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Ponderosa pine, nearly 1,000 years old, in the Wah Wah Mountains: photo provided by Doug Page, BLM Cedar City District Office

Genetic Assessment of Ponderosa Pine (*Pinus ponderosa*) Sites within the Proposed Wah Wah Mountains Area of Critical Environmental Concern (ACEC), Millard County and Iron County, Utah

Part of NFGEL Project #232

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The Wyoming BLM and the USDA Forest Service National Forest Genetics Laboratory (NFGEL) are collaborating 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 63 populations across the range of the species in the western United States, including two in the proposed Wah Wah Mountains Area of Critical Environmental Concern (ACEC) in western Utah.

The Wah Wah Mountains contain the oldest known living ponderosa pine individuals in the world. One tree has been stump dated to the year 1075 (Kitchen 2010), and another tree in close proximity was dated to plus or minus 5 years of the 1075 date. The ponderosa pine in this area exists in small stands, with small groups of trees and individuals scattered in a landscape primarily dominated by Single leaf pinyon (*Pinus monophylla*) and juniper (*Juniperus* spp). Other components include white fir (*Abies concolor*), Curlleaf mountain mahogany (*Cercocarpus ledifolia*), with a small component of bristlecone pine (*Pinus longaeva*).

The two sampled ponderosa pine populations from the Wah Wah Mountains bracket the northern and southern portions of this south-to-north-running mountain range. The Steamboat Mountains stand (32 sampled trees) is located near the southern end of the range, in Iron County, Utah, while the Northern Wah Wah Mountains stand (24 sampled trees) is located near the northern end of the range, in Millard County, Utah. The Northern Wah Wah stand contains scattered ponderosa pine saplings, and no evidence of reproduction. The Steamboat stand was partially logged in the late 19th century. Post-harvest regeneration was good and the stand continues to regenerate. Both stands are part of small and isolated disjunct populations separated from larger concentrations of ponderosa pine to the southeast and from other isolated disjunct populations to the north, west and south. The findings below are preliminary and may change with further analysis. When the final report is issued any changes in the Northern Wah Wah and Steamboat Mountain results will be addressed.

Preliminary results from analyses of 20 isozyme loci and of a single microsatellite molecular marker locus suggest that the Steamboat Mountain population exhibits higher-than-average genetic variation by several measures, and that the Northern Wah Wah population, with some exceptions, possesses an average level of genetic variation compared to other populations across the range of the species. An analysis of mitochondrial DNA sequences suggests that these populations belong to a highly differentiated ponderosa pine evolutionary lineage that is relatively limited in extent.

Sequencing 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 (Figure 1). Specifically, the Steamboat Mountain and Northern Wah Wah trees possess an mtDNA haplotype that exists only in central and western Utah, eastern Nevada and northwestern Arizona. This haplotype is markedly longer than the haplotypes present nearby in other parts of Nevada, Arizona and Utah. While more work remains to determine the exact relationship of this evolutionary lineage with others in ponderosa pine, this pattern suggests that ponderosa pine in the region underwent considerable genetic differentiation, perhaps as a result of Pleistocene, or earlier, isolation and migration patterns.

Analyses of 20 allozyme loci detected higher-than-average genetic variation by several measures for the Steamboat Mountain population, and roughly average levels of genetic variation for the Northern Wah Wah population. Steamboat Mountain, for example, was in the highest quartile among the 63 sampled ponderosa pine populations for allelic richness, which is calculated as the number of alleles standardized to the smallest sample using rarefaction (El Mousadik and Petit 1996). This value was 1.74 compared to a mean of 1.53 for all sampled populations. The Northern Wah Wah population was approximately average for this measure of diversity (1.56).

A similar pattern was apparent for observed heterozygosity: Steamboat Mountain was in the highest quartile (0.133 compared to the mean of 0.095), and Northern Wah Wah was about average (0.098). Both populations expressed a higher-than-average percent of polymorphic loci (55 percent for Steamboat Mountain and 60 percent for Northern Wah Wah, compared to the 47 percent mean for all populations). Finally, both populations possessed at least one rare allozyme allele. Steamboat Mountain contained two unique alleles and one additional allele present in fewer than 10 percent of the ponderosa pine populations; Northern Wah Wah also had one allele present in fewer than 10 percent of populations.

A separate analysis encompassed a single highly polymorphic microsatellite molecular marker locus (*PtTX2146*), and again found higher-than-average genetic variation at Steamboat Mountain, but detected lower-than-average variation at Northern Wah Wah by some measures. For example, 9 alleles were amplified from Northern Wah Wah and 11 were amplified from Steamboat Mountain, compared to an average of 9.9 alleles for all the sampled populations. The allelic richness of the Northern Wah Wah population also was relatively low compared to other populations, in the second lowest quartile for allelic richness (allelic richness of 6.51 compared to the mean of 6.72). The Steamboat Mountain was in the second highest quartile (allelic richness of 7.16). At the same time, both populations had higher-than-average observed heterozygosity: 0.938 for Steamboat Mountain (highest quartile), and 0.833 for Northern Wah Wah (second highest quartile), compared to the mean of 0.769. The Northern Wah Wah population contained one rare microsatellite allele that was present in fewer than 10 percent of the 63 sampled ponderosa pine populations. Steamboat Mountain had no rare microsatellite alleles compared to other populations.

Despite their isolated disjunct status, both the Steamboat Mountain population and the Northern Wah Wah population were less inbred than average compared to other sampled ponderosa pine populations. In fact, at the microsatellite locus, both populations were outbred (inbreeding coefficient of -0.185 and -0.069, respectively) while populations were on average inbred (inbreeding coefficient of 0.014). In the allozyme analysis, both populations were found to be inbred (inbreeding coefficient of 0.136 and 0.121, respectively), although less so than on average (0.159 mean inbreeding coefficient for all populations).

Additional analyses could further quantify ponderosa pine genetic variation in the Steamboat Mountain and Northern Wah Wah populations and could clarify their evolutionary relationships with other ponderosa pine populations throughout the range of the species.

Management and Silvicultural Considerations:

The documented age of the oldest ponderosa pine in the Northern Wah Wah indicates that the establishment date of the oldest cohorts in the stand would be approximately the year 1075 (936 BP). This date is significant in that the Medieval Climate Anomaly (MCA) (a warm and dry period from 1050 to 650 BP which included widespread severe multi-decadal droughts in the western US) was ending and the Little Ice Age (a cool and wet period from 500 to 100 BP) was beginning that lasted until the late 1800's to early 1900's. If the oldest cohorts are not the first generation then the actual stand establishment date could stretch farther back, predating the MCA.

The Northern Wah Wah stand, due to the lack of ponderosa pine reproduction, may be undergoing localized extinction. Alternatively, the conditions for successful reproduction may be so sporadic that it possibly will be decades (or more) between successful regeneration events. The Steamboat stand does not exhibit a lack of regeneration. Potential causes of reproductive differences in the regeneration observed at the Steamboat and Northern Wah Wah sites might also have been influenced by relatively recent human actions at the Steamboat site, specifically disturbance of the site caused by logging, and the lack of disturbance at the Northern Wah Wah site. The sampled area on Steamboat Mountain where most of the regeneration has taken place, had been logged. An adjacent sampled area on Steamboat Mountain where there had been no logging had little to no regeneration. Soil parent material is also different as are local weather (rainfall) patterns. The northern Wah Wah stand is substantially drier than the Steamboat stand.

Biondi and others (2011) studied Mt. Irish, a similar mountain range, in eastern Nevada. Their findings based on dendrochronology and climate analysis indicates that reduced wildfire fire frequency was attributable to climatic conditions, not post-settlement fire management activities. Their findings may have significance on the management decision-making process for the proposed ACEC. Brown and others (2008) and Heyerdahl and others (2006) provide more valuable information on climate impacts on fire regimes in the Utah and eastern Nevada region.

Rehfeldt and others (2006) work indicates that this area will become more climatically conducive to ponderosa pine in the near future (2030). If this prediction is accurate, then it is important to manage both the Wah Wah Mountain stands to keep them on the landscape, so the locally adapted ponderosa pine genotype is 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 Wah Wah populations of ponderosa pine. Until then, it is recommended that the Wah Wah ponderosa pine stands be managed as interesting and important populations of ponderosa pine that **(1)** have the oldest known living ponderosa pine individuals, **(2)** possess relatively high allelic diversity by some measures, and **(3)** represent a specific genetic haplotype that is only found in western Utah and eastern Nevada and in the Black Rock Mountains of northwestern Arizona.

The Northern Wah Wah population may have experienced the effects of isolation and small population size, while the Steamboat stand suggests that it has not necessarily experienced the loss of genetic diversity often associated with genetic drift and inbreeding in small and isolated populations (Jaramillo-Correa and others 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 and others 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 and others 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 Wah Wah ponderosa pine populations, 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. Due to successional processes and changing disturbance patterns, these stands have a high aerial fuel loading and fuel ladders with the juniper and pinyon as well as other woody species growing in close proximity to the ponderosa pine. 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. This "thinning from below" management strategy would also reduce resource competition and allow the ponderosa pine to release for better growth and resistance to the periodic droughts in the region.
2. Maintain as much structural and age diversity within the ponderosa pine as possible.
3. Maintain a ponderosa pine basal area of less than 60 to reduce the potential for mountain pine beetle infestations.
4. In areas where prior disturbances have allowed pockets of natural ponderosa pine regeneration, thin these pockets to reduce the impacts of potential wildland fire and to allow for the release of the younger aged cohorts.
5. The area surrounding the oldest trees in the Northern Wah Wah stand should emphasize protection of these individuals.
6. Because of the differences in stand regeneration, these areas need to be evaluated for different potential management actions. The difference between the regeneration of the Northern Wah Wahs and the Steamboat areas indicate that they may be working under different sets of environmental constraints or previous disturbance events.
7. Collect seeds locally and store them in long term storage, so locally adapted seed can be used for planting after a disturbance or to expand the stand size and populations.
8. Prescribed fire activities should be undertaken with care and follow the recommendations listed in Hood (2010) for areas involving older cohort trees.

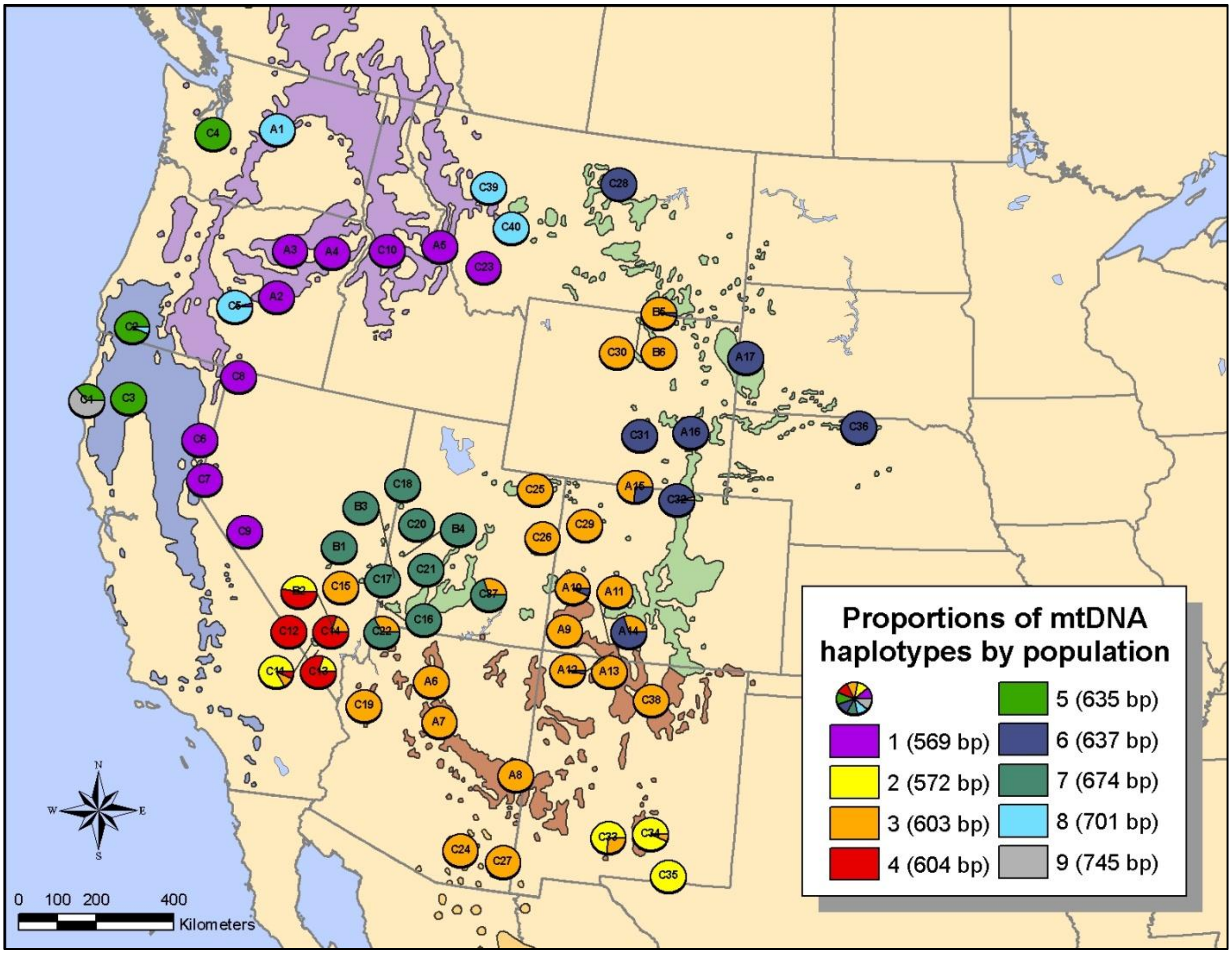

Figure 1: Distribution of haplotypes across the range of ponderosa pine following sequencing of a variable mitochondrial DNA region. The Steamboat Mountains population is B3 and the Northern Wah Wah Mountains population is B4, both located in western Utah.

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