Evaluating Showy Milkweed (*Asclepias speciosa*) ecotypic variation in adaptive traits using a genecological approach

Why should you care?

Ecosystem services include direct and indirect benefits that humans get from the natural environment. These include provisioning (products from ecosystems), regulating (regulation of ecosystem products; think clean water, air, soil fertility), supporting (services necessary for production services) and cultural/aestetic (non-material cultural benefits) services. Intact and functional ecosystems are essential to the life supporting properties of Earth that humans depend on. Ecosystem stewardship and management is essential for sustaining natural systems and the services they provide.

Background

Interactions between climate change and anthropogenic (human-caused) habitat destruction are reducing the extent and diversity of organismal communities. In many instances, climate and anthropogenic drivers create in acute thresholds that when crossed, result in rapid losses of biodiversity and community structure. Losses of community structure are correlated with changes in ecosystem resistance to change and resilience to persist unchanged under dynamic conditions.

To address/mitigate climate and anthropogenic issues and to preserve the functionality of ecosystems, land managers, scientists, and conservationists are developing and deploying next generation tools to maximize restoration success. Habitat restoration seeks to augment compromised abiotic and biotic communities with (most often native) primary producers in efforts to produce: structural habitat features, improve food resources for heterotrophs, and to improve ground cover and stability. In terrestrial habitat restoration, vascular plants are the key focus of restoration efforts, yet increasing emphasis is being placed on soil communities and keystone heterotrophs.

Classic restoration approaches have overlooked the value of selecting restoration propagules (seeds, cuttings, tubers) with traits that are adapted to the unique climate selection pressures of restoration sites. Genecology is the study of genetic variation within species in relationship to their population distribution and environmental selection pressures environment. Local variations in environmental conditions (particularly abiotic ones) produce variations in genes that influence morphological, physiological, behavioral, or phenological characters. These adaptive traits (traits that have benefits to survival and reproduction) vary across populations due to differences in selection pressures. Species with a broad geographic distribution thus tend to show greater levels of phenotypic variation in traits than to restricted ones. These changes in phenotypes across the landscape reflect the changing nature of selection pressures. Interdisciplinary tools in the fields of ecology (genecology), Geographic Information Systems (GIS) and statistics provide a synthesis of powerful tools that can be used to understand the patterns of genetic diversity and variation in adaptive traits among prospective populations of seed sources and the climate variables that those patterns.

Lab focus

Western monarch butterflies (*Danaus plexippus*) have declined ~97% from historic abundances in the early 1980s and are being evaluated for listing under the federal Endangered Species Act. Habitat loss and fragmentation in wintering, migratory, and breeding areas are considered key drivers of declines. Since monarchs depend on milkweeds (*Asclepias sp.)* for reproduction, there is increased interest among conservationists to plant milkweeds as habitat restoration. Among milkweeds in the Western US, showy milkweed (*Asclepias speciosa)* has a broad ecological niche and is the most abundant; making it a good candidate for restoration activities. Successful habitat restoration will require germplasm that is adapted for target restoration environments and seasonal phenology. Morphological traits are adaptive, easily observable, and shaped by climate; making them helpful in genecological applications for determining seed transfer zones (common areas where seeds or propagules can be translocated with minimal risk to being poorly adapted).

Objectives: In **part one** of this lab, you will help collect data in a common garden approach that will be used to evaluate morphological variation among 36 showy milkweed populations from across the Intermountain West (**Fig 1**). Common gardens are experimental designs where various ecotypes (populations of the same species from different environments) are grown under the same experimental conditions to identify variation in adaptive traits. This data will be used to describe geographic patterns of adaptive traits and their relationships to local climate and to construct species specific seed zones for showy milkweed with intention to maximize the success of restoration plantings for monarch butterfly conservation.

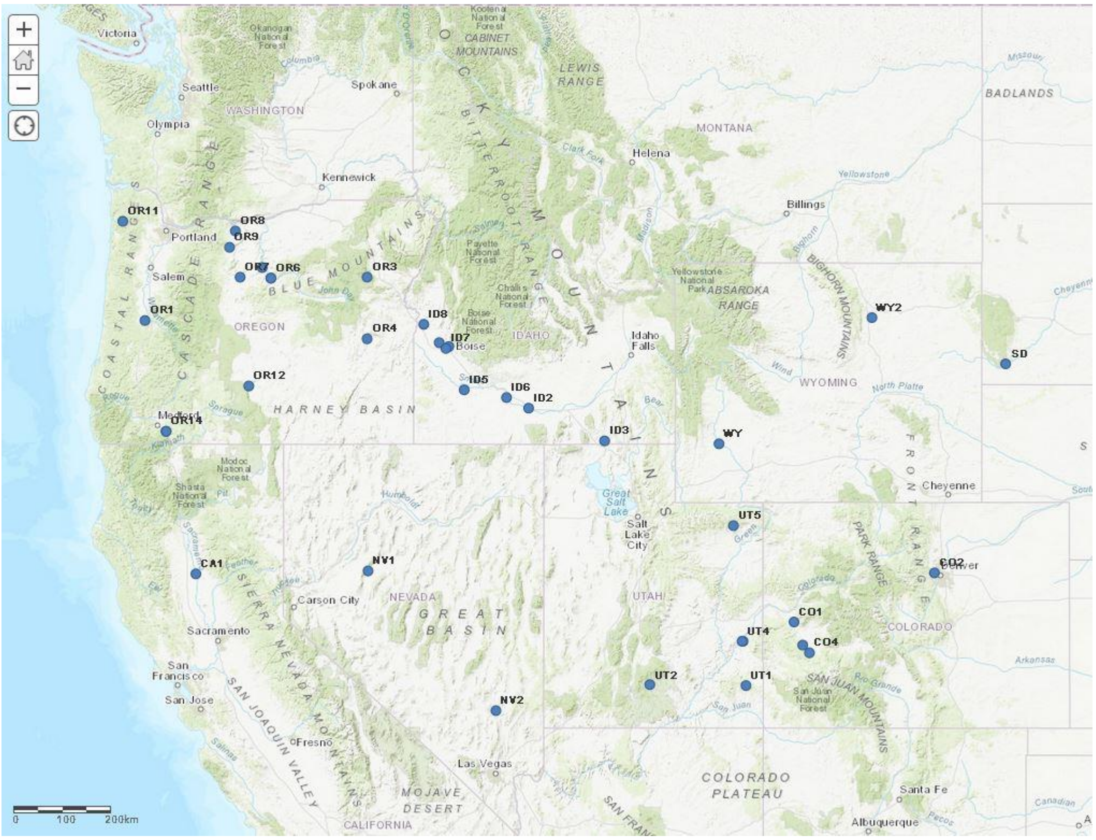


Fig 1A.

Fig. 1: **A.** Seed source locations for 35 populations of milkweed and **B.** their provisional seed zones; common colors represent similar annual temperature regimes and heat stress indexes.

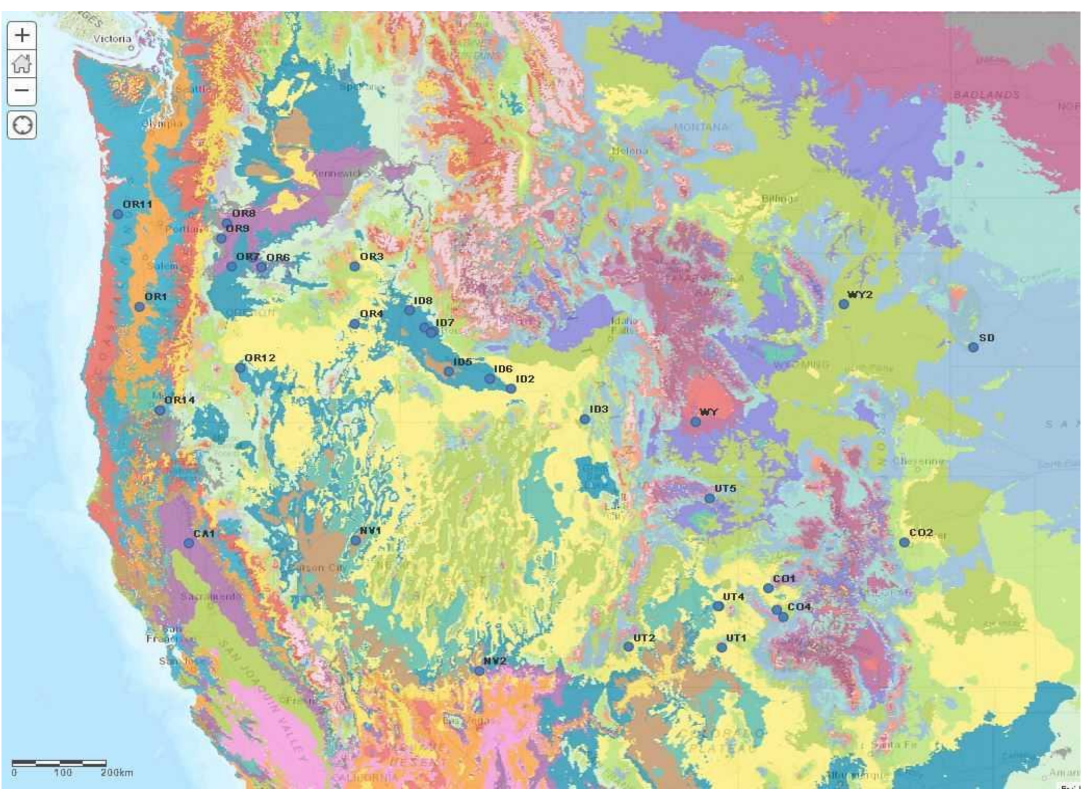


Fig 1B.

In **part two** of this lab, you will construct and test hypotheses about how climate and other abiotic factors shape putative adaptive traits in western showy milkweed populations. This effort will rely testing correlation among remotely sensed climate data (Climate WNA; Wang et al. 2012) in conjunction with measured traits from the CWI milkweed common garden. Along the way, you'll be exposed to some additional methods that researchers use to analyze, simplify, and interpret multivariate data sets.

The data you collect in the field will be used in the progression of this long-term experiment and the eventual development of species specific seed transfer zones for showy milkweeds in the west to benefit monarch butterfly and pollinator conservation efforts. Further, your inquiry and analyses will also help shed light on potential explanations for how climate and elevation drive adaptation in local milkweed ecotypes of this species.

Field Procedure

Organization

1. Your instructor will provide you with a brief orientation about the layout and best practices of work in the experimental garden.
2. Work with a partner
   a. Each group will receive:
      i. bundles of labelled collection envelopes
      ii. matching data sheet
      iii. a baggie with a moist towel for wiping up latex
      iv. one storage box with envelope dividers
      v. two sharpie markers
      vi. loose printer paper
3. NOTE: the garden contains both experimental units (plants we measure) and non-experimental units (plants that fill-in the grid so al plants have equal completion). **Fig 2** shows the garden layout.
   a. Non-experimental (referred to as "randoms") should not be measured or collected from
   b. "Randoms" are located in the following locations:
      i. The first three plants at the top of the garden
      ii. The last two plants at the end of the garden (exception is row J, where it's only the last plant)
      iii. Rows A and N (every plant on these rows are randoms).

4. Envelopes are ordered from the top of the row to the bottom and exclude all random plants.
5. Use the data sheet to confirm the order of envelopes

Safety precautions:

- While not necessarily allergenic, dust and particulates from plants can be irritating to skin, eyes or respiratory systems. Please use caution when working with dry plants. It is advised that participants wear long sleeves and pants and closed toed shoes.
- Milkweed latex is sticky and can be a skin irritant for some. If you get latex on your skin, you are advised to wipe it off with a moist towel before it can dry.
- Milkweed latex contains low levels of cardenolides. When ingested in large quantities, these toxins can cause cardiac failure.

You may choose to wear the following OPTIONAL personal protective equipment-

- Nitrile gloves
- Dust mask

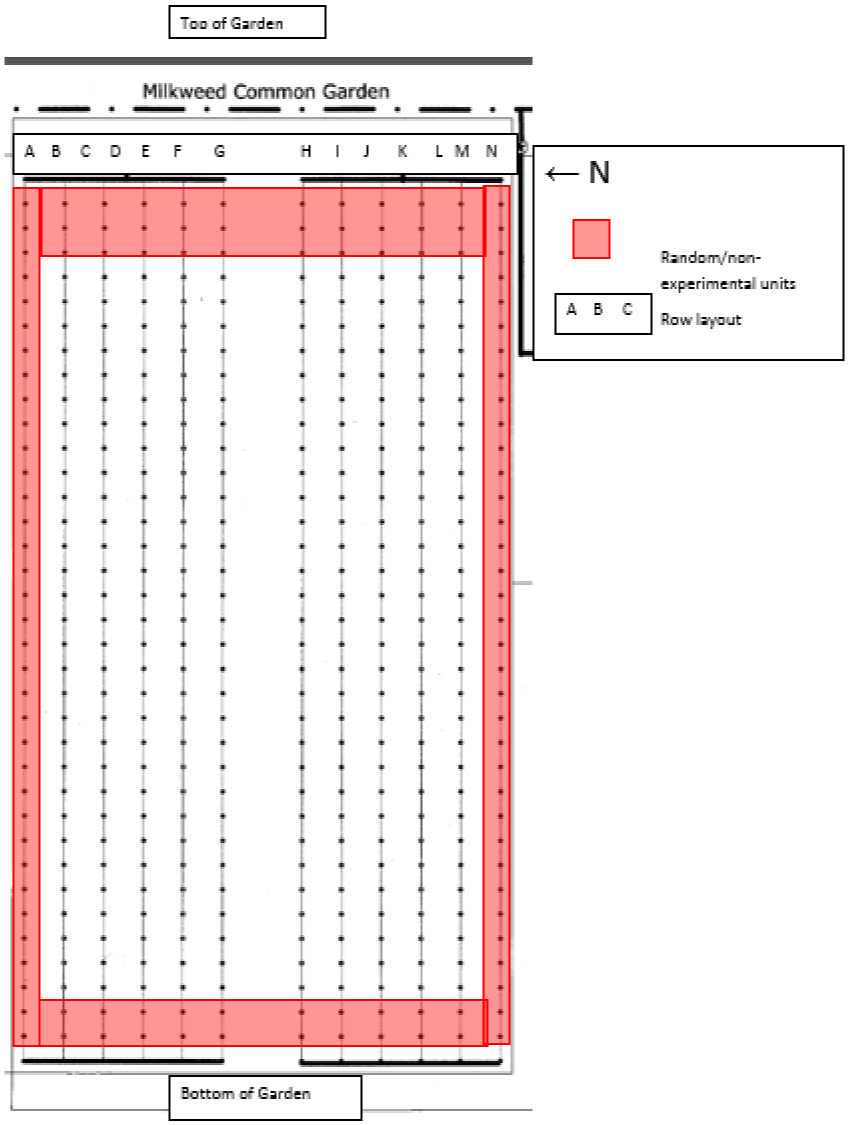


Fig 2: CWI Experimental Milkweed Garden Layout

Harvesting leaves to determine specific leaf area

1. For each experimental unit, harvest one representative leaf
    a. This leaf should be:
        i. green/living- no dead/yellow/brown leaves
        ii. Selected at random
        iii. Mature- not the youngest leaves at the top
        iv. represent an "average leaf"
            1. not be the largest or smallest leaf on the plant
    b. to harvest:
        i. Grasp the leaf at the petiole and gently but firmly pull down (Fig 3).
            1. The plant will release latex-like sap
            2. Let the sap drip for 2-3 seconds and then wipe with a moist towel
        ii. Place the leaf in the labelled envelope
        iii. Ensure that the leaf is as flat as possible, taking care that leaves remain flat during transport
        iv. collate vertically into the storage box
            1. Separate rows with labeled envelope dividers (e.g.: A, B, C, D).

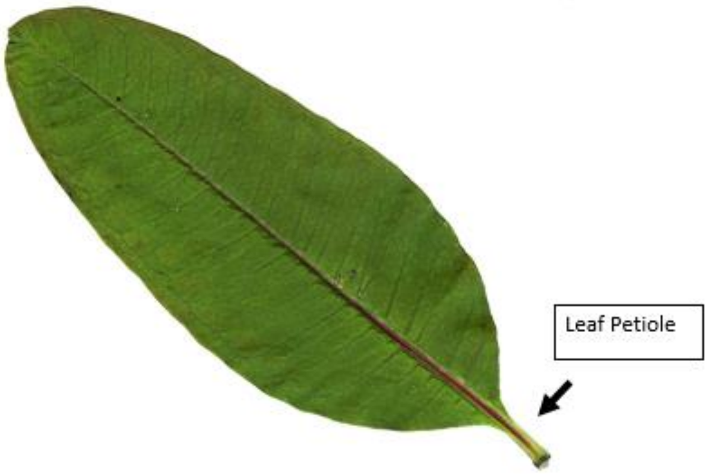
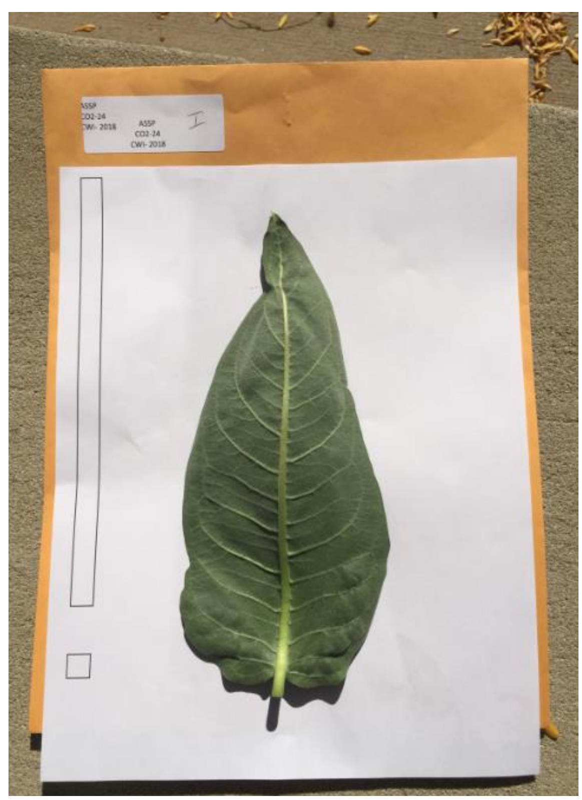


Fig 3: Milkweed leaf with petiole highlighted

Massing, Photographing, drying and massing leaves to determine SLA

2. Photographing leaves
    a. Carefully remove the leaf from its envelope
    b. Arrange the sample envelope, size standard sheet, and leaf as shown in **Fig. 4**
    c. using the class iPads,take a single clear picture of the leaf that shows:
        i. The entire leaf (ventral side facing up/ or whatever arrangement makes the leaf flat as possible.)
        ii. The entire size standard scale
        iii. The entire printed identification label that is on the envelope
    d. Return leaves back to storage envelopes, taking care that leaves remain flat
    OR
    e. Pass the leaf to a partner for leaf fresh mass measurements



3. Fresh leaf mass leaf dry matter content (LDMC)
    a. Carefully remove the leaf from its envelope
    b. Place and tare a large weigh boat on the digital balance
    c. Place leaf tissue on the weigh boat.
    d. Record the mass in grams to the nearest 0.00 g (or whatever the minimum decimal place of the device) on your paper data sheet.
    e. Return leaves back to storage envelopes, taking care that leaves remain flat
    f. collate vertically into the storage box
        1. Separate rows with labeled envelope dividers (e.g.: A, B, C, D).

4. Your instructor will transfer envelopes to drying ovens

5. Dry leaf mass for specific leaf area (SLA) and LDMC

NOTE: dried leaves are fragile, friable, and delicate. Handle with care when measuring mass.

a. Carefully remove the leaf from its envelope
b. Place and tare a large weigh boat on the digital balance
c. Place leaf tissue on the weigh boat*.
g. Record the mass in grams to the nearest 0.00 g (or whatever the minimum decimal place of the device) on your paper data sheet.

*If the leaf overhangs the weigh boat considerably, record the initial mass data

i. You may opt to carefully break the leaf so it fits entirely within the weigh boat
ii. Update recorded mass if necessary