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

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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
Species Included in the Genetic Survey

Shrubs
- Artemesia tridentata
- Atriplex canescens
- Ericameria (Chrysothamnus) nauseosa
- Purshia tridentata

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

Grasses
- Bromus carinatus
- Hesperostipa (Stipa) comata
- Achnatherum (Oryzopsis) hymenoides

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)

- **Organelllar 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 $\sim$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)

- **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’

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

** Horning and Cronn, in review
Indices of diversity

- **Allozymes**: % polymorphic loci (P), average alleles per locus (A), expected heterozygosity (He)
- **cpDNA**: Total haplotypes, expected heterozygosity (He)

Index of differentiation: $F_{ST}$

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

- Computed using ANOVA ($\Theta$), 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

- $H_0$: $F_{ST}$ nuclear genes = $F_{ST}$ maternal organelles when $N_e$ included
  $$\text{Exp } F_{ST_{cp}} = 6 \cdot F_{ST_{nuc}} / [2 + (4 \cdot F_{ST_{nuc}})]$$
- Significance of observed difference tested by bootstrapping approach (Hamilton and Miller, 2002)
## Genetic diversity across species examined

<table>
<thead>
<tr>
<th>Species</th>
<th>Pops</th>
<th>% P</th>
<th>A</th>
<th>H&lt;sub&gt;e&lt;/sub&gt;</th>
<th>N&lt;sub&gt;Hap&lt;/sub&gt; / Pop (N&lt;sub&gt;Hap&lt;/sub&gt;)</th>
<th>H&lt;sub&gt;e&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Astragalus utahensis</em></td>
<td>4</td>
<td>63</td>
<td>2.09</td>
<td>0.217</td>
<td>1.25 (5)</td>
<td>0.690</td>
</tr>
<tr>
<td><em>Atriplex canescens</em></td>
<td>9</td>
<td>61</td>
<td>1.92</td>
<td>0.139</td>
<td>1.67 (15)</td>
<td>0.835</td>
</tr>
<tr>
<td><em>Bromus carinatus</em></td>
<td>2</td>
<td>19</td>
<td>1.24</td>
<td>0.062</td>
<td>2.0 (4)</td>
<td>0.709</td>
</tr>
<tr>
<td><em>Crepis acuminata</em></td>
<td>4</td>
<td>58</td>
<td>1.75</td>
<td>0.117</td>
<td>1.0 (5)</td>
<td>0.610</td>
</tr>
<tr>
<td><em>Eriogonum umbellatum</em></td>
<td>4</td>
<td>67</td>
<td>2.11</td>
<td>0.195</td>
<td>1.25 (5)</td>
<td>0.835</td>
</tr>
<tr>
<td><em>Hesperostipa comata</em></td>
<td>3</td>
<td>20</td>
<td>1.22</td>
<td>0.078</td>
<td>1.67 (5)</td>
<td>0.685</td>
</tr>
<tr>
<td><em>Lupinus argenteus</em></td>
<td>7</td>
<td>100</td>
<td>2.8</td>
<td>0.272</td>
<td>1.57 (11)</td>
<td>0.870</td>
</tr>
<tr>
<td><em>Vicia americana</em></td>
<td>3</td>
<td>54</td>
<td>1.71</td>
<td>0.119</td>
<td>1.0 (3)</td>
<td>0.667</td>
</tr>
</tbody>
</table>
## Genetic differentiation across species

<table>
<thead>
<tr>
<th>Species</th>
<th>Nuclear $F_{ST}$</th>
<th>cpDNA $F_{ST}$</th>
<th>cpDNA $F_{ST,(exp)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Atriplex canescens</em></td>
<td>0.285</td>
<td>&gt;</td>
<td>0.280</td>
</tr>
<tr>
<td><em>Astragalus utahensis</em></td>
<td>0.357</td>
<td>&lt;</td>
<td>0.394</td>
</tr>
<tr>
<td><em>Crepis acuminata</em></td>
<td>0.276</td>
<td>&lt;</td>
<td>0.580</td>
</tr>
<tr>
<td><em>Eriogonum umbellatum</em></td>
<td>0.318</td>
<td>&lt;</td>
<td>0.685</td>
</tr>
<tr>
<td><em>Bromus carinatus</em></td>
<td>0.634</td>
<td>&lt;</td>
<td>0.694</td>
</tr>
<tr>
<td><em>Lupinus argentatus</em></td>
<td>0.314</td>
<td>&lt;</td>
<td>0.863</td>
</tr>
<tr>
<td><em>Hesperostipa comata</em></td>
<td>0.885</td>
<td>&lt;</td>
<td>0.938</td>
</tr>
<tr>
<td><em>Vicia americana</em></td>
<td>0.299</td>
<td>&lt;</td>
<td>0.958</td>
</tr>
</tbody>
</table>
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*

<table>
<thead>
<tr>
<th>Population</th>
<th>Allozyme Data</th>
<th>cpDNA Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% P</td>
<td>A</td>
</tr>
<tr>
<td>San Juan UT (2581)</td>
<td>52.2</td>
<td>1.7</td>
</tr>
<tr>
<td>White Pine NV (2733)</td>
<td>65.2</td>
<td>1.9</td>
</tr>
<tr>
<td>Washington UT (2608)</td>
<td>52.2</td>
<td>1.7</td>
</tr>
<tr>
<td>Lincoln NV (2720)</td>
<td>91.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Juab UT (2586)</td>
<td>56.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Power ID (2718)</td>
<td>65.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Utah UT (2703)</td>
<td>47.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Population means</td>
<td>100</td>
<td>2.8</td>
</tr>
</tbody>
</table>
Eleven haplotypes identified

- One shared among populations: population 2581 (freq = 1.0) and population 2733 (freq = 0.26)
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: $H_e = 0.0$
  - 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*

**NJ phenogram based on Nei’s genetic distances**

Is this a different species?

\[ F_{ST} \] Nuclear Data

\[ P \leq 0.0036 \]

(5% nominal level, with sequential Bonferroni correction for multiple tests)
Cytoplasmic differentiation in Lupinus argenteus

Cytoplasmic differentiation significant among sources showing non-significant allozyme differentiation

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

*P* ≤ 0.0036
(5% nominal level, with sequential Bonferroni correction for multiple tests)
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*

<table>
<thead>
<tr>
<th>Location</th>
<th>FST Nucleus</th>
<th>FST Cytoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corro NM 2737</td>
<td>0.31 0.40 0.27 0.20 0.27 0.02 0.41 0.29</td>
<td>0.18 0.06 0.42 0.33 0.18 0.16 0.53 0.42</td>
</tr>
<tr>
<td>Nye NV 2730</td>
<td>0.18</td>
<td>0.19</td>
</tr>
<tr>
<td>Washington UT 2725</td>
<td>0.15 0.19 0.54 0.54 0.25 0.30 0.73 0.51</td>
<td>0.15 0.32 0.09 0.30 0.25 0.13 0.23 0.27</td>
</tr>
<tr>
<td>Juab UT 2704</td>
<td>0.15</td>
<td>0.32</td>
</tr>
<tr>
<td>Lincoln NV 2721</td>
<td>0.11 0.08 0.17 0.15 0.26 0.13 0.23 0.31</td>
<td>0.05 0.17 0.08 0.02 0.09 0.10 0.18 0.36</td>
</tr>
<tr>
<td>Sanpete UT 2729</td>
<td>0.05 0.17 0.08 0.02 0.09 0.10 0.18 0.36</td>
<td>0.14 0.17 0.20 0.27 0.17 0.17 0.22 0.13</td>
</tr>
<tr>
<td>Taos NM 2738</td>
<td>0.14</td>
<td>0.17</td>
</tr>
<tr>
<td>Juab UT 2702</td>
<td>0.10</td>
<td>0.02</td>
</tr>
<tr>
<td>Corro NM 2736</td>
<td>0.06</td>
<td>0.33</td>
</tr>
</tbody>
</table>

*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 ___________________________)
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