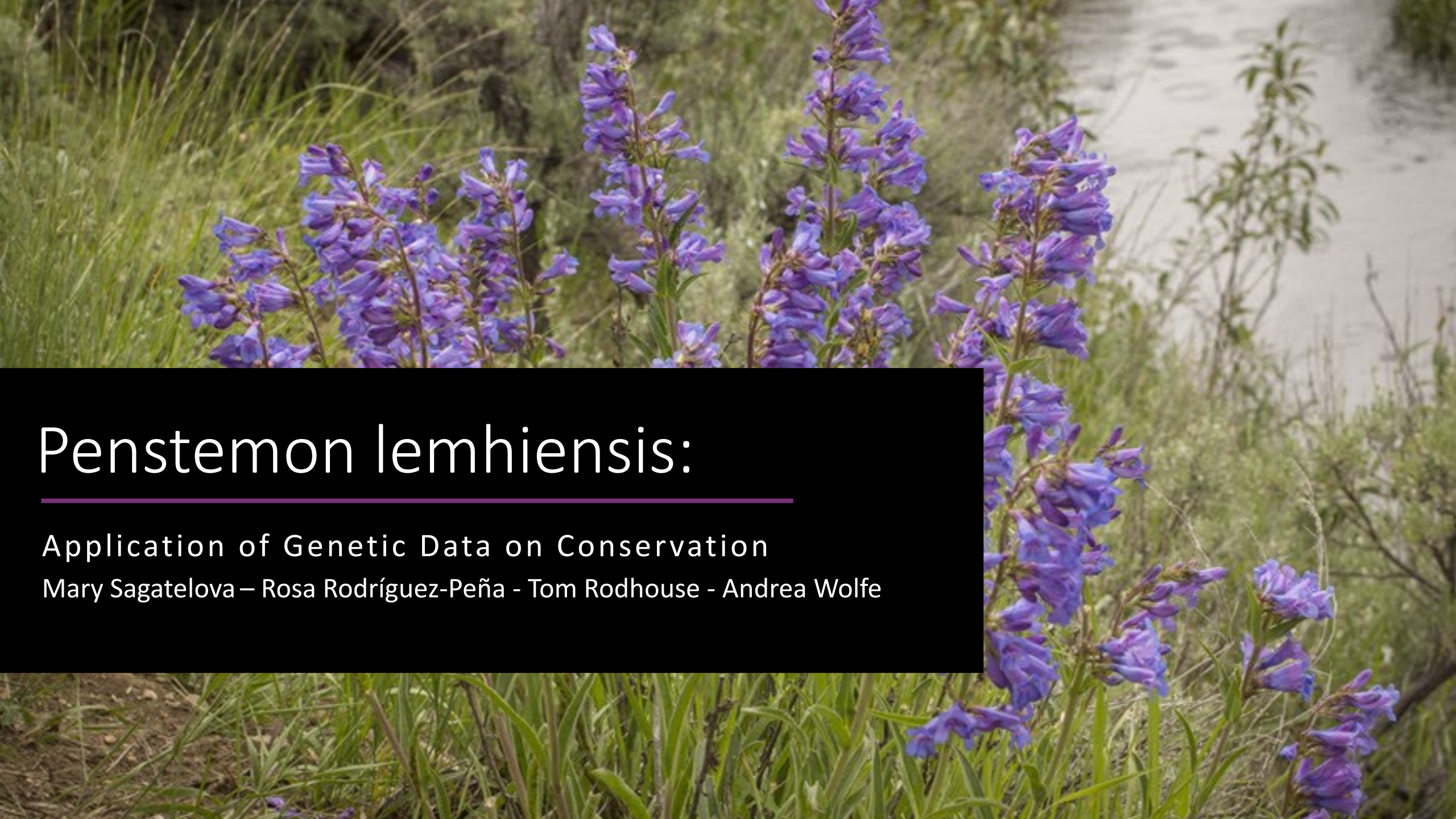


Penstemon lemhiensis:

Application of Genetic Data on Conservation

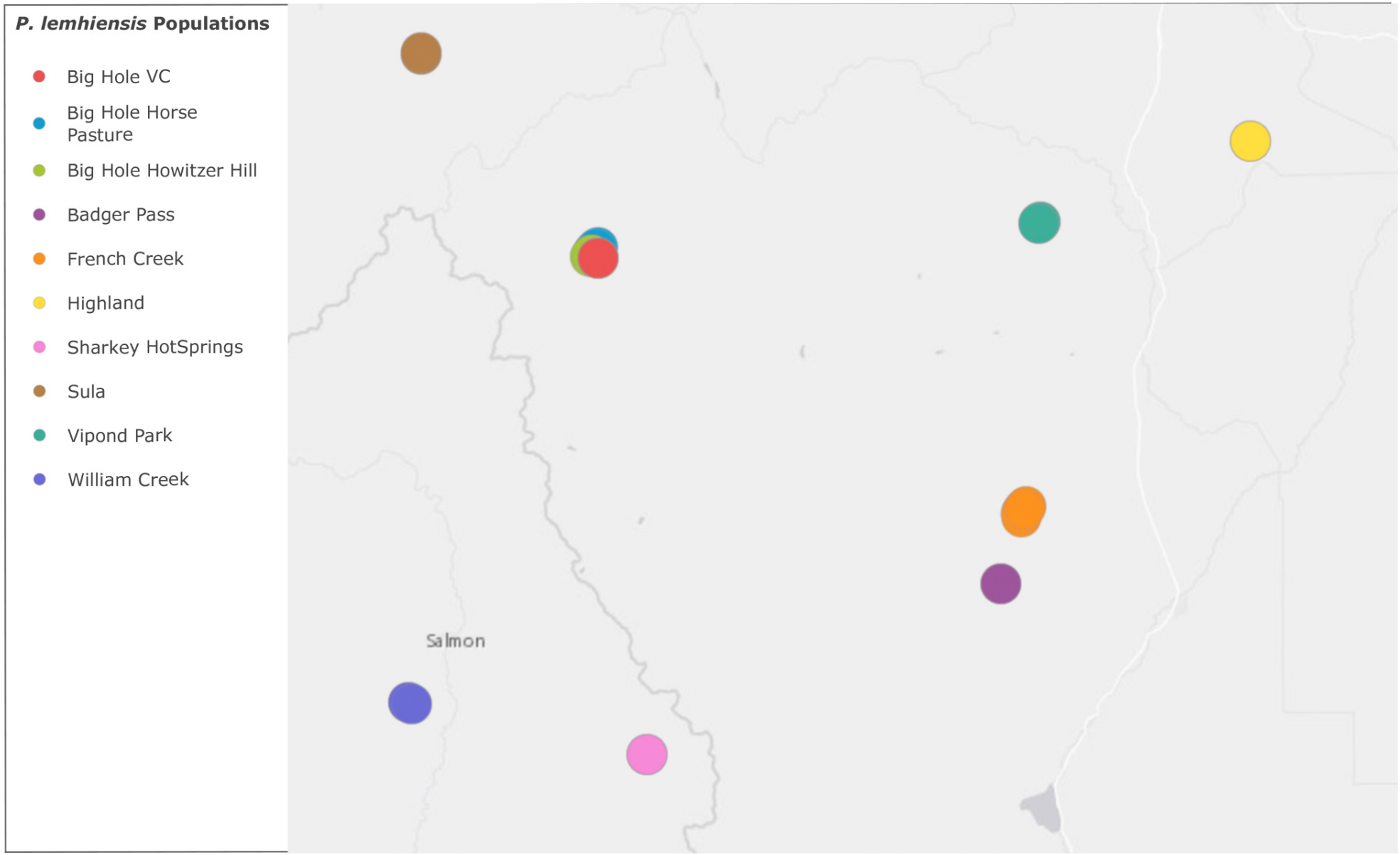
Mary Sagatelova – Rosa Rodríguez-Peña - Tom Rodhouse - Andrea Wolfe

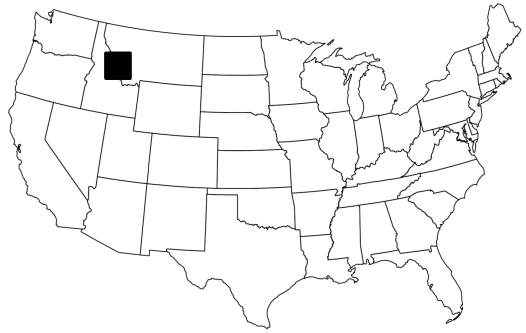
Goal of the Study

Assess the genetic diversity and structure within and among 10 populations of *P. lemhiensis* in Idaho and Montana to infer on species vulnerability.

Use findings to inform on conservation initiatives.



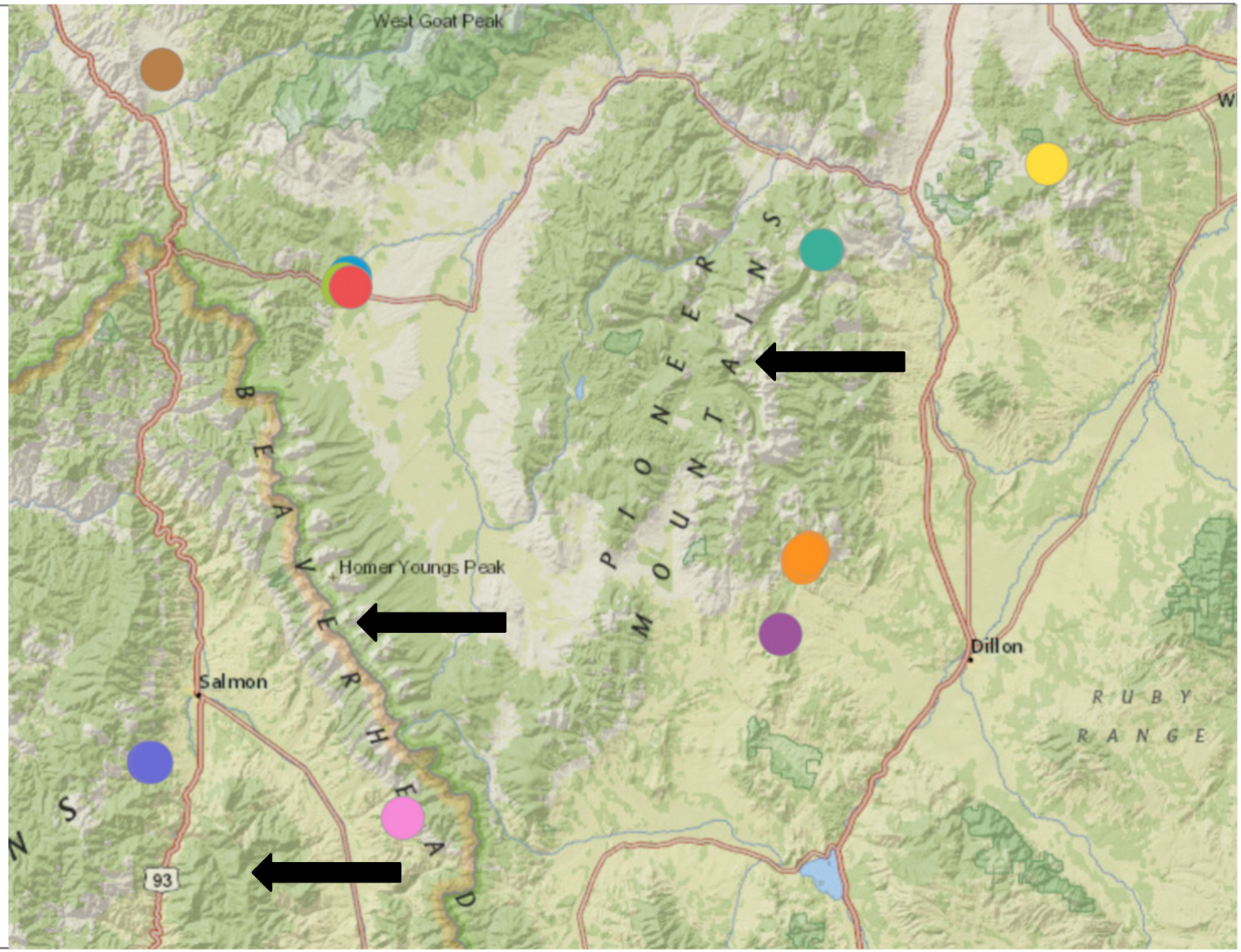


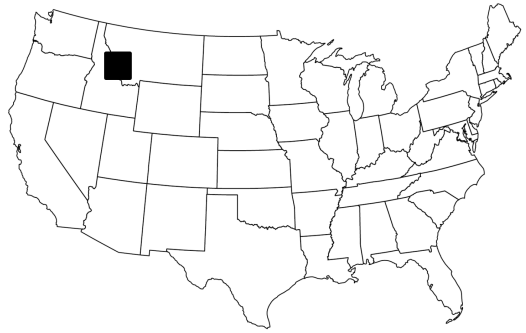


- 10 populations across Montana and Idaho
- 2 populations in Idaho and 8 in Montana

***P. lemhiensis* Populations**

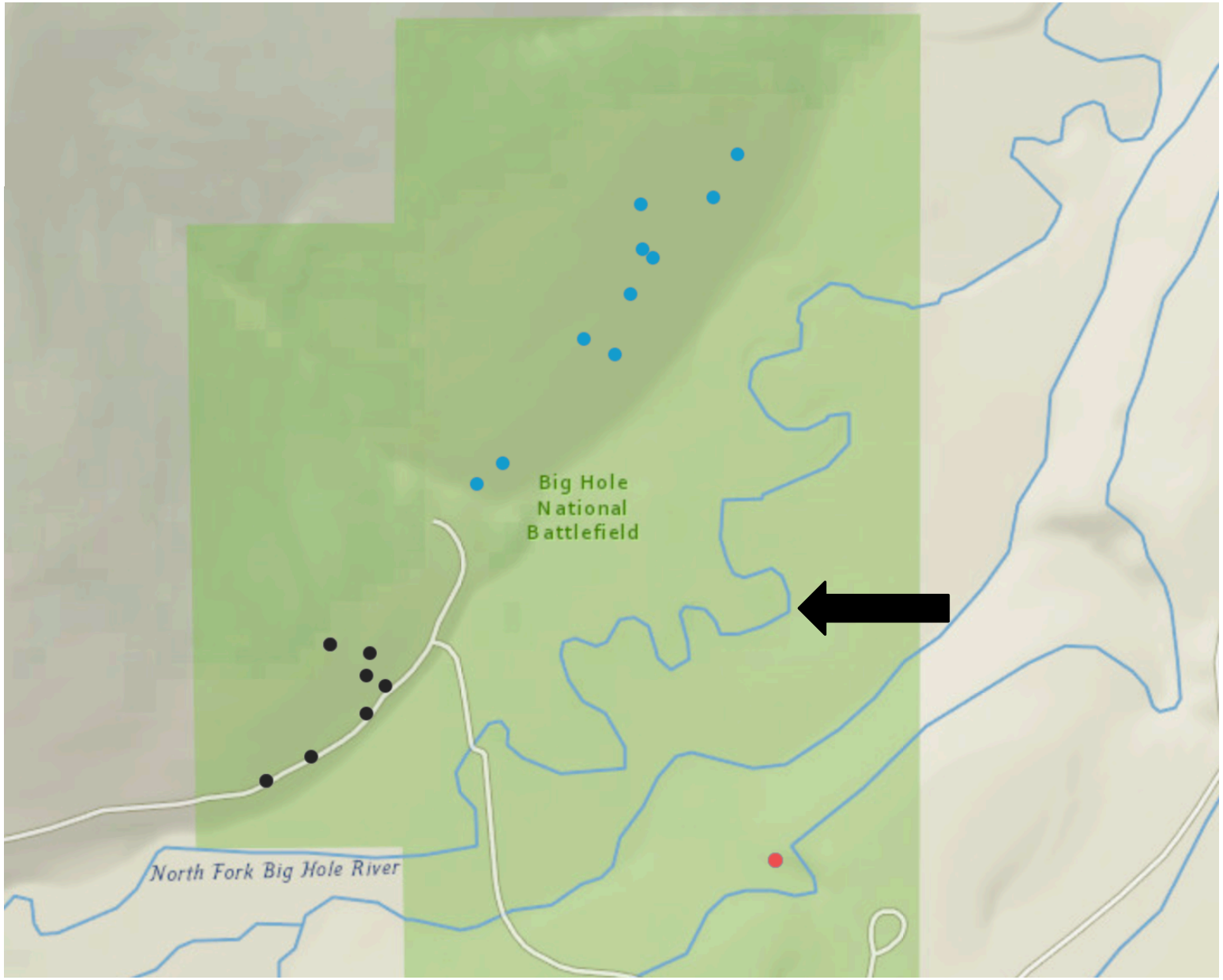
- Big Hole VC
- Big Hole Horse Pasture
- Big Hole Howitzer Hill
- Badger Pass
- French Creek
- Highland
- Sharkey HotSprings
- Sula
- Vipond Park
- William Creek





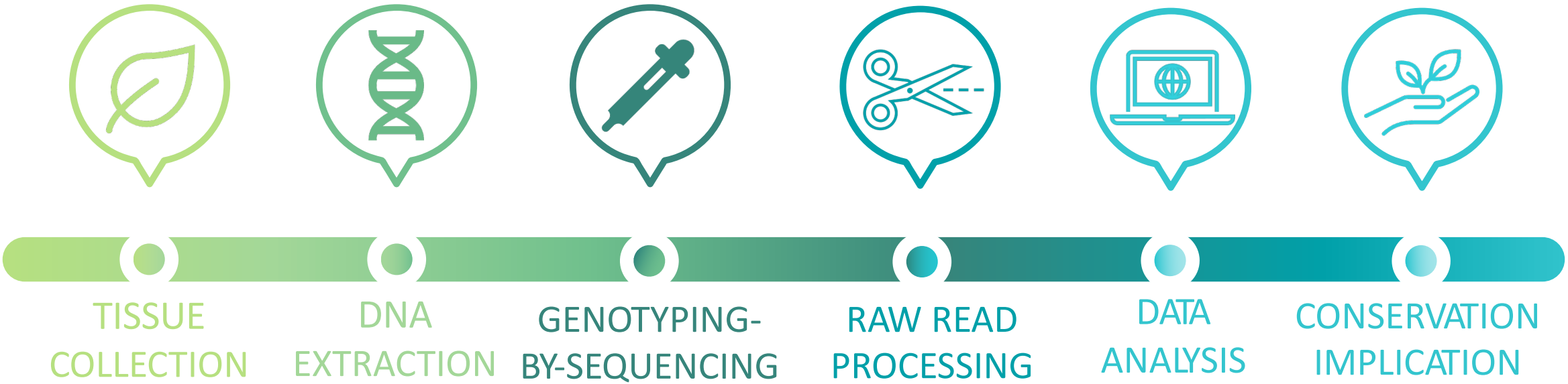
- 10 populations across Montana and Idaho
- 2 populations in Idaho and 8 in Montana
- There are notable natural barriers

- Big Hole Visitor Center
- Big Hole Horse Pasture
- Big Hole Howitzer Hill



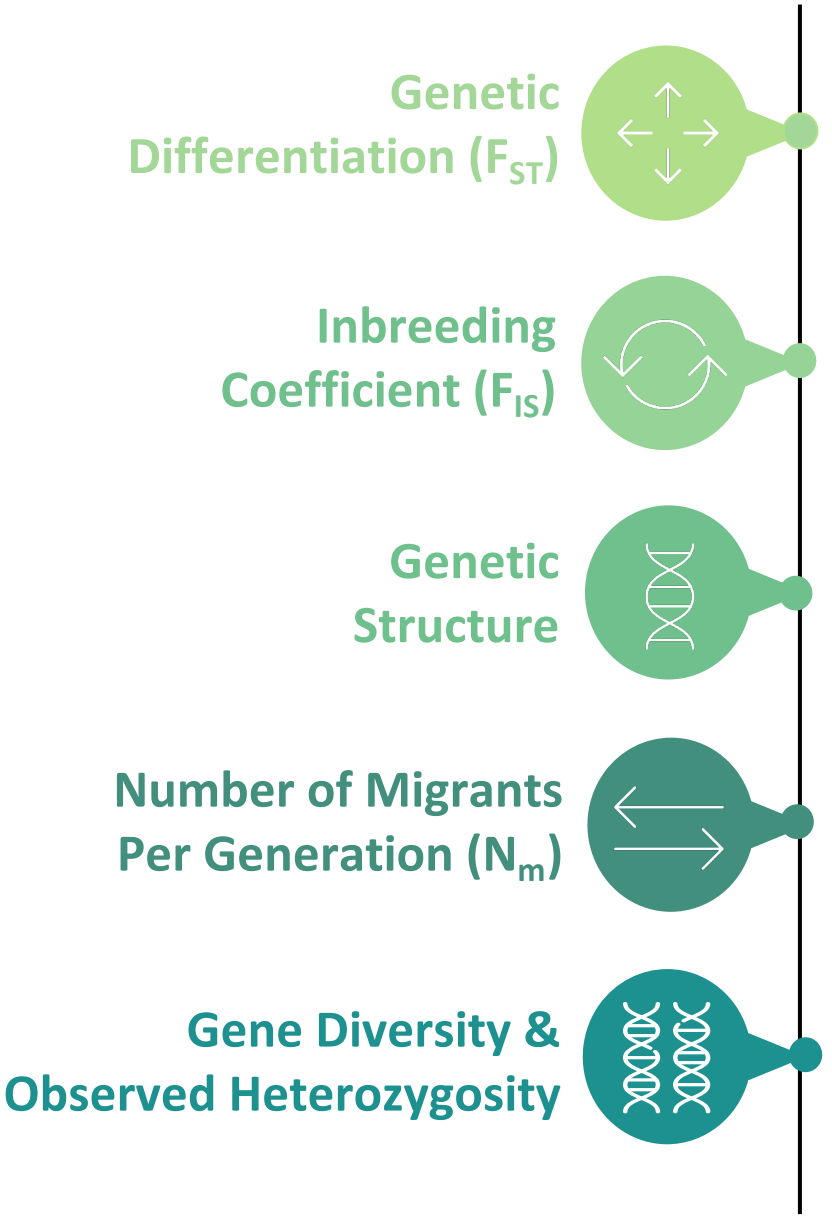
1 notable natural barrier

METHODOLOGY



Sequencing Statistics

- After raw data was cleaned, 93 out of 96 individuals remained.
- 795 loci utilized for population genetics analysis – much coverage when compared to the 225 loci used in a previous AFLP analysis!
- Cleaned data was used to conduct analyses.



F_{ST} – Genetic Differentiation

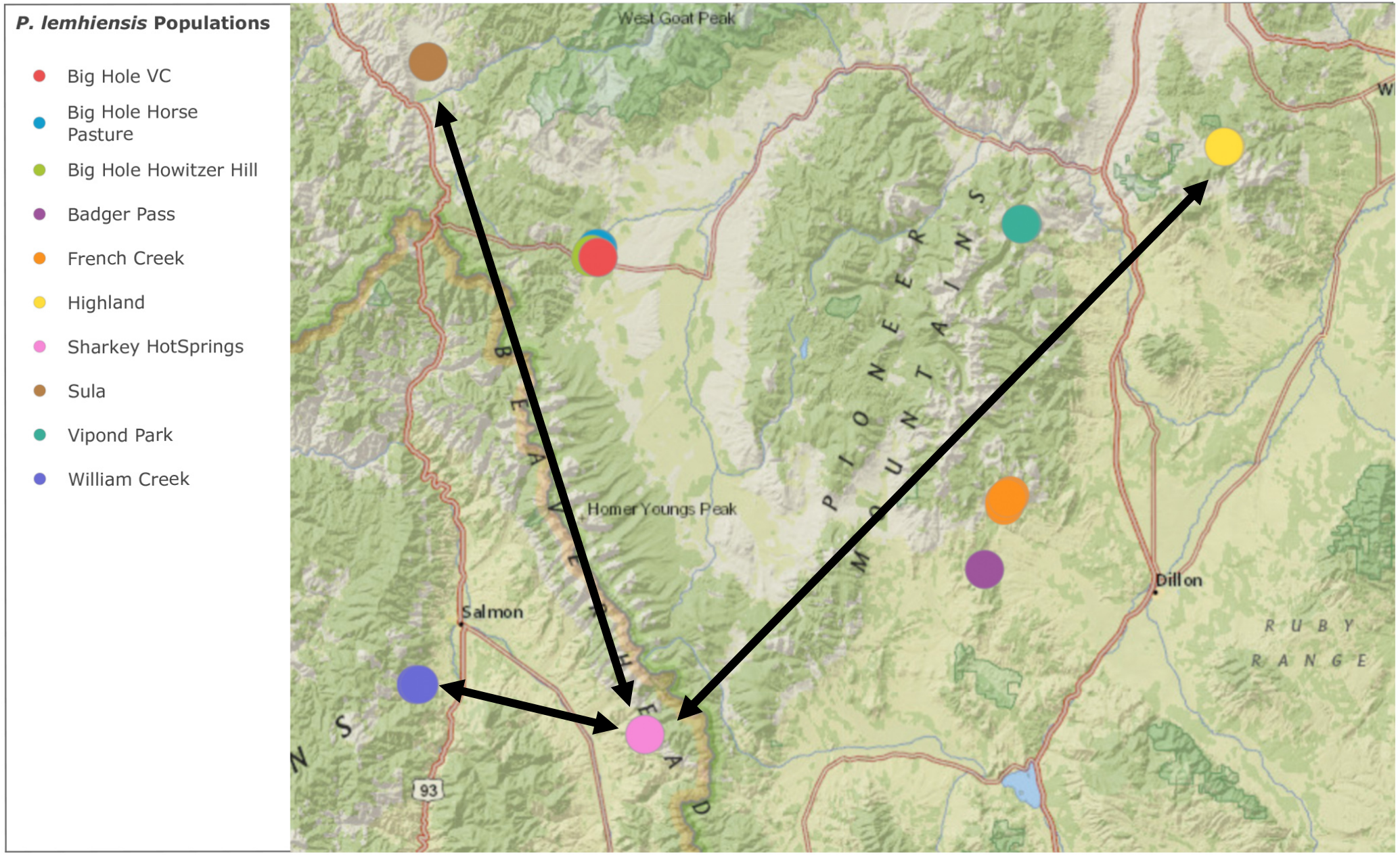
- Measure the degree of genetic differentiation.
- Ranges 0-1 with higher values indicating higher degrees of variation.
- Conflicting opinions on threshold values and their implications for conservation assessments.
  - F_{ST} values >0.15 are considered to represent significant genetic differentiation (Frankham et al. 2002).
  - Lowe & Allendorf (2010) suggests that:
    - F_{ST} values <0.02 indicates sufficient gene flow and maintenance of similar allelic frequencies.
    - F_{ST} values ≥0.20 indicate insufficient gene flow such that harmful effects of inbreeding could be seen in small populations.

F_{ST} values

	Big Hole	Sharkey Hot Springs	Badger Pass	Highland	Vipond Park	French Creek	William Creek	Sula
Big Hole	0							
Sharkey Hot Springs	0.1830813538	0						
Badger Pass	0.1238784205	0.1710562158	0					
Highland	0.1316174246	0.2541139003	0.1375298361	0				
Vipond Park	0.1071924586	0.2394010545	0.09512410877	0.1108514684	0			
French Creek	0.1312882962	0.194111655	0.02506466868	0.1518384454	0.1005431759	0		
William Creek	0.1555108811	0.2493429682	0.1791015636	0.2219020052	0.1708782925	0.1878133849	0	
Sula	0.1232422255	0.2803090552	0.2103420566	0.2256931903	0.207213723	0.218437887	0.2115942122	0

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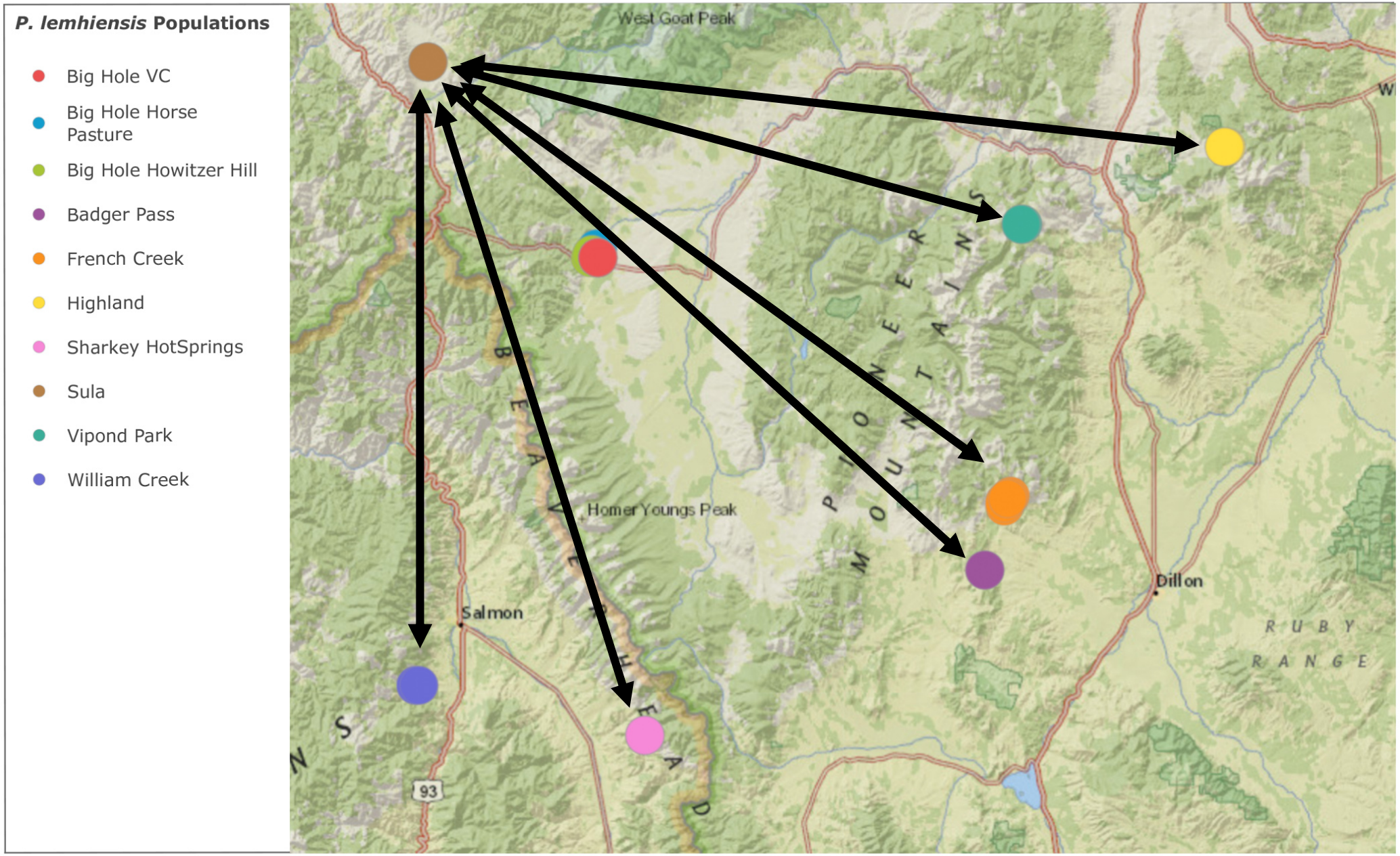


Highest degree of differentiation between Sula and Sharkey Hot Springs populations

Second highest degree of differentiation between Sharkey Hot Springs and Highland populations

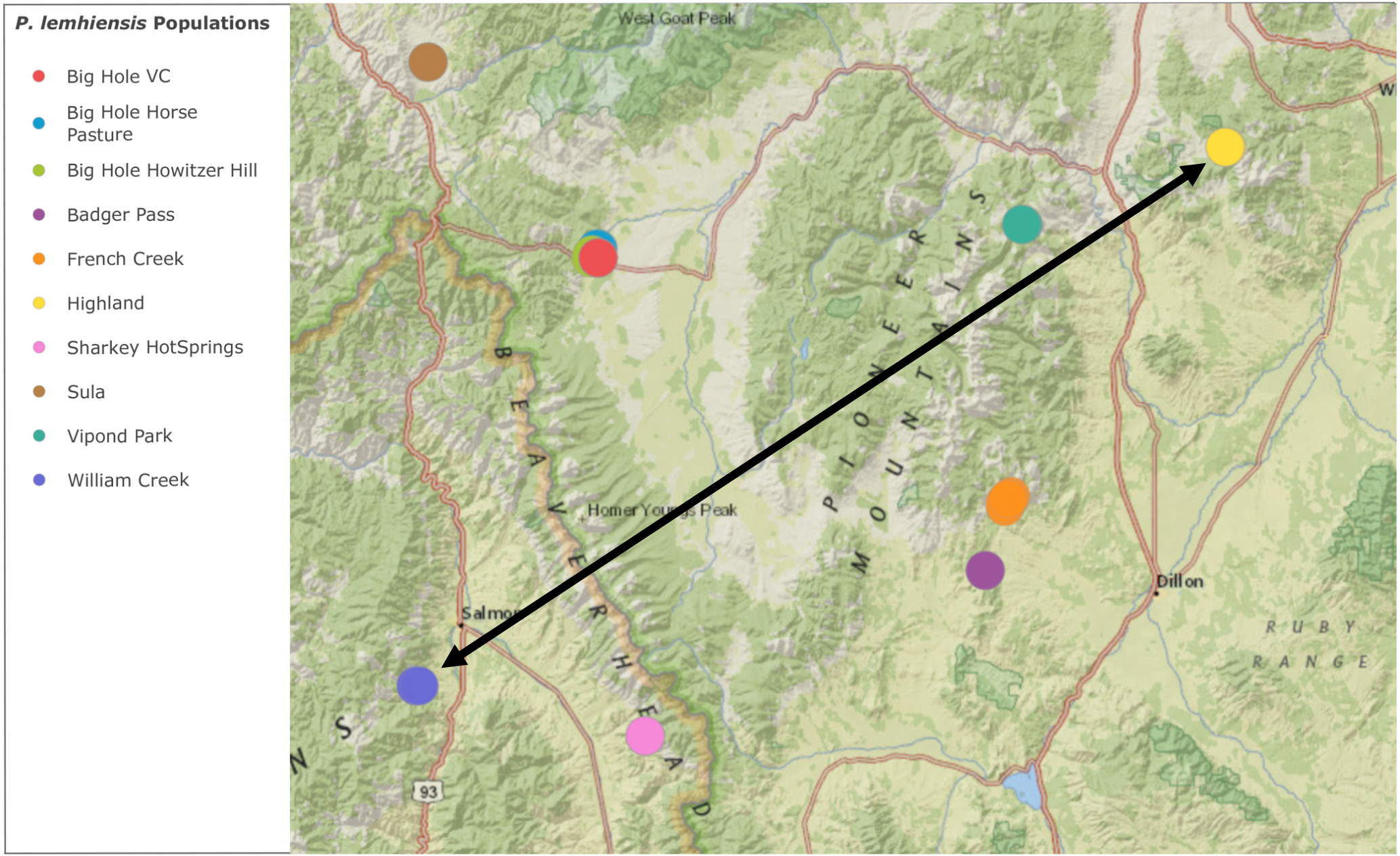
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P. lemhiensis Populations
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Big Hole Horse Pasture
Big Hole Howitzer Hill
Badger Pass
French Creek
Highland
Sharkey HotSprings
Sula
Vipond Park
William Creek
West Goat Peak
Homer Youngs Peak
Salmon
Dillon
BEAVERHEAD MOUNTAINS
PIONEER MOUNTAINS
RUBY RANGE
93

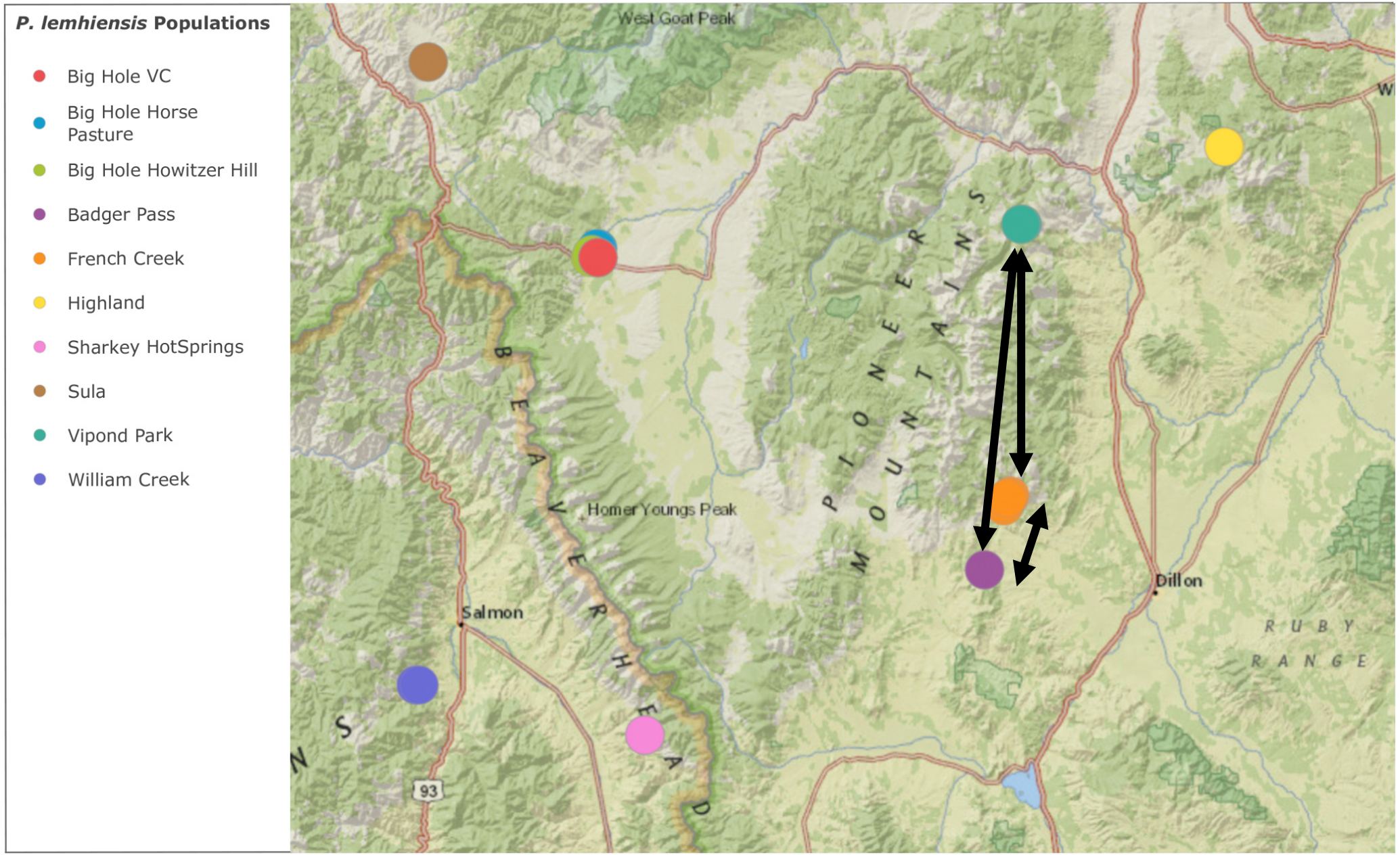
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Sula	0.1232422255	0.2803090552	0.2103420566	0.2256931903	0.207213723	0.218437887	0.2115942122	0



Lowest degree of genetic differentiation between Badger Pass and French Creek Populations

Generally, low differentiation between Bader Pass, French Creek, and Vipond Park

F_{ST} – Genetic Differentiation General Conclusions

Genetic differentiation is highest between geographically distant populations as well as those with natural barriers.

Genetic differentiation is lowest between geographically close populations.

F_{IS} value: Measure of inbreeding. Ranges 0-1 with higher values indicating higher degrees of inbreeding within populations.

Population	F_{IS}	99% CI
Sula	0.01329218107	-0.02, 0.07
French Creek	0.0005514705882	-0.07, 0.04
Vipond Park	-0.02071174377	-0.06, 0.03
Badger Pass	-0.0054375	-0.05, 0.04
Highland	-0.039140625	-.10, -0.01
Sharkey Hot Springs	-0.01914414414	-0.07, 0.03
William Creek	-0.03435294118	0.01, 0.05
Big Hole	0.03174081238	0.1, 0.15

Negative values of F_{IS} indicate that individuals in a population are less related than you would expect under a model of random mating.

- Could indicate larger ancestral population size that is now fragmented into smaller populations.

Highest value at Big Hole populations, but collectively these populations represent 1,300 alleles, so this high of a value is not unexpected.

P. lemhiensis inbreeding coefficients in comparison to other Penstemon:

Population	F_{IS}
Sula	0.01329218107
French Creek	0.0005514705882
Vipond Park	-0.02071174377
Badger Pass	-0.0054375
Highland	-0.039140625
Sharkey Hot Springs	-0.01914414414
William Creek	-0.03435294118
Big Hole	0.03174081238

Species	Estimated Population Size	F_{IS} Value
P. scariosus var. albifluvis	12,215	0.230
P. scariosus var. cyanomontanus	N/A	0.200
P. scariosus var. garrettii	N/A	0.290
P. scariosus var. scariosus	N/A	0.370
P. debilis	4100	0.23
P. albomarginatus	100,000-200,000	0.16

Rodríguez-Peña et al. 2018; Wolfe et al. 2014; Wolfe et al. 2016

Low F_{IS} values reflect the likelihood of gene flow among these populations.

F_{IS} – Inbreeding General Conclusions

Low F_{IS} values are indicative of little inbreeding occurring within each population.

However, trends due to small, fragmented populations could be undetectable currently.

Outbreeding detected with each population implies that gene flow between populations is occurring.

Observed Heterozygosity and Gene Diversity

Population	H_O	H_S
Sula	0.09488020177	0.09813366961
French Creek	0.1128050314	0.1116645649
Vipond Park	0.1109445844	0.1096469105
Badger Pass	0.115813367	0.1150315259
Highland	0.1117254408	0.1061209068
Sharkey Hot Springs	0.09271807838	0.0907079646
William Creek	0.1338413098	0.1287153652
Big Hole	0.1136649874	0.1181738035

H_O is a measure of genetic variation and H_S is a measure of gene diversity.

Discrepancies in these two values point at other forces affecting the populations (i.e. inbreeding)

- No significant differences between values for respective populations

This is consistent with the F_{IS} values – populations that indicate outbreeding.

Values of gene diversity highest in William Creek and Big Hole populations

H_O and H_S General Conclusions

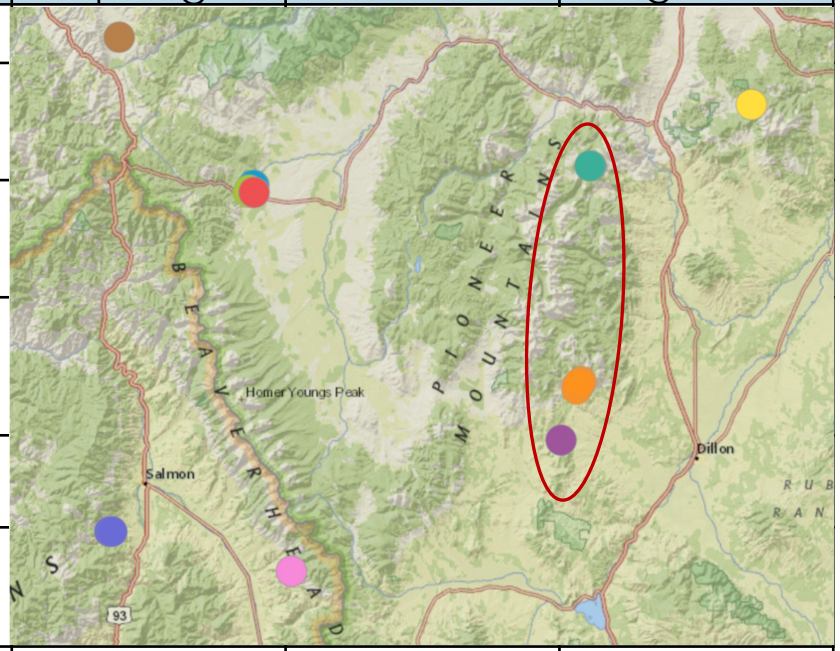
Broadly similar values across populations – gene flow is occurring between populations.

Larger ancestral population that has fragmented into smaller populations and we are not able to capture major effect on gene diversity yet.

Greatest gene diversity is seen in the Big Hole National Battlefield and William Creek populations

Number of Migrants Per Generation Between Populations. Higher values indicate higher rate of gene flow between populations .

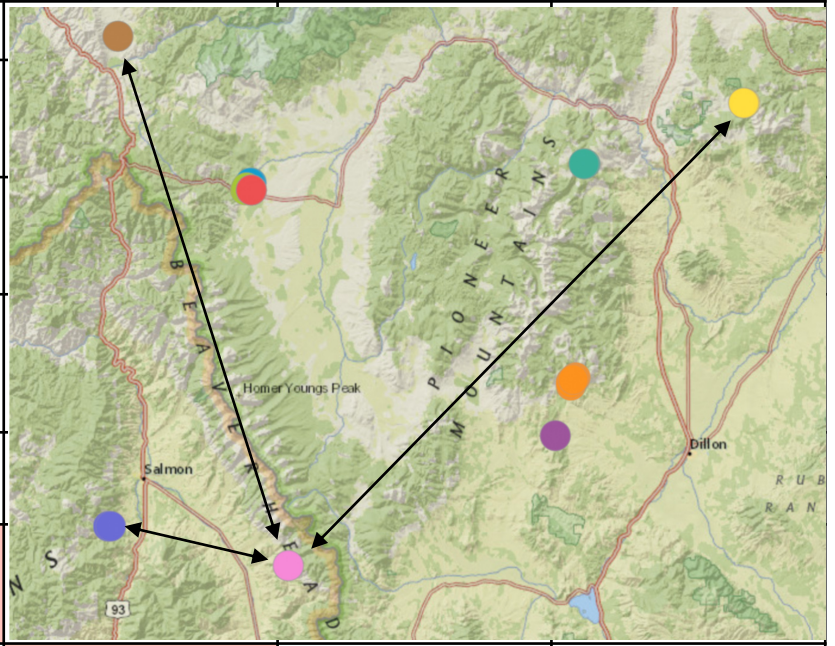
	Sula	French Creek	Vipond Park	Badger Pass	Highland	Sharkey Hot Springs	William Creek	Big Hole
Sula								
French Creek	1.73035							
Vipond Park	1.89179	4.52447						
Badger Pass	1.86151	18.87961	4.76018					
Highland	1.70739	2.82039	4.01759	3.15764				
Sharkey Hot Springs	1.26644	2.01433	1.57324	2.41305	1.46359			
William Creek	1.8519	2.15768	2.54617	2.29205	1.83034	1.48644		
Big Hole	3.50584	3.27735	4.11397	3.49479	3.26715	2.20799	2.84623	



Highest degree of gene flow between French Creek, Badger Pass, and Vipond Park
Lowest degree of gene flow between Sula, Sharkey Hot Springs, Highland, and William Creek.
However, all values are above 1, so gene flow between all populations is occurring

Number of Migrants Per Generation Between Populations. Higher values indicate higher rate of gene flow between populations .

	Sula	French Creek	Vipond Park	Badger Pass	Highland	Sharkey Hot Springs	William Creek	Big Hole
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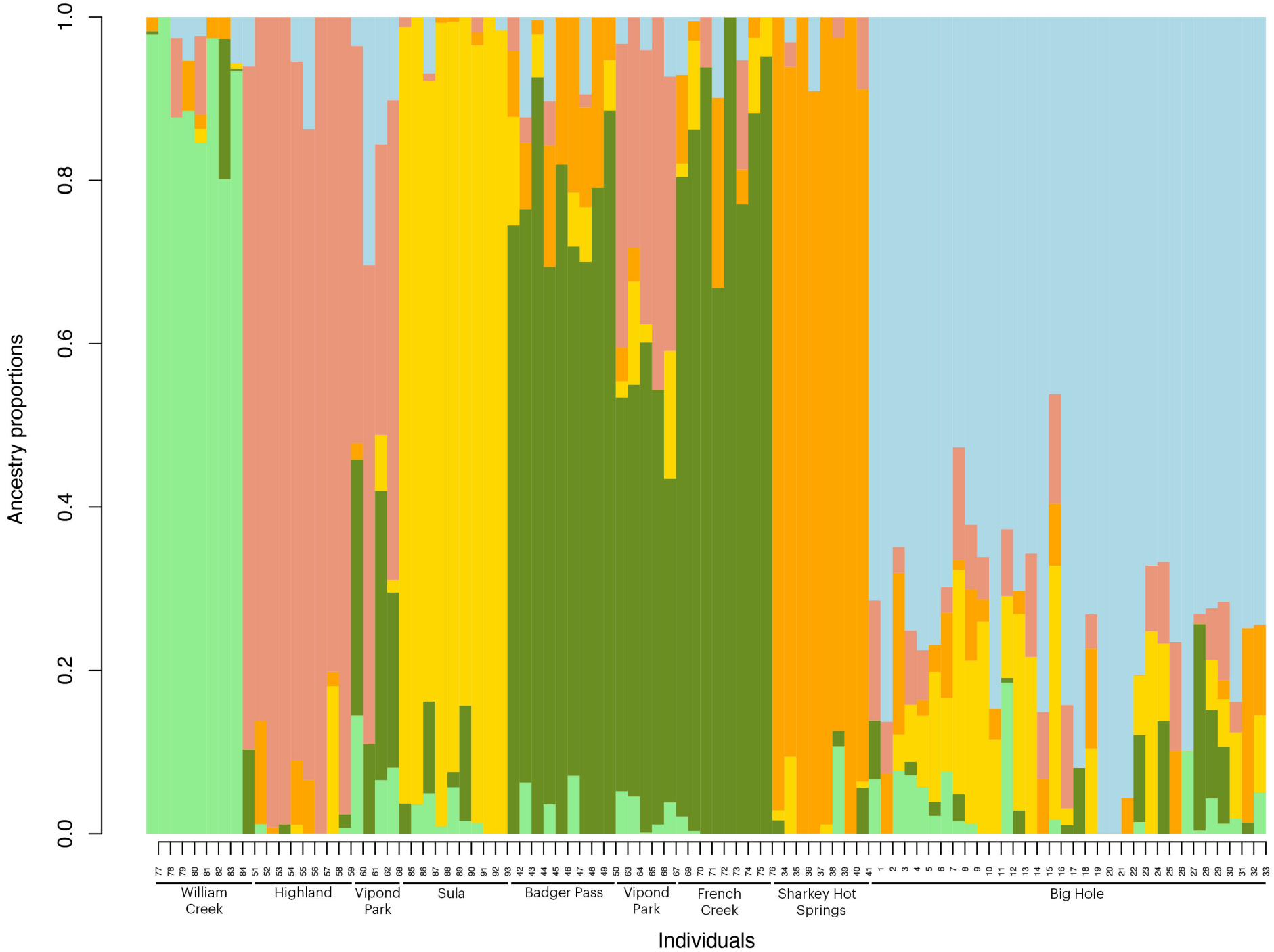
Number of Migrants Per Generation General Conclusions

Gene flow is occurring between populations.

- Corresponds with the F_{ST} values

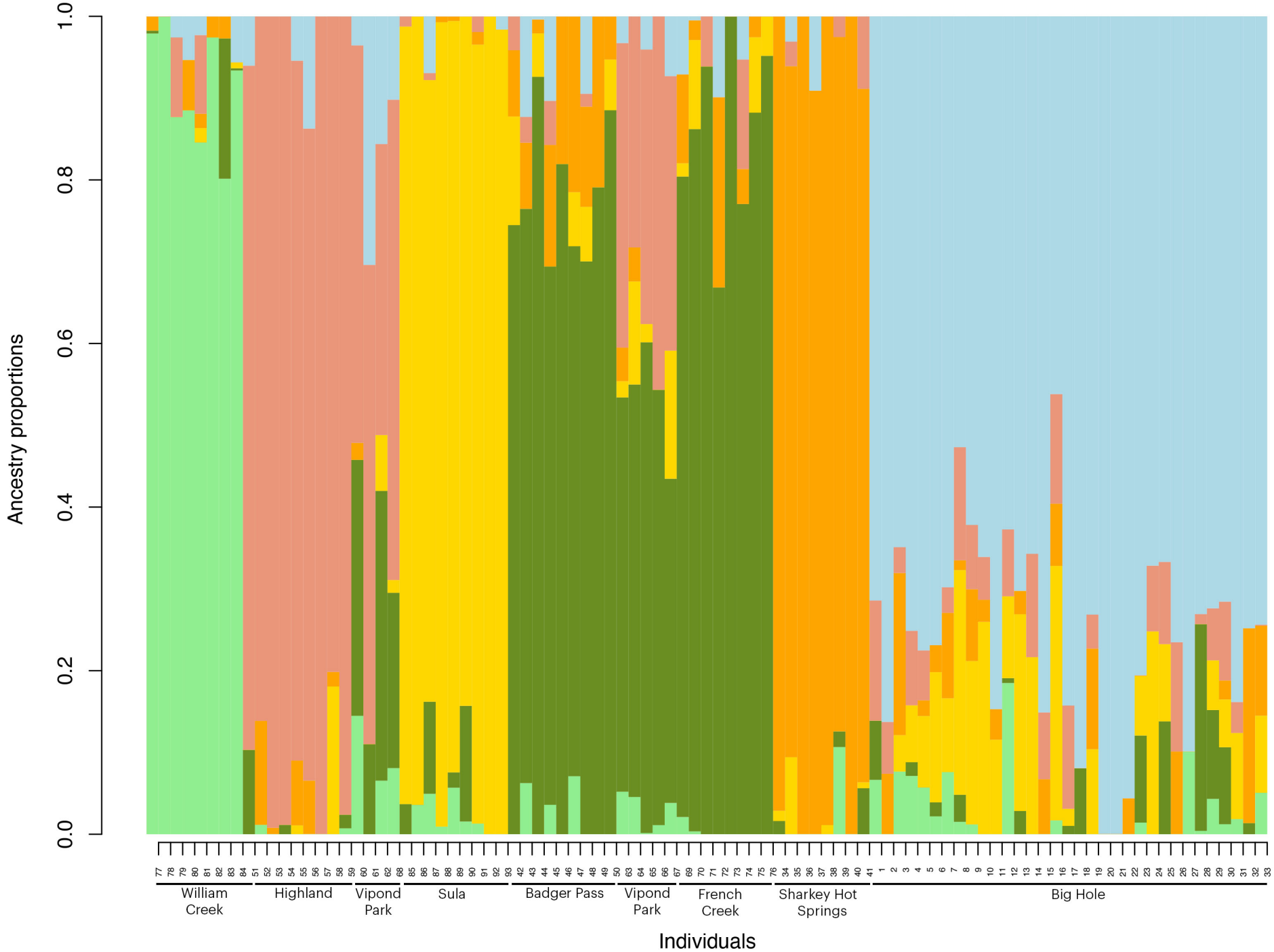
Highest degree of immigration is occurring between geographically close populations.

Lowest degree of immigration is occurring between geographically distant populations *but* it is occurring!



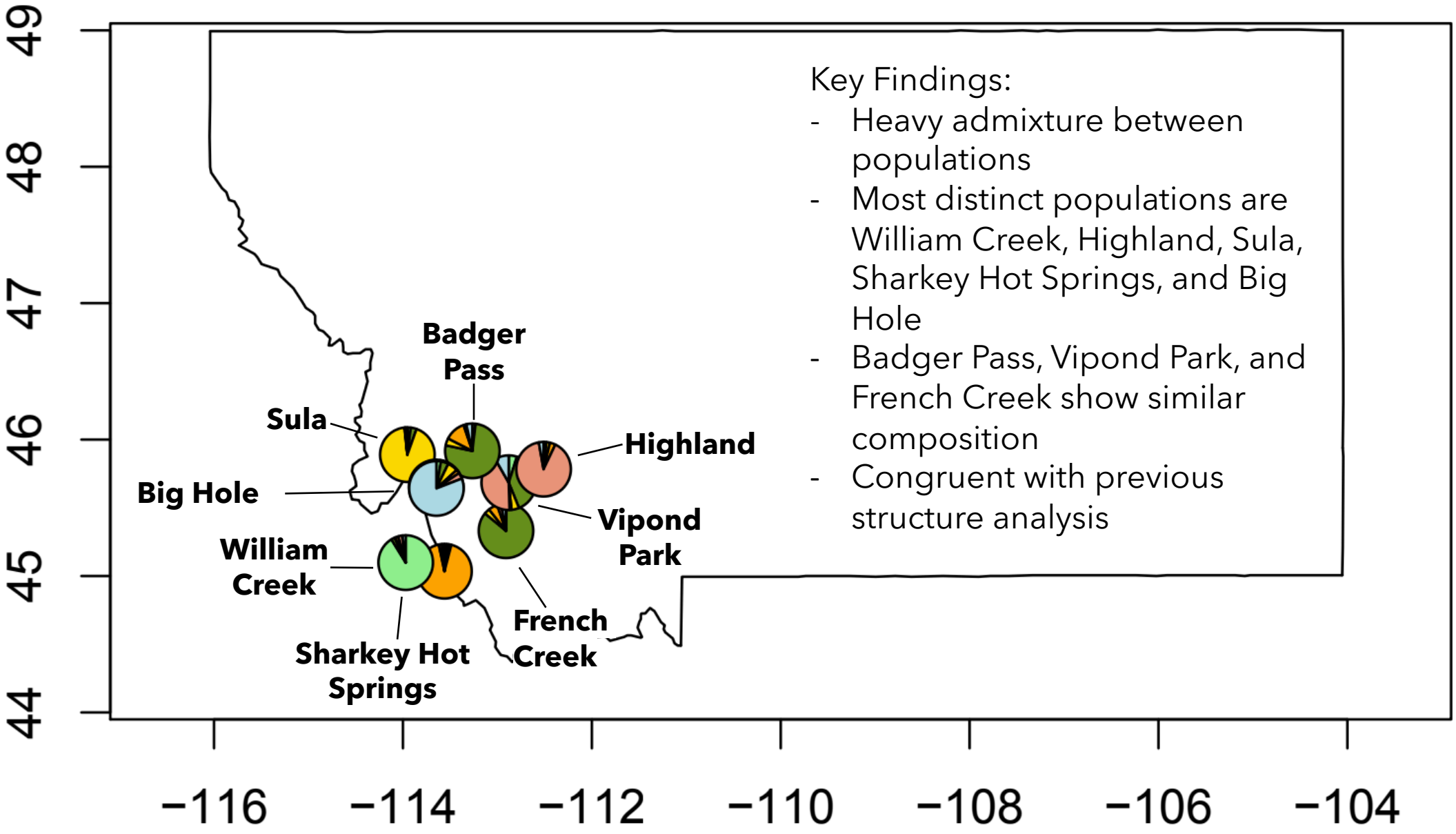
Ancestry Matrix:

- Detects the underlying genetic populations among a set of individuals genotyped at multiple markers using nonnegative matrix factorization (NMF) algorithms
- Computes the proportion of the genome of an individual originating from each inferred ancestral population
- In this graph, each column represents individuals



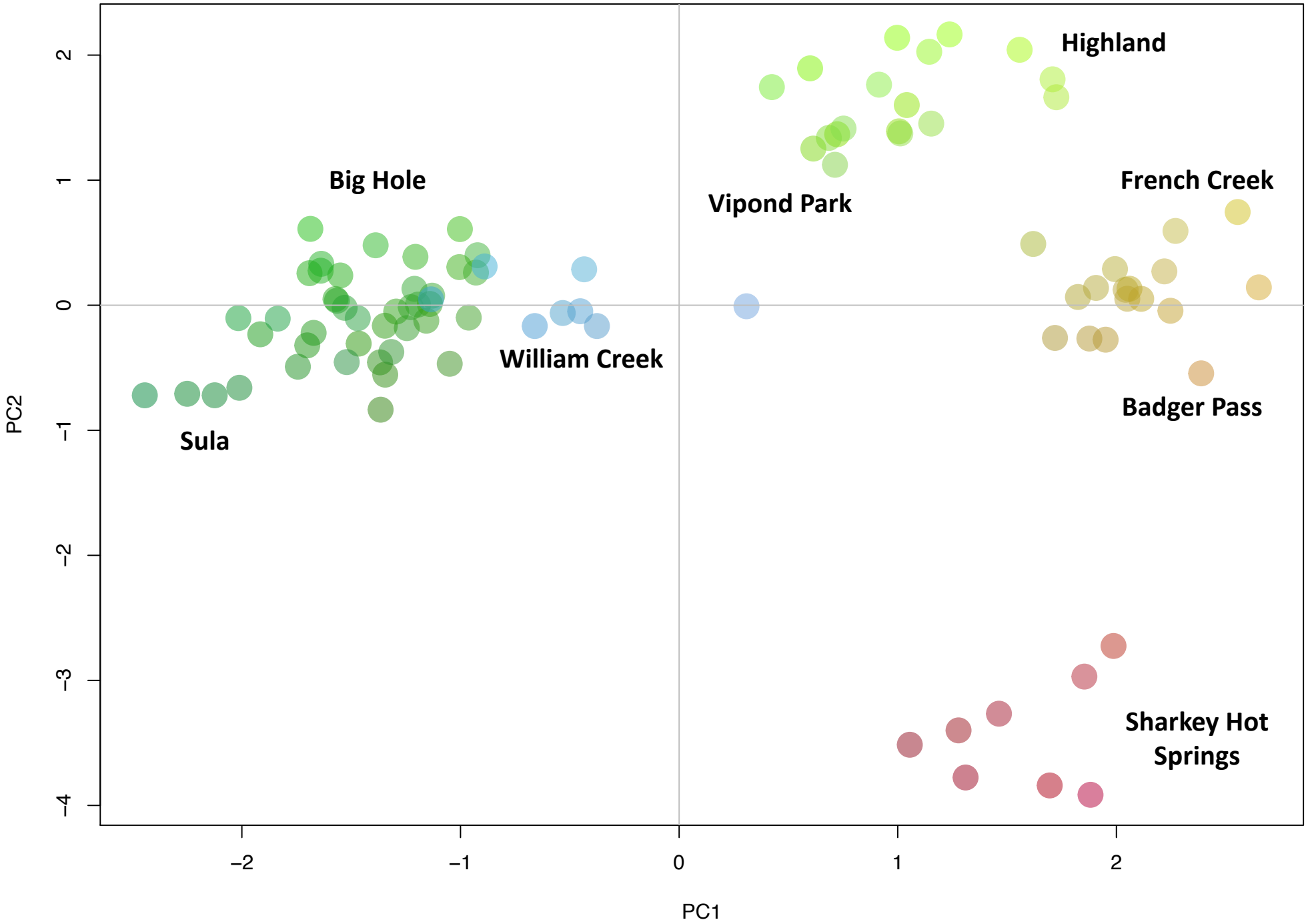
Key Findings:

- Heavy admixture across most individuals
- Big Hole populations cluster together. No major distinctions between three subpopulations
- Most distinct populations are William Creek, Highland, Sula, and Sharkey Hot Springs
- Badger Pass, Vipond Park, and French Creek cluster together



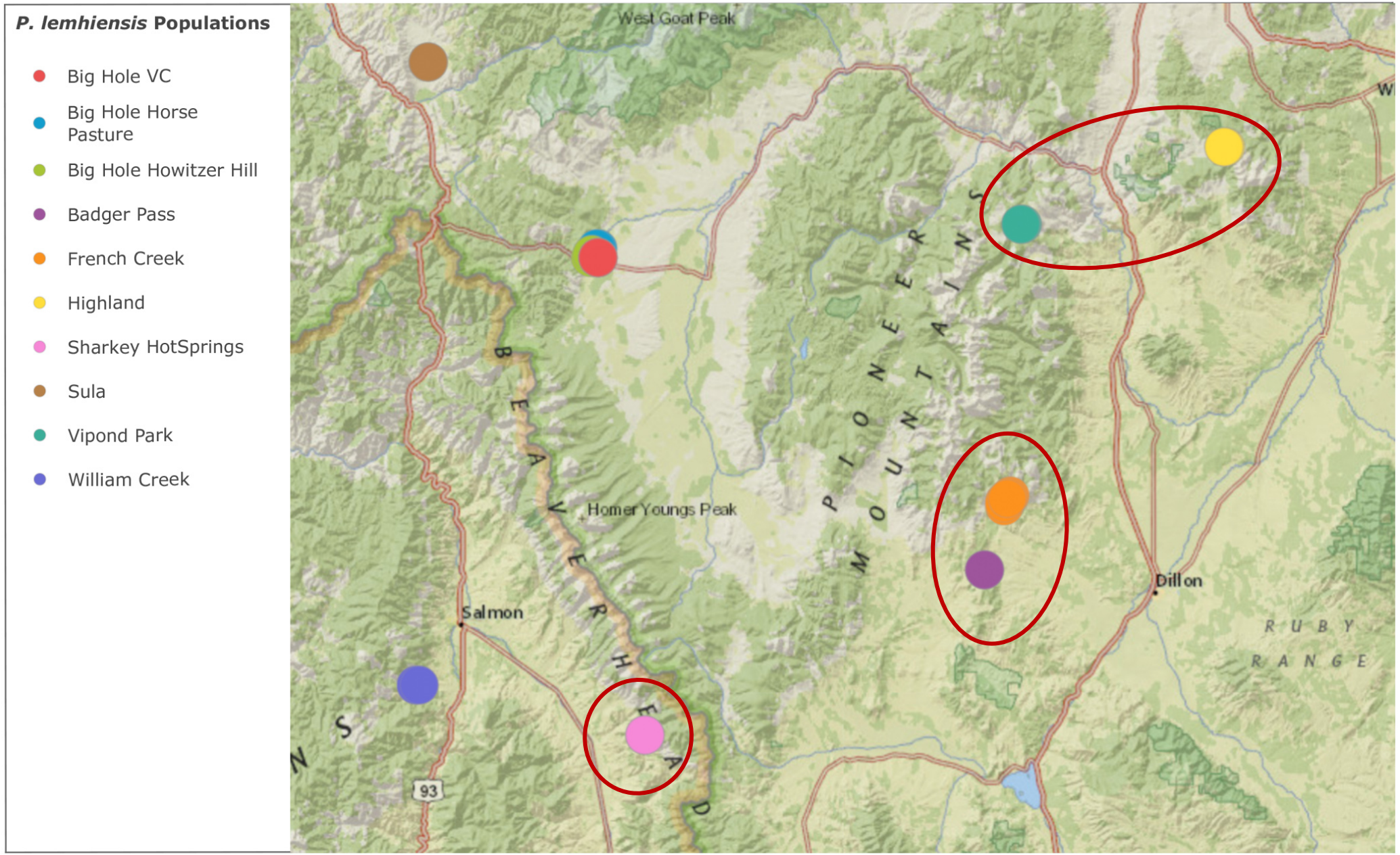
Key Findings:

- Heavy admixture between populations
- Most distinct populations are William Creek, Highland, Sula, Sharkey Hot Springs, and Big Hole
- Badger Pass, Vipond Park, and French Creek show similar composition
- Congruent with previous structure analysis

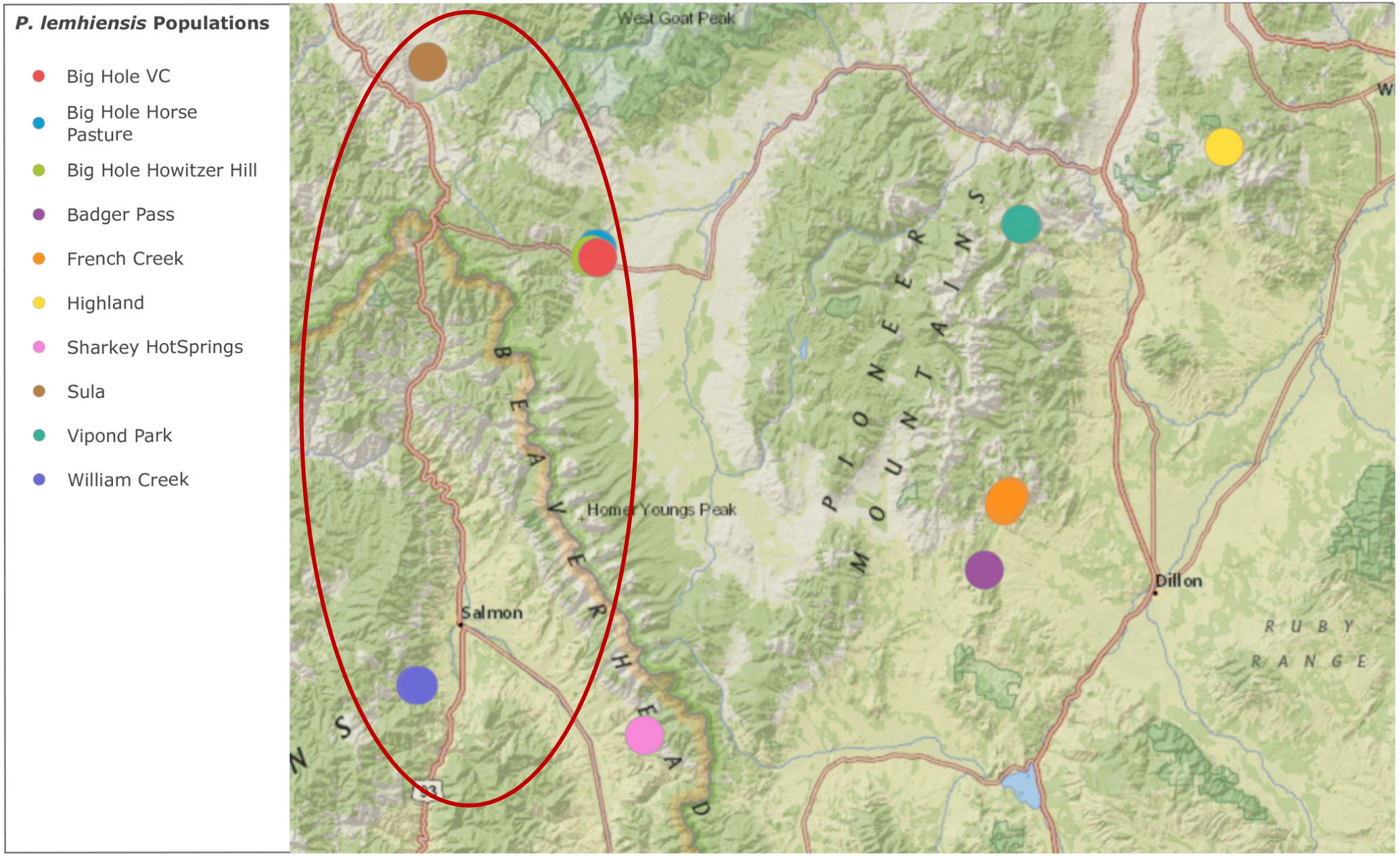


Principal Component Analysis:

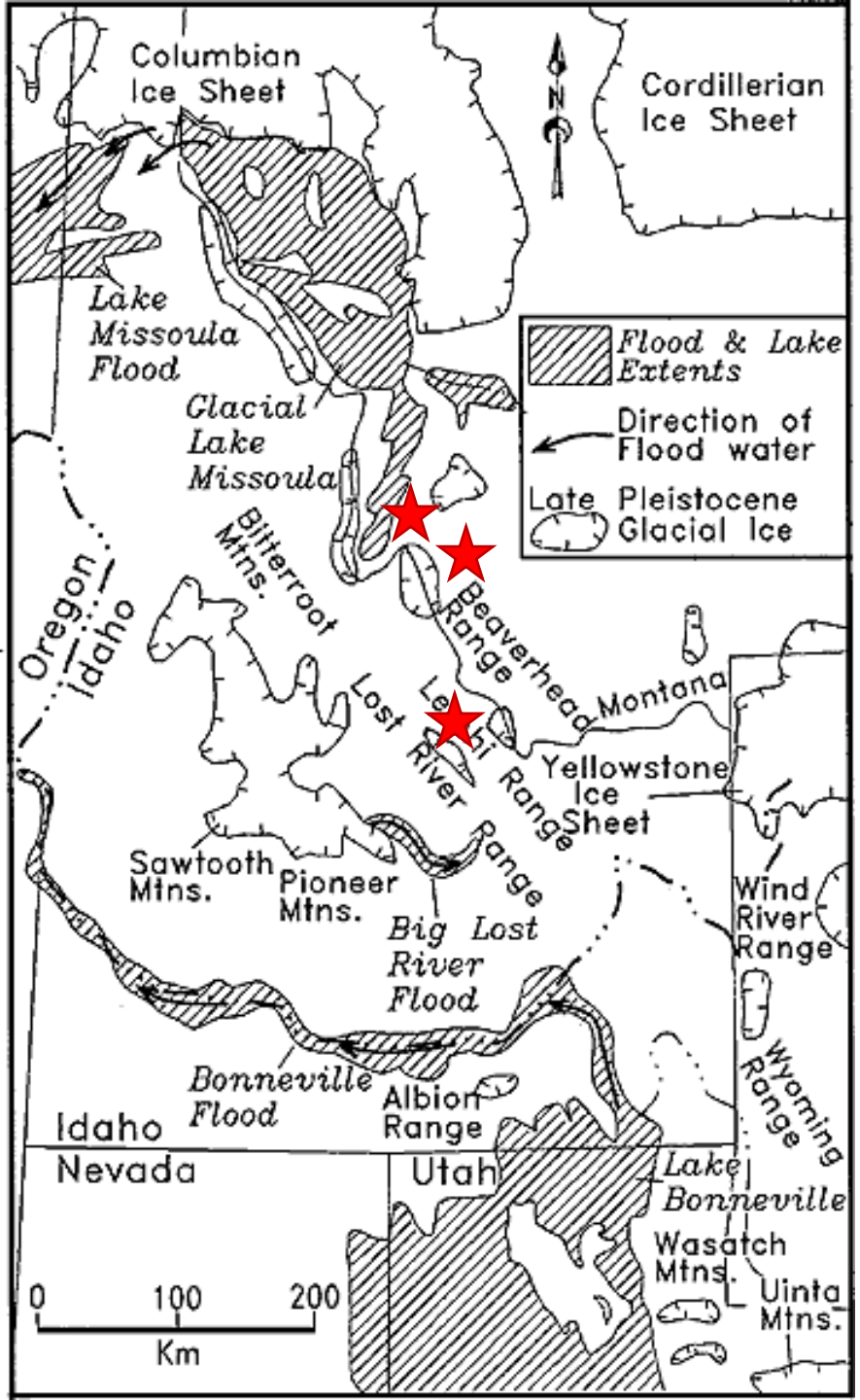
- Algorithm that reduces the dimensionality of the data but retains most of the variation.
- Calculate principal components (PC) that explain the variation among individuals within genetic data due to genetic ancestry.
- PCs are displayed as continuous axes that reflect genetic variation due to ancestry.
- Individuals with similar values for a particular PC will group together

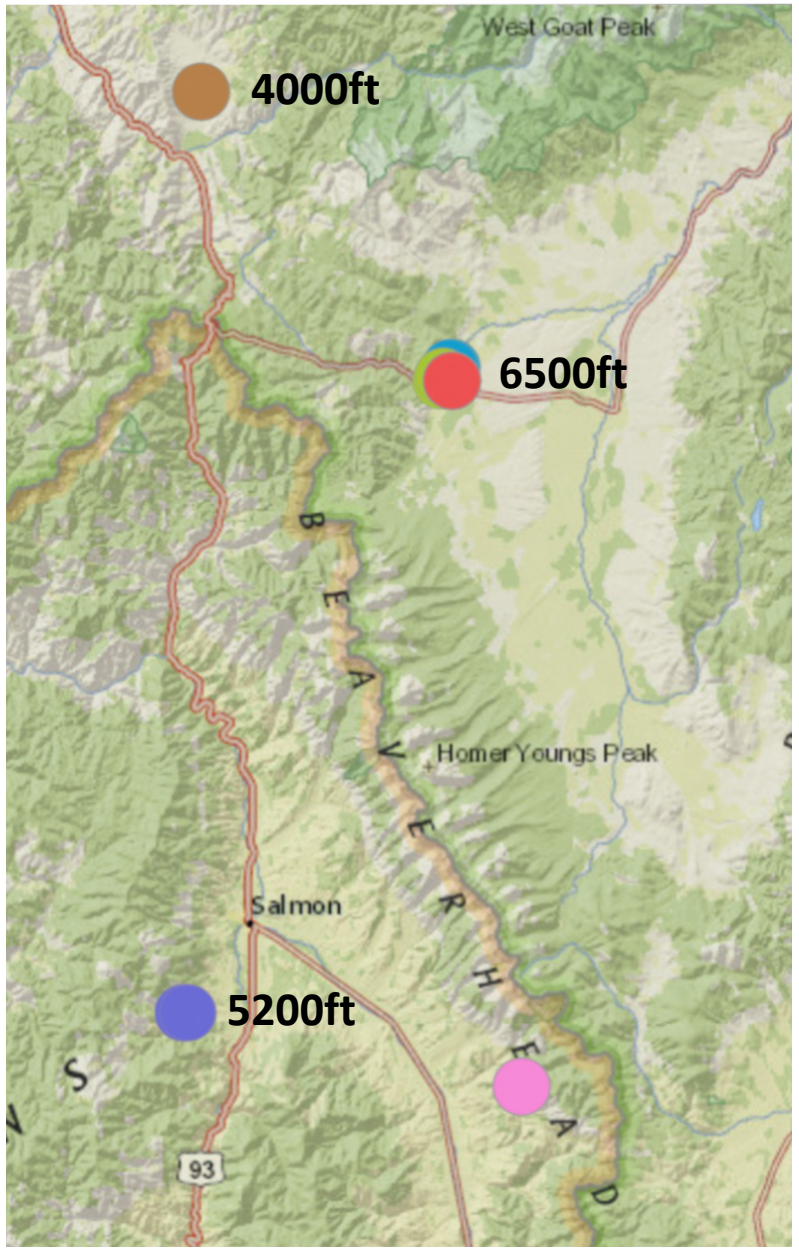


Clustering makes sense given geographic distance between some populations!



However, some clustering is not explained by geographic proximity.

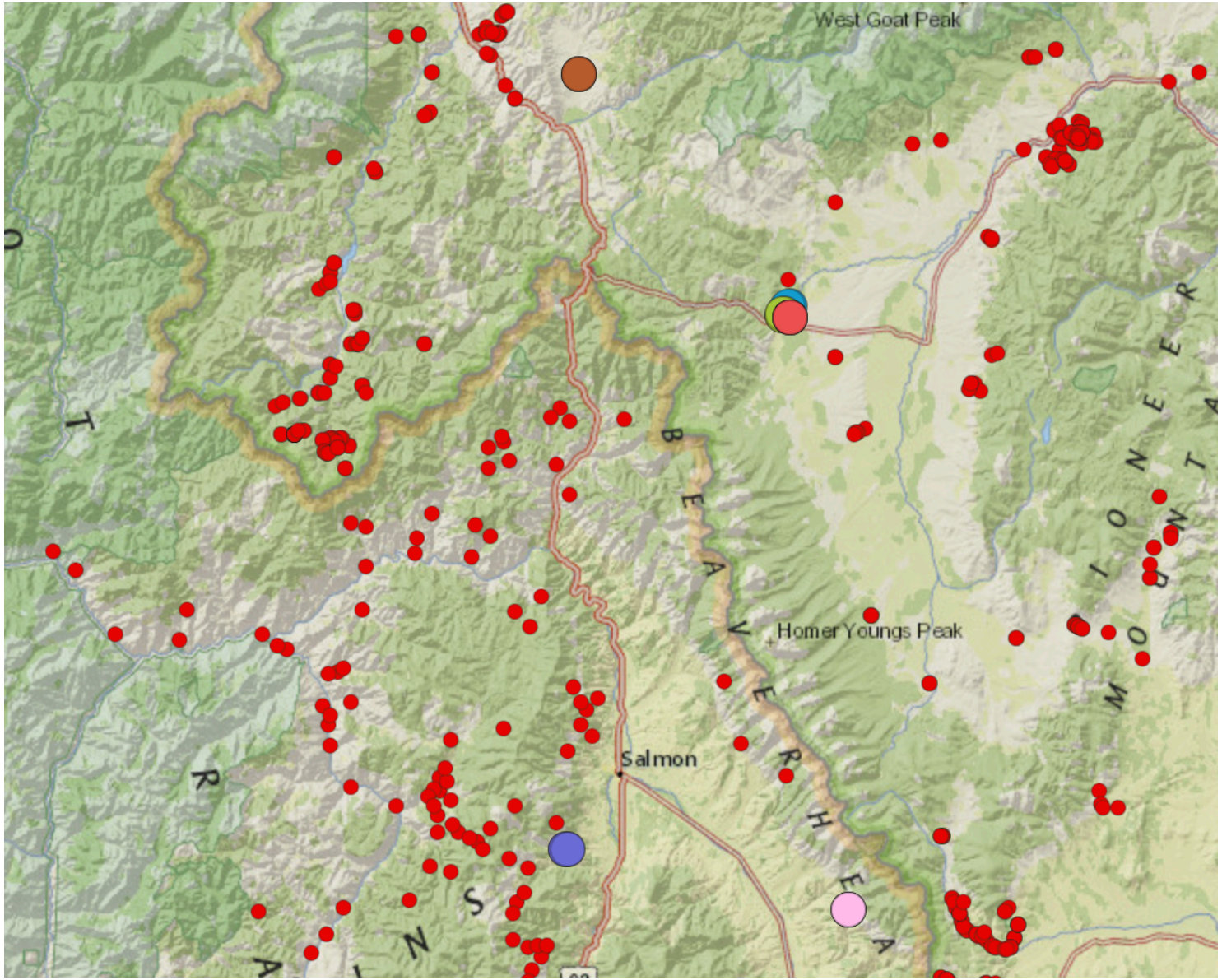


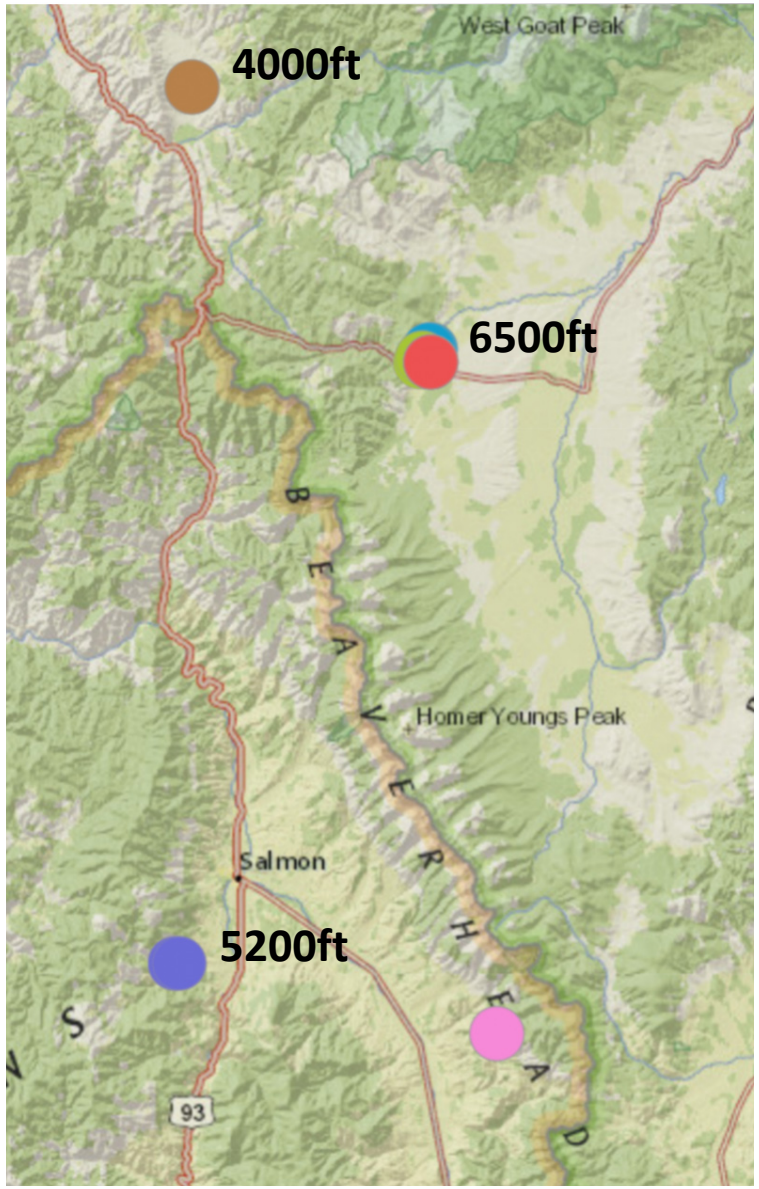


Pleistocene Glaciation:

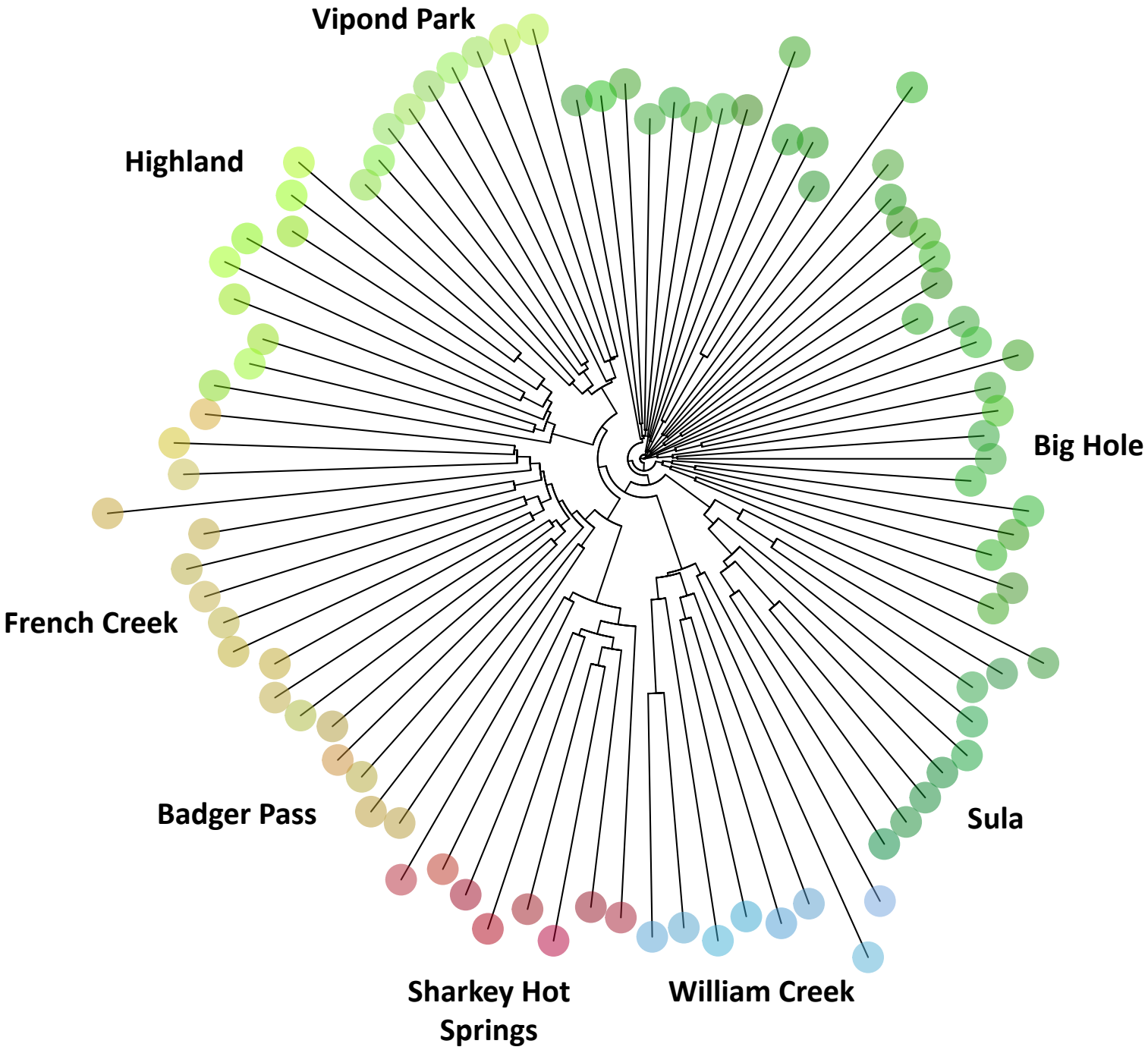
- Clade of *Penstemon* with *P. lemhiensis* is only 1-1.2 mya
  - 13 major and 9 minor glaciation events in this time
- Alpine glaciers and glacial ice sheets retreat likely shifted populations back north

Connectivity and ancestral diversity detected by PCA is likely from populations forced south during glaciation and then retreated north as glaciers melted.





Observed localities of *P. lemhiensis* by Montana Natural Heritage Program



Neighbor Joining Tree:

- Genetic distance matrix is used to produce a representation of population relationships in the form of a tree.
- Not a representation of evolutionary history for the populations.
- Same relationships as reflected in the PCA

- Badger Pass and French Creek populations form one group
- Highland and Vipond Park populations are genetically close to one another but form independent branches.
    - Reflective of the relationship seen in the PCA.
- Individuals at Big Hole are genetically more similar to one another than any other population, forming 1 large branch.

Major Conclusions as Applicable to Conservation Measures

If species are fragmented into genetically isolated subpopulations, there is a greater risk of extinction. **This degree of genetic isolation is not *currently* evident in *P. lemhiensis*.**

Geographically close populations are experiencing higher degrees of gene flow and migration.

However, with more isolated populations, given the distribution of the populations and the geographic separation by barriers, there is likely the retention of ancestral polymorphic conditions. While some populations are not currently undergoing gene flow, there was contact in the past and we are still seeing that signal in the analysis.

There has been insufficient time for differences to accrue among separate subpopulations.

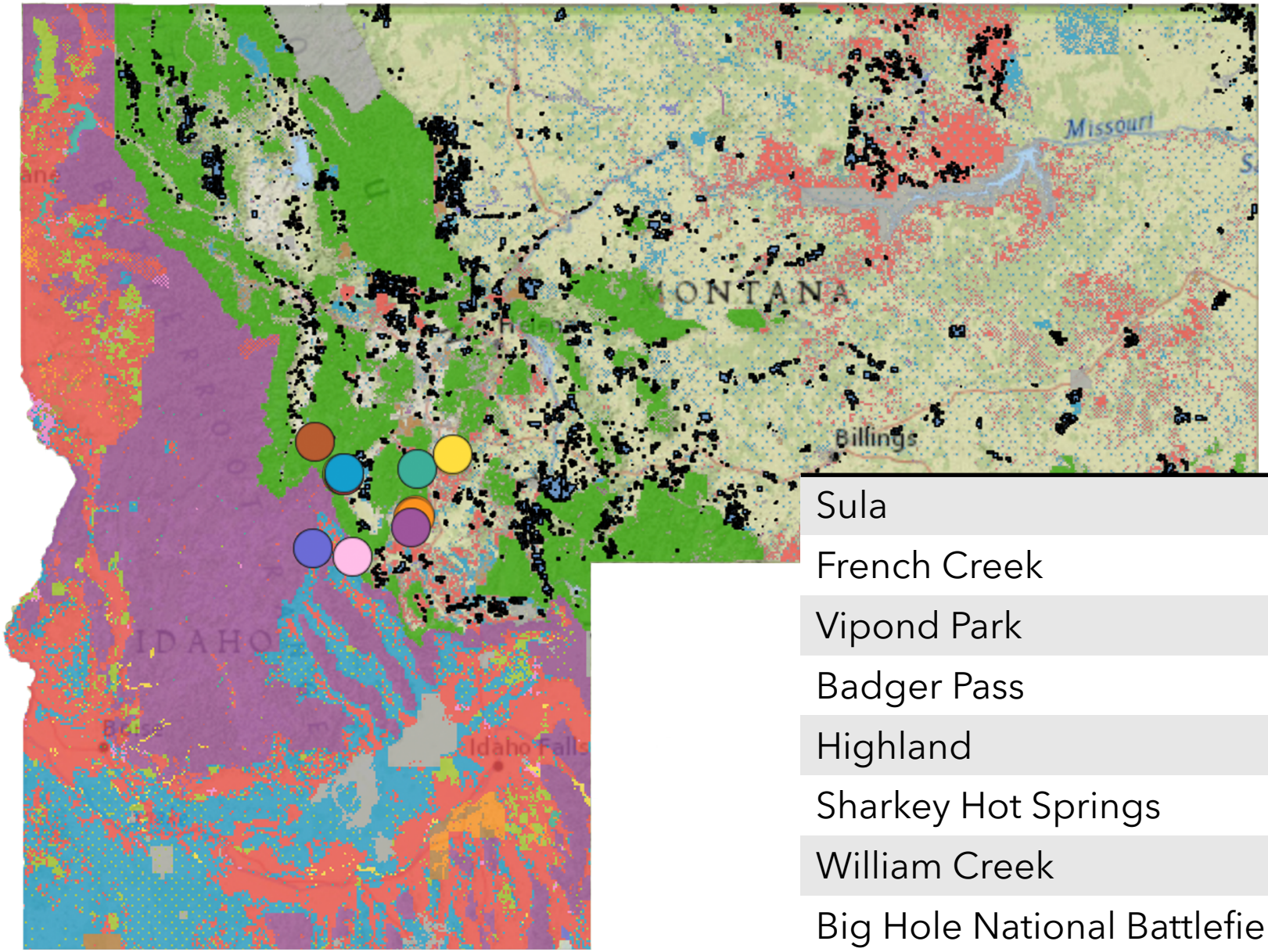
Conservation Efforts

Less Differentiated Populations – Populations not very isolated from one another and gene flow is maintained. Low population size is still a major threat to diversity moving forward.

- Manage ecological and demographic threats within these populations.
- Facilitate migration via pollen or seed immigration (Ottewell et al. 2016).

Highly Differentiated Populations – Populations more isolated from one another and gene flow is maintained but more restricted.

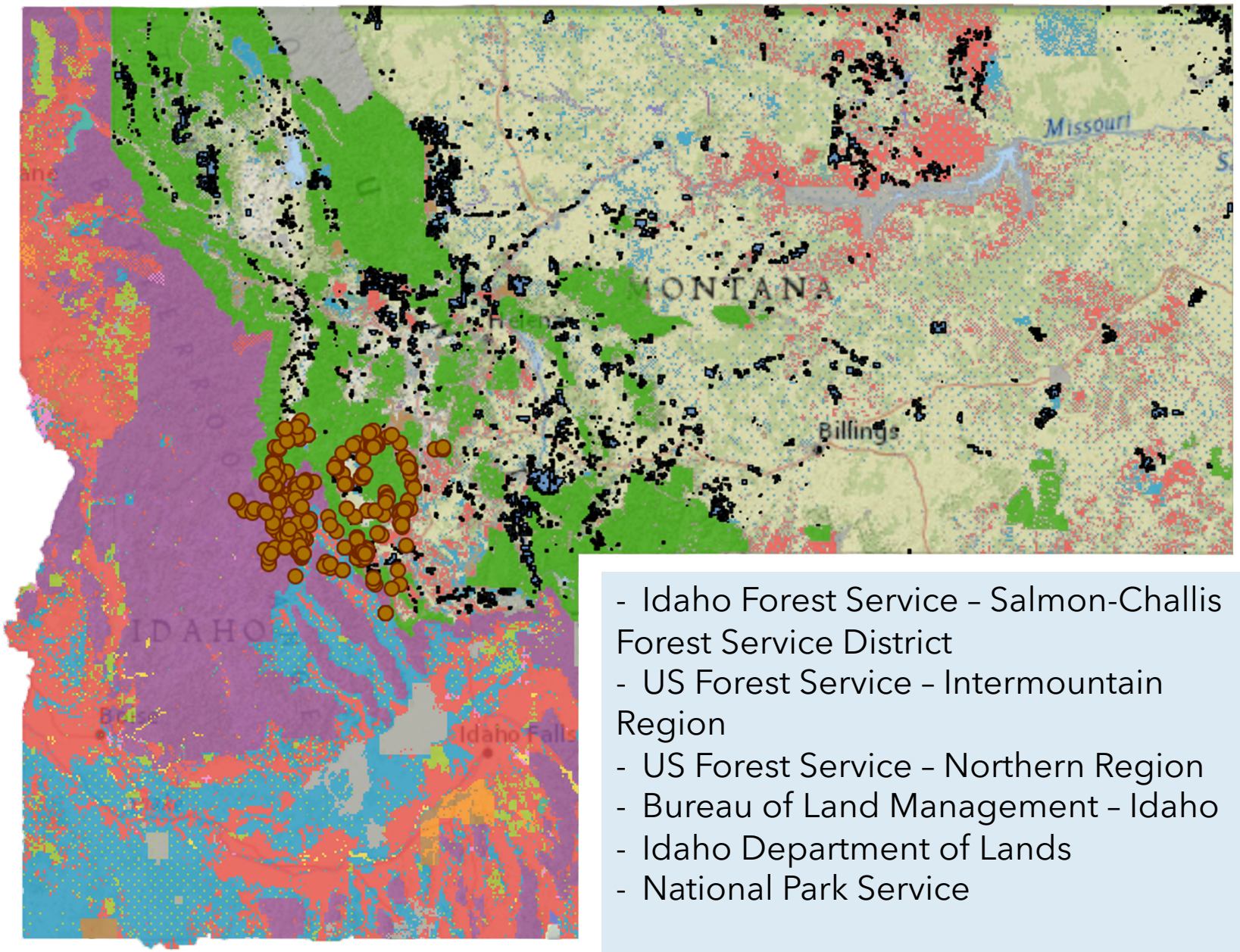
- Focused germplasm conservation efforts
- Manage ecological and demographic threats within these populations.
- Preserve populations with most diversity – Big Hole and William Creek



Institutional Barriers:

- Distinct agency management of land across species range for *P. lemhiensis* will influence collaborative conservation efforts

Sula	US Forest Service
French Creek	US Forest Service
Vipond Park	US Forest Service
Badger Pass	US Forest Service
Highland	US Forest Service
Sharkey Hot Springs	Bureau of Land Management
William Creek	Bureau of Land Management
Big Hole National Battlefield	National Park Service



Considering all known *P. lemhiensis* localities reported by the Idaho and Montana Natural Heritage Program, the number of agencies that would be involved in species wide conservation efforts is vast

- Idaho Forest Service – Salmon-Challis Forest Service District
- US Forest Service – Intermountain Region
- US Forest Service – Northern Region
- Bureau of Land Management – Idaho
- Idaho Department of Lands
- National Park Service
- Idaho Depart. of Env. Quality
- Idaho Fish and Game
- Montana Natural Heritage Program
- Montana EPA
- Private Land
- Bureau of Land Management – Montana/Dakotas
- Montana Department of Natural Resources and Conservation



NATIONAL PARK SERVICE

The Organic Act of 1916 (16 U.S.C. §1) dictates a land management approach that **prioritizes land preservation, recreation, and fish and wildlife conservation**.

Park Service rules, regulations, and policies place a heavy emphasize on the conservation of park resources. The Park Service's managed lands and resources generally receive a higher degree of protection than those of BLM and FS.



BUREAU OF LAND MANAGEMENT

The Federal Land Policy and Management Act of 1976 (43 U.S.C. §§1701) dictates **a multi-purpose land management approach** by the Bureau of Land Management.

BLM is authorized to facilitate multiple uses for land, including recreation, grazing, timber, watershed, wildlife and fish habitat, and conservation. Historic extraction management practices.

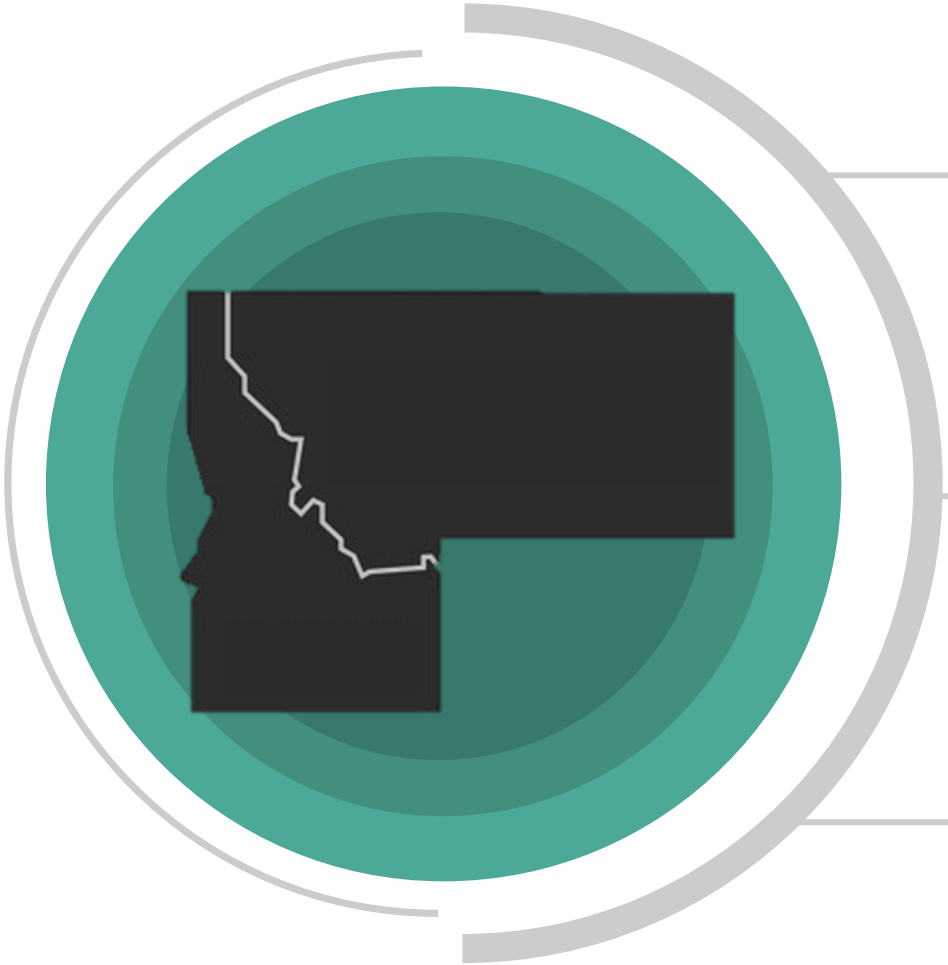


U.S. FOREST SERVICE

The Multiple-Use Sustained-Yield Act of 1960 (16 U.S.C. §§528-531) dictates a **multi-purpose land management approach** by the United States Forest Service.

Authorized to protect the lands, preserve water flows, provide timber, and to facilitate recreation, livestock grazing, and wildlife and fish habitat. Significant amounts of private or state-owned land exists within USFS boundaries that USFS has little or no control over.

COOPERATIVE CONSERVATION STRATEGIES



Utilize Landscape Conservation Cooperatives
Developed by the Dept. of the Interior to work collaboratively to meet shared natural resource priorities.

Develop Collaborative Organizational Capacity
Establish supportive infrastructure for effective collaborative efforts. Conduct gap analysis and fill existing information gaps through aligned data sharing. Identify individual agency needs.

Foster Participatory Process
Initiate communication strategy between federal, state, tribal, private, and non-governmental stakeholders to keep all stakeholders engaged and informed