Population genetic analysis of disjunct Ozark and Appalachian populations of tall larkspur (*Delphinium exaltatum*)

Rebekah A. Mohn
Miami University, Oxford, Ohio
mohnra@miamioh.edu
Mentor: Christine E. Edwards
Species Concept

• “A separately evolving metapopulation lineage” where lineage refers to an “ancestor-descendent lineage.” (De Queiroz, 2007)
Disjunct plant distributions

- The Appalachian and Ozark disjunction. (400 miles)
- Causes of disjunction:
  - Long-distance dispersal
  - Glaciation
  - Recolonization from refugia
  - A hot and dry period following glacial retreat

**Delphinium exaltatum** Aiton

- Perennial herb with disjunct distribution.
- Flowers insect pollinated and self-compatible.
- Seeds are gravity or water dispersed.
- Conservation status: vulnerable.
Research Questions

• How is genetic variation structured in Delphinium exaltatum?

• What biogeographical factors have affected patterns of genetic structure?

• Do patterns of genetic structure reflect the disjunction between the two groups of Delphinium exaltatum?

• Is there evidence that D. exaltatum may contain more than one species?
Hypothesis

• Null hypothesis: Genetic structure does not reflect the disjunction between the two groups of *Delphinium exaltatum*.

• Genetic structure reflects the disjunction between the two groups of *Delphinium exaltatum* supporting distinction taxonomically of the two groups.
### Sampling

<table>
<thead>
<tr>
<th>Abbr.</th>
<th>Voucher</th>
<th>County, State</th>
<th>(n_s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MO1</td>
<td>S. Farrington, et al. 12-16 (MO)</td>
<td>Howell, MO</td>
<td>22</td>
</tr>
<tr>
<td>MO2</td>
<td>E. Haglund, et al. OZAR 43809 (ONSR*)</td>
<td>Shannon, MO</td>
<td>24</td>
</tr>
<tr>
<td>MO3</td>
<td>E. Haglund, et al. OZAR 43810 (ONSR*)</td>
<td>Shannon, MO</td>
<td>24</td>
</tr>
<tr>
<td>MO4</td>
<td>G. Yatskievych, et al. 12-054 (MO)</td>
<td>Shannon, MO</td>
<td>17</td>
</tr>
<tr>
<td>MO5</td>
<td>G. Johnson, et al. s.n. (MO)</td>
<td>Shannon, MO</td>
<td>10</td>
</tr>
<tr>
<td>NC1</td>
<td>Y. Johnson s.n. (MO)</td>
<td>Watauga, NC</td>
<td>22</td>
</tr>
<tr>
<td>NC2</td>
<td>Y. Johnson s.n. (MO)</td>
<td>Durham, NC</td>
<td>22</td>
</tr>
<tr>
<td>PA1</td>
<td>S.P. Grund 6219 (MO)</td>
<td>Huntington, PA</td>
<td>13</td>
</tr>
<tr>
<td>TN1</td>
<td>R. Cook s.n. (MO)</td>
<td>Anderson, TN</td>
<td>23</td>
</tr>
<tr>
<td>VA1</td>
<td>A. Hyduke, et al. 11 (MO)</td>
<td>Montgomery, VA</td>
<td>21</td>
</tr>
<tr>
<td>WV1</td>
<td>P. J. Harmon, et al. 3490 (MO)</td>
<td>Mecer, WV</td>
<td>23</td>
</tr>
</tbody>
</table>
**Microsatellites**

**Microsatellite**: Small base pair repeats in the nuclear DNA.

**Locus**: A specific microsatellite (or other genetic fragment) that is usually isolated with a primer.

**Diploid**: every individual has two alleles per locus.

**Allele**: The number of base pairs at a microsatellite.

**Homozygote**: an individual with two of the same allele.

**Heterozygote**: an individual with two different alleles.

**PCR**: Polymerase Chain Reaction.
Sample collection

DNA extraction
  • CTAB

DNA amplified
  • PCR
  • Yale analyzed

Data Scored
  • Microsatellites scored

Data Analysis
  • Structure
  • Clumpak
  • MSA
  • Genepop
  • BOTTLENECK
  • INEST
  • Arlequin
  • FSTAT
  • Genodive

Results
Population Genetics
Results
Genetic Divergence

Structure
Genetic Divergence

Structure

**Figure A:**
Distribution map with structure results.

**Figure B:** K=2 through K=5
Structure results.
Genetic Divergence

Population Genetic Patterns

An unrooted tree calculated with Nei’s genetic distance
Patterns of pairwise genetic structure in *D. exaltatum*. The genetic distance calculated by Jost’s D is reported above the diagonal (P67 was not included in the Jost’s D calculation).
Patterns of pairwise genetic structure in *D. exaltatum*. The genetic distance calculated by Jost’s D is reported above the diagonal (P67 was not included in the Jost’s D calculation).
### Genetic Divergence

#### Analysis of Molecular Variance (AMOVA)

**Percent of Variation**

<table>
<thead>
<tr>
<th></th>
<th>Among groups</th>
<th>Among populations within groups</th>
<th>Within populations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>{MO1, MO2, MO3, MO4, MO5} vs. {VA1, WV1, NC1, NC2, PA1, TN1}</strong></td>
<td>20.06</td>
<td>32.28</td>
<td>47.66</td>
</tr>
<tr>
<td></td>
<td><strong>40.15</strong></td>
<td><strong>17.7</strong></td>
<td><strong>42.15</strong></td>
</tr>
</tbody>
</table>
Genetic Divergence and Implications
• Genetic variation and structure is not according to the biogeographical boundaries and taxonomic distinction based on the disjunction is not supported.

• Divergence potentially to the level of speciation may have occurred between \{PA1, WV1, VA1, NC1\} and \{MO1, MO2, MO3, MO4, MO5, NC2, TN1\}.

• \{NC2\} has also diverged from \{MO1, MO2, MO3, MO4, MO5, TN1\}, possibly to the point of distinction.
Mode of Disjunction and Divergence

- {MO1, MO2, MO3, MO4, MO5}
  - Southern
  - Lower Elevations
  - Western

- {PA1, WV1, VA1, NC1}
  - Northern
  - Higher Elevations
  - Eastern

- {NC2, TN1}
  - Southern
  - Lower Elevations
  - Eastern
East-West Disjunction
Long-Distance Dispersal
Recolonization from coastal refugia
Hot and dry climate

- {MO1, MO2, MO3, MO4, MO5}
  - Western
- {PA1, WV1, VA1, NC1, TN1, NC2}
  - Eastern
Mode of Genetic Divergence

- {PA1, WV1, VA1, NC1}
  - Northern
  - Higher Elevations

- {MO1, MO2, MO3, MO4, MO5, NC2, TN1}
  - Southern
  - Lower Elevations

Genetic Divergence
Appalachian uplift
Glaciation
Conclusions and Future Research

• Genetic Divergence
  • The genetic structure does not reflect the biogeographical distribution and does not support taxonomic separation of Missouri from the Appalachian populations of *Delphinium exaltatum*.
  • Speciation may have occurred between the northern and southern populations.

• Future Research
  • Determine whether morphometric analyses support distinguishing the two groups as separate species.
  • Add an Ohio population to the genetic analysis to further explain the biogeographic and genetic distributions of various groups.
Acknowledgments

• Special thanks to Joel Swift, Burgund Bassüner, Alex Linan, George Yatskievych, Peter Hoch, David Bogler, Allison Miller, and the many other individuals who made this program possible. Thanks to NSF for funding and the Missouri Botanical Garden for hosting the REU.

• Thank you to Chrstopher T. Frye, Maile Neel, Tanja Schuster, Kerry Wixted, Donald Rohrback, Susan Farrington, Randall J. Evans, Grace Johnson, Dan Drees, Paul McKenzie, Liz Olson, Randy Evans, Erin Haglund, Yari Johnson, Steven Grund, Rebecca Cook, Abigail Hyduke, Tom Wieboldt, Paul J. Harmon, and Jeff Hajenga for collecting the vouchers and samples for DNA.