

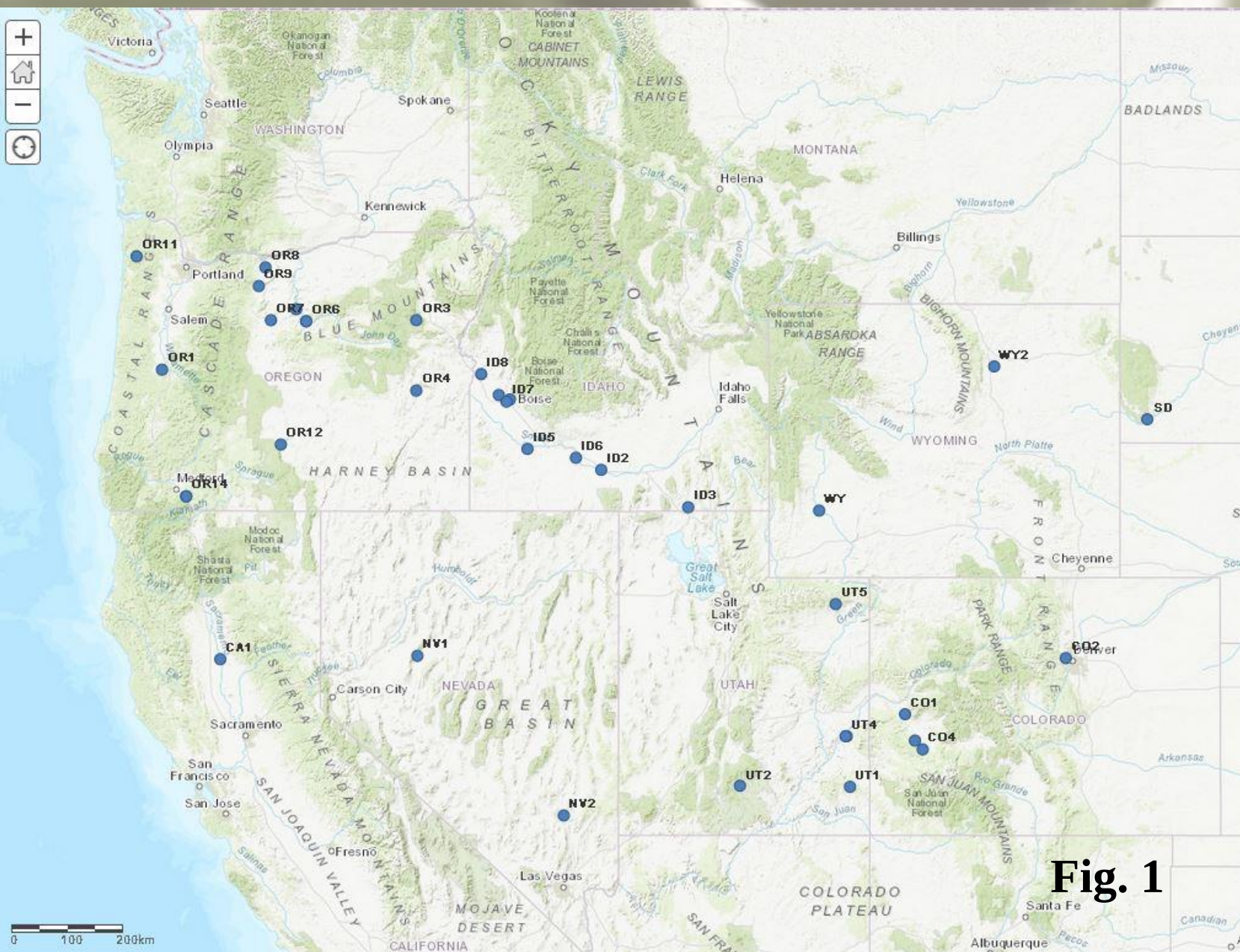
Phenotypic Variation in Adaptive Traits Among Western Populations of Showy Milkweed (*Asclepias speciosa*)

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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.



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).

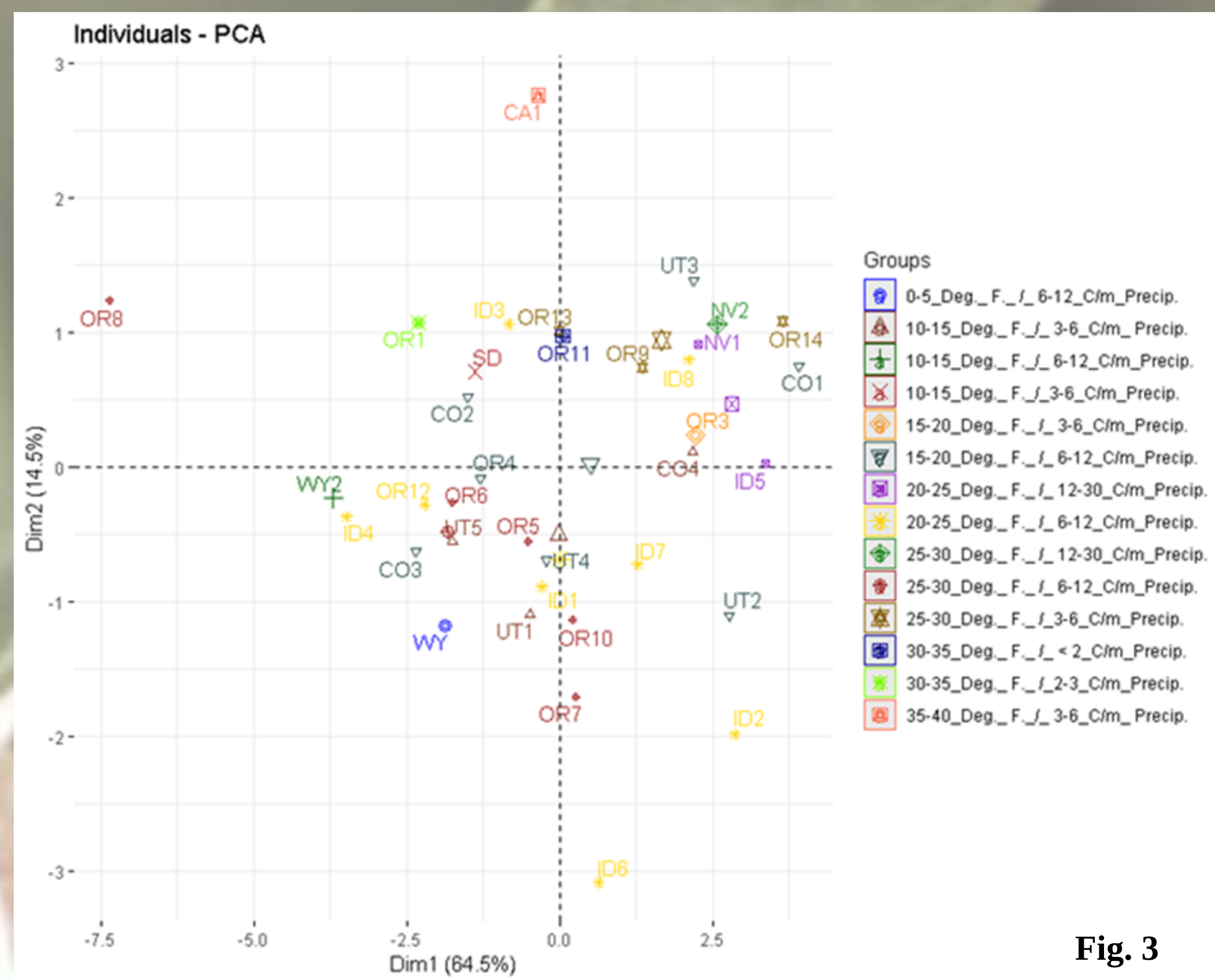


Fig. 3

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.

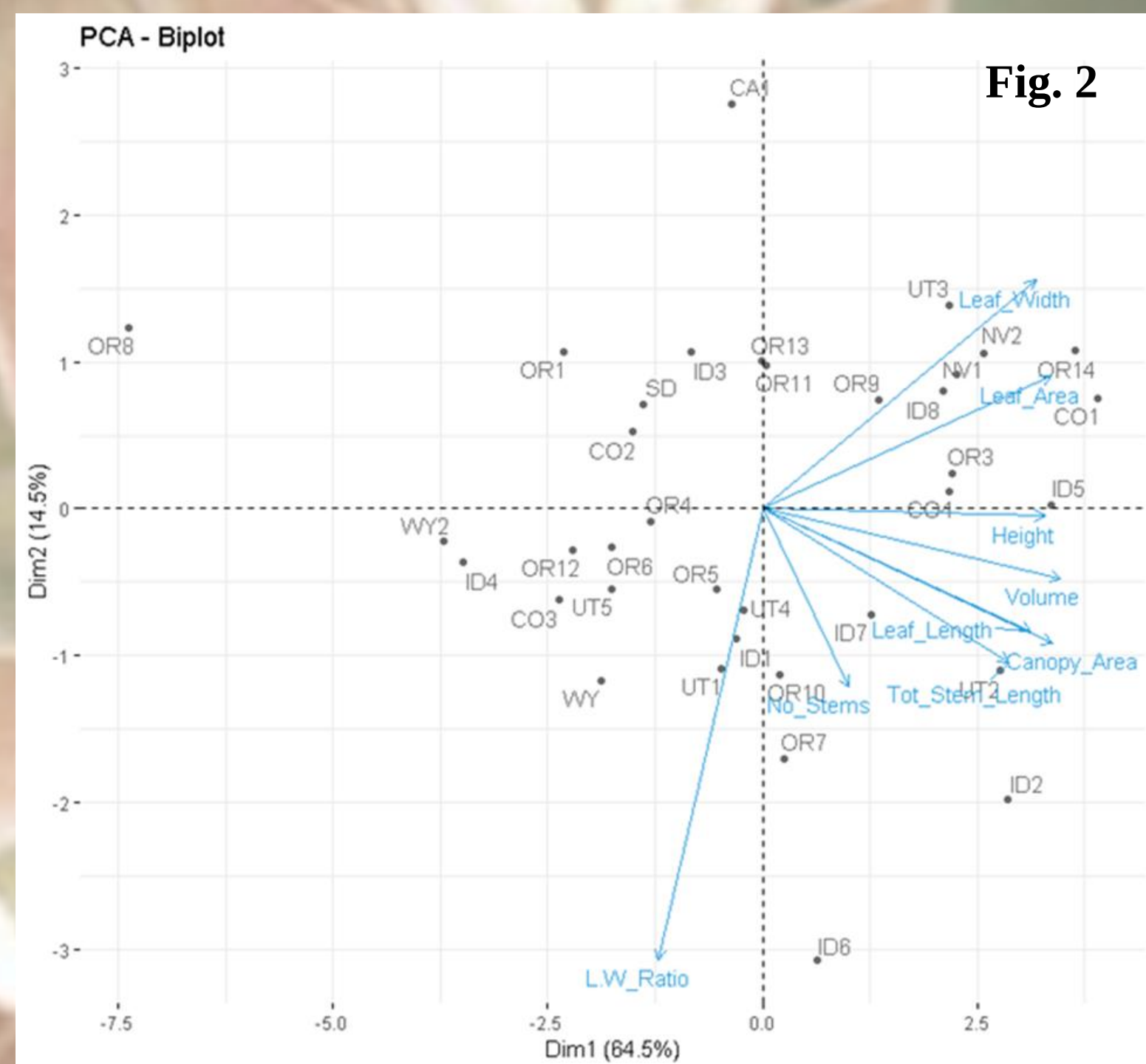


Fig. 2



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

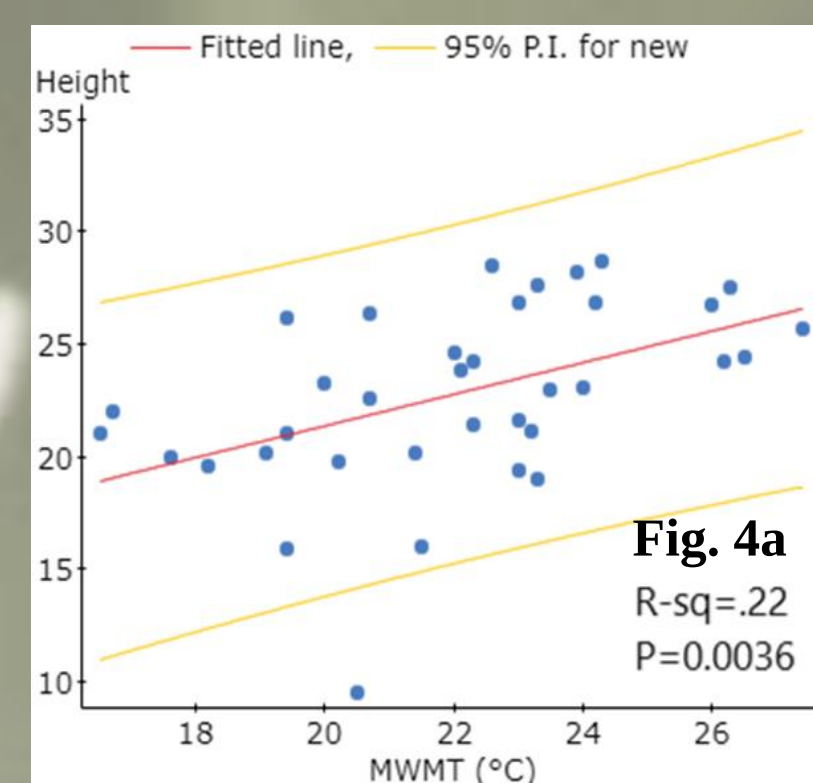


Fig. 4a

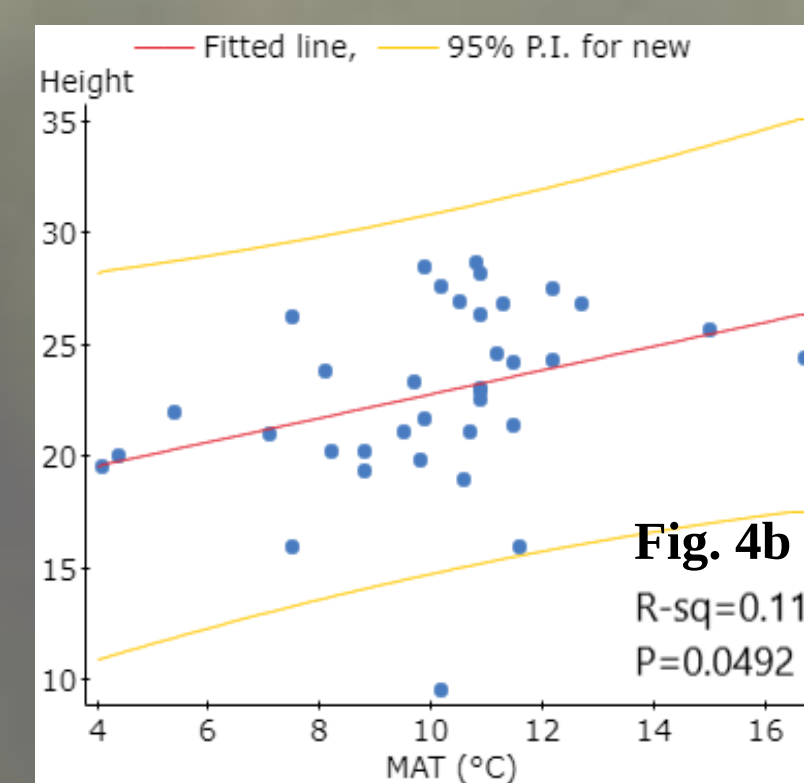


Fig. 4b



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



- 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 ($\leq$ 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.

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