Genetic changes associated with native plant propagation: case study in *Castilleja levisecta*

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Study species

*Castilleja levisecta*

golden paintbrush

- short-lived perennial
- Orobanchaceae
- hemiparasitic
- **effectively self-incompatible**
- pollinated by *Bombus*
- federally threatened

Photo: Tom Kaye
Historic range

Pacific Northwest

• British Columbia
• Washington
• Oregon

Threatened due to habitat loss:
• urbanization
• non-native species
• forest encroachment
Current range

- 11 extant populations
- most occur in islands of the Puget Sound
Reintroduction

- began in 2005
- all in this study occurred in 2010-2011
- Two planting techniques
  - seed
  - plug
Reintroduction

Wild seed collected

Grown in seed-increase beds

Outplanted in reintroduction sites

Genetic Change?
Genetic diversity…

Why is it important?

Restorations using plants with higher genetic diversity have:

✓ faster recovery after climactic extremes
   (Hughes and Stachowicz 2004)

✓ increased resistance to pests and pathogens
   (Tooker and Frank 2012)

✓ improved establishment success
   (Crawford and Whitney 2011)

✓ more potential to respond to a changing climate
   (Jump et al. 2009)
Genetic diversity…

What is it?

“The extent of genetic variation in a population or species” (Frankham 2013)

- variants and proportion of genes
  (number of alleles, Na; number of effective alleles, Ne)
- pairing of alleles within an individual
  (observed heterozygosity, Ho; expected heterozygosity, He)
- differences between individuals (inbreeding, F)
- differences between populations (structure, Fst)
Genetic diversity...

What is it... for this study?

- inbreeding depression
- "local" adaptation
- seed transfer zones

✔ microsatellites: non-adaptive (neutral)
Potential means of genetic change through propagation

Potential means of genetic change through propagation

- Each grey box represents an action
- Each action has inherent potential to change genetic diversity
- As plants are held in production longer, there is greater risk of genetic change

Nursery design

Wild:
- Naas (N)
- Rocky Prairie (R)
-Ebey’s Landing (E)
-Fort Casey (C)

Corvallis Plant Materials Center (ORPMC)

Webster Nursery
Nursery design

Wild:
- Naas (N)
- Rocky Prairie (R)
- Ebey’s Landing (E)
- Fort Casey (C)

Corvallis Plant Materials Center (ORPMC)

Webster Nursery
Study populations

- **Wild**
  - N
  - R
  - E
  - C

- **Nursery**
  - ORPMC
    - NS
    - RS
    - ES
    - CS
  - Web

- **Reintroduction**
  - Plug from ORPMC

- **Seed from ORPMC**
- **Seed from Webster**
- **Smith Prairie**

- **pop n = 21**
- **sample n = 617**
- nursery material = 1\textsuperscript{st} generation
- reintroduction = 2\textsuperscript{nd} generation
Study question

- Does propagation in the nursery change genetic diversity?

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Bottleneck</th>
<th>Outbreeding</th>
<th>Status quo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of alleles</td>
<td>↓</td>
<td>↑</td>
<td>no change</td>
</tr>
<tr>
<td>Inbreeding value</td>
<td>no change</td>
<td>↓</td>
<td>no change</td>
</tr>
<tr>
<td>Genetic structure (Fst)</td>
<td>high structure, genetically similar to one source</td>
<td>low structure, genetically variable</td>
<td>structure will mirror wild populations</td>
</tr>
</tbody>
</table>
Methods: data collection

**DNA extraction**

**DNA amplification**

**leaf collection**

**genetic analysis**

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| UFP28 | 14 | 101 | 101 | 182 | 182 | 225 | 225 | 241 | 241 | 210 | 210 | 165 | 165 | 241 | 247 | 202 | 241 |
| UFP30 | 14 | 101 | 101 | 182 | 191 | 225 | 227 | 247 | 253 | 210 | 216 | 174 | 174 | 241 | 244 | 202 | 202 |
| UFP32 | 14 | 101 | 110 | 182 | 200 | 225 | 227 | 247 | 253 | 210 | 216 | 171 | 174 | 244 | 247 | 217 | 235 |
| UFP34 | 14 | 101 | 101 | 191 | 191 | 227 | 242 | 247 | 247 | 210 | 210 | 165 | 174 | 244 | 247 | 205 | 220 |
| UFP36 | 14 | 107 | 107 | 191 | 191 | 227 | 227 | 247 | 247 | 210 | 210 | 174 | 174 | 247 | 247 | 238 | 244 |
| UFP37 | 14 | 101 | 101 | 182 | 182 | 219 | 225 | 250 | 253 | 210 | 210 | 165 | 165 | 247 | 247 | 202 | 205 |
| UFP38 | 14 | 104 | 107 | 182 | 191 | 221 | 242 | 247 | 253 | 213 | 216 | 168 | 174 | 244 | 247 | 211 | 220 |
| UFP39 | 14 | 101 | 107 | 182 | 191 | 219 | 227 | 247 | 250 | 210 | 213 | 174 | 180 | 244 | 247 | 214 | 235 |
Methods: data analysis

- **GenAlEx**
  - number of alleles (Na)
  - number of effective alleles (Ne)
  - observed heterozygosity (Ho)
  - expected heterozygosity (He)
  - inbreeding coefficient (F)
  - population differentiation (Fst)

- **R statistical software**
  - analyses of variance (ANOVA)
  - Tukey HSD

- **Structure**
  - visualize genetic similarity
## Results: diversity

<table>
<thead>
<tr>
<th>Analysis of Variance (ANOVA) results</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>Diversity Index</td>
<td>p value</td>
</tr>
<tr>
<td>Number of alleles (Na)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number of effective alleles (Ne)</td>
<td>0.02</td>
</tr>
<tr>
<td>Observed heterozygosity (Ho)</td>
<td>0.006</td>
</tr>
<tr>
<td>Effective heterozygosity (He)</td>
<td>0.01</td>
</tr>
<tr>
<td>Inbreeding (F)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Yes, the groups differ!
Results: diversity

Wild:
- vary considerably

Nursery:
- looks similar to wild populations… BUT!
- Higher inbreeding

Reintroductions:
- all were higher in number of alleles
- differ from nursery inbreeding
- seed and plug techniques do not differ

<table>
<thead>
<tr>
<th>Location</th>
<th>Code</th>
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<tbody>
<tr>
<td>Naas Seed Bed</td>
<td>NS</td>
</tr>
<tr>
<td>Rocky Prairie Seed Bed</td>
<td>RS</td>
</tr>
<tr>
<td>Ebey’s Landing Seed Bed</td>
<td>ES</td>
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Number of alleles

Inbreeding coefficient
## Results: diversity

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</tr>
<tr>
<td>(Na,Ne)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inbreeding</td>
<td>no change</td>
<td>↓↓</td>
<td>no change</td>
</tr>
<tr>
<td>(Ho,He,F)</td>
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<td>(Fst)</td>
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Results: structure

High gene flow – low structure

Low gene flow – high structure
Results: structure

Rocky Prairie differs from the rest

Naas and Fort Casey more similar than Ebey’s Landing are to either

Each cluster well represents a population
Results: structure

- nursery populations mirror wild sources
- reintroductions from ORPMC display admixture of wild sources
- reintroductions from Webster show less consistency
Smith Prairie

- The only population that was not allowed to cross-pollinate within the nursery design
And...

- The only population where the reintroduction site showed a decrease in the number of alleles from the wild source population
Steps in nursery propagation within study

- Smith Prairie results highlight the influence of cross-pollination in this study.
- Other studies looking at nursery propagation without this step may have very different results.
### Results

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Conclusions

- Nursery production successfully increased genetic diversity
  - reintroduction sites show higher number of alleles and lower inbreeding
  - nursery production provides a more relaxed selection than in the wild

- Wild communities show unique genetic signatures

- Nursery populations tended to have higher inbreeding
  - could impact reintroduction sites if:
    - inbred plants survive through outplanting
    - inbred plants are held through multiple generations in the nursery, compounding the effects

- Source-identified nursery rows benefit production results
Success! How was this accomplished?

- careful site selection
- defined seed-collection protocol

- beneficial germination conditions
- source-identified rows allows control over seed mix
- plants grown in seed increase beds for sing
Acknowledgments

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Questions?

Photo: Dave Skaar
References

Frankham, R. et al. Predicting the probability of outbreeding depression. Conservation Biology. 25(3) 465-475