Determining monarch (*Danaus plexippus*) natal site distribution in Nevada using stable isotope analysis and wing morphometrics

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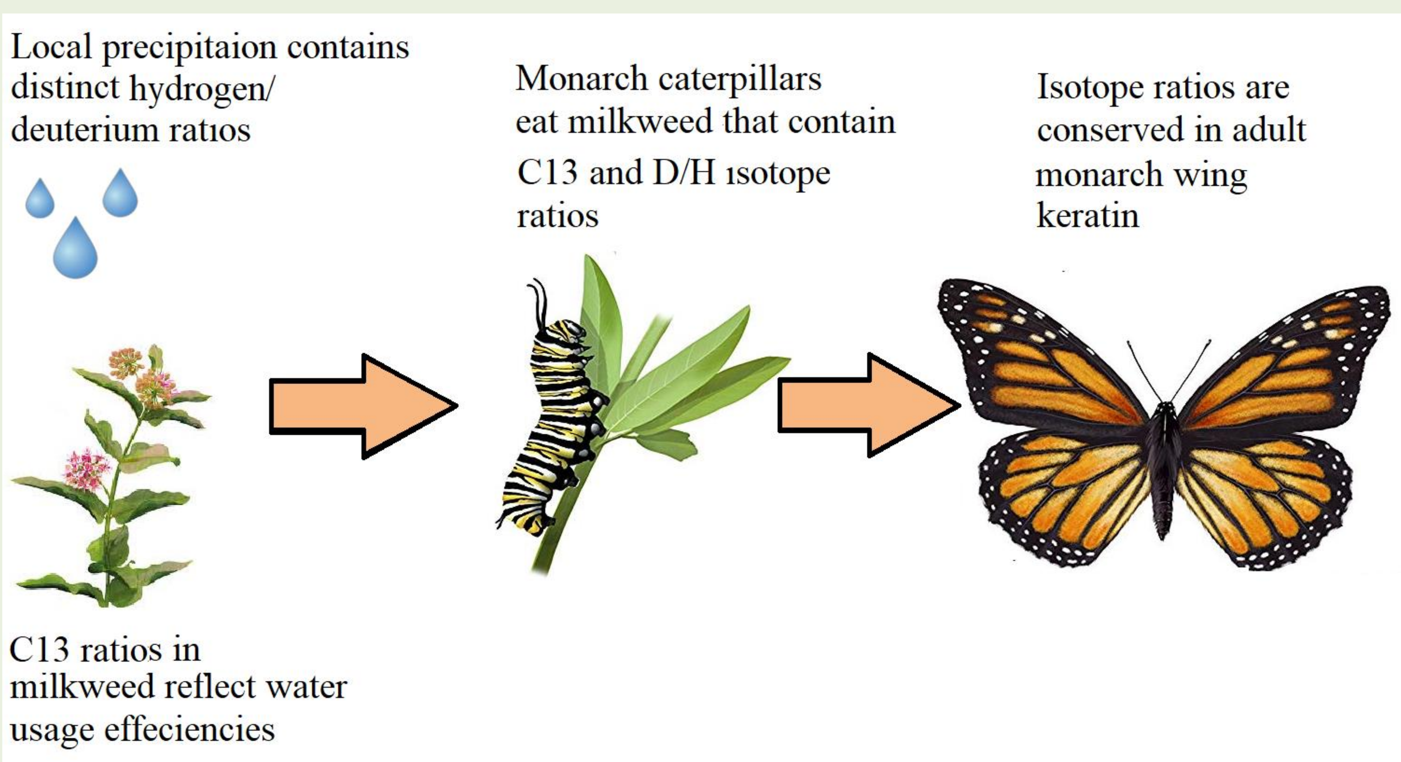
Introduction

Western monarch butterflies (*Danaus plexippus*) have significantly declined in the last 30 years but breeding locations in the Western United States are not well known. Within a natal site, some plants may be more preferred than others—knowing where monarchs successfully breed is critical for understanding monarch population biology and movement.

Hydrogen isotope (deuterium) ratios are produced by local precipitation, and carbon isotope ratios (δ13C) indicate plant water relations. Higher δ13C values reflect better water use efficiency. Plants incorporate these isotopes, and when eaten caterpillars eat plants, the isotopes are transmitted to adults.

Overwintering monarchs are predicted to have more elongated forewings and higher length:width ratios to support sustained gliding. Summer generations are predicted to have rounder wings, which should allow for greater maneuverability[2].



Objective: To investigate the natal sites and preferred food plants of monarchs and to test whether differences in plant isotopes predict monarch forewing shape.

Methods

Milkweeds

- 150 milkweed leaf samples were collected from 30 sites across a precipitation gradient in Nevada and Northern California from May through September.
- Leaf samples were freeze dried, then homogeneously ground.
- $\delta^{13}C$ isotope analysis was performed at UC Davis Stable Isotope Facility.

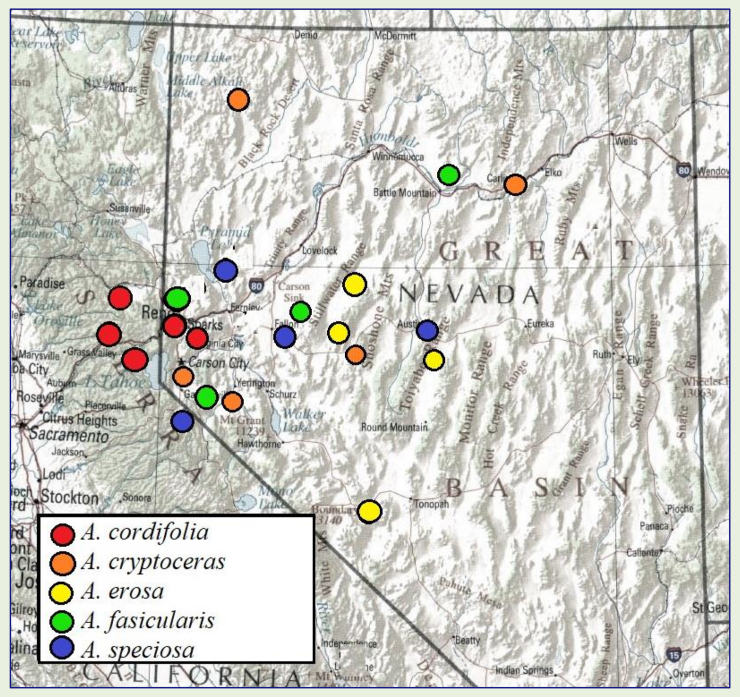


Fig 1. Milkweed Populations: Distribution of milkweeds sampled across Nevada and Northern California.

Methods

Monarchs

- A total of 16 adult monarchs were collected from across Nevada from July through December of 2018.
- Wings were removed, cleaned of surface oils using 2:1 chloroform:methanol, then analyzed for $\delta^{13}C$ and deuterium isotope ratios.
- Forewing morphometrics linked to flight capabilities were measured using a scanner and Image J.

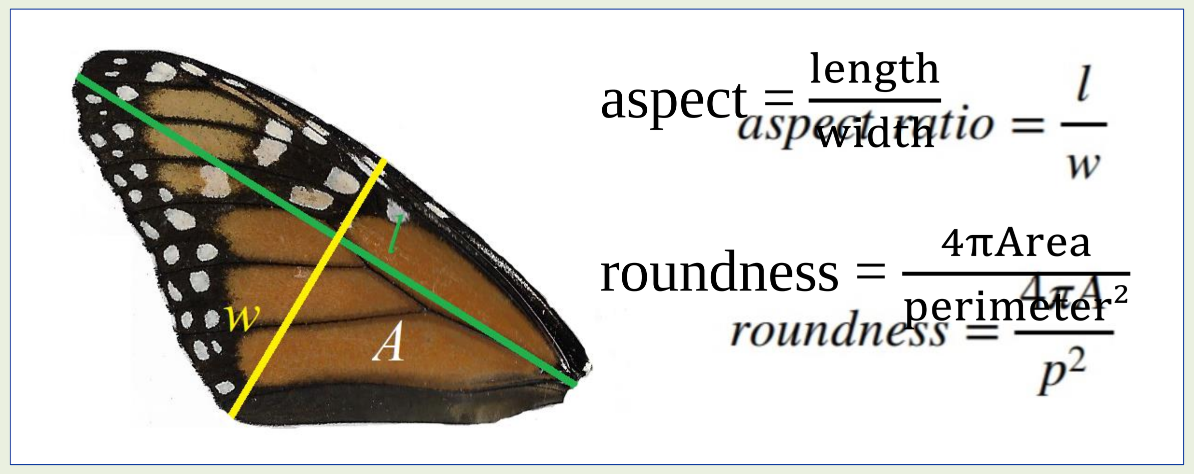


Fig 2. Wing shape: Forewing morphometrics included the length of the wing from apex to thorax, the width of the wing at the line perpendicular to the length, the aspect ratio of the wing and the overall roundness.

Results

Isotopes

- Deuterium concentration in wings decreased with increasing latitude. Wing deuterium also increased over the summer breeding season.
- Plant samples showed higher $\delta^{13}C$ values in drier sites for *A. speciosa* ($F_{1,38} = 5.72$, $P < 0.03$)
- Monarch $\delta^{13}C$ values were more consistent with $\delta^{13}C$ of plants that received irrigation (not shown)

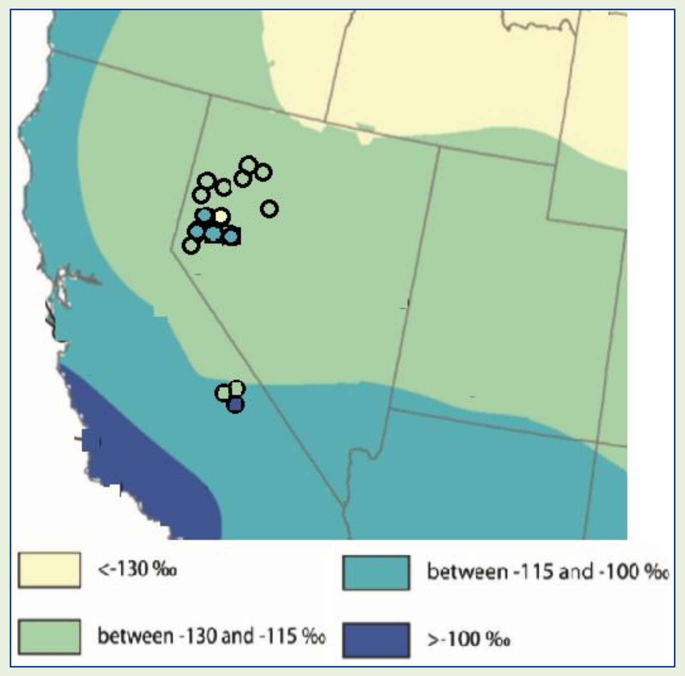


Fig 4. Map of monarch netting site and deuterium values: Location of monarch netting with color specific to the region the monarch originated from as suggested by deuterium values. Map adjusted from Yang *et al.* (2016).

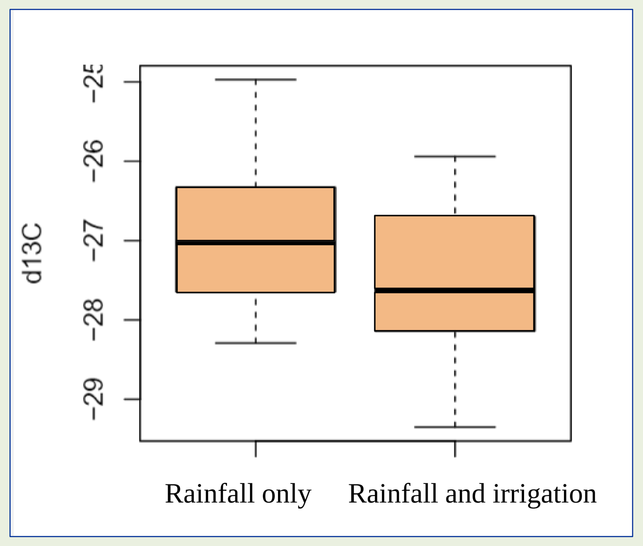


Fig 5. $\delta^{13}C$ values in agricultural populations: $\delta^{13}C$ isotopic ratios of in *A. speciosa* populations that receive only rainfall versus those supplemented by irrigation water.

Results

Morphometrics

- Forewing aspect (length:width) decreased over the summer breeding season ($X^2 = 51.8$, df = 8, $P < 0.03$). There were also marginal decreases in forewing area and roundness.
- For a given monarch body size, wing size increased from mid-summer to early fall, then decreased.

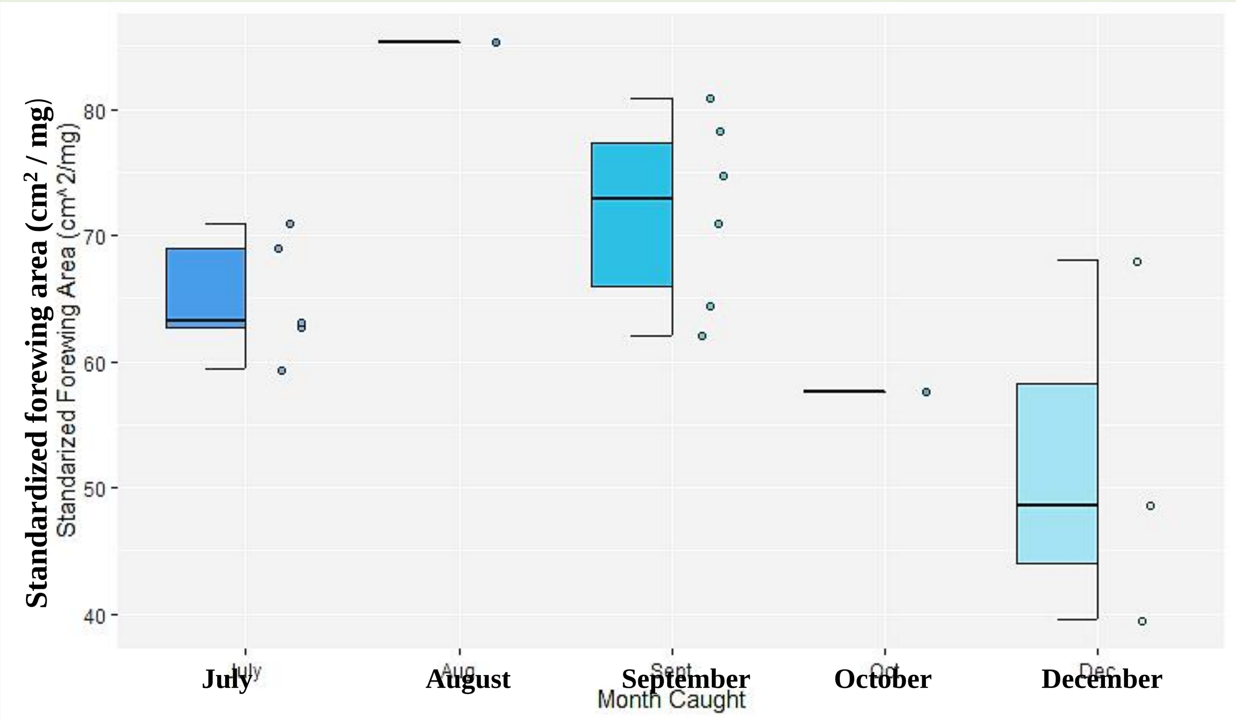


Fig 6. Forewing area by month caught: Graphs show monarch forewing area standardized by the butterfly's dry weight. Boxes show medians and quartiles. Points are individual monarchs.

- There was a marginal, negative relationship between forewing roundness and $\delta^{13}C$ when excluding one late-season outlier ($F_{1,13} = 2.67$, $P = 0.1$)

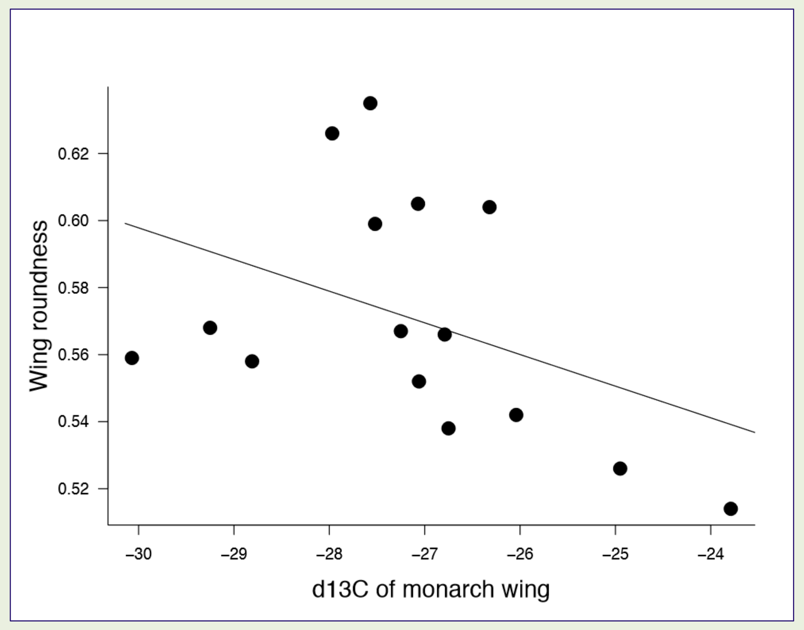


Fig 7. Monarch forewing roundness and C13 isotopes: Level of wing roundness as predicted by $\delta^{13}C$ of the wing in a linear model.

Conclusions

- Our results suggest that there are important monarch natal sites in Nevada.
- Milkweeds that receive only rainfall are more efficient at water usage and may produce monarchs more efficient at long-distance flight.
- $\delta^{13}C$ isotopes of monarchs are more consistent with plant samples that receive irrigation.
- Monarch wing shape changes over the breeding season and may depend on the water relations of the food plant.
- Understanding milkweed's role in monarch development and migration will be essential for studying this threatened species.

Acknowledgments

This research was supported by the Mead Undergraduate Research Award and the Nevada Undergraduate Research Award. Additional thanks to: Fabiane Mundim, the Reno Herbarium at UNR, and the Western Monarch Milkweed Mapper from the Xerces Society.

References

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