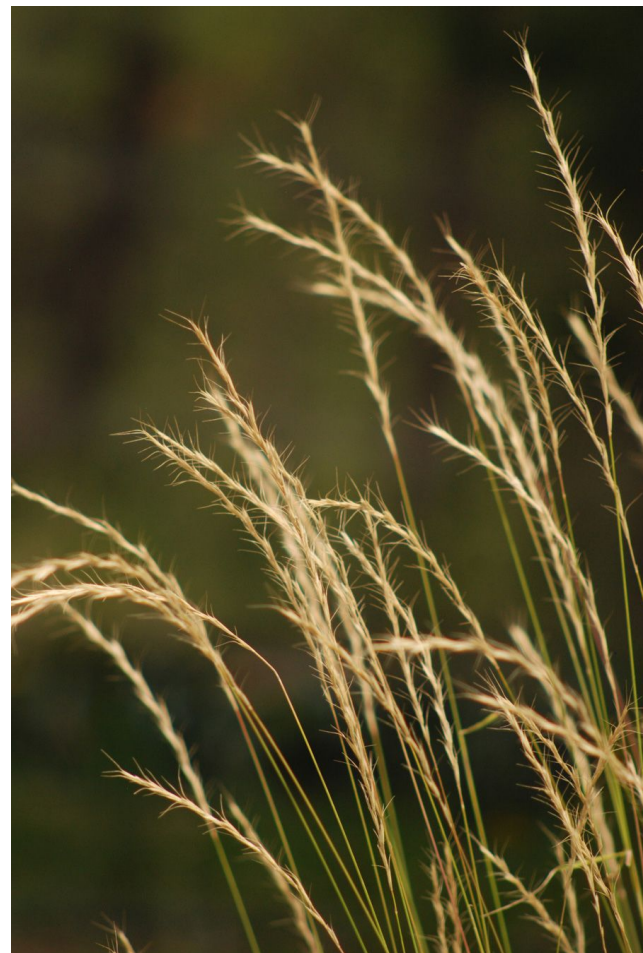
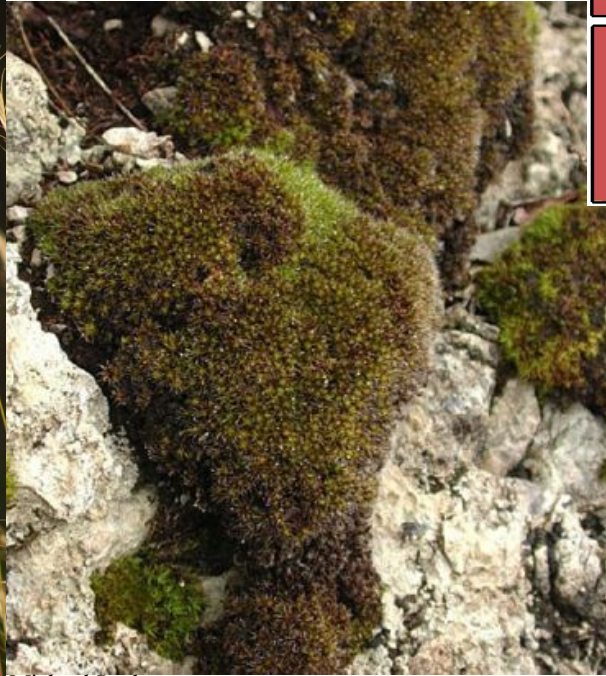


# The applicability of next-generation sequencing to native plant materials development

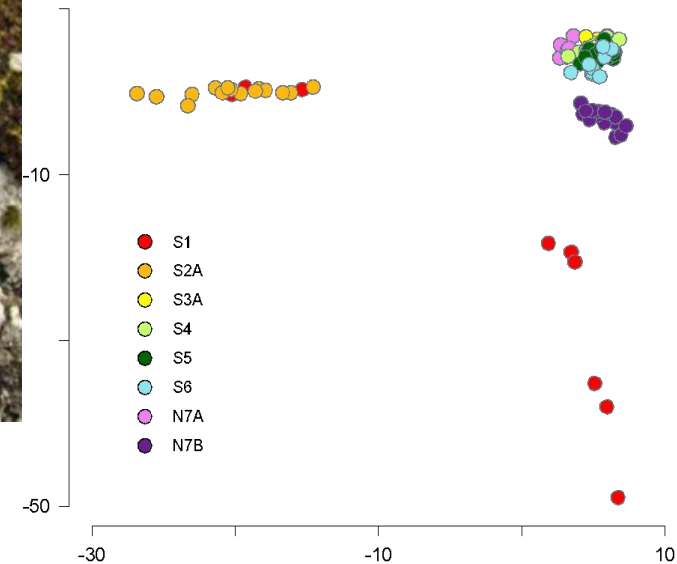
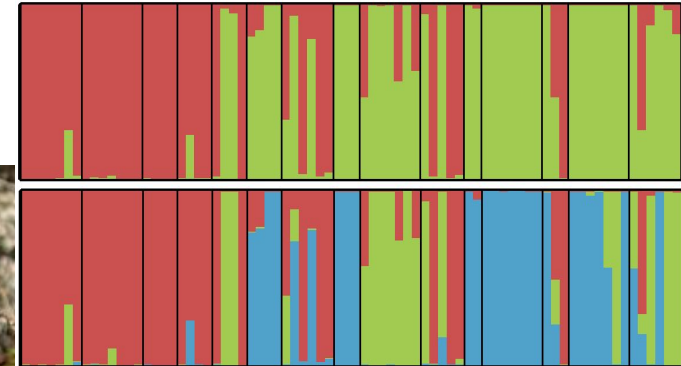
Rob Massatti, USGS-Southwest Biological Science Center  
Flagstaff, AZ



[www.blackfootnativeplants.com](http://www.blackfootnativeplants.com)



Michael Luth



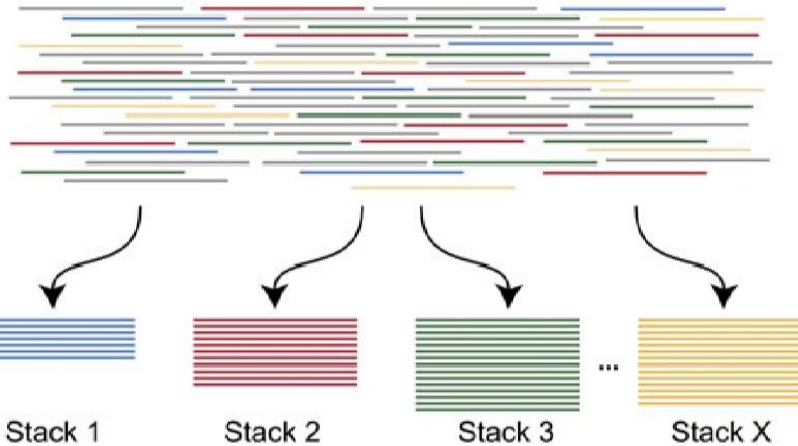
# Next-generation sequencing data collection

Library prep protocols: RADseq; ddRAD; GBS; commercial

Sequencers: Illumina (HiSeq, MiSeq, etc.); PacBio; Ion Torrent; 454

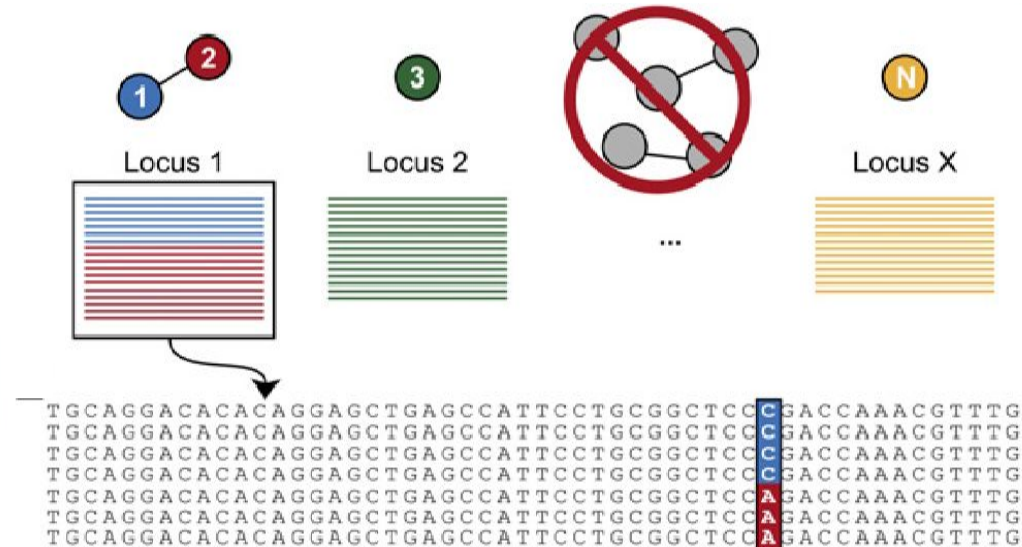
Assembly methods: reference; *de novo*

(1) – Fragment genome



(2) – Cluster within individuals

(3) – Cluster among individuals



(4) – Resolve variation

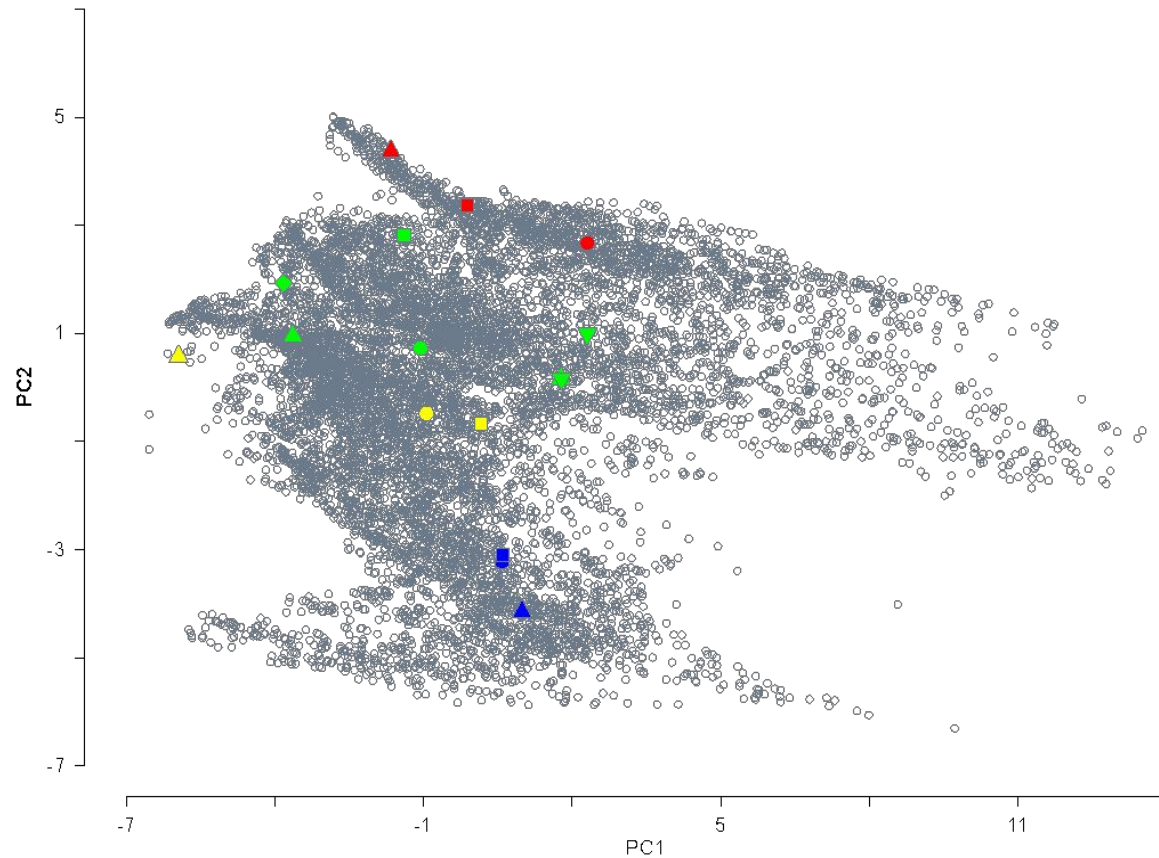
# Example 1: Evidence for local adaptation in *Syntrichia ruralis*, a common biocrust component across the Colorado Plateau

## Motivating questions

- 1) Do mosses show geographic structuring of their genetic variation?
- 2) Do mosses show signs of adaptation to local environmental conditions?
- 3) When we generate propagules for restoration, where should we source them from to maximize our success?



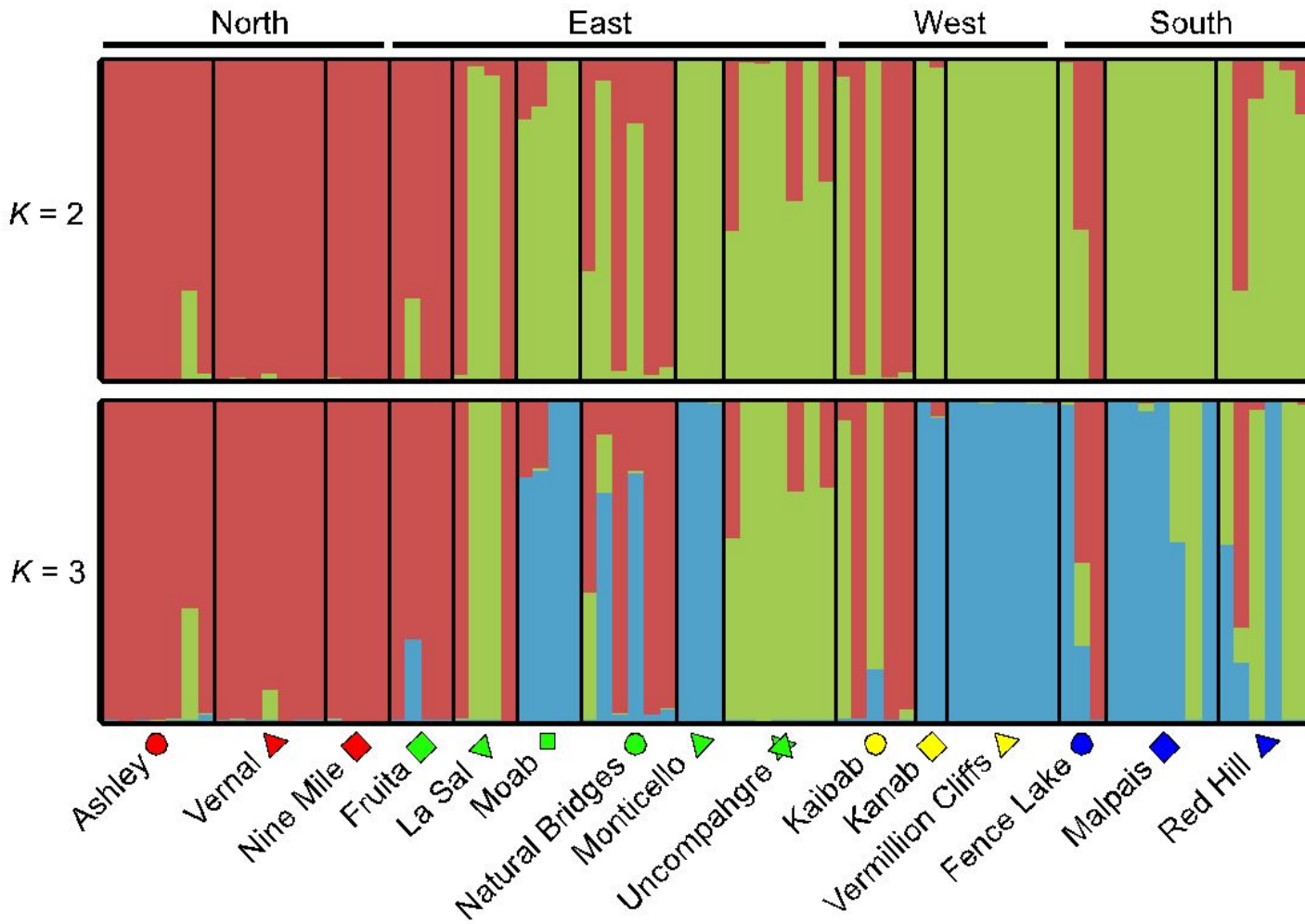
# Environmental space across the Colorado Plateau



**PC1:** Decreasing temperature, more total precipitation  
(i.e., higher elevation)

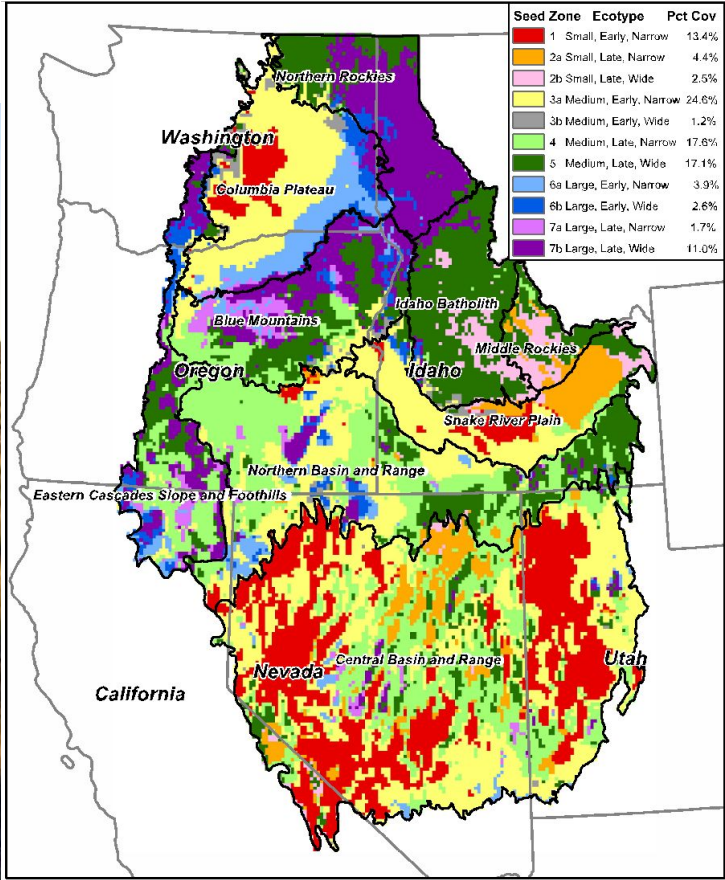
**PC2:** Larger difference between summer high temps and winter  
low temp; reduced monsoonal intensity (i.e., higher latitude)

# Results: STRUCTURE analyses



- Propagules may need to be sourced from inside and outside of monsoonally affected areas, as well as from higher and lower elevations

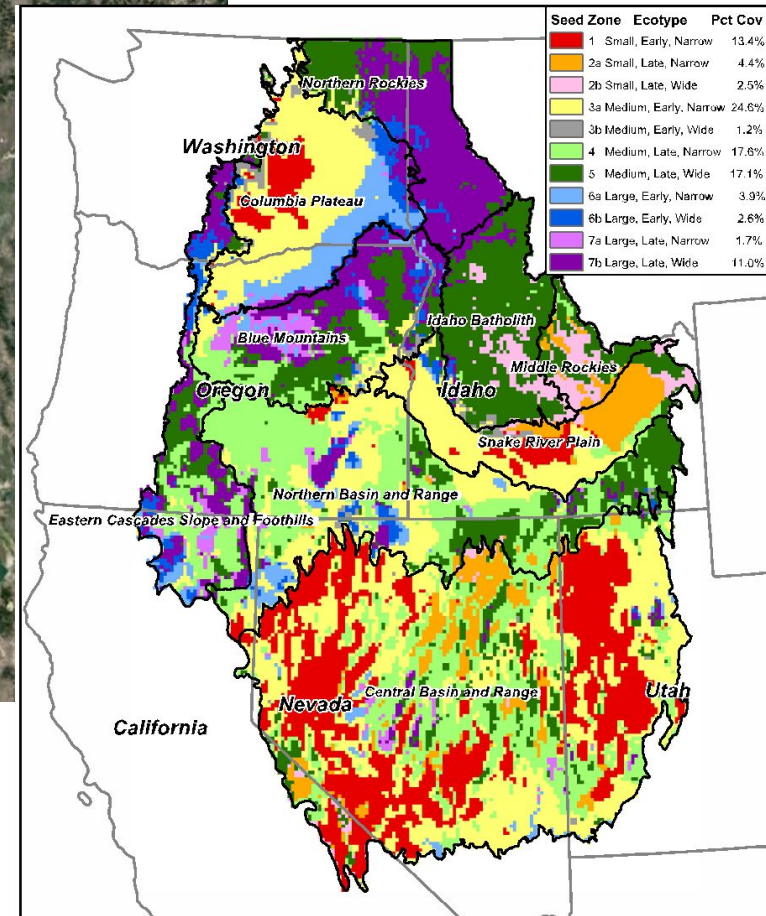
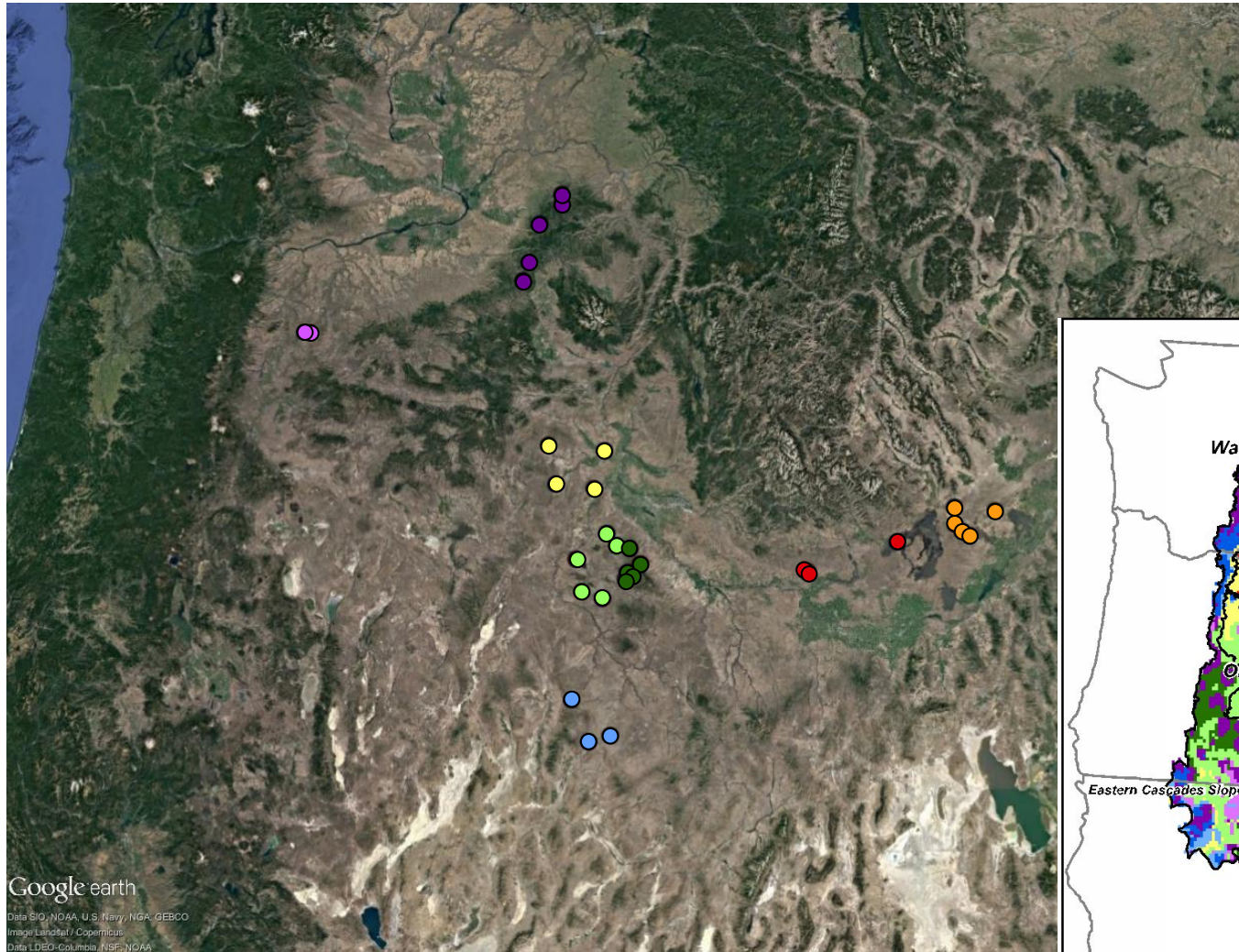
# Example 2: Bluebunch wheatgrass anonymous genomic data vs. empirical seed transfer zones



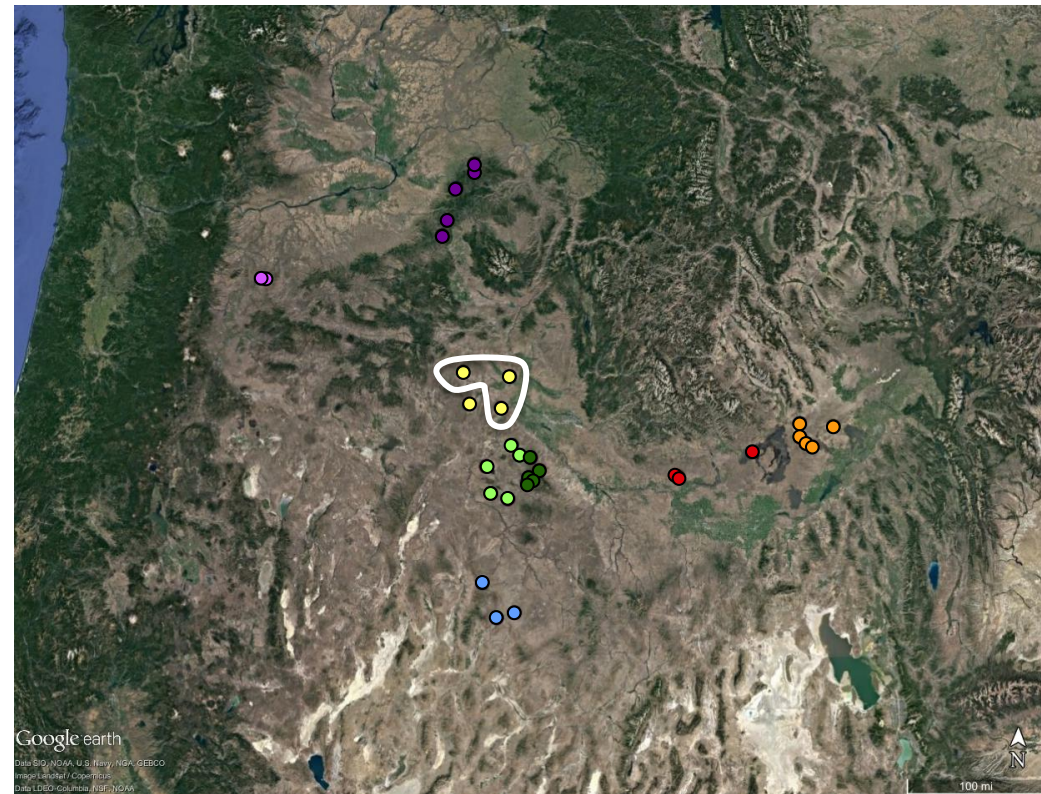
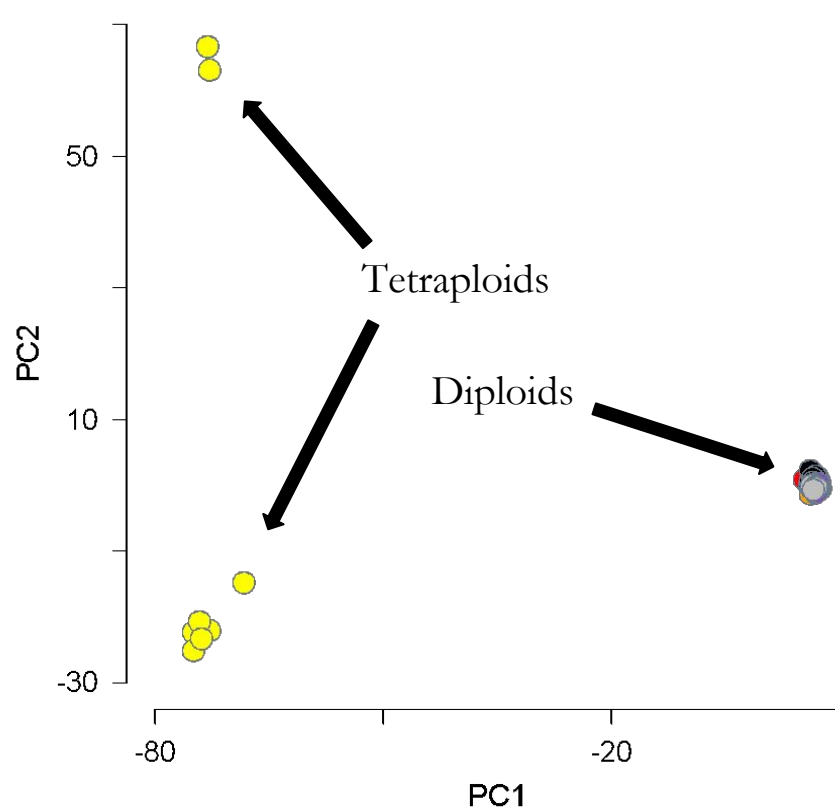
St. Clair et al. 2013

Photo: PRBO Conservation Science Shrubsteppe Monitoring Program

# Geographic sampling for NGS Library 2



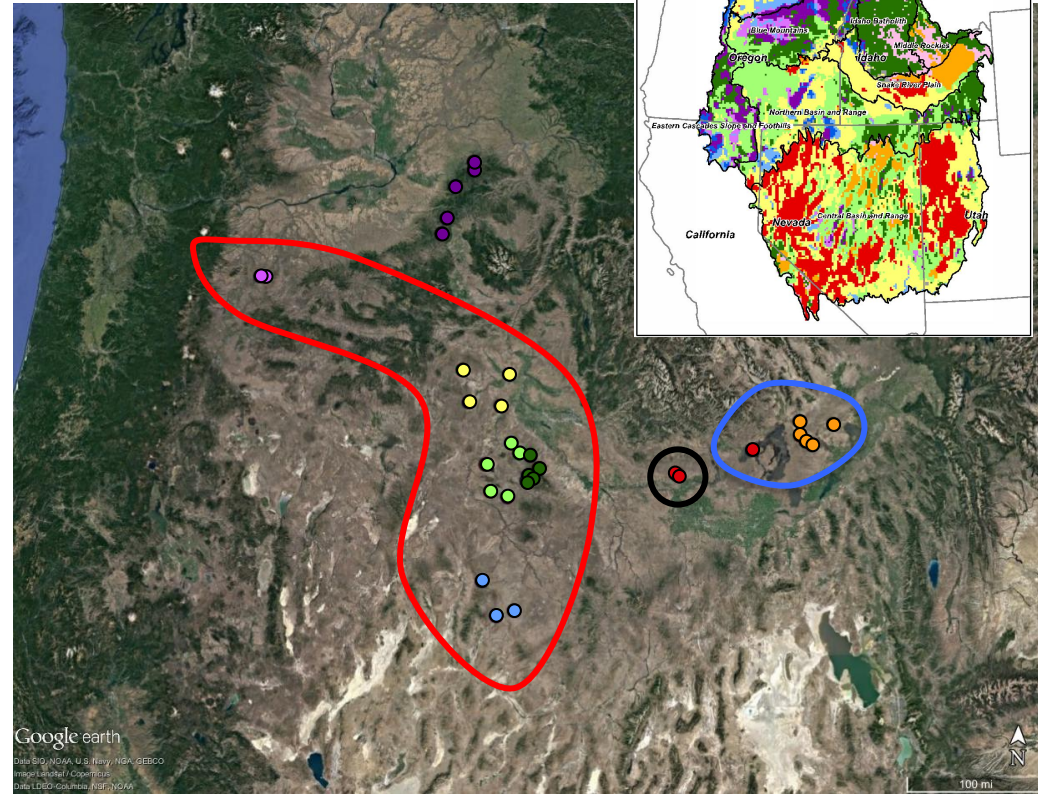
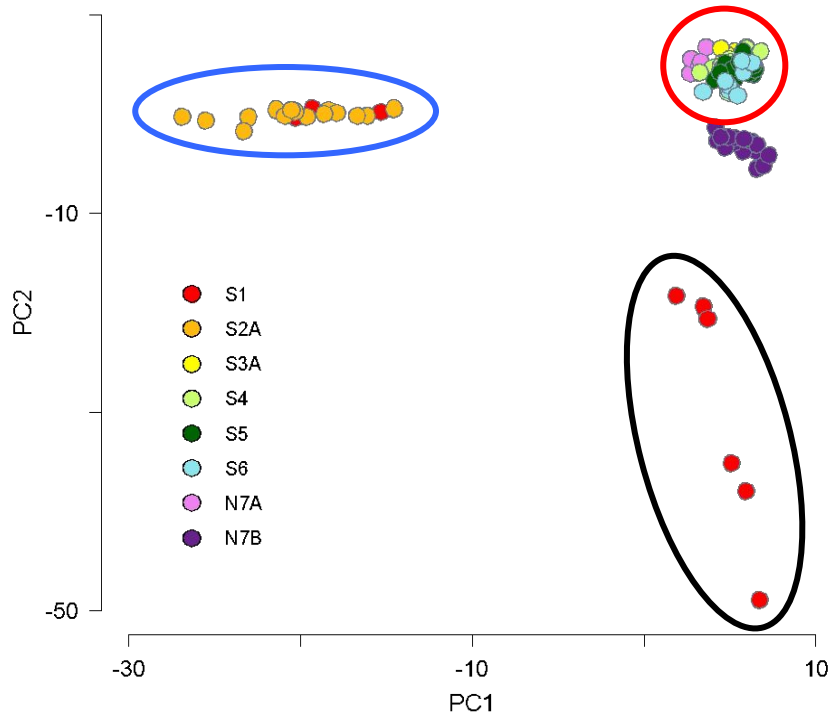
# Initial PCA of bluebunch data



- Polyploid populations easily discriminated from diploid populations
- Is there a biological difference between polyploid populations?

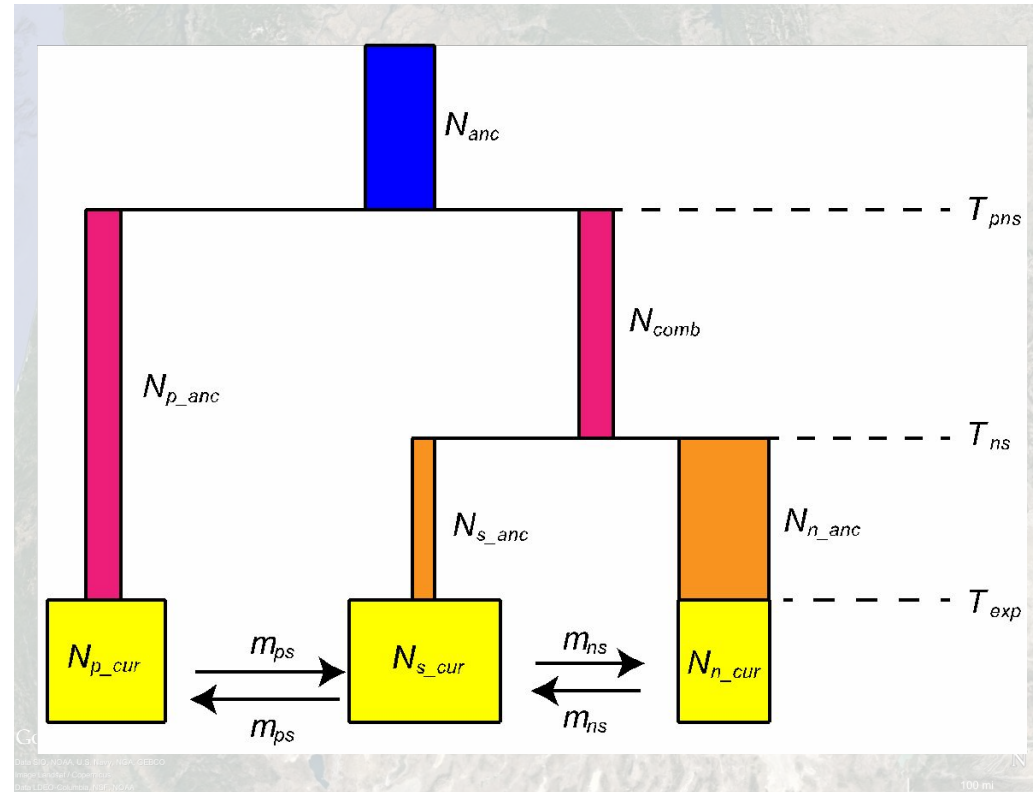
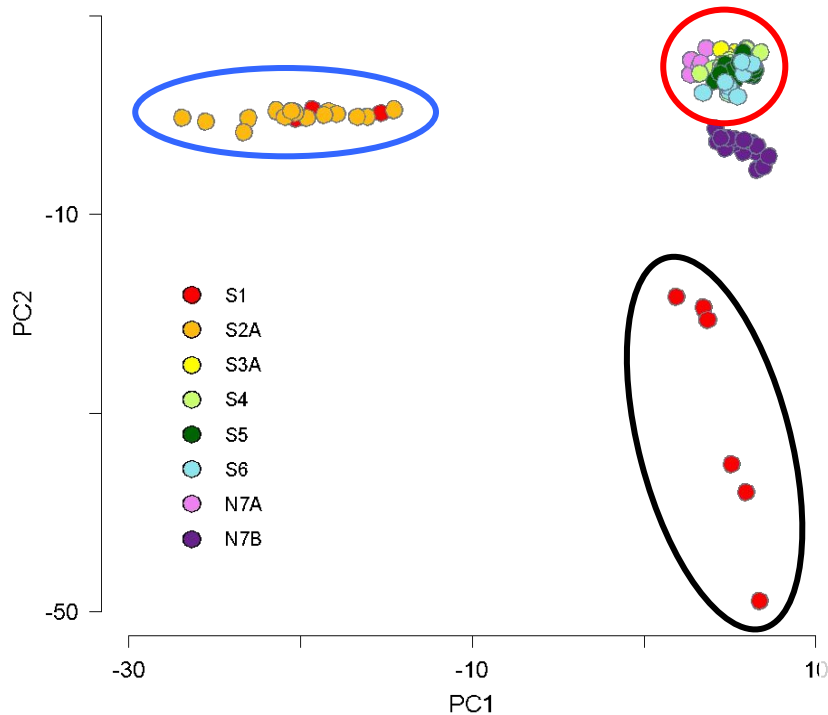


# PCA excluding polyploid individuals



- Standing genetic variation clusters in unpredictable ways, likely due to historical processes (e.g., refugia during Pleistocene)
- Mismatch between anonymous genomic variation and seed transfer zones – how do we incorporate this information into conservation/restoration planning?

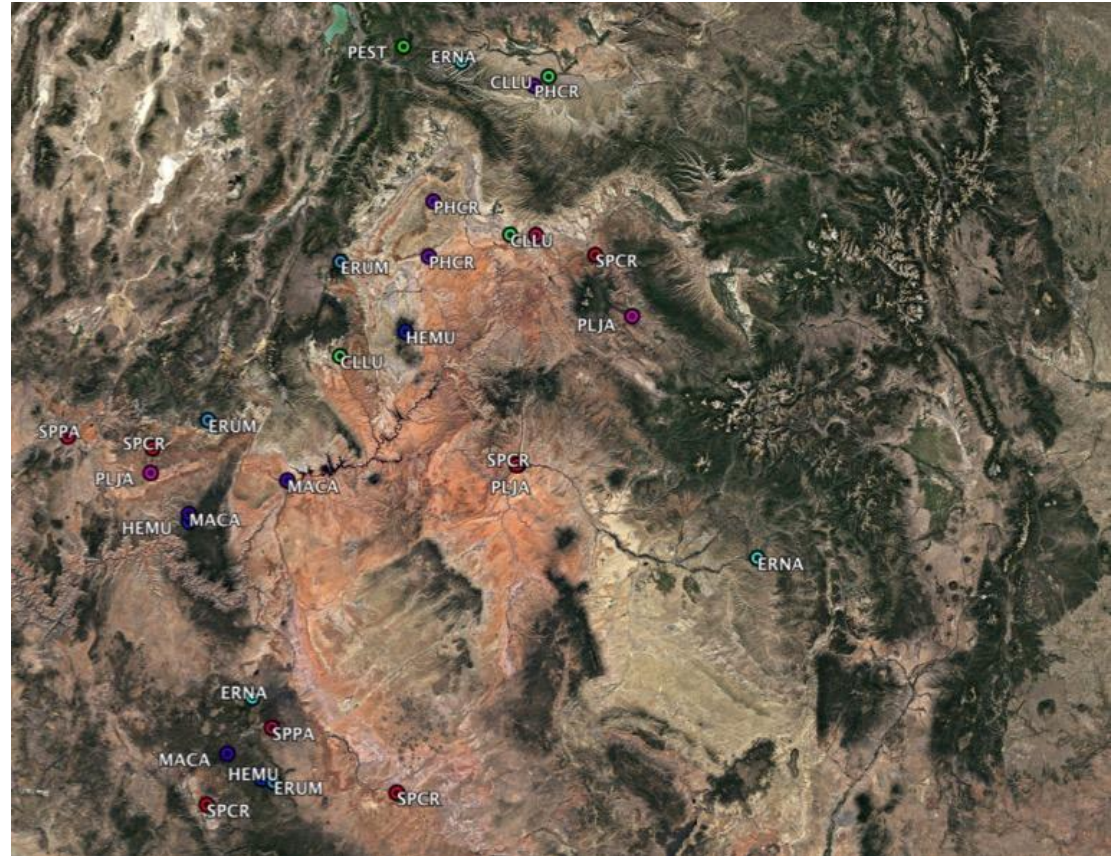
# PCA excluding polyploid individuals



- Standing genetic variation clusters in unpredictable ways, likely due to historical processes (e.g., refugia during Pleistocene)
- Mismatch between anonymous genomic variation and seed transfer zones – how do we incorporate this information into conservation/restoration planning?

# Example 3: Comparative studies to elucidate impact of species-specific traits & historical processes on geographic patterns of genetic variation

## Ongoing work at the Colorado Plateau Native Plant Program



Species for preliminary  
data generation:

*Cleome lutea*

*Eriogonum umbellatum*

*Machaeranthera canescens*

*Phacelia crenulata*

*Sphaeralcea parvifolia*

*Ericameria nauseosa*

*Heliomeris multiflora*

*Penstemon strictus*

*Pleuraphis jamesii*

*Sporobolus cryptandrus*

# Summary

## **Example 1: Assessing local adaptation**

- Design sampling strategies to represent environmental space
- Environmental gradients may inform propagule sourcing

## **Example 2: Informing seed transfer zones**

- NGS may be a simple way to elucidate geographic patterns of polyploidy
- While morphological traits may vary with environmental space in predictable ways, standing genetic variation may have independent geographic patterns

## **Example 3: Utilizing comparative studies**

- Discerning geographic patterns of genomic diversity across species with a diversity of life history characteristics may help us construct a regional framework for propagule sourcing

The success of applying this technology to restoration and conservation issues will be proportional to the knowledge we apply concerning the biology and ecology of the organisms and the natural history of their habitats. It is critical for practitioners to be involved in designing these studies and interpreting the resulting patterns.

# Acknowledgements

## Moss work:

Kyle Doherty

Troy Wood

Wayne Padgett

Elizabeth Milano

Matthew Bowker

Andrew Krohn

Tom Juenger

Forest-Rangeland Soil Ecology Lab –  
Northern Arizona University

## Bluebunch work:

Francis Kilkenny

Holly Prendeville

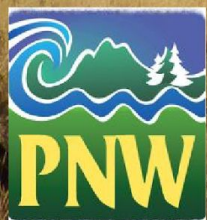
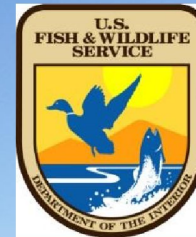
Bryce Richardson

Steve Larson

Jeff Ott

Josh Udall

## Funding sources:





The preceding presentation was delivered at the

## **2017 National Native Seed Conference**

Washington, D.C. February 13-16, 2017

This and additional presentations available at <http://nativeseed.info>

