

Restoration Effects on Genetic Diversity of *Castilleja levisecta*

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Castilleja levisecta

- Golden paintbrush
- Perennial
- Hemiparasitic
- Endemic to Pacific Northwest
- Threatened by competition with native and non-native species and human encroachment



Castilleja levisecta

- Only known sites in Washington and British Columbia
- Federally threatened in the United States
- Endangered in Washington
- Former populations in Oregon, not seen for 40 years
- In 2009 given global ranking of G1 (critically imperiled)
- Restorations ongoing

Populations Studied

- Nursery population grown from 5 wild populations as seed sources
- Restoration population grown from nursery as seed source
- 3 wild populations

Microsatellites

- Simple Sequence Repeats (SSRs)
- “Neutral” genetic marker
- Tandem base pair repeats (i.e. (GTT)₈)
- Highly conserved flanking region
- Variable number of repeats
- Tend to be species specific
- Codominant marker
- Can be amplified by PCR

Fitness and Genetic Diversity

- Neutral genetic diversity is not a measure of fitness
- Several studies have shown high positive correlation between genetic diversity and fitness¹

1: Fischer et al. 2003; Reed and Frankham 2003; Dostalek et al. 2010

Question

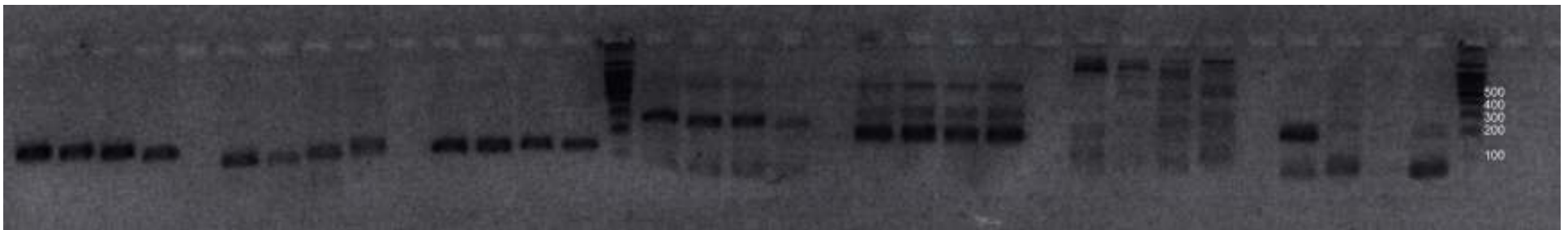
How does the restoration of *Castilleja levisecta* to former habitats, affect genetic diversity?

Methods

- CTAB Extraction
- Nanodrop
- PCR
- Electrophoresis (presence/absence)
- Fluorescent tag
- CEQ™ 8000 Genetic Analysis System-
fragment analysis

Primer Selection

- Primers tested based on successful amplification of other *Castilleja* species²
- Selected based on verification from gel electrophoresis (1.5% agarose)



Fant, J.B., H. Weinberg-Wolf, D.C. Tank, K.A. Skogen. 2012. Characterization of Microsatellite Loci in *Castilleja Sessiliflora* and Transferability to 24 *Castilleja* Species (Orobanchaceae). *Applications in Plant Sciences* 2013 1(6): 12000564.

PCR

Step 1:

- 95° - 3 min
 - 94° - 40 sec
 - 53° - 40 sec
 - 72° - 1 min
 - 72° - 10 min
-) x 15

Step 2:

- 95° - 3 min
 - 94° - 40 sec
 - 55° - 40 sec
 - 72° - 1 min
 - 72° - 10 min
-) x 25

Sample Fragment Analysis

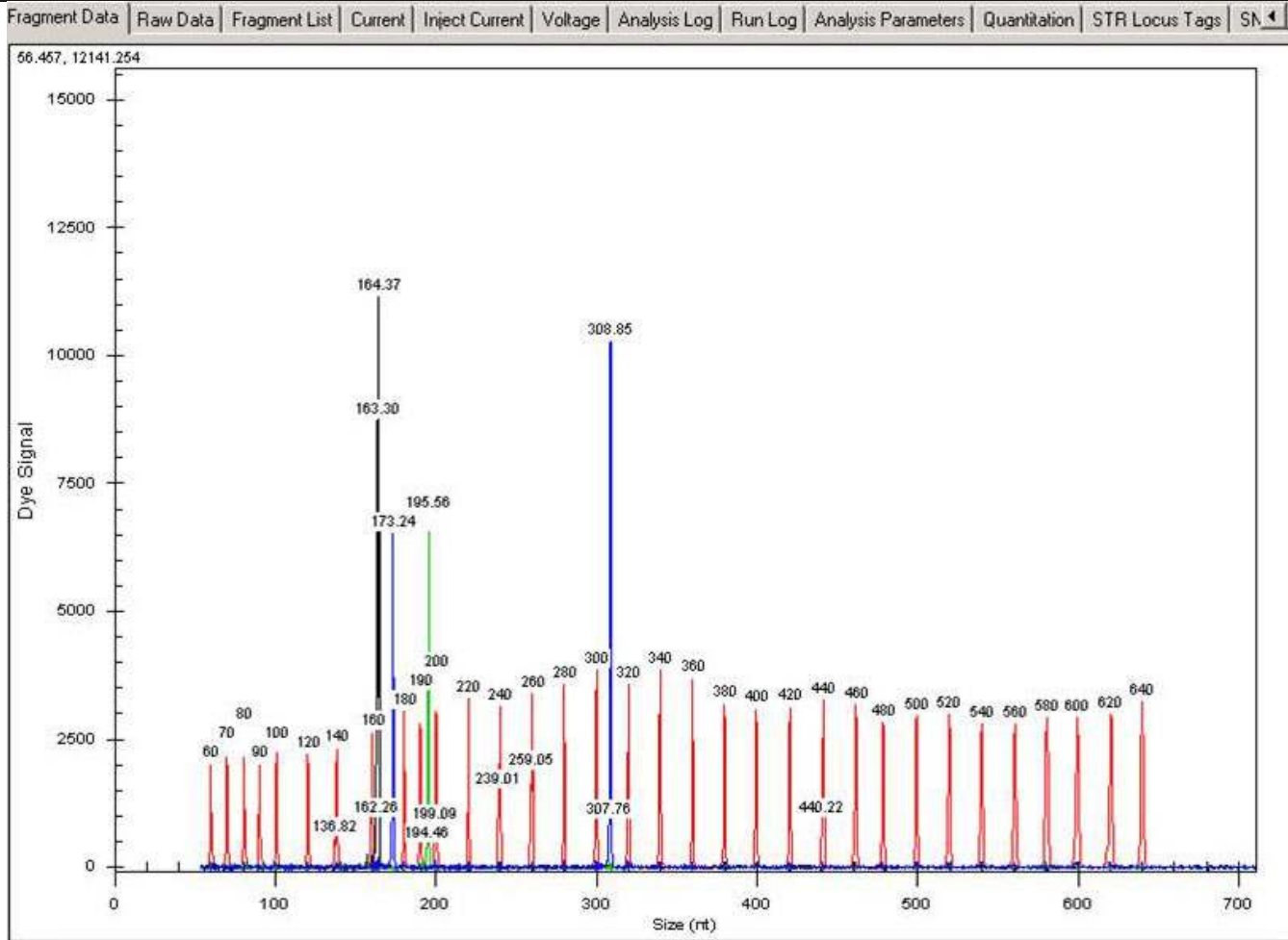


Figure 1: Sample fragment analysis - CEQ™ 8000 Genetic Analysis System

Results

- N- mean number of samples per locus
- Na- (allelic richness) mean number of alleles per locus
- Ho- mean observed heterozygosity per locus
- He- mean estimated heterozygosity per locus
- He: $(1-\sum p_i^2)$

Table 1: Measures of genetic diversity per population averaged over all loci

| | N | Na | Ho | He |
|--------------------|----------|-----------|-----------|-----------|
| Wild 1 | 21.333 | 2.556 | 0.452 | 0.367 |
| | | | | |
| Wild 2 | 24.667 | 6.667 | 0.504 | 0.721 |
| | | | | |
| Wild 3 | 22.444 | 2.889 | 0.569 | 0.425 |
| | | | | |
| Nursery | 20.333 | 4.000 | 0.409 | 0.467 |
| | | | | |
| Restoration | 22.222 | 4.778 | 0.527 | 0.478 |

% Association per Population

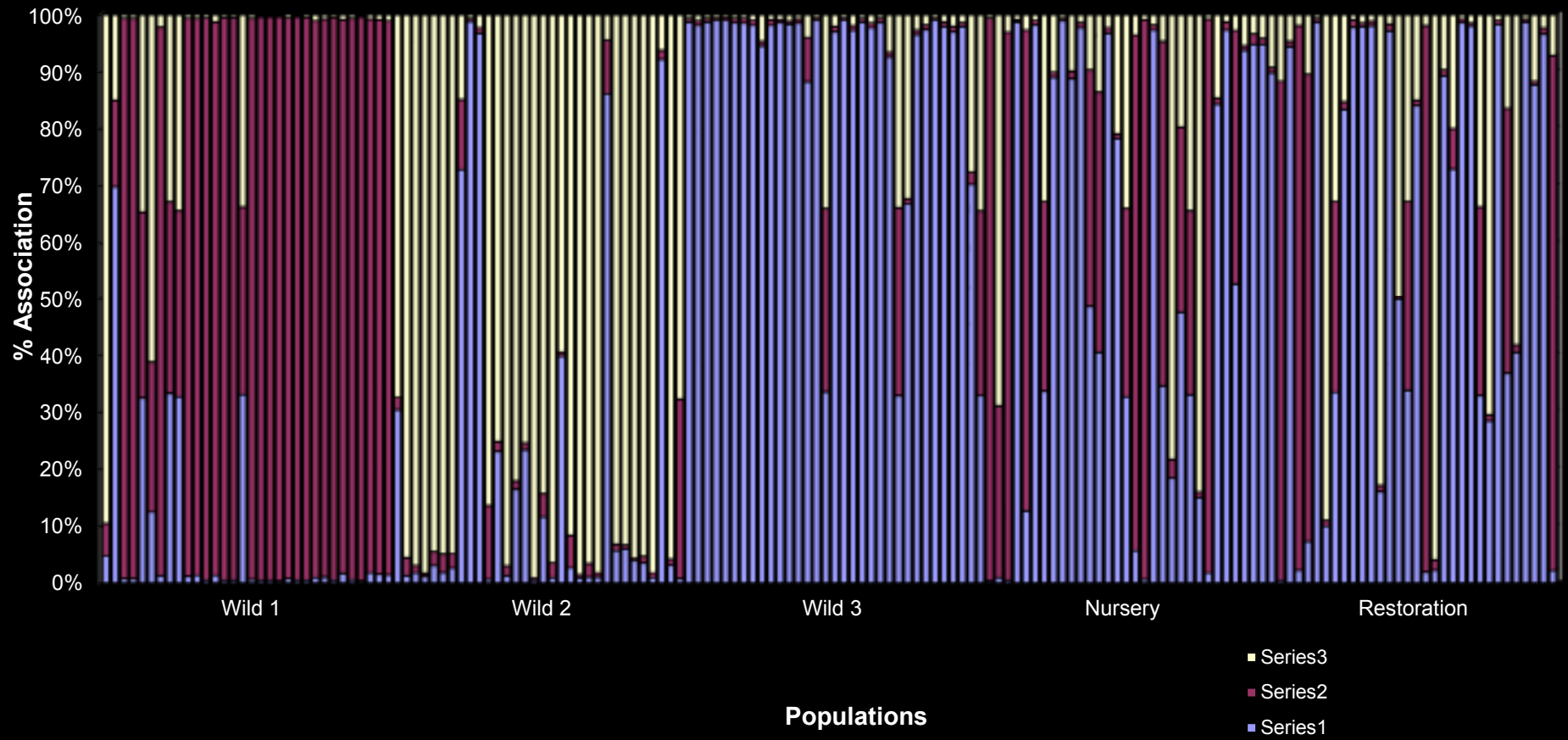


Figure 2: Bayesian admixture proportions for individual plant samples and populations for 3 wild populations, a nursery and a restoration population

Genetic Diversity Compared to Measure of Fitness

Fitness

Diversity

Table 2: Empirical measures of fitness³ compared to measure of genetic diversity (expected heterozygosity)

| | Proportion producing flowers ³ | Proportion producing fruit ³ | Proportion of Transplants surviving to 2005 ³ | He |
|--------|---|---|--|-------|
| Wild 1 | 0.21 ± 0.05 | 0.12 ± 0.04 | 0.16 ± 0.04 | 0.367 |
| Wild 2 | 0.65 ± 0.03 | 0.34 ± 0.03 | 0.22 ± 0.03 | 0.721 |

Lawrence, B.A., T.N. Kaye. Reintroduction of *Castilleja levisecta*: Effects of Ecological Similarity, Source Population Genetics, and Habitat Quality. *Society for Ecological Restoration International* doi: 10.1111/j.1526-100X.2009.00549.x.

Discussion

- Nursery and restoration populations show higher allelic richness (N_a) and estimated heterozygosity (H_e) than 2 wild populations
- Highest allelic richness and estimated heterozygosity in Wild 2 population, due to presence of unique private alleles
- Nursery and restoration populations' statistics show the effects of mixing seed sources
- Correlation and trend of genetic diversity and fitness
- Wild 3 population a dominant founder in nursery and restoration populations

Conclusion

- Good sampling of seed sources for genetic variation of nursery and restoration populations
- Gain in genetic diversity from two of the wild populations in the restoration
- Possible exclusion of Wild 3 population from use as seed source due to outbreeding depression and “swamping” of alleles
- No loss of genetic diversity present in restoration of *Castilleja levisecta*

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References

- Fischer, M., M. Hock, and M. Paschke. 2003. Low genetic variation reduces cross-compatibility and offspring fitness in populations of narrow endemic plant with a self-incompatibility system. *Conservation Genetics* 4:325-36.
- Reed, D. H., and R. Frankham. 2003. Correlation between fitness and genetic diversity. *Conservation Biology* 17:230-37.
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