Phenotypic Variation in Adaptive Traits Among Western Populations of Showy Milkweed (*Asclepias speciose*)

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INTRODUCTION

- Losses of milkweeds in monarch butterfly (*Danaus plexippus*) breeding habitats and migratory corridors are hypothesized to be a chief contributor to widespread declines in monarch populations across North America (Pleasants and Oberhauser 2013, Flockhart et al. 2015).
- Successful habitat restoration requires germplasm that is adapted to target restoration environments.
- Genecology evaluates variation in putative traits related to survival among populations in relation to source environments and local selection pressures (St. Claire et al. 2013).
- Previous approaches suggest local seeds are best, but may not be available in suitable quantities, or may not be adapted to projected climate change scenarios (Kilkenny 2015).
- We evaluated phenotypic variation in growth form traits among 36 populations of showy milkweed (*Asclepias speciosa*) to evaluate genetically appropriate adaptive traits among local climates to aid in identifying seed sources for monarch habitat restoration.

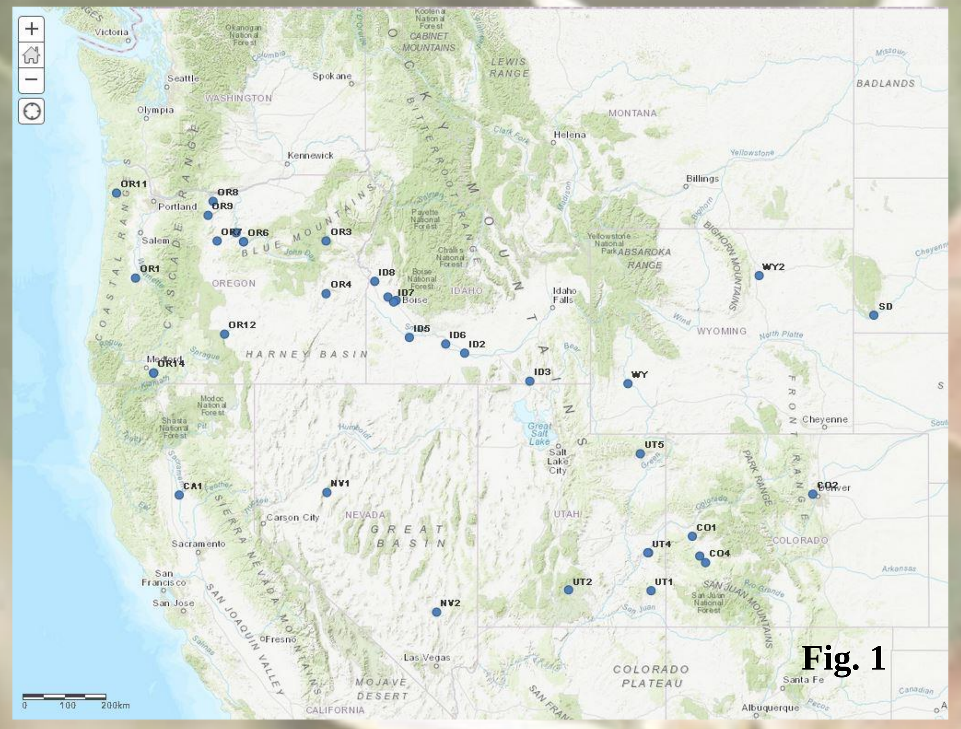
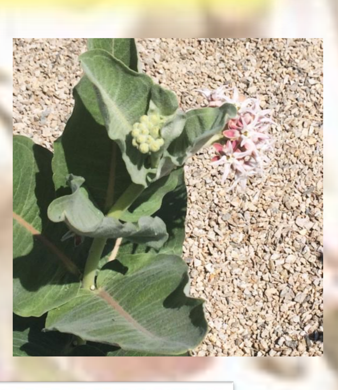


Fig. 1



METHODS

Plant Propagation and Transplanting

- Procured seeds from 36 populations (Fig. 1)
  - BLM, USFWS germplasm accessions
  - Citizen scientist collections
- Seeds were sowed in February in plug trays. Seedlings were transplanted to pots in April and transplanted in the common garden in late May.
  - 0.5 m between plants, 1 m between rows
- Prior to planting, the site was treated with herbicide, and covered in weed barrier and crushed gravel to reduce weed competition and heat loads.
- After planting, the garden received supplemental irrigation and was weeded on a weekly basis.
- Among the 360 experimental plants (ten per a population), we used 158 non-experimental units as buffer rows and columns, and to spaces in the experimental layout.

Data Collection

- Weekly collection of morphological measurements and plant growth data (Table 1).
- Remotely sensed climate data retrieved from ClimateWNA v5.30 (Wang et al. 2012).

Analysis

- Principal components analysis:
  - Conducted in program R version 3.5.1 using prcomp () function in package (stats)
- We used single factor ANOVAs to determine which measured traits significantly varied among populations.
  - Tukey's HSD was used to determine where the variance was among populations at a significance level of $\alpha = 0.05$.
- Relationships between collections site characteristics and measured traits were analyzed with a simple linear regression models (Table 1).

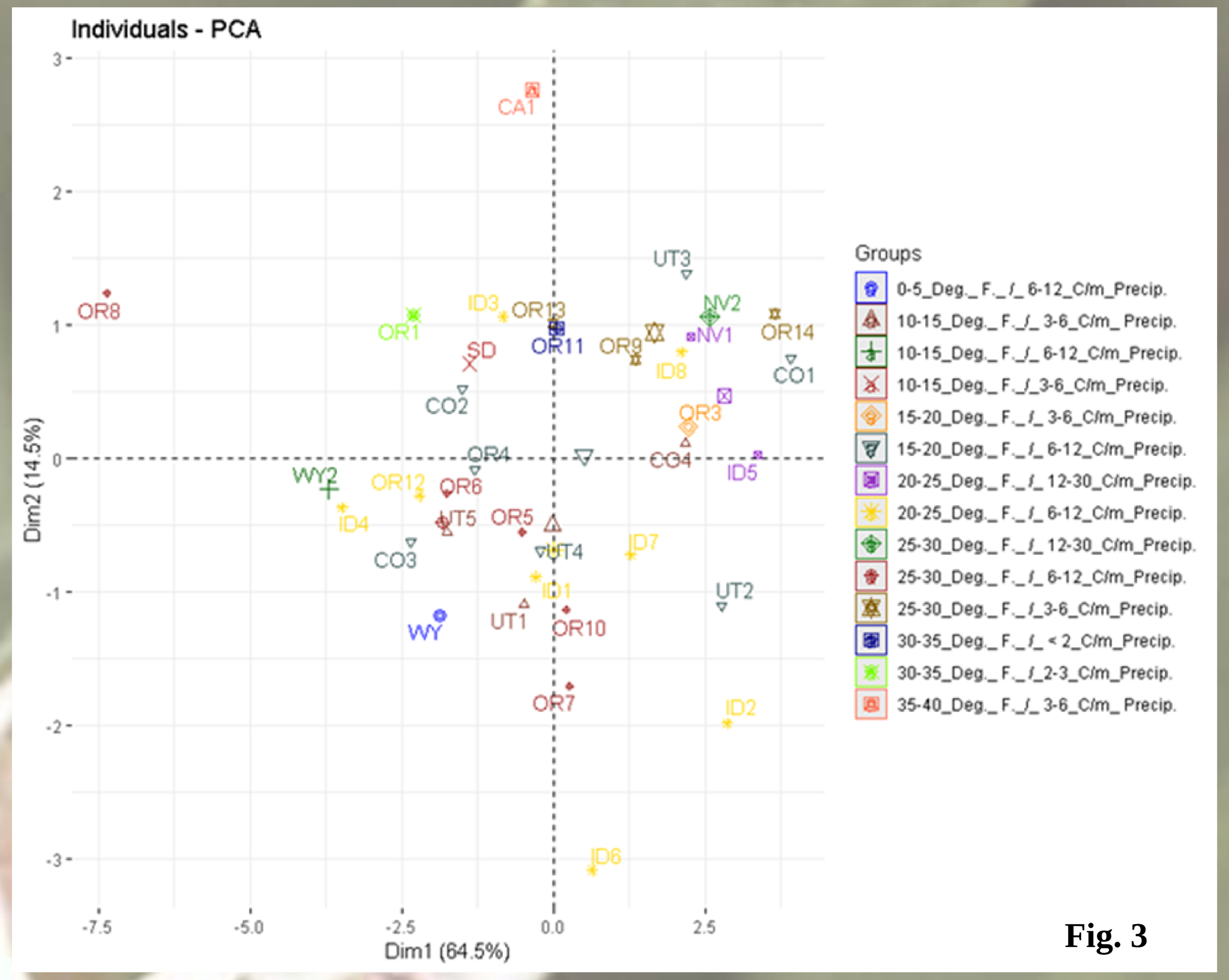


Fig. 3

Fig. 1- Locations of 36 populations of showy milkweed used in this study. **Fig. 2-** Principal components analysis separations among 36 populations of Showy Milkweed. **Fig. 3-** Biplot with principal components analysis separations with dimensional loading factors among 36 populations of Showy Milkweed. **Table 1-** Environmental variables and phenotypic characteristics used in analyses. **Fig. 4a-** Regression analysis of MWMT (°C) and plant height. **Fig. 4b-** Regression analysis of MAT (°C) and plant height. **Table 2-** Relative contributions of plant phenotypic characteristics (%) to top two principle components.

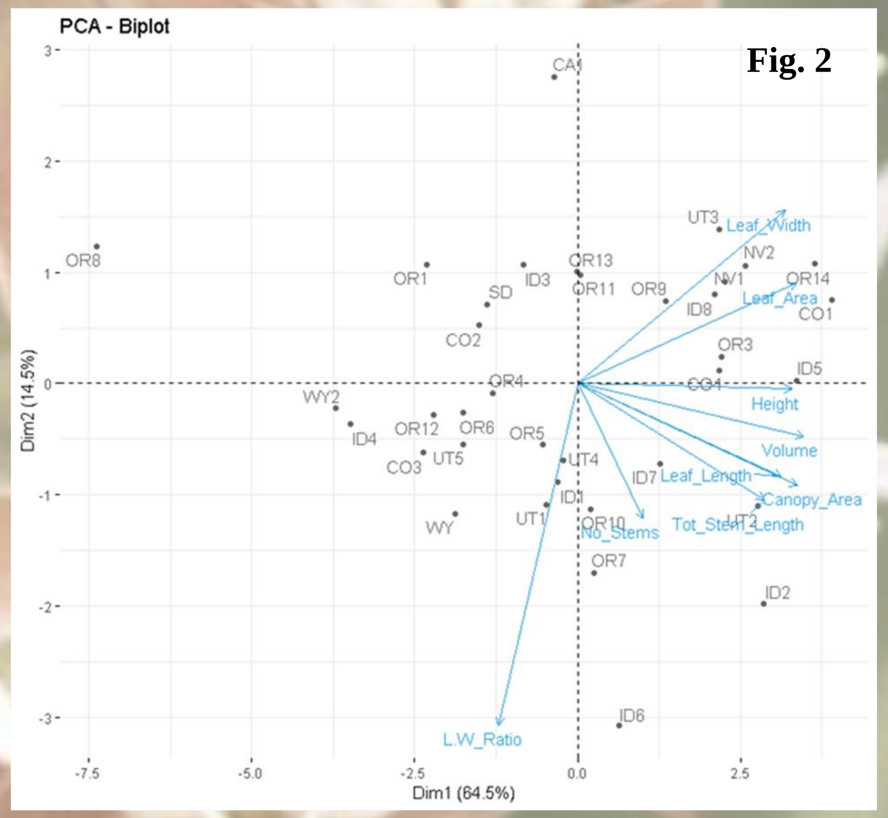


Fig. 2

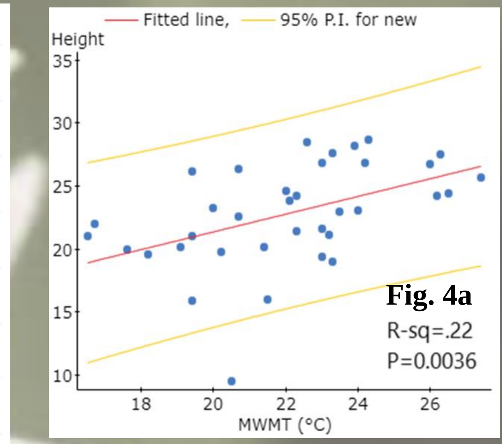


Fig. 4a

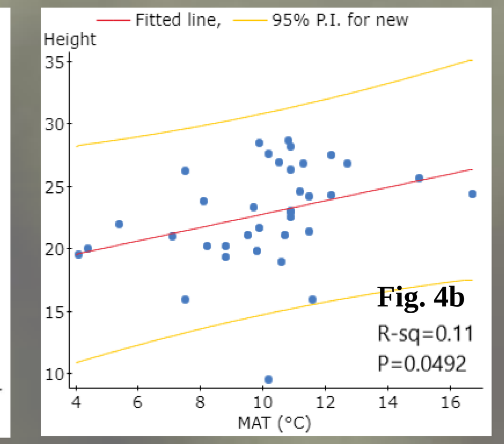
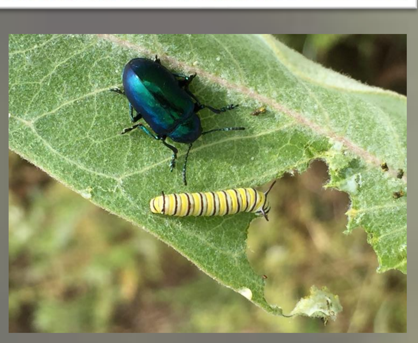


Fig. 4b

Table 1

Environmental Variables	Description
MAT (°C)	Mean average temperature
MWMT (°C)	Mean warmest month temperature
MSP (mm)	Mean annual summer (May to Sept.) precipitation
MAP (mm)	Mean annual precipitation
Elevation (m)	Site elevation
Phenotypic Characteristics	**Description**
Leaf Length (cm)	Length of the most mature leaf from petiole to terminal tip
Leaf Width (cm)	Widest point of the most mature leaf
Est. Leaf Area (cm^2)	Product of Leaf length x Leaf width
Leaf L/W Ratio	Quotient of Leaf length / Leaf width
Plant Height (cm)	Height measurement from the emergence from soil to the top of the plant
Canopy Area (cm^2)	Maximum distance of the canopy extent (cm) multiplied by the maximum canopy extent of perpendicular axis.
Volume (cm^3)	Plant Height multiplied by canopy area.
Total Stem Length (cm)	Sum length of the three longest stems
Number of stems	Total number of basal and axillary stems



RESULTS

- 79% of variation among populations is explained by two Principal Components (Fig. 2)
  - PC1 associated with larger and greater surface areas, volumes and growth traits
  - PC2 associated with lower LW ratio, reduced leaf width, and fewer stems.
- Mixed evidence that populations from common provisional seed zones (Bower et al. 2014) have similar traits (Fig. 3)
  - Some populations from common seed zones cross both PCs
    - 20-25 °C, 6-12 mm
    - 15-20°C, 6-12mm
  - Some populations cluster closely
    - 25-30°C, 6-12mm
    - 25-30°C, 3-6mm
    - 20-25°C, 12-30mm

Table 2

	Volume	No. of Stems	Total Stem Length	Height	Leaf Length	Leaf Width	LW Ratio	Leaf Area	Canopy Area
Dimension 1	15.8	1.3	10.8	14.2	12.8	13.3	1.9	14.8	15
Dimension 2	1.3	8.6	6.4	0.01	4.1	14.3	55.5	4.8	4.9

- ANOVAs revealed significant ($\alpha = 0.05$, df=35) effect of populations on all measured traits
  - Statistical differences driven mostly by 3-4 populations with significantly different growthforms.
    - OR8, OR1, WY2, CA1
- MWMT(°C) and MAT (°C) show significant positive relationships with plant height (Figs. 4a, 4b).

DISCUSSION

- Preliminary support that Showy milkweed exhibits genecological differences across western US.
- Patterns do not clearly correspond to provisional seed zones, suggesting that species specific seed zones should be constructed for showy milkweed.
- Predominate divisions among populations due to differences in growth form and leaf characters (Fig. 2).
- Regressions across individuals nested within populations (not shown) indicate that the bulk of variation in traits exists among individuals.
  - Phenotypic plasticity may play an important role in showy milkweed responses to annual weather conditions (Sultan 2000).
- Environmental maternal effects may also serve as a source of variation in evaluating trait responses between generations(Galloway 2005, Donohue 2009).
- Regression analyses indicated that plant height may be adaptive.
  - Higher MWMT(°C) and MAT (°C) correlated with increased height
  - Plant size often correlates to competitive ability (Westoby 1998).
  - Cool, wet spring in 2018 may be responsible for large divisions in growth form.
- Variation among principal components does not strongly correspond to provisional seed zones.
  - Closely clustered populations suggest some populations from common seed zones may have similar character traits (Fig. 3).

FUTURE WORK

- Multi-year study (≤ three years)
  - Incorporate measures of pubescence, above ground biomass, precise leaf surface area, surface area, emergence and flowering phenology.
  - Explore correlations with additional climate variables in ClimateWNA.
  - Examine population genetics.
  - Explore variations in cardenolide toxins with respect to ecotypes and stress.

ACKNOWLEDGEMENTS

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