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

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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.

Images below: Justine E. Hausheer / TNC, 2015


Image: Wikimedia Commons

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[8]

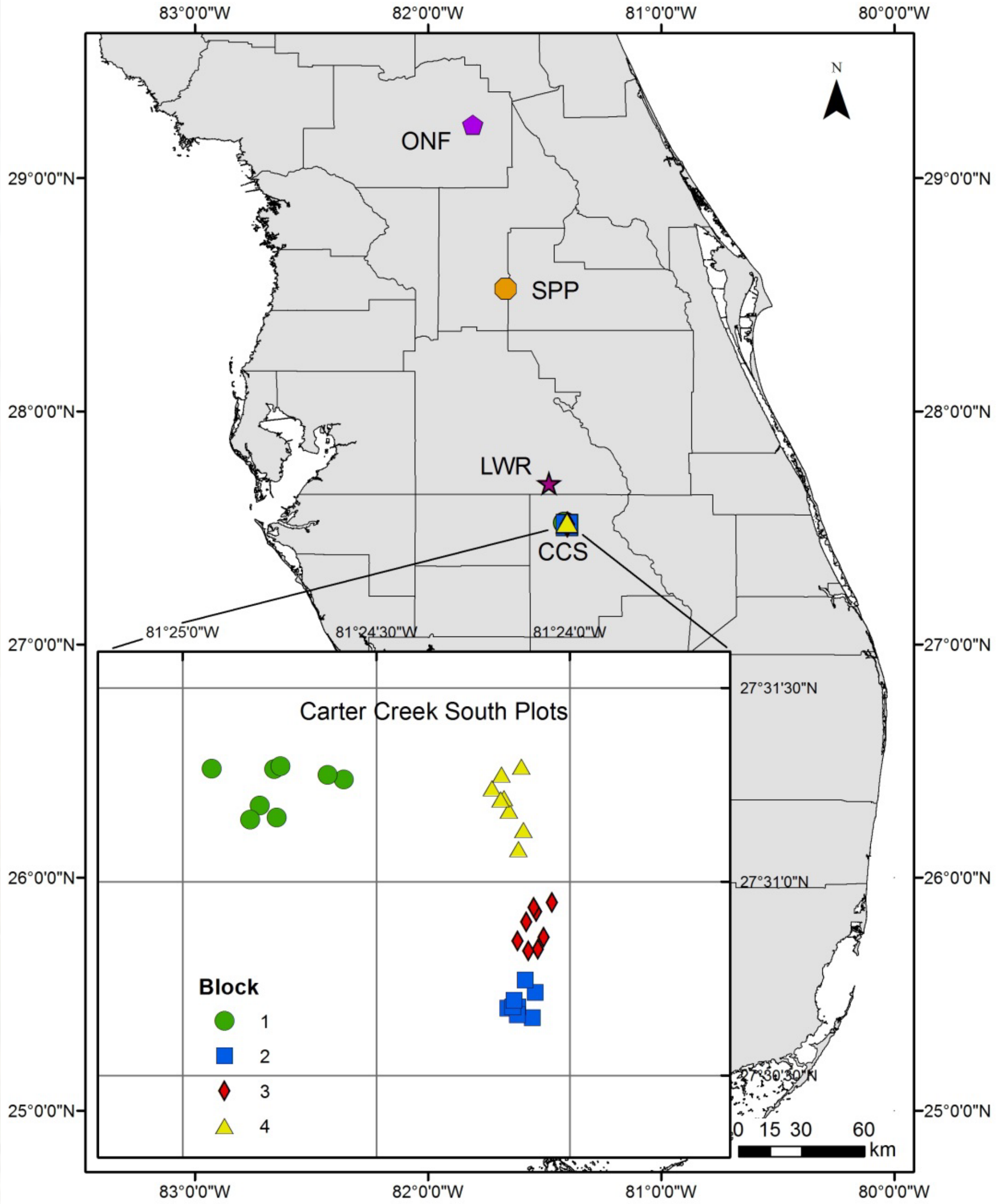
Swift et al investigated patterns of genetic structure by sampling *P. lewtonii* at both fine-scale and range-wide scales.

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 *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?

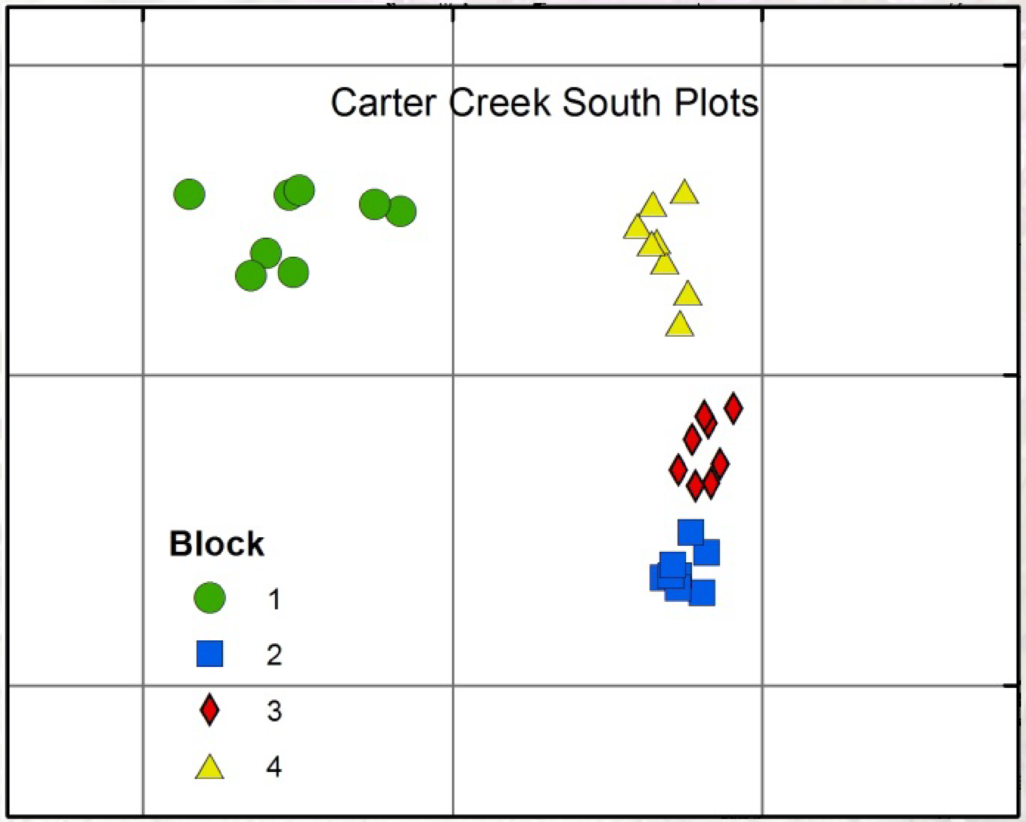
Top left image: Wikimedia Commons

Current research questions

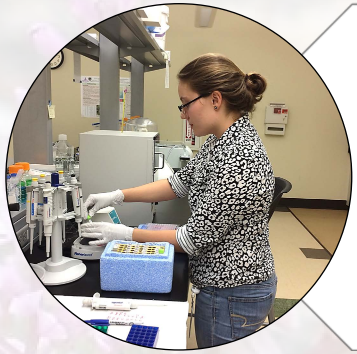
- What are the effects of fire on the genetic diversity, favored mating system, and the genetic structure of populations of *P. lewtonii?*
- Do changes in ecological conditions following fire stimulate germination of outcrossed seeds or inbred seeds?
- Are outcrossed seeds even being produced by the 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.

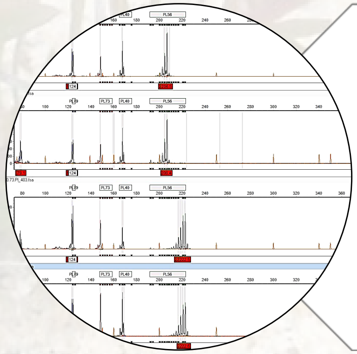


Molecular Methods



Preformed DNA extraction using a modified CTAB protocol

PCR amplification utilizing the same 11 microsatellite loci used in Swift et al to genotype individuals



Microsatellite peak scoring, data analysis, and comparison with pre-fire results

Goal: To examine the effects of fire on the **favored mating system**

Post-fire Analysis Results

__Heterozygosity__

Expected heterozygosity (H_E) and observed heterozygosity observed (H_0) remained low overall.

	H_0	H_e	F_{IS}
Pre-fire	**0.040**	**0.436**	**0.909**
Post-fire	**0.052**	**0.425**	**0.878**

This shows that the selfing rate remains very high, even after fire.

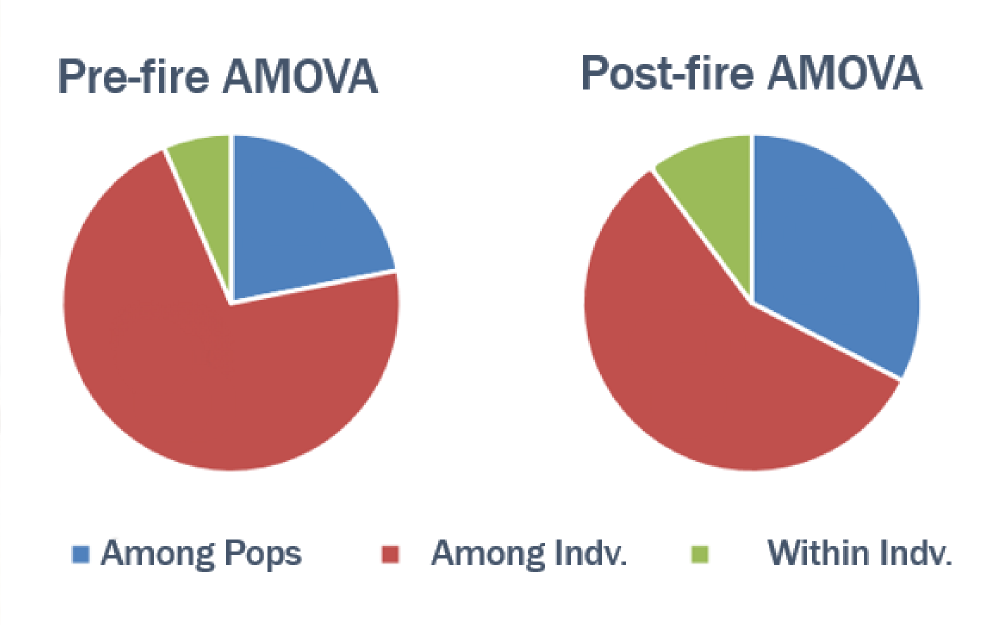
Goal: To examine the effects of fire on the **genetic diversity**

Post-fire Analysis Results

Private Alleles within Carter Creek Population								
	Locus	Allele	Freq			Locus	Allele	Freq
Pre-fire	PL56	194	0.011		Post-fire	PL80	152*	0.031
Pre-fire	PL56	210	0.005		Post-fire	PL80	156*	0.021
Pre-fire	PL80	134	0.005		Post-fire	PL18	234*	0.007
Pre-fire	PL18	219	0.003		Post-fire	PL18	244*	0.027
Pre-fire	PL18	242	0.013		Post-fire	PL82	173*	0.004
Pre-fire	PL18	246	0.007		Post-fire	PL82	175*	0.013
Pre-fire	PL82	150	0.007					
Pre-fire	PL54	131	0.005		* = New Allele to Range-Wide Population			

Goal: To examine the effects of fire on the **genetic structure**

Post-fire Analysis Results



<u>AMOVA analysis (shown left):</u>

Both at population and plot level, both the amount of genetic variation within individuals and the amount of genetic structure among populations increased.

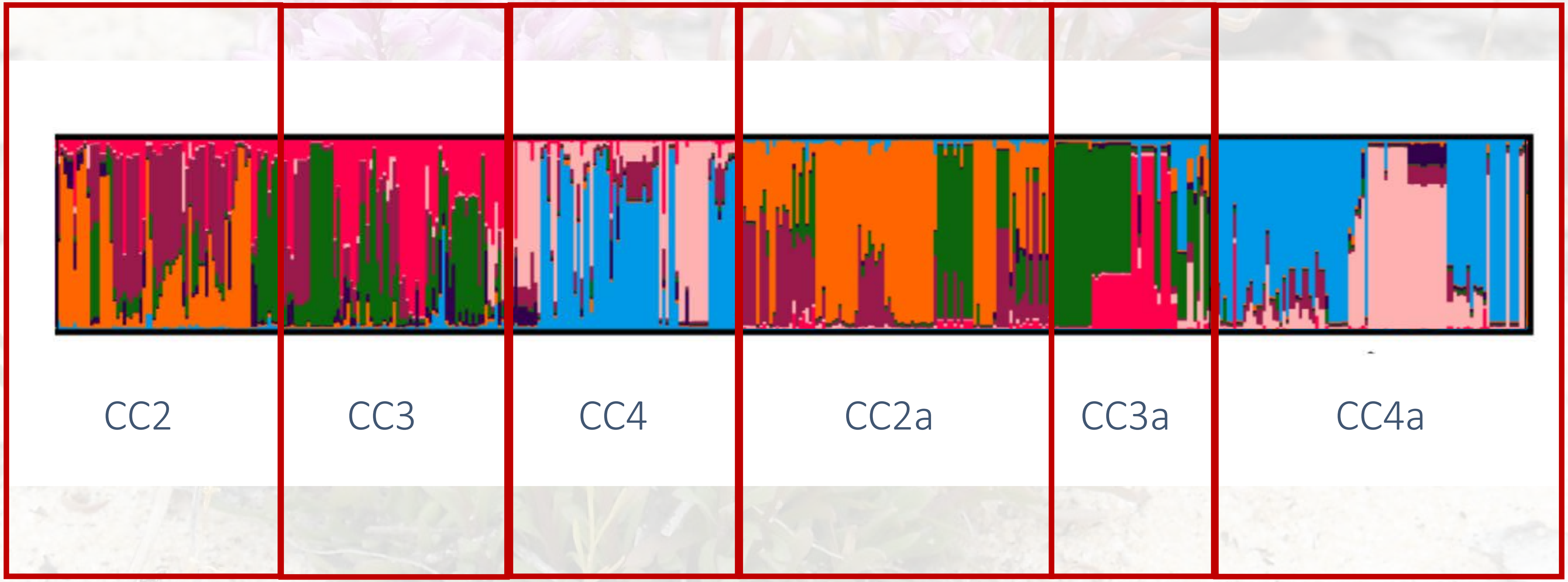
This demonstrates an increased representation of selfed seeds, likely from the below-ground flowers.

Goal: To examine the effects of fire on the **genetic structure**

Post-fire Analysis Results

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

InStruct run at K=7



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

Seed Data Analysis Results

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

	Pre-fire	Post-fire	Seeds
H_0	0.040	0.052	0.092
H_e	0.436	0.425	0.350

This suggests that outcrossed seeds are not being produced frequently.

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

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Thank you!