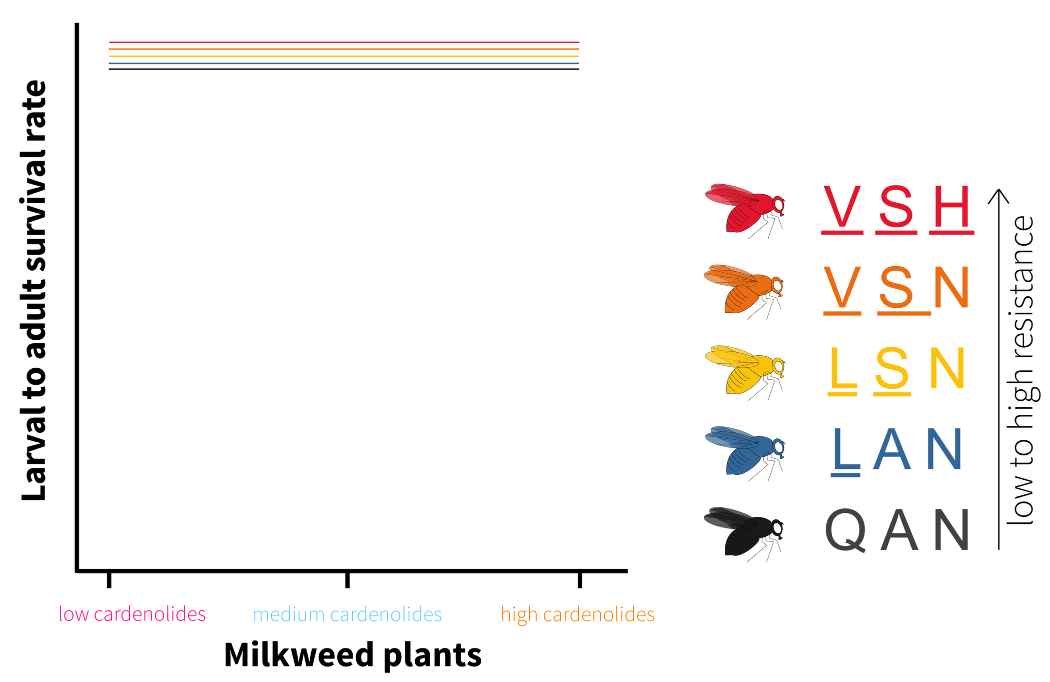
Bottom-up ✓

Resistance mutations aid feeding on toxic milkweeds



Bottom-up ×

Resistance mutations do not aid feeding on toxic milkweeds



Conclusions

The present set of experiments will allow us to characterize whether resistance substitutions in the sodium pump of milkweed butterflies primarily evolved to aid them in feeding on toxic milkweeds.