Patterns of Nuclear and Cytoplasmic Differentiation in Intermountain Restoration Species: Tales From Two Genomes

Rich Cronn[1], E. Durant McArthur[2], Valerie Hipkins[3]

[1] Pacific Northwest Research Station, Corvallis, OR

[2] Rocky Mountain Research Station, Provo, UT

[3] Pacific Southwest Station, Placerville, CA

Genetic survey of Great Basin restoration species

RMRS Shrub Lab, National Forest Genetic Electrophoresis Laboratory (NFGEL) and PNW Genetics are working to:

- **Characterize baseline genetic diversity in ~ 20 different Great Basin natives**
  - **Most species understudied and underrepresented in collections and databases**
- **Evaluate genetic differentiation within and among partitions for all species**
- **Determine genetic identity of seed sources used in post-fire revegetation**

Photo: Vicky Erickson

Photo: Durant MacArthur

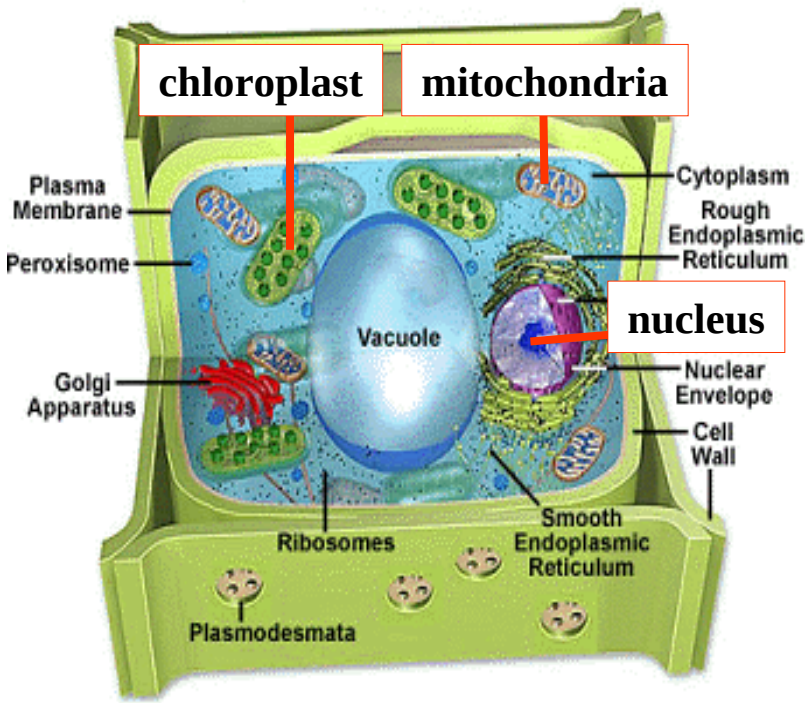
Species Included in the Genetic Survey

- **Shrubs**
  - *Artemesia tridentata*
  - *Atriplex canescens*
  - *Ericameria (Chrysothamnus) nauseosa*
  - *Purshia tridentata*
- **Grasses**
  - *Bromus carinatus*
  - *Hesperostipa (Stipa) comata*
  - *Achnatherum (Oryzopsis) hymenoides*
- **Legumes / Forbs**
  - *Astragalus utahensis*
  - *Balsalmorhiza sagittata*
  - *Crepis acuminata*
  - *Erigeron pumilus*
  - *Eriogonum umbellatum*
  - *Heliomeris (Viguiera) multiflora*
  - *Lupinus argenteus/sericeus*
  - *Vicia americana*
  - *Ceratoides lanata*
  - *Lomatium grayi/dissectum*
  - *Penstemon deustus*
  - *Phlox longifolia*

2001 – 2004 collections

- *≥ 5 populations*
- *< 5 populations*

Cellular genomes and their properties



- **Nuclear Genome -**
  - **Chromosomes = 2 (diploid) or more (polyploid) per cell**
  - **Biparental inheritance**
  - **Survey using protein polymorphism (allozymes) DNA methods (AFLP)**
- **Organellar Genomes –**
  - **Circular haploid chromosome**
  - **Uniparental (maternal) inheritance**
  - **Survey either cpDNA or mtDNA (effectively linked via single parent transmission)**

Why study both genomes?

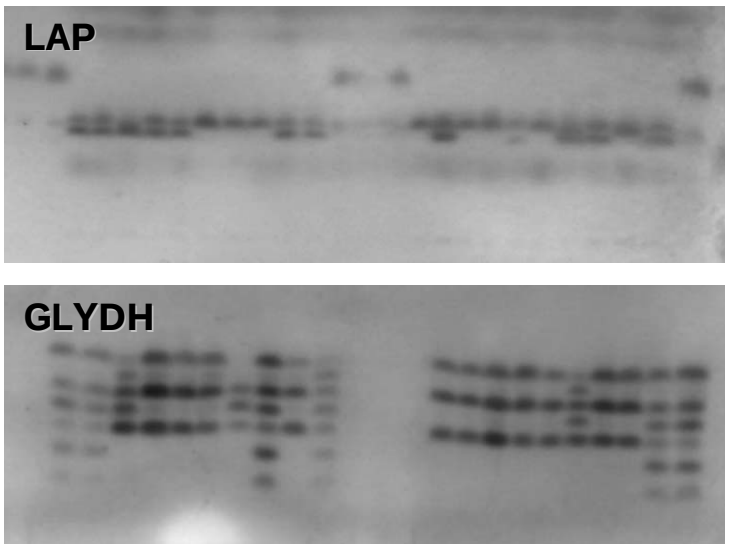
- **Potential to reveal different patterns of diversity**
  - Nuclear loci di- to polyploid, biparental inheritance; organelle genomes haploid, seed transmitted*
  - Alone: heterozygosity, rate of inbreeding, size of maternal neighborhood, gene flow from seed; Combined, gene flow via pollen
- **Potential for contrasting differentiation**
  - Independent unlinked partitions; can record different histories
  - N_e of nuclear genes ~4X larger than cpDNA; drift and migration influence organellar genes more than nuclear genes.
- **Nucleo-cytoplasmic interactions and fitness consequences**
  - Sterility/fertility often under cytoplasmic control (e.g., CMS)
  - Alloplasmic replacement: altered disease susceptibility, reduced fitness (maize, wheat)

** usually!*

Genetic Analysis I: Evaluating Variation

Nuclear Variation

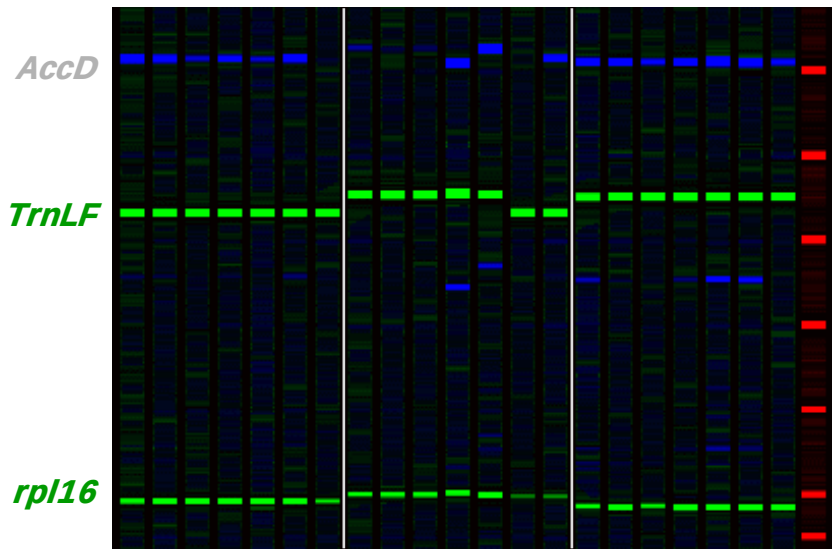
- Starch electrophoresis*, AFLP
- 18 enzyme activities screened; up to 28 loci identified (Lupinus)
- Loci/alleles inferred from known patterns in other species (e.g., Wendel and Weeden, 1991)



SOP: www.fs.fed.us/psw/programs/nfgel/publications.shtml

Organellar Variation

- Length polymorphism in PCR amplified non-coding cpDNA**
- Products fluorescently labeled; multiplex six loci (~3500 bp)
- ID variants in 1 bp intervals
- Code as 'haplotypes'



*** Horning and Cronn, in review*

Genetic Analysis II: Analytical Approaches

- **Indices of diversity**
  - **Allozymes:** % polymorphic loci (P), average alleles per locus (A), expected heterozygosity (He)
  - **cpDNA:** Total haplotypes, expected heterozygosity (He)
- **Index of differentiation: F_{ST}**

$$F_{ST} = \sigma^2_{\text{AMONG}} / \sigma^2_{\text{AMONG}} + \sigma^2_{\text{WITHIN}}$$

  - Computed using ANOVA *(Θ)*, pairwise and across all populations
  - Significance evaluated by permutation; contingency tables of alleles x s tested for goodness of fit (*G* statistic; Goudet et al., 1996).
- **Test of neutral differentiation: nucleus vs. organelle**
  - H_o: F_{ST} nuclear genes = F_{ST} maternal organelles when N_e included

$$Exp\ F_{ST_{cp}} = 6 \bullet F_{ST_{nuc}} / [2 + (4 \bullet F_{ST_{nuc}})]$$

  - Significance of observed difference tested by bootstrapping approach (Hamilton and Miller, 2002)

Genetic diversity across species examined

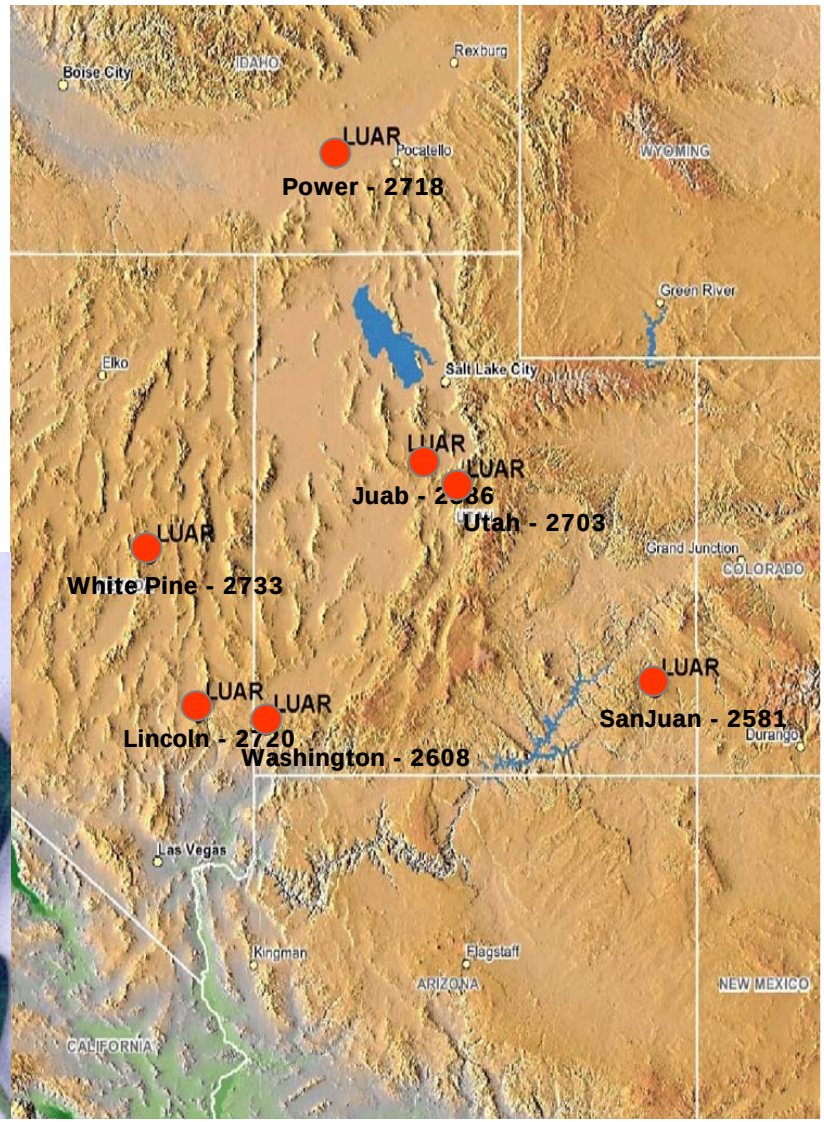
		Allozyme			cpDNA	
Species	**Pops**	**%P**	**A**	**H_e**	**N_{Hap} /Pop (N_{Hap})**	**H_e**
Astragalus utahensis	**4**	**63**	**2.09**	**0.217**	**1.25 (5)**	**0.690**
Atriplex canescens	**9**	**61**	**1.92**	**0.139**	**1.67 (15)**	**0.835**
Bromus carinatus	**2**	**19**	**1.24**	**0.062**	**2.0 (4)**	**0.709**
Crepis acuminata	**4**	**58**	**1.75**	**0.117**	**1.0 (5)**	**0.610**
Eriogonum umbellatum	**4**	**67**	**2.11**	**0.195**	**1.25 (5)**	**0.835**
Hesperostipa comata	**3**	**20**	**1.22**	**0.078**	**1.67 (5)**	**0.685**
Lupinus argenteus	**7**	**100**	**2.8**	**0.272**	**1.57 (11)**	**0.870**
Vicia americana	**3**	**54**	**1.71**	**0.119**	**1.0 (3)**	**0.667**

Genetic differentiation across species

Species	Nuclear F_{ST}		cpDNA F_{ST}	cpDNA $F_{ST\ (exp)}$
Atriplex canescens	0.285	>	0.280	0.545*
Astragalus utahensis	0.357	<	0.394	0.625*
Crepis acuminata	0.276	<	0.580	0.533
Eriogonum umbellatum	0.318	<	0.685	0.583
Bromus carinatus	0.634	<	0.694	0.839
Lupinus argenteus	0.314	<	0.863	0.579*
Hesperostipa comata	0.885	<	0.938	0.916
Vicia americana	0.299	<	0.958	0.561*

Nuclear and cytoplasmic diversity in Lupinus

- *L. argenteus* (and *L. sericeus?*)
- Allozyme diversity at 23 loci, 64 alleles
- cpDNA diversity: 6 loci (3450 bp)
- 7 populations, 20 individuals each
- Mean pairwise distance: 353 km
  - Range: 40 – 640 km)

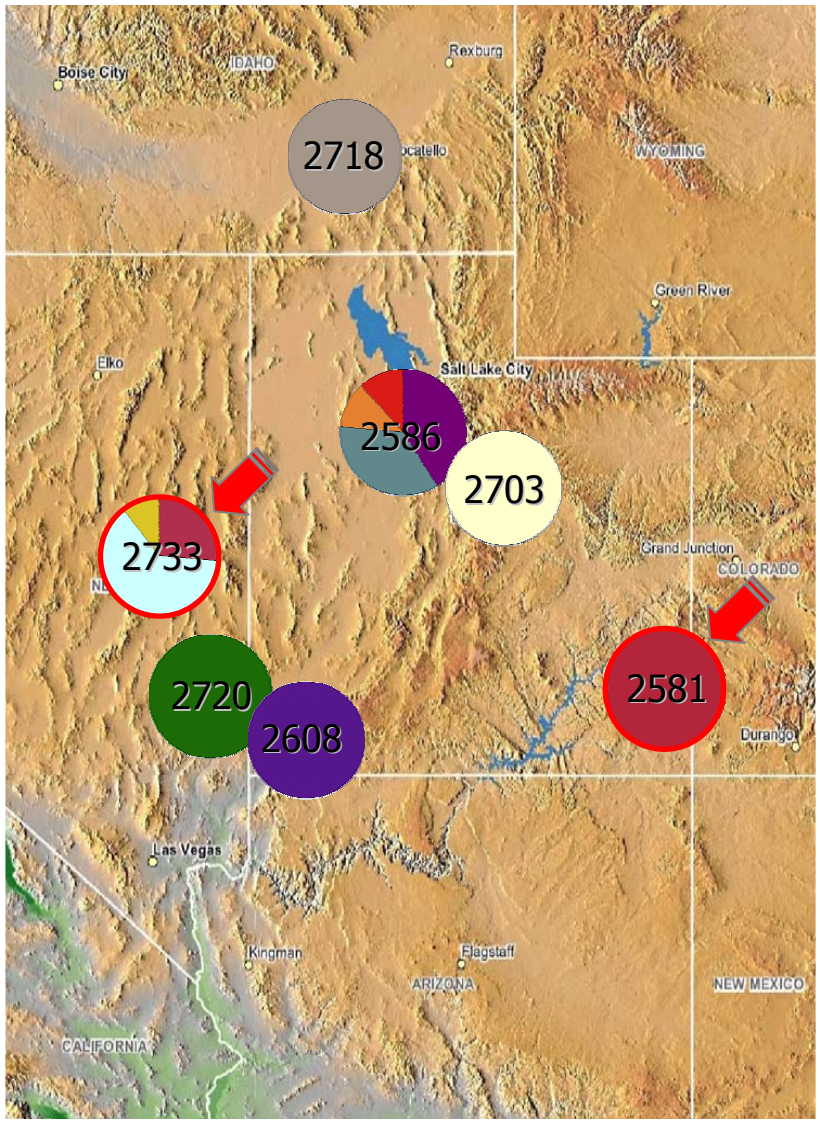


Genetic diversity in Lupinus argenteus

Population	Allozyme Data			cpDNA Data	
	%P	A	H_e	N_{Hap}	H_e
San Juan UT (2581)	52.2	1.7	0.154	1	0
White Pine NV (2733)	65.2	1.9	0.205	3	0.521
Washington UT (2608)	52.2	1.7	0.144	1	0
Lincoln NV (2720)	91.3	2.3	0.273	1	0
Juab UT (2586)	56.5	1.7	0.169	4	0.678
Power ID (2718)	65.2	2.1	0.255	1	0
Utah UT (2703)	47.8	1.7	0.200	1	0
Population means	100	2.8	0.272	1.7	0.870

Cytoplasmic differentiation in Lupinus argenteus

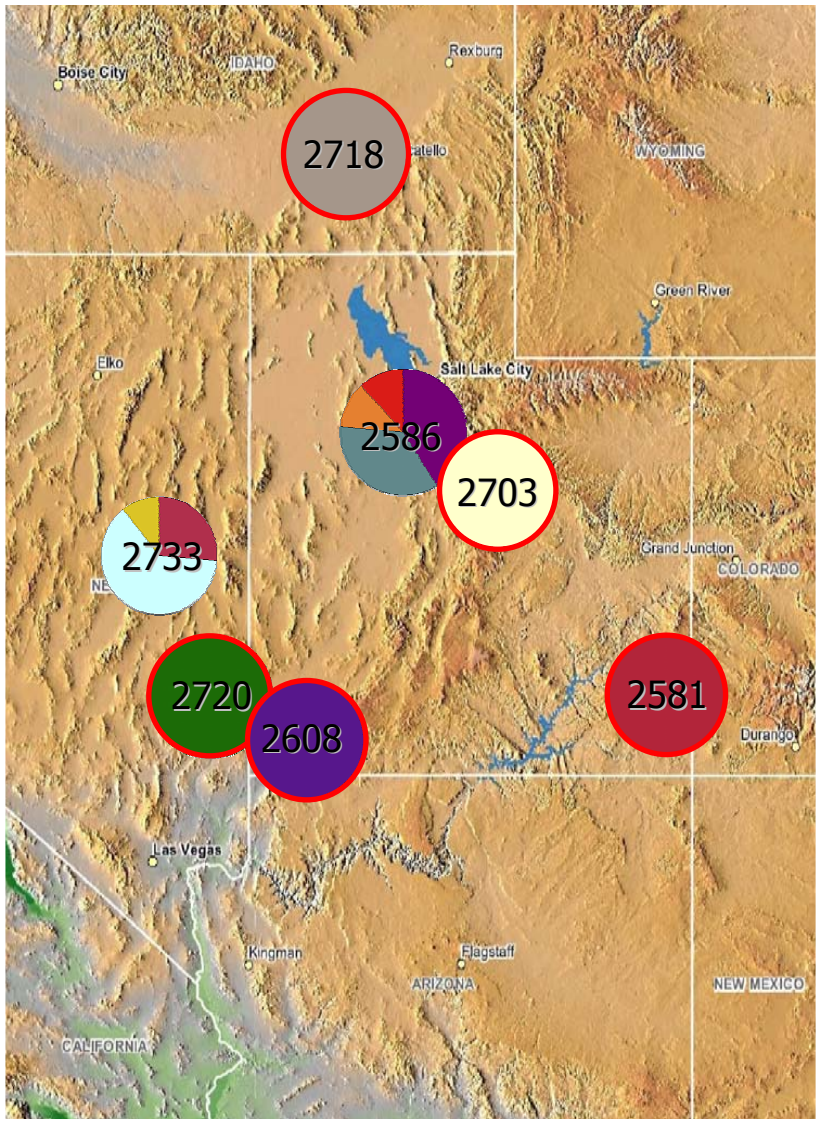
- **Eleven haplotypes identified**
  - One shared among populations: population 2581 (freq = 1.0) and population 2733 (freq = 0.26)



Cytoplasmic differentiation in Lupinus argenteus

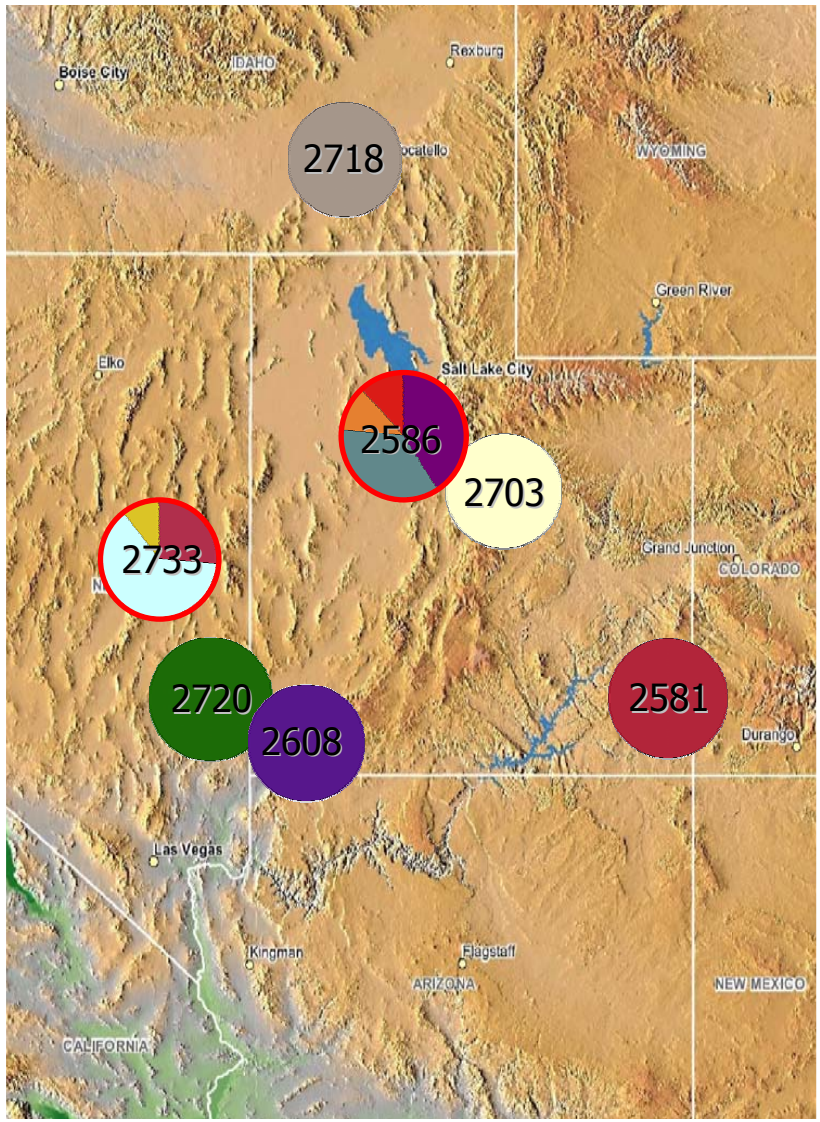
- **Eleven haplotypes identified**
  - One shared among populations: population 2581 (freq = 1.0) and population 2733 (freq = 0.26)

- **Five of seven populations fixed for unique haplotype**
  - 1 haplotype: H_e = 0.0
  - Fixation occurs in proximal populations (e.g., 2720 vs. 2608)

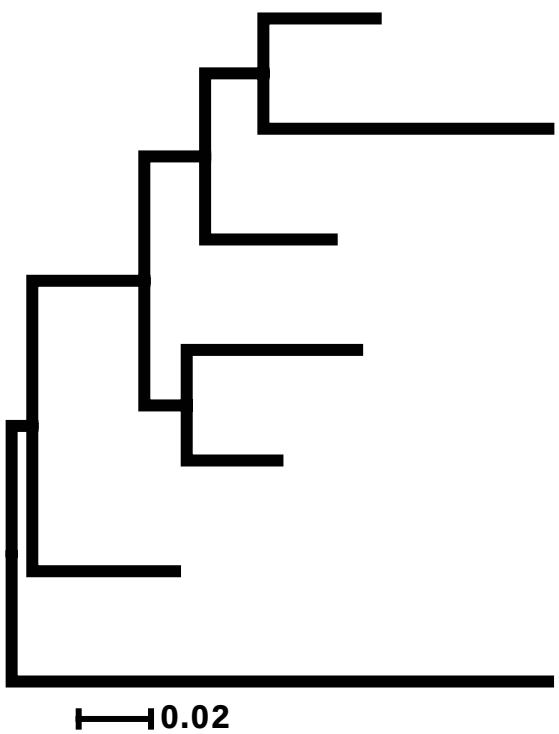


Cytoplasmic differentiation in Lupinus argenteus

- **Eleven haplotypes identified**
  - One shared among populations: population 2581 (freq = 1.0) and population 2733 (freq = 0.26)
- **Five of seven populations fixed for unique haplotype**
  - 1 haplotype: He = 0.0
  - Fixation occurs in proximal populations (e.g., 2720 vs. 2608)
- **Two populations showed high diversity**
  - 2733: 3 haplotypes, H_e = 0.521
  - 2586: 4 haplotypes, H_e = 0.671



Nuclear differentiation in Lupinus argenteus



	2718	2733	2608	2581	2720	2586	2703
Power ID 2718	-						
White Pine NV 2733	0.19	-					
Washington UT 2608	0.24	0.26	-				
SanJuan UT 2581	0.25	0.25	0.26	-			
Lincoln NV 2720	0.12	0.15	0.08	0.22	-		
Juab UT 2586	0.28	0.29	0.31	0.33	0.25	-	
Utah UT 2703	0.42	0.48	0.51	0.53	0.42	0.42	

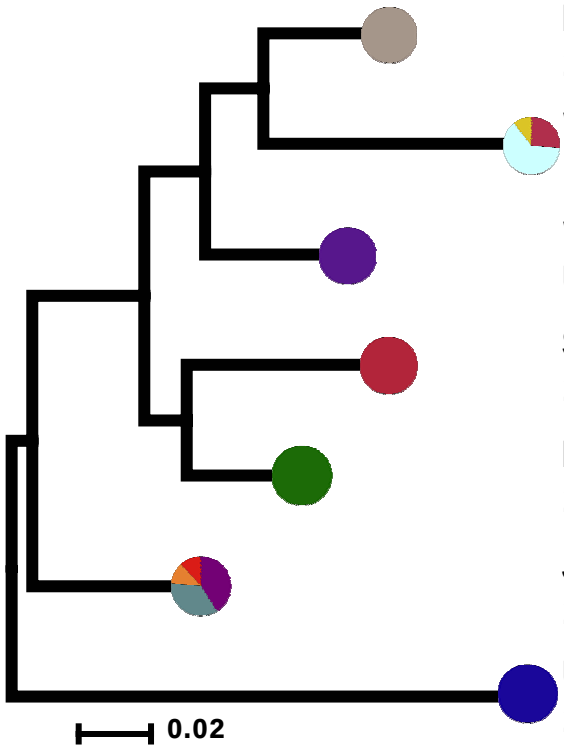
Is this a different species?

F_{ST} **Nuclear Data**

$P \leq 0.0036$
(5% nominal level, with sequential Bonferroni correction for multiple tests)

NJ phenogram based on Nei's genetic distances

Cytoplasmic differentiation in Lupinus argenteus



	2718	2733	2608	2581	2720	2586	2703
Power ID 2718	-	0.71	1	1	1	0.64	1
White Pine NV 2733	0.19	-	0.71	0.62	0.73	0.36	0.73
Washington UT 2608	0.24	0.26	-	1	1	0.64	1
SanJuan UT 2581	0.25	0.25	0.26	-	1	0.63	1
Lincoln NV 2720	0.12	0.15	0.08	0.22	-	0.66	1
Juab UT 2586	0.28	0.29	0.31	0.33	0.25	-	0.66
Utah UT 2703	0.42	0.48	0.51	0.53	0.42	0.42	-

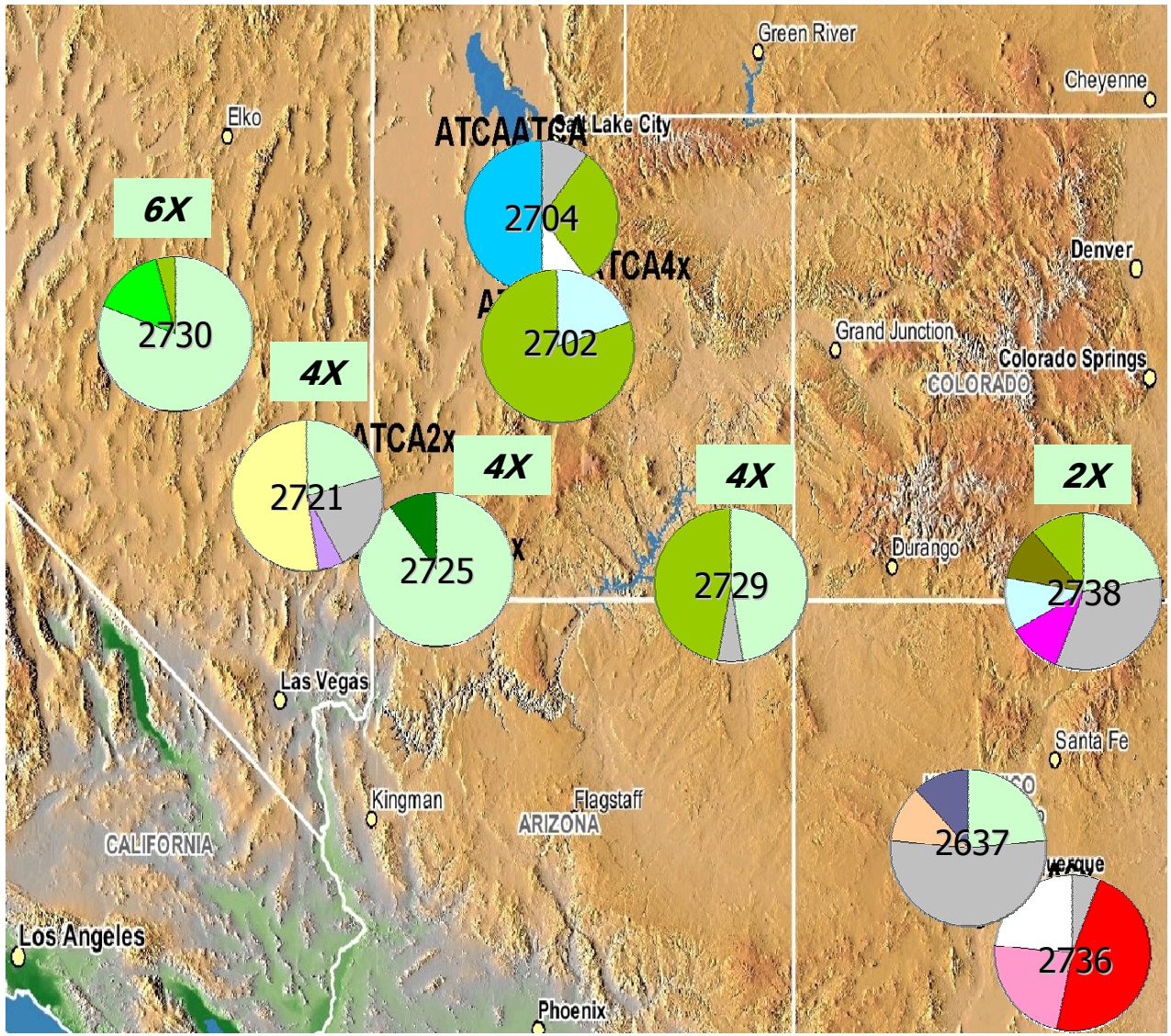
F_{ST} Cytoplasm

$P \leq 0.0036$
(5% nominal level, with sequential Bonferroni correction for multiple tests)

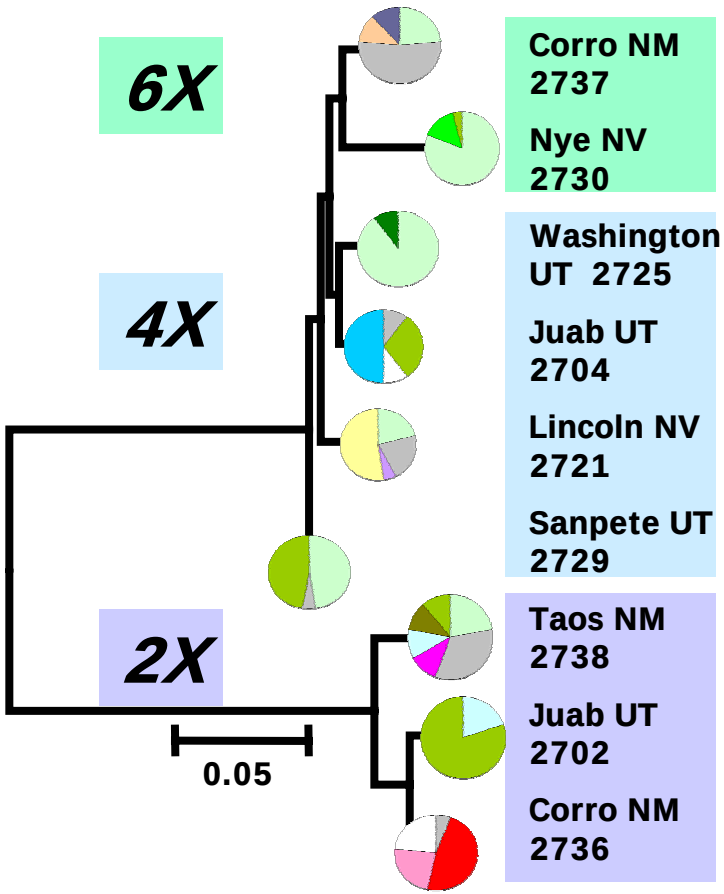
Cytoplasmic differentiation significant among sources showing non-significant allozyme differentiation

Cytoplasmic differentiation in Atriplex canescens

- **Outstanding cpDNA diversity relative to other species**
  - 15 haplotypes across 9 wild populations
  - 4.1 haplotypes per population (N = 20)
- **Low differentiation across populations**
  - Two haplotypes common across study
- **Haplotypes shared across ploidy levels**



Genetic differentiation in Atriplex canescens



	2737	2730	2725	2704	2721	2729	2738	2702	2736
Corro NM 2737		0.31	0.40	0.27	0.20	0.27	0.02	0.41	0.29
Nye NV 2730	0.18		0.06	0.42	0.33	0.18	0.16	0.53	0.42
Washington UT 2725	0.15	0.19		0.54	0.54	0.25	0.30	0.73	0.51
Juab UT 2704	0.15	0.32	0.09		0.30	0.25	0.13	0.23	0.27
Lincoln NV 2721	0.11	0.08	0.17	0.15		0.26	0.13	0.23	0.31
Sanpete UT 2729	0.05	0.17	0.08	0.02	0.09		0.10	0.18	0.36
Taos NM 2738	0.14	0.17	0.20	0.27	0.17	0.17		0.22	0.13
Juab UT 2702	0.10	0.02	0.25	0.39	0.13	0.24	0.19		0.40
Corro NM 2736	0.06	0.33	0.19	0.32	0.08	0.23	0.18	0.02	

F_{ST} Cytoplasm $P \leq 0.0022$

F_{ST} Nucleus $F_{ST} \geq 0.25$

Summary of findings from the survey

- **Nucleus and cytoplasm differ in magnitude, pattern of genetic diversity**
  - **Plants with low nuclear diversity may show abundant cytoplasmic diversity (and vice-versa)**
  - **Non-significant nuclear differentiation and significant cytoplasmic differentiation can be seen in same plants**

Important for germplasm evaluation, characterization – cpDNA may provide a region-specific marker

- **Cytoplasmic differentiation >> nuclear differentiation***
  - **In most cases, difference accounted for by smaller *Ne* of cpDNA**
  - **Atriplex and Lupine are striking exceptions! Why is $F_{ST_{cp}}$ so low / high?**
    ...unusual seed movement... biparental cp transmission... selection...

Important for considering the geographic scale of non-neutral adaptive traits

Remaining questions, analyses, ideas…

- How consistent are these trends?
  - More populations, species being added
- What is the pattern/magnitude of diversity in seeded populations? What can we say about the apparent rate of seed diffusion? Of pollen flow?
  - Case studies for *Artemesia, Atriplex, Purshia*
- How frequently are misidentified materials used in restoration?
  - Unusual genotypes present at a measurable frequency
  - Critical need for a reference database that integrates taxonomic and genetic information
- (your good ideas go here _____)

ACKNOWLEDGEMENTS

Shrub Sciences Laboratory

Stewart C. Sanderson, Jeffrey E. Ott, Jeffrey R. Taylor,, Nancy L. Shaw, Bracken Davis, Gary Jorgensen, Ann Debolt, Melissa Scholten, and Jane Struttmann

National Forest Genetics Laboratory

Barbara Wilson, Jennifer DeWoody, Ashley Lindstrom, Pat Guge, Randy Meyer, Robert Saich, Ricardo Hernandez, Suellen Carroll

PNW Genetics Team

Matt Horning, Brian Knaus, Rebecca Huot, Susan Huber

Funding Support

USDI Bureau of Land Management Native Plant Program

USDA Forest Service National Fire Plan

PNW Research Station