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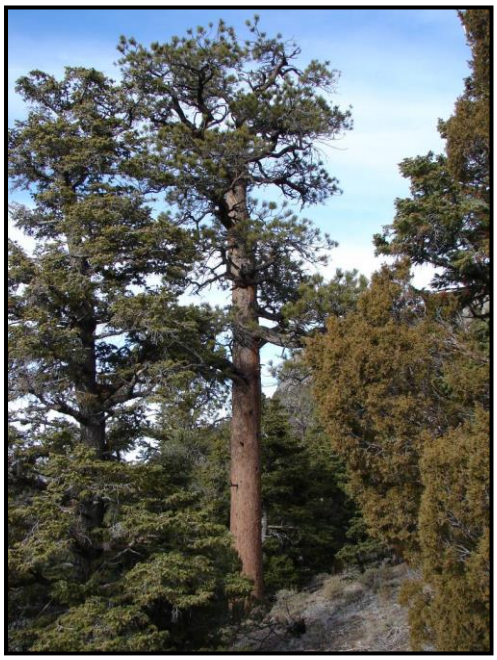

Ponderosa pine, nearly 1,000 years old, in the Wah Wah Mountains: photo provided by Doug Page, BLM Cedar City District Office

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) 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 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 Mountain 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 (38.599 N, -113.553 W) contains scattered ponderosa pine saplings, and no evidence of reproduction. The Steamboat Mountain stand (38.091 N, -113.691 W) 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 (Little 1971).

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). Both the Steamboat Mountain and the Northern Wah Wah populations consist entirely of Haplotype 7, which was found to exist almost exclusively in eastern Nevada, southern Utah, and northwestern Arizona (Figure 1). The evolutionary relationship between Haplotype 7 and other haplotypes, and the geographic arrangement of the haplotypes, suggest that Haplotype 7 (possibly with Haplotypes 2 and 4) may belong to a lineage that is sister to the rest of *P. ponderosa* (Potter et al. 2013). This haplotype may have weathered the most recent glacial maximum in the Great Basin region.

Results of analyses of seven highly polymorphic nuclear microsatellite markers and 19 isozyme markers (Potter et al. in review) indicate that the Steamboat Mountain population contains high genetic variation compared to the average across all 104 sampled populations, while the Northern Wah Wah population has average to below-average genetic variation by most measures (Figures 2 and 3).

For example, an average of 9.43 alleles was detected across the seven microsatellite loci in the Steamboat Mountain population, compared to 7.37 alleles for all the sampled populations

and 7.00 for the Northern Wah Wah population. Steamboat Mountain also had 3.14 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 and 2.29 for Northern Wah Wah. The same pattern was the case for microsatellite observed heterozygosity (0.624 and 0.501 compared to the across-population average of 0.561), but only Steamboat Mountain was out of Hardy-Weinberg Equilibrium with a deficiency of observed heterozygosity compared to expected heterozygosity. Steamboat Mountain had one unique microsatellite allele that was detected in no other population.

Analyses of the 19 isozyme loci detected above-average levels of genetic variation for Steamboat Mountain and generally average levels of genetic variation for Northern Wah Wah. Allelic richness was 2.16 for Steamboat Mountain and 1.84 for Northern Wah Wah (compared to the mean across populations of 1.79). The proportion of polymorphic loci was 0.58 and 0.53, respectively (compared to the average of 0.47), and observed heterozygosity was 0.141 and 0.104 (compared to the average of 0.102). The Northern Wah Wah population had a higher value for rare alleles per locus, however (0.37 compared to 0.26 for Steamboat Mountain and the mean of 0.18). Both populations were out of Hardy-Weinberg Equilibrium for isozymes, with a deficiency of observed compared to expected heterozygosity (0.157 for Steamboat Mountain and 0.118 for Northern Wah Wah).

Unlike most of the 104 ponderosa pine populations, and despite their isolated disjunct status, neither of the two western Utah populations was inbred across the microsatellite loci (inbreeding coefficients of -0.012 and -0.033 for Steamboat Mountain and Northern Wah Wah, compared to the mean across populations of 0.093). For the isozyme loci, both populations were inbred, although Steamboat Mountain was less inbred than average (0.077 compared to 0.177) and Northern Wah Wah was more so (0.209).

Locations closer to Pleistocene refugia are expected to have greater genetic variation than those colonized later (Hewitt 1996), and areas with consistently high allelic richness across the two marker systems employed in this analysis, and with one or more unique alleles, may be the most likely candidates. The vicinity of the proposed Wah Wah Mountains ACEC, therefore, may have harbored a Pleistocene refuge for ponderosa pine, given the high levels of genetic variation and the existence of a unique allele in the Steamboat Mountain population, and the fact that both populations were outbred rather than inbred across the microsatellite loci.

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 U.S.) was ending and the Little Ice Age (a cool and wet period from 500 to 100 BP) was beginning that lasted until the mid-1800s. 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 materials are also different between the sites, 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, indicate that reduced wildfire 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 additional information on climate impacts on fire regimes in the Utah and eastern Nevada region.

Work by Rehfeldt and others (2006) predicts 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 and Steamboat Mountain stands to keep them on the landscape, so 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 Wah Wah Mountains populations of ponderosa pine. Until then, it is recommended that ponderosa pine stands in the proposed Wah Wah Mountains ACEC be managed as interesting and important populations of ponderosa pine that **(1)** contain the oldest known living ponderosa pine individuals; **(2)** possess relatively high genetic diversity by some measures that may indicate proximity to a Pleistocene refuge; **(3)** represent a specific mitochondrial haplotype that is only found in western Utah and eastern Nevada and in the Black Rock Mountains of northwestern Arizona, along with an outlier population in northwestern Colorado; and **(4)** are susceptible to potential future inbreeding depression and loss of genetic variation because of their isolation from larger concentrations of the species, while also possessing the potential to evolve unique adaptations and novel combinations of alleles.

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 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 Wah Wah Mountains 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. Continue maintaining these populations as un-even aged stands, maintaining structural and age diversity.
3. In the Steamboat Mountain stands, maintain a basal area of between 40 and 60 to reduce the potential for mountain pine beetle infestations if mountain pine beetle is present.
4. In the Steamboat Mountain stand(s) where it is possible to pro-active forest management, dense pockets of natural ponderosa pine regeneration should be thinned 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. Seeds should be collected from the Wah Wah Mountain population, including the oldest trees. These seeds will provide invaluable genetic reference material and seed source from the oldest known ponderosa pine survivors of the Medieval Climate Anomaly.
7. It is also recommended that seeds be collected from the Steamboat Mountain stand, so there is a locally adapted seed source in case of widespread disturbance which would require planting of seedlings to maintain the stand on the landscape (see Bonner and Karrfalt 2008).
8. Prescribed fire activities should be undertaken with care and follow the recommendations listed in Hood (2010) for areas involving older cohort trees.
9. Wildland Fire for Resource Benefit planning efforts need to take into account the uniqueness of these stands, both from an age (oldest known living ponderosa pine) as well as from a genetic diversity aspect. These characteristics make both the Wah Wah and Steamboat Mountain populations unique in the world.

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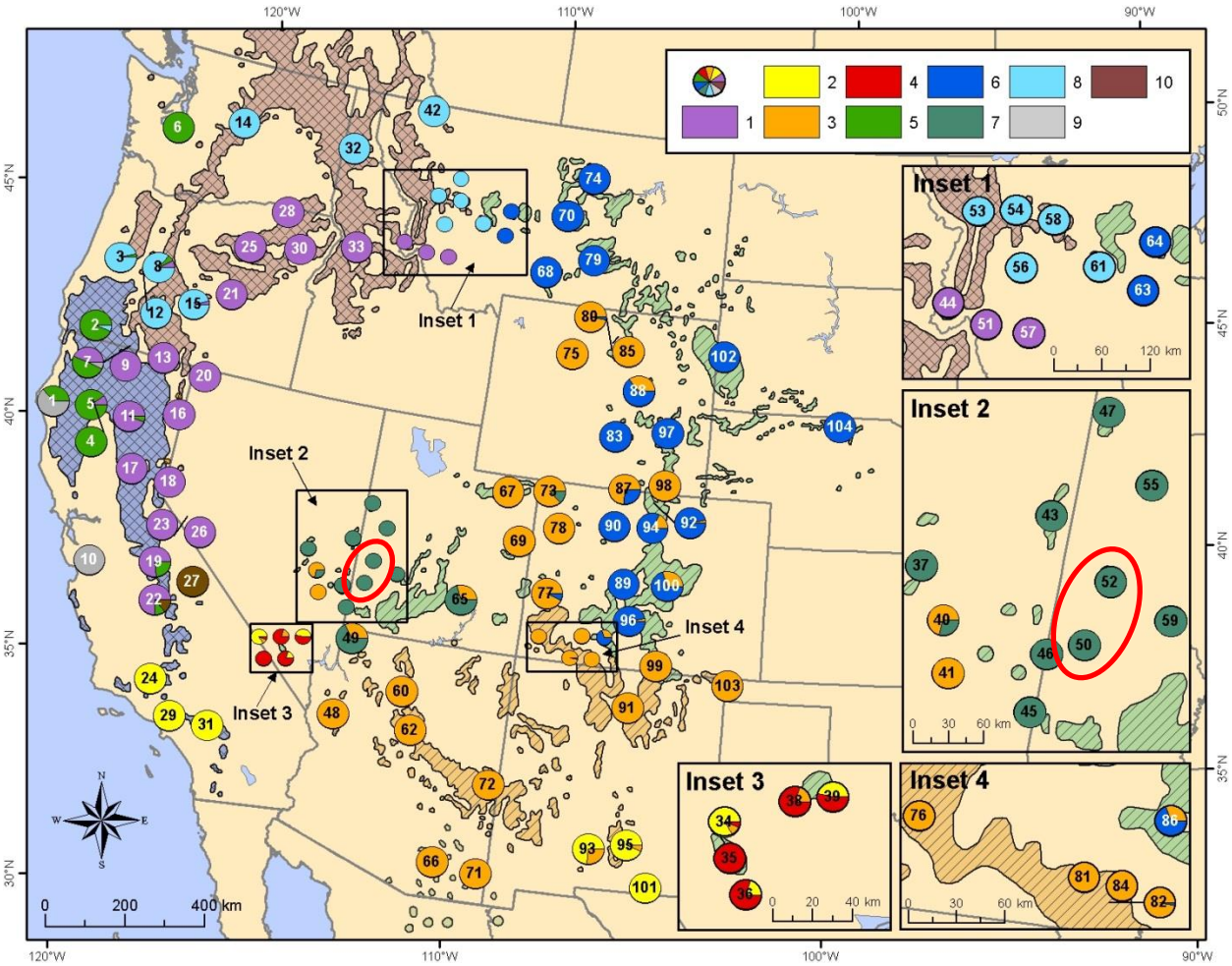

Figure 1: Distribution of haplotypes across the range of ponderosa pine following sequencing of a variable mitochondrial DNA region (Potter *et al.* 2013). The Steamboat Mountain population is #50 and the Northern Wah Wah population is #52; both consist entirely of haplotype 7.

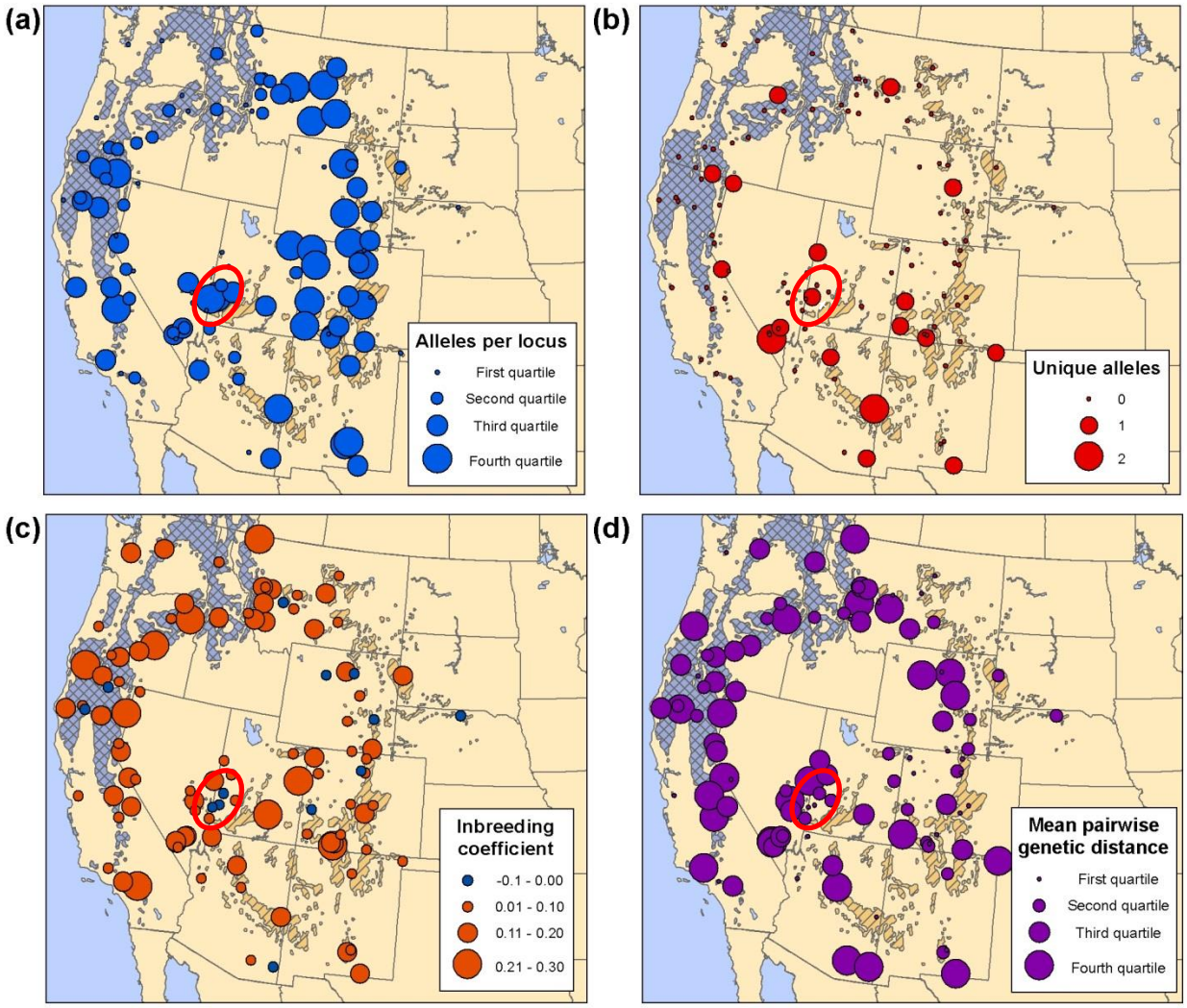

Figure 2: *Pinus ponderosa* classifications of *(a)* alleles per locus (A), *(b)* unique alleles (A_U), *(c)* inbreeding coefficient (F_{IS}), and *(d)* mean pairwise chord distance (D_C), based on seven polymorphic microsatellite loci (Potter *et al.* in review). The Steamboat Mountain and Northern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

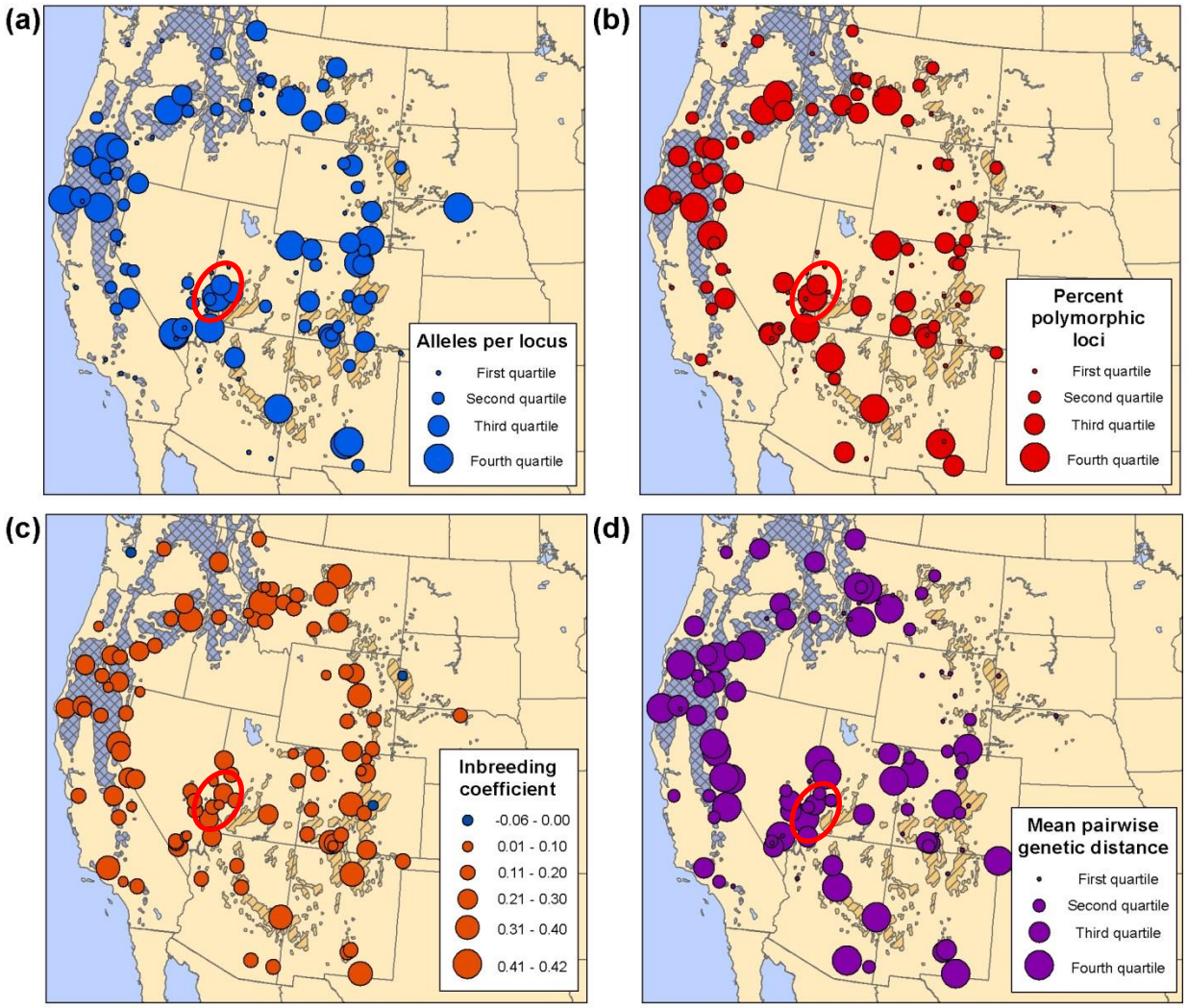

Figure 3: *Pinus ponderosa* classifications of *(a)* alleles per locus (A), *(b)* percent polymorphic loci (P_P), *(c)* inbreeding coefficient (F_{IS}), and *(d)* mean pairwise chord distance (D_C), based on 19 isozyme loci (Potter *et al.* in review). The Steamboat Mountain and Northern Wah Wah populations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