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Genetic Assessment of Ponderosa Pine (*Pinus ponderosa*) within Glacier National Park, Montana

Part of NFGEL Project #254

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The Wyoming BLM and the USDA Forest Service National Forest Genetics Laboratory (NFGEL) have collaborated to complete the first range-wide genetic sampling and analysis of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) using standardized field and laboratory protocols. This project encompasses 104 populations across the range of the species in the western United States, including the farthest north sampled population in Glacier National Park in Montana (48.825 N, -114.324 W). Ponderosa pine in Glacier National Park is separated from larger concentrations of ponderosa pine to the west and south, and is defined as an isolated disjunct population in our analyses (Potter et al. in review) because it is located more than 25 km from the nearest ponderosa pine distributional area greater than 1,000 km², as defined by Little (1971). Ponderosa pine is concentrated in the southwestern part of the park. Associated species include lodgepole pine (*Pinus contorta* Dougl.), western larch (*Larix occidentalis* Nutt.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco).

The ponderosa pine sample in Glacier National Park consisted of 30 trees from the North Fork Subdistrict in Flathead County, Montana, from which short branch tips were collected in 2011. Where possible, study sampling was limited to mature trees that were at least 100 meters apart. The focus of the study was on trees located in stands that were established with natural regeneration before 1900 and with no evidence of reforestation activities through 2012. Nearly all of the sampled trees in Glacier National Park were estimated to be at least 375 years old. Some of the samples were taken from the area of the 1988 Red Bench fire. The samples were collected from three small stands in close proximity to each other, at elevations ranging from 3572 to 3692 feet. The sampled pines were estimated to be between approximately 3 and 62 inches diameter at breast height, and between 12 and 125 feet in height.

Sequencing and analysis of a fragment of haploid mitochondrial DNA (containing the second intron of the *nad1* gene) has yielded results illuminating evolutionary relationships among ponderosa pine populations across the distribution of the species (Potter et al. 2013). Specifically, the Glacier National Park population possesses an mtDNA haplotype (#8) that exists in an arc from southern Oregon northeast into central Washington and northern Idaho and east into Montana (Figure 1). This haplotype appears to correspond with Haplotype B described in Latta and Mitton (1999) and Johansen and Latta (2003), and is probably closely related to the other haplotypes that constitute the Pacific variety of ponderosa pine (*P. ponderosa* var. *ponderosa*) (Potter et al. 2013). The ancestors of the Glacier National Park ponderosa pines may have weathered one or more of the Pleistocene glacial maxima in northwestern California and southwestern Oregon, the location of Pleistocene refugia for multiple species (Shafer et al. 2010). Haplotypes 5, 8 and 9 may have existed here during a recent glacial period, with Haplotype 8 possibly diverging from Haplotype 5 at that time and later dispersing north and east.

Results of analyses of seven highly polymorphic nuclear microsatellite markers and 19 isozyme markers (Potter et al. in review) suggest that the Glacier National Park population exhibits low genetic variation by some measures compared to the average across all 104 sampled populations, and an average level of genetic variation for other measures (Figures 2 and 3).

For example, an average of 6.71 alleles was detected across the seven microsatellite loci in the Glacier National Park population, compared to 7.37 alleles for all the sampled populations. It had 1.86 rare alleles per locus (locally common alleles found in fewer than 25 percent of the

populations) compared to the mean of 2.63 across populations. Microsatellite observed heterozygosity was slightly higher than average (0.608 compared to 0.561), but the population was out of Hardy-Weinberg Equilibrium with a deficiency of observed heterozygosity compared to expected heterozygosity (0.639). Glacier National Park had no unique microsatellite alleles compared to other populations.

Analyses of the 19 isozyme loci detected roughly average levels of genetic variation for the Glacier National Park population. Allelic richness was 1.84 (compared to the mean across populations of 1.79), mean number of rare alleles was 0.21 (compared to 0.18), proportion of polymorphic loci was 0.47 (compared to 0.47), and observed heterozygosity was 0.102 (compared to 0.102). The population was out of Hardy-Weinberg Equilibrium for isozymes, with a deficiency of observed compared to expected heterozygosity (0.145).

As expected given its isolated disjunct status, the Glacier National Park population was more inbred than average compared to other sampled ponderosa pine populations for the microsatellite loci (inbreeding coefficient of 0.219 compared to the mean across populations of 0.093). For the isozyme loci, however, the population was neither more or less inbred than average (0.180 compared to 0.177).

Management and Silvicultural Considerations:

Work by Rehfeldt and others (2006) predicts that the Glacier National Park area will become more climatically conducive to ponderosa pine in the medium-term (2060). If this prediction is accurate, then it is important to manage ponderosa pine stands in Glacier National Park to keep them on the landscape, so the locally adapted ponderosa pine genotypes are available to fill the future fundamental and realized niche for ponderosa pine.

Current techniques allow for the cost-effective analysis of neutral markers such as microsatellites and isozymes. Future work may identify specific adaptively significant genes or gene complexes present in the Glacier National Park population of ponderosa pine. Until then, it is recommended that ponderosa pine stands in Glacier National Park be maintained as populations that **(1)** contain very old ponderosa pine individuals, and **(2)** are susceptible to inbreeding depression and loss of genetic variation because of their isolation from larger concentrations of the species, but also have the potential to evolve unique adaptations and novel combinations of alleles.

The Glacier National Park population may have experienced the effects of isolation and small population size, including the loss of genetic diversity often associated with genetic drift and inbreeding in small and isolated populations (Jaramillo-Correa et al. 2009), and which is expected to reduce overall population fitness (Reed and Frankham 2003). Individuals in small populations are generally less fit as a result of environmental stress and inbreeding, forces that can substantially increase the probability of population extirpation under changing environmental conditions (Willi et al. 2006), but rare alleles in particular can act as a reservoir of gene forms that may be of adaptive benefit under new environmental or competitive conditions (Schaberg et al. 2008).

Conservation biology emphasizes the maintenance of native gene pools as an important function in maintaining ecosystem and species integrity. Jackson and Betancourt (NSF-DEB-9815500) state that “Finally, our results underscore the growing need to focus more on genotypes than species in biogeographic modeling and ecological forecasting.” With the growing awareness of climate change and its potential impacts on the outliers of a species, such as the Glacier National Park ponderosa pine population, the above statement underscores the long term need for appropriate management action.

Silvicultural Recommendations:

1. These stands are within a region that has significant wildland fire activity. To reduce the potential for uncharacteristic wildland fire within the ponderosa pine stands, it is recommended that management activities to reduce the fuel ladders and aerial fuel loadings be conducted. Prescribed fire can be used at low intensities to keep these stands on the landscape in a seral condition.
2. The area surrounding the oldest trees in Glacier National Park should emphasize protection of these individuals.
3. Because of the differences in stand regeneration and site condition, each stand in Glacier National Park needs to be evaluated for different potential management actions because each may be influenced by different sets of environmental constraints or previous disturbance events.
4. If any reseedling activity is contemplated, seeds should be collected locally and stored in long term storage (see Bonner and Karrfalt 2008), so locally adapted seed can be used for planting after a disturbance or to expand stand size and number of populations.
5. Prescribed fire activities should be undertaken with care and follow the recommendations listed in Hood (2010) for areas involving older cohort trees.

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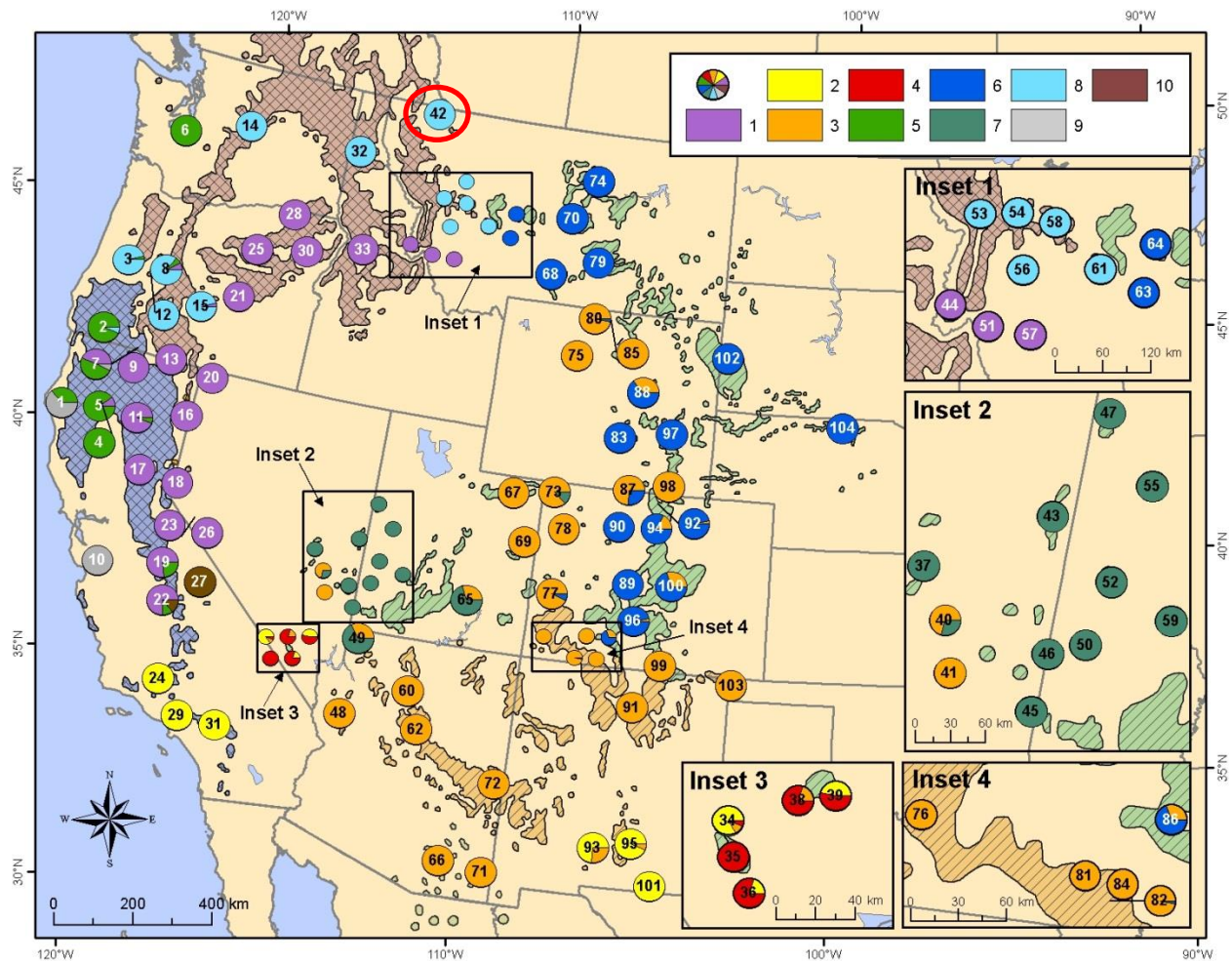


Figure 1: Distribution of haplotypes across the range of ponderosa pine following sequencing of a variable mitochondrial DNA region (Potter *et al.* 2013). The Glacier National Park population is #42, and consists entirely of haplotype 8.

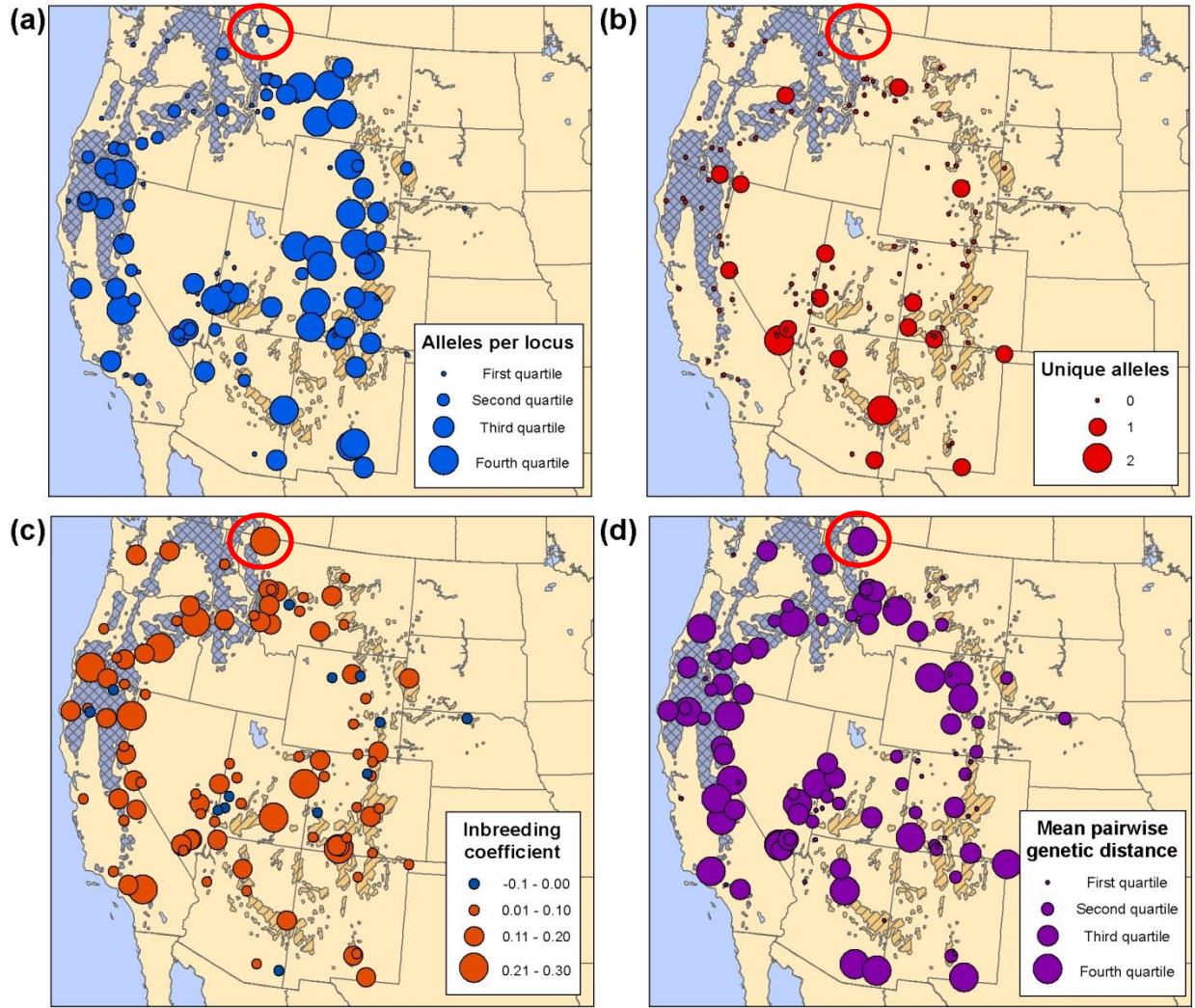


Figure 2: *Pinus ponderosa* classifications of (a) alleles per locus (A), (b) unique alleles (A_U), (c) inbreeding coefficient (F_{IS}), and (d) mean pairwise chord distance (D_C), based on seven polymorphic microsatellite loci (Potter *et al.* in review). The Glacier National Park population is circled in red.

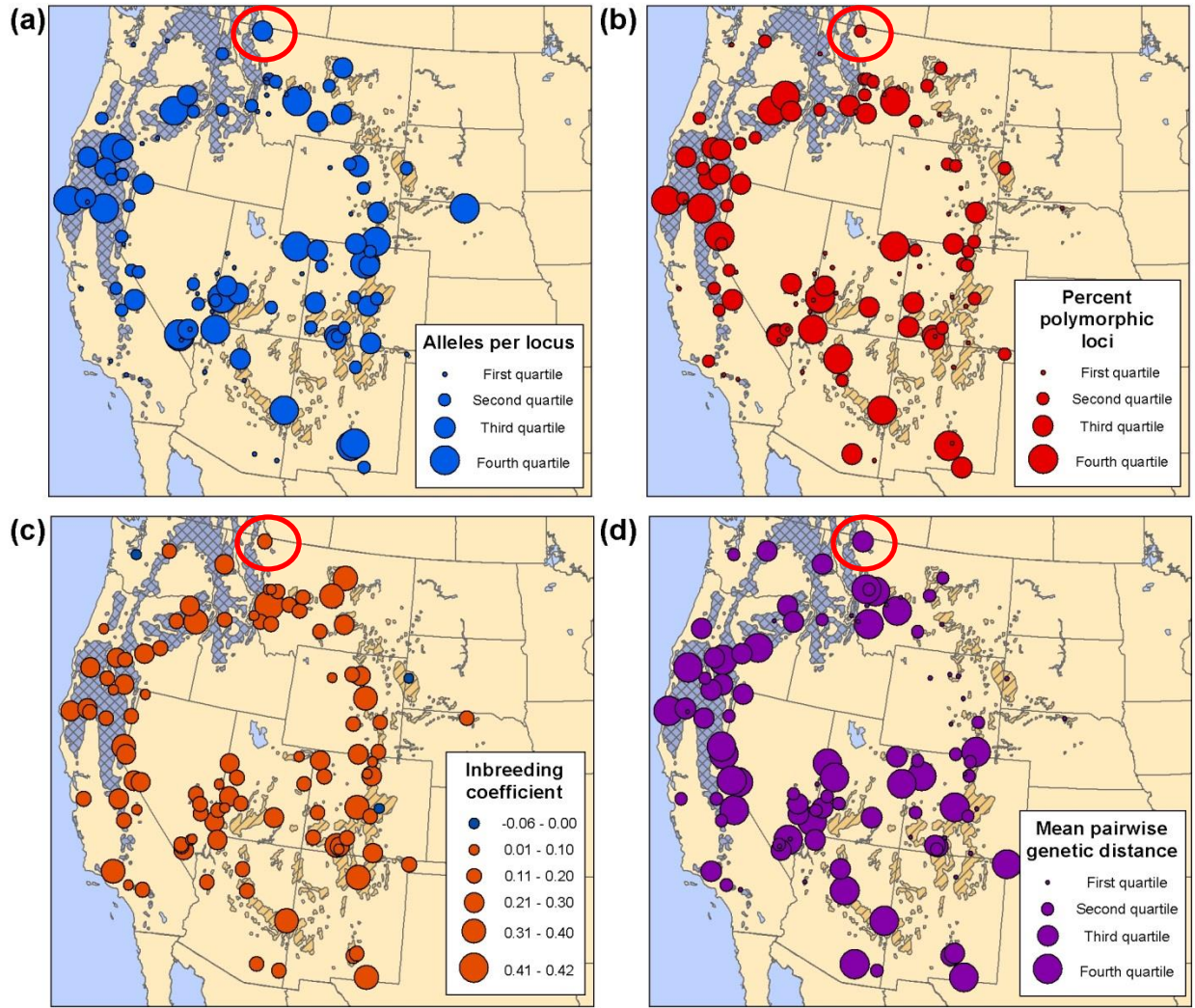


Figure 3: *Pinus ponderosa* classifications of (a) alleles per locus (A), (b) percent polymorphic loci (P_P), (c) inbreeding coefficient (F_{IS}), and (d) mean pairwise chord distance (D_C), based on 19 isozyme loci (Potter *et al.* in review). The Glacier National Park population is circled in red.