



# Where do they come from? Using microsatellite and ddRADseq markers to infer encroachment dynamics of *Juniperus virginiana* in the Lakeside Daisy Nature Preserve



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## INTRODUCTION

**Background:** *Juniperus virginiana* (eastern red cedar) is a common dioecious coniferous tree native to the eastern United States (Fig. 1) that is frequently used for landscaping in urban areas and is often planted as a windbreaker around agricultural fields. Although native, eastern red cedar (ERC) is expanding its range by encroaching into sensitive areas forming dense monocultures, and shading out smaller plants, thus causing harm to the local flora. The impacts of encroaching plants are similar to those of invasive species and include causing biodiversity loss, modifying plant communities, and affecting ecosystem functions.

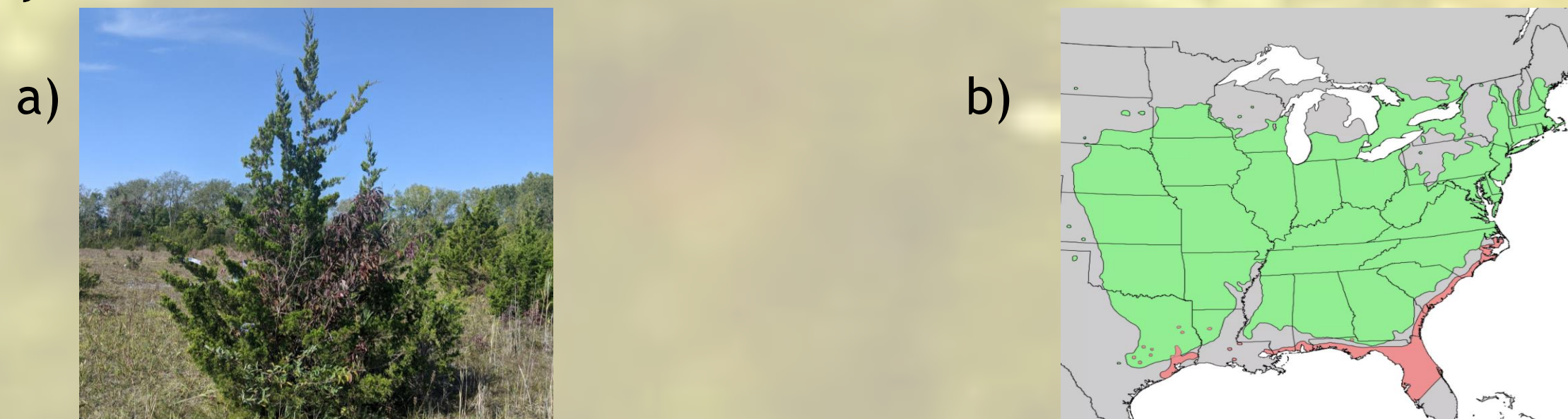


**Figure 1.** a) An adult ERC and b) current distribution of two subspecies of *Juniperus virginiana*, *Juniperus virginiana* var. *virginiana* (eastern red cedar) (green) and *Juniperus virginiana* var. *silicicola* (southern red cedar) (red) across North America.

**Study site:** Lakeside Daisy Nature Preserve has been set aside to protect the federally threatened *Tetaneuris herbacea* but is now home to an encroaching population of ERC. Historically covered with short grasses and wildflowers, Lakeside Daisy Nature Preserve is currently being colonized by ERC, which could put the native species at this site at risk.

**Hypotheses:** 1) Genetic variation among ERC at Lakeside Daisy Nature Preserve will increase in younger temporal subsets of the population compared to older ones if novel genetic diversity is entering the preserve. 2) Alternatively, genetic diversity may remain stable over time, resulting in younger generations that are genetically similar to the “founder trees” of the population, suggesting that they are descendants of the founders and not outside trees.

## METHODS

**Sample Collection:** Leaf tissue was taken from a representative sample of 192 trees at Lakeside Daisy Nature Preserve and in the surrounding area. These trees are grouped by age into decade age groups based on stem cross-sections or wood cores taken from each tree by counting rings. DNA was extracted from each sample and diluted to 10 ng/μL.

**Microsatellite Analysis:** I performed a genetic analysis of ERC using ten polymorphic microsatellite loci and will soon use nine more as well. All samples were sent to MCLab in San Francisco, CA for sequencing and analyzed using Peak Scanner software by ThermoFisher Scientific to determine the multi-locus genotype of all 192 individuals. Once analyzed, I will use GenAlEx 6.5 to calculate common genetic diversity indicators like allele richness and allele frequencies among ERC of different ages.

**ddRADseq:** I will send my DNA samples to Admera Health Biopharma Services to perform double digest restriction-site associated DNA sequencing (ddRADseq) in order to reduce any biases resulting from the use of microsatellite analyses. I will analyze the sequenced samples using open-source software in a UNIX-like environment to find SNP genetic markers.

**Preliminary Analysis:** Individuals were first separated by DBH, or base diameter when DBH was unavailable, into 5 populations. The “founder” population that was formed in or before the 1990s, F, and the roadside population, R, were separated from the remaining individuals. These remaining individuals were separated into LD1, LD2, and LD3 with LD1 having the smallest individuals and LD3 having the largest. GenAlEx 6.5 was used to perform the microsatellite analysis.

## RESULTS

**Table 1.** Pairwise population Fst values demonstrating similarities between pairs of populations within sample group. The lower Fst values between LD1, LD2, and LD3 indicate closeness in allele frequencies whereas the larger values seen when comparing F and R populations to other populations indicates fewer genetic similarities.

| LD1   | LD2   | LD3   | F     | R     |     |
|-------|-------|-------|-------|-------|-----|
| 0.000 |       |       |       |       | LD1 |
| 0.003 | 0.000 |       |       |       | LD2 |
| 0.004 | 0.004 | 0.000 |       |       | LD3 |
| 0.013 | 0.017 | 0.015 | 0.000 |       | F   |
| 0.011 | 0.014 | 0.013 | 0.016 | 0.000 | R   |

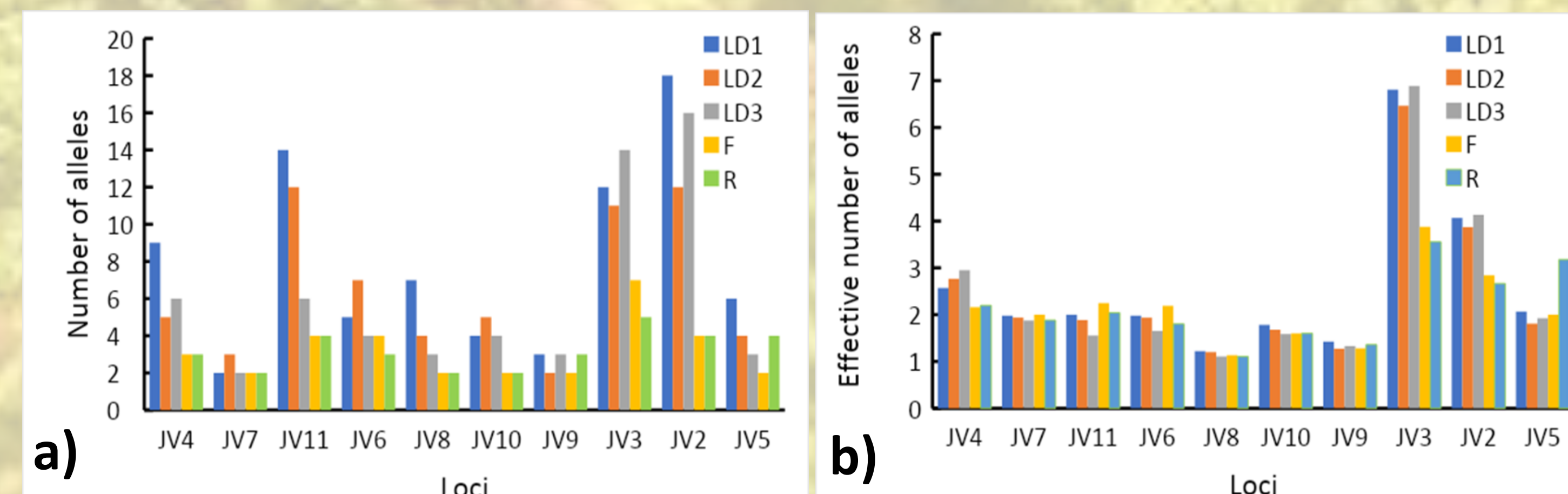


**Figure 2.** a) Number of unique alleles per locus and population with loci labeled arbitrarily from JV2-JV11. b) Number of effective alleles per locus and population with loci labeled arbitrarily from JV2-JV11. Calculating the number of effective alleles at a locus considers the frequency at which alleles are expressed in the population.

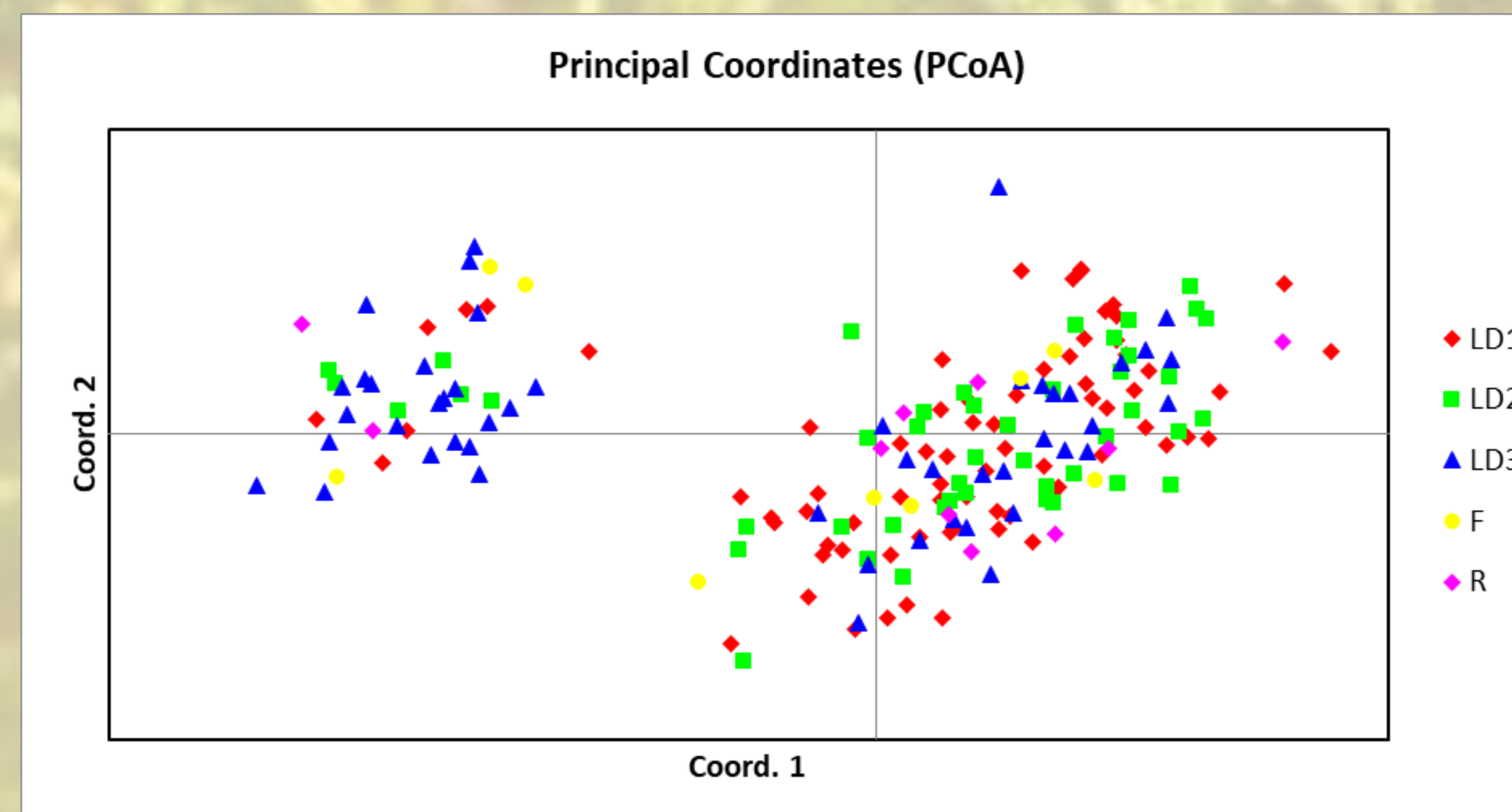


**Figure 3.** The principal coordinate analysis of samples taken from Lakeside Daisy Nature Preserve based on ten polymorphic microsatellite markers shows two clusters of individuals. It is currently unknown what is causing the clustering seen.

## CONCLUSIONS

Based on the preliminary results, the genetic variation in younger generations may be elevated compared to the genetic variation among the older stands of trees in the “founder” area and along the roadside (Table 1; Figure 2). Furthermore, trees within the LD1, LD2, and LD3 populations appear to be more closely related to one another than to the R and F populations (Table 1). There appears to be two groups of more closely related trees comprised of individuals from all populations, but it is not currently understood what may be causing this division (Figure 3). This preliminary analysis has allowed me to identify microsatellite markers that are reliable and some that are not reliable, which will change the markers that I choose to use for this analysis.



**Figure 4.** Satellite imagery of Lakeside Daisy Nature Preserve showing “founder” area circled and roadside population along road shown.

## EXPECTED RESULTS

If Hypothesis I is correct, I expect that genetic variation will increase in the younger subsets of trees compared to older generations, indicated by increased observed heterozygosity in younger temporal subsets and novel alleles that were not present in older generations. These novel alleles may come in the form of differentially sized microsatellite fragments, present in microsatellite analysis, or SNPs, which will be elucidated using ddRADseq. Alternatively, if Hypothesis II is correct, I do not expect to see significant changes in genetic variation in the younger subsets of ERC in the population. In this case, the diversity of the younger trees should resemble that of the “founder” and roadside trees. If Hypothesis II is correct, I expect to see similar numbers of unique and effective alleles in all temporal groups as well as similar levels of heterozygosity.

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