Analysis of the effect of fire on the genetic diversity and structure of *Polygala lewtonii* (Polygalaceae)

Elena M. Meyer¹,⁴, Joel F. Swift², Burgund Bassünér², Stacy A. Smith³, Eric S. Menges³, and Christine E. Edwards²

¹New College of Florida, Sarasota FL; ²Missouri Botanical Garden, St. Louis MO; ³Archbold Biological Station, Venus FL; ⁴Correspondence: elena.meyer15@ncf.edu
A dynamic, endangered ecosystem: Florida scrub

Florida scrub is a endangered ecosystem.

Fire is its dominant mode of ecological disturbance.

Fire events maintain these ecosystems.

Disturbance events can have a profound effect on the genetic makeup of a population.
Three Flower Types

Above-ground, closed, self pollinated (cleistogamous)

Above-ground, open, cross pollinated (chasmogamous)

Below-ground, closed, self pollinated (cleistogamous)
Two Seed Types
Below-ground seeds produced by the cleistogamous flowers are larger than the above-ground seeds, and generally less-numerous.
• These contribute to a below-ground seedbank, and are spread along the rhizome, germinating very close to the parental plant.

Above-ground seeds can be produced either by selfing, or outcrossing.
• They are smaller and more numerous, and can be dispersed by ants.
Background study by Swift et al\textsuperscript{8}

Swift et al investigated patterns of genetic structure by sampling \textit{P. lewtonii} at both fine-scale and range-wide scales.

At Carter Creek South:
- Four blocks
- 8 randomized 1 m plots within blocks

Found that:
1. Self-fertilization or inbreeding occurs with very limited reproduction via outcrossing.
2. Populations of \textit{P. lewtonii} are genetically differentiated at very small spatial scales.
This leads to another question: If outcrossing plays such a small role in the reproduction of the species, why does the plant invest in its plentiful, showy flowers?

Are outcrossed seeds failing to germinate?

Is this due to an ecological reason, such as lack of fire?

Is outcrossing not occurring, period?
Current research questions

• What are the effects of fire on the genetic diversity, favored mating system, and the genetic structure of populations of *P. lewtonii*?

• Do changes in ecological conditions following fire stimulate germination of outcrossed seeds or inbred seeds?

• Are outcrossed seeds even being produced by the above-ground flowers?
Sampling Methods

1. Re-sampling of post-fire leaf tissue from Carter Creek South using the same plots from Swift et al.
2. Sampling of seeds from above-ground open-pollinated flowers.

Molecular Methods

1. Preformed DNA extraction using a modified CTAB protocol.
2. PCR amplification utilizing the same 11 microsatellite loci used in Swift et al to genotype individuals.
3. Microsatellite peak scoring, data analysis, and comparison with pre-fire results.
Post-fire Analysis Results

Heterozygosity

Expected heterozygosity ($H_e$) and observed heterozygosity ($H_0$) remained low overall.

<table>
<thead>
<tr>
<th></th>
<th>$H_0$</th>
<th>$H_e$</th>
<th>$F_{IS}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-fire</td>
<td>0.040</td>
<td>0.436</td>
<td>0.909</td>
</tr>
<tr>
<td>Post-fire</td>
<td>0.052</td>
<td>0.425</td>
<td>0.878</td>
</tr>
</tbody>
</table>

This shows that the selfing rate remains very high, even after fire.
**Goal:** To examine the effects of fire on the *genetic diversity*

### Post-fire Analysis Results

#### Private Alleles within Carter Creek Population

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>Freq</th>
<th>Locus</th>
<th>Allele</th>
<th>Freq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-fire</td>
<td>PL56</td>
<td>194</td>
<td>0.011</td>
<td>Post-fire</td>
<td>PL80</td>
</tr>
<tr>
<td>Pre-fire</td>
<td>PL56</td>
<td>210</td>
<td>0.005</td>
<td>Post-fire</td>
<td>PL80</td>
</tr>
<tr>
<td>Pre-fire</td>
<td>PL80</td>
<td>134</td>
<td>0.005</td>
<td>Post-fire</td>
<td>PL18</td>
</tr>
<tr>
<td>Pre-fire</td>
<td>PL18</td>
<td>219</td>
<td>0.003</td>
<td>Post-fire</td>
<td>PL18</td>
</tr>
<tr>
<td>Pre-fire</td>
<td>PL18</td>
<td>242</td>
<td>0.013</td>
<td>Post-fire</td>
<td>PL82</td>
</tr>
<tr>
<td>Pre-fire</td>
<td>PL18</td>
<td>246</td>
<td>0.007</td>
<td>Post-fire</td>
<td>PL82</td>
</tr>
<tr>
<td>Pre-fire</td>
<td>PL82</td>
<td>150</td>
<td>0.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-fire</td>
<td>PL54</td>
<td>131</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = New Allele to Range-Wide Population
Goal: To examine the effects of fire on the genetic structure

Post-fire Analysis Results

AMOVA analysis (shown left):
Both at population and plot level, both the amount of genetic variation within individuals and the amount of genetic structure among populations increased.

This demonstrates an increased representation of selfed seeds, likely from the below-ground flowers.
Goal: To examine the effects of fire on the **genetic structure**

### Post-fire Analysis Results

<table>
<thead>
<tr>
<th>CC2</th>
<th>CC3</th>
<th>CC4</th>
<th>CC2a</th>
<th>CC3a</th>
<th>CC4a</th>
</tr>
</thead>
</table>

CC 1-4 = Pre-fire, CC 2a-4a = Post-fire  

InStruct run at K=7
To provide better resolution on these issues, we examined genetic data from 30 *P. lewtonii* seeds from above-ground, open pollinated seeds.

### Seed Data Analysis Results

<table>
<thead>
<tr>
<th></th>
<th>Pre-fire</th>
<th>Post-fire</th>
<th>Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_0$</td>
<td>0.040</td>
<td>0.052</td>
<td>0.092</td>
</tr>
<tr>
<td>$H_e$</td>
<td>0.436</td>
<td>0.425</td>
<td>0.350</td>
</tr>
</tbody>
</table>

This suggests that outcrossed seeds are not being produced frequently.

Question: Are outcrossed seeds being produced by the above-ground flowers?
Conclusions

We see a clear shift in alleles present in the population. AMOVA and InStruct analysis shows an increase in population structure.

We don’t see evidence of significantly more outcrossing in above-ground open-pollinated seeds.

Why?
• An extinct pollinator?
• Bi-parental inbreeding?
• Habitat fragmentation?
Implications

Hand-pollination studies could show if the outcrossing mechanism is functional.

We already know fire is good for this species.

Over this two year period, we see no genetic decline.

Understanding the complex mating system and genetic structuring of this species is vital to inform conservation efforts.
Acknowledgments

A special thanks to the Edwards lab for help and support; to REU coordinators Dr. Peter Hoch, Dr. Monica Carlsen-Krause, and Dr. Wendy Applequist; and to Dr. Richard Abbott for providing images used. This work was supported by the Florida Department of Agriculture and Consumer Services Division of Plant Industry (Grant number 020159) and the Research Experiences for Undergraduates Program of National Science Foundation.
Thank you!