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September 25, 2009

Genetic evaluation of population structure and individual movement of rainbow trout in the lower South Fork Boise River and associated tributaries

Study description

The lower South Fork of the Boise River (SFB) from Anderson Ranch Reservoir downstream to Arrowrock Reservoir is the premier rainbow trout (*Oncorhynchus mykiss*) fishery in southwest Idaho (Figure 1). Rainbow trout are native to the Boise River, although hatchery rainbow trout have been introduced throughout the last century with unknown impact. The Idaho Department of Fish and Game has regularly monitored rainbow trout in the lower SFB over the past decade or more. Recent surveys have yielded reduced numbers in general and a skewed age-class distribution, with an unusually high proportion of large (>400mm) fish relative to the number of small fish. One potential explanation for this unusual age distribution is that the lower SFB does not represent a closed population, but that these large fish may migrate in from spawning reaches elsewhere in the system. The North and Middle Fork Boise Rivers are connected to the lower SFB through Arrowrock Reservoir, but the lower SFB is isolated from Anderson Ranch Reservoir and the upper SFB by Anderson Ranch Dam; there are a handful of small tributaries that connect to the SFB between the two reservoirs (Figure 1). Within this larger area rainbow trout can potentially migrate through the relatively unobstructed mainstem rivers and access those tributaries that are not blocked with road culverts. Such movement has been observed in bull trout (*Salvelinus confluentus*), where fish radio-tagged in the headwaters of the North and Middle Fork Boise River were later found in Arrowrock Reservoir and the lower SFB (Monnot et al 2008). Alternatively, the "missing" smaller rainbow trout may be rearing in the tributaries to the lower SFB or in the walled-canyon reach just above the entrance to Arrowrock Reservoir that has not been sampled previously. A better understanding the dynamics of the lower SFB in relation to its immediate tributaries and tributaries in the North and Middle Fork Boise Rivers will improve our ability to conserve this valuable rainbow trout fishery.

Analysis of patterns of genetic diversity has become an important tool for the recovery and management of recreational species and native species of conservation concern. Genetic data can be used to make inferences about the size and stability of populations and relationships among them, and to determine the most likely origin of individuals by 'assigning' them back to probable source populations. Individual assignment is well suited for determining likely sources of the large, potentially migratory, individuals observed in the lower SFB, particularly in that it does not require following the physical movement of individuals – which is often time and cost-prohibitive. Using individual assignments effectively, however, requires the genetic characterization of all or most potential source populations. A recent genetic study of 36 headwater populations of rainbow trout in the North and Middle Fork Boise Rivers (Neville et al,

In press, referred to below as "the headwater study") provides this necessary foundation, having genetically characterized a large number of populations that may be sources of migratory rainbow trout in the lower SFB.

Methods and results

Rainbow trout tissue samples were collected in tributaries to the lower SFB (Rock, Pierce, Dixie, and Granite creeks, in red in Figure 1) by the US Forest Service in May 2008 by electroshocking. Tissue samples were collected from the lower SFB river (red line in Figure 1) in the summer of 2008 by angling by members of the Trout Unlimited Ted Trueblood chapter and the Boise Valley Fly Fishermen, as well as the Idaho Department of Fish and Game. These samples from the lower SFB and immediate tributaries are referred to below as the "SFB complex". Genotypes were obtained for twelve of the thirteen microsatellite loci used in the headwater study (Table 1, see protocol and details in the headwater study). Markers were not used to evaluate the influence of hatchery rainbow trout for this study (but see below), but because non-native cutthroat trout have also been introduced into the Boise River basin genotypes were obtained for seven markers developed for rainbow/cutthroat trout hybrid detection (see details in the headwater study).

Three hundred eighteen individuals were genotyped from the SFB complex. From these, thirty six rainbow/cutthroat trout hybrids were identified: 3 hybrids were in Dixie Creek, one was in Pierce Creek, and 32 were in Rock Creek. All of these individuals were removed and the Rock Creek sample as a whole was dropped, leaving 282 individuals in the final dataset.

In a first round of analyses using FSTAT (version 2.9.3.2; Goudet 2001), individuals were grouped based on their sampling location, i.e., the lower SFB (all samples from throughout this mainstem reach were combined), Pierce, Granite and Dixie creeks. Adherence to Hardy-Weinburg expectations was evaluated based on F_{IS} estimation and randomization tests. Two loci (Omm1236 and Omm1220) had significant F_{IS} values suggesting a deficit of heterozygotes in several populations (Table 1). These loci were maintained in subsequent analysis because, when previously evaluated across 55 rainbow trout populations in the Boise and Payette River basins in the headwater study, they did not have consistent patterns of H-W deficit or excess. The F_{IS} values observed in the SFB complex are thus more likely due to biological processes (e.g., the presence and sampling of distinct groups within a tributary, known as a 'Wahlund effect') as opposed to null alleles at these loci. Unfortunately, many of the fish from Granite and Dixie creeks were young-of-year fish, so this effect is likely to be from sampling related siblings. These individuals were retained in these analyses but this 'sibling effect' would be an issue for further use of these data. In general, this issue would be expected to bias downward estimates of genetic diversity and increase estimates of differentiation.

Overall levels of genetic diversity (based on gene diversity, H_E) were normal, if not relatively high, in the SFB complex. Gene diversity averaged across loci for the lower SFB was 0.76, with the three tributaries ranging from 0.72-0.75 (Table 2). As a comparison, this metric for 55 headwater tributaries of the Boise and Payette Rivers ranged from 0.45-0.84 (based on the same set of microsatellite loci plus one additional locus). Average allelic richness (R_s), a measurement of the number of alleles standardized by sample size, ranged from 8.19 in Granite creek to 10.55 in the lower SFB (R_S = 3.4 to 10.71 in the 55 populations referenced above). A low but

significant degree of differentiation was observed among the lower SFB and its 3 tributaries (theta = 0.017; 95% CI = 0.015-0.019).

The clustering algorithm STRUCTURE (Pritchard et al 2001) was used to estimate the number of genetic clusters (k) within the lower SFB and its immediate tributaries and, for each individual, to determine the proportion of ancestry in these clusters (i.e., perform individual assignments). As opposed to the above evaluation of genetic differentiation, where individuals were grouped *a priori* by sampling site, this approach uses no information about individual location but groups individuals into clusters that maximize the fit to theoretically-expected patterns. Based on preliminary runs I evaluated k = 1-10, with four runs for each k and a burn-in length of 100,000 and 100,000 MCMC replicates for each run. The analysis suggested that 6 genetic clusters (k=6) were most likely in the SFB complex. In Figure 2, each vertical bar represents an individual fish and the 6 colors represent the 6 genetic clusters created by STRUCTURE. Colors within individuals represent the proportional membership of that individual to each cluster, such that individuals that are mostly one color were estimated to have almost complete ancestry in one cluster (high assignment), while individuals with many colors had ancestry mixed among clusters. Results corroborated the observation of low but significant genetic differentiation among the tributaries in the SFB complex, in that tributaries were characterized by a certain degree of genetic autonomy suggested by the distinctive colors within tributaries (e.g., the green and aqua in Granite creek and the green and red in Dixie creek; Figure 2). The fact that groups of individuals within the same tributary clearly assigned to different clusters (e.g., some individuals in Pierce creek are almost all green and some are almost all aqua) also corroborates the suggestion above of a Wahlund effect (or 'sibling effect' as it may be in this case) within the samples, i.e., that samples from these tributaries did not comprise a random collection of individuals from the population but individuals from two genetically distinctive clusters. The fact that fish from different tributaries were assigned to the same cluster (as evidenced by the shared aqua color in Pierce and Granite creeks, and the shared green color in Granite and Dixie creeks in Figure 2) also suggests some interchange between pairs of tributaries. Only two individuals caught in the lower SFB (arrows in Figure 2 showing dark blue individuals) assigned to the adjacent tributaries with relatively high probability (>=70% assignment), both assigning to Pierce creek (partly characterized by the dark blue cluster).

The clustering analysis was performed again with the samples from the SFB complex and including the samples from 36 headwater populations from the North and Middle Fork Boise Rivers ("the headwater tributaries") analyzed previously (Figure 1; see Neville et al, In Press, for details on these populations); these samples added an additional 1213 individuals to the dataset. In STRUCTURE, the same parameters as above were used, but based on preliminary assessments this analysis evaluated k = 17-34. Of 43 sample locations, the most likely number of clusters was 29 (Figures 3 and 4). Several clusters contained individuals from the both the SFB complex and several headwater tributaries (Figure 4), suggesting some interchange between the SFB complex and these other streams. These outside tributaries were Rattlesnake and Little Rattlesnake creeks, which flow into the southeast tip of Arrowrock Reservoir; Cottonwood creek, which flows into the northeast finger of Arrowrock Reservoir, and several streams further up the Middle and North Forks of the Boise River (Granite, Big Owl, and Sheep creeks; Figure 1). There was no obvious spatial pattern in terms of the fish captured in the lower SFB that assigned with high affinity to clusters including headwater tributaries, i.e., there was no evidence

that migratory fish from the headwaters were using certain parts of the lower SFB but rather these fish were spread throughout the mainstem reach.

A final clustering analysis was performed where STRUCTURE was forced to make 2 groups (assumedly the 'SFB complex' vs. 'the headwaters'). This analysis showed two additional headwater tributaries with genetic ties to the SFB complex, in that many individuals in Pine and Evans creeks had higher proportional ancestry in the cluster loosely identifying the SFB complex (green in Figure 5) than the cluster generally identifying the headwaters (red in Figure 5). Pine creek is a tributary to More's creek near Idaho City, and Evans creek flows into Anderson Ranch Reservoir.

Discussion and Conservation Implications

Perhaps the most cut-and-dry implication of this work was the finding that the population in Rock creek contained mostly cutthroat trout/rainbow trout hybrids. Some hybridization with non-native cutthroat trout had been detected previously in several of the headwater tributaries (see Neville et al, In Press) and was evident to a limited degree in two of the SFB complex tributaries, but the extent to which native rainbow trout have been replaced by cutthroat-rainbow hybrids in Rock creek was surprising. The population in Rock creek is isolated above a culvert that was potentially slated for removal, but based on these results culvert removal would provide little benefit and would potentially be harmful in that it would reconnect these hybrids to the rest of the system. In contrast, there were no cutthroat/rainbow trout hybrids detected in our sample from the lower SFB; this was also surprising, given that mainstem rivers provide corridors for the spread of hybrids from sources of introductions to other tributaries. In addition to cutthroat trout, hatchery rainbow trout have been planted in the Boise River basin; IDFG has recently developed markers to evaluate introgression between hatchery and native rainbow trout (Campbell et al, In prep), which could be used to determine the degree of hatchery introgression in the Boise River basin if this is of interest.

Excluding the issue of cutthroat-rainbow trout hybridization discussed above, these results suggest the SFB complex is genetically 'healthy', with normal levels of genetic diversity and no indication that any suspected demographic declines have impacted these fish from a genetic perspective (although formal tests for genetic bottlenecks were not performed).

Clustering analyses and individual assignments are generally assumed to capture current dispersal patterns, but the clarity of these patterns and the ability to assign individuals with confidence is often muddied by low differentiation and/or signals from historical genetic relationships among populations. Because genetic differentiation among tributaries was low and relevant genetic clusters generally were not defined by single tributaries but rather comprised individuals from several tributaries and the lower SFB at once, unfortunately it was impossible to assign individuals from the lower SFB back to specific tributaries in most cases. It is also likely that some of the relationships uncovered here reflect a lingering genetic signal from the history of this system as an interconnected whole. For instance, given that dam at the base of Anderson Reservoir is impassible (Meyer 1999), the clustering of Evans creek individuals with SFB complex in the 2-cluster analysis (Figure 5) is likely to be a relic signal from before this dam was built. In general, however, these results did show evidence of interchange between the lower SFB and its adjacent tributaries as well as with several headwater tributaries. It is likely that

some individuals captured in the lower SFB originated in one of the adjacent tributaries or more distant headwater tributaries, and that there is a migratory component in rainbow trout the lower SFB river that relies on connectivity to the headwaters.

Genetic results from this study do not give clear insight as to the mechanisms behind the skewed age distribution observed in the lower SFB. It is noteworthy, however, that during the sampling effort in 2008, IDFG captured many smaller (age 1-2) fish in the previously-unsampled canyon just above Arrowrock dam. The possibility of age segregation within the lower SFB may explain the skewed age distribution and even account for suggested population declines, and could be investigated further to improve our understanding of the demographic dynamics of this river reach. In the meantime, the migratory connectivity to headwater tributaries indicated by these genetic results emphasize that the lower SFB should be viewed as one component of a larger, interconnected system.

Acknowledgements

Funding for this work was provided by Trout Unlimited as support to HN and through an Embrace-A-Stream Grant awarded to the Ted Trueblood chapter of Trout Unlimited, as well as donations from the Boise Valley Fly Fishermen (BVFF), Magic Valley Fly Fishermen (MVFF), Ted Trueblood TU (TTTU) and many of their members. Logistical and field support was provided by volunteers of the TTTU, BVFF, MVFF, the Idaho Department of Fish and Game, and the Boise National Forest. The headwater study that provided the foundation for this work was funded by the National Fire Plan, the U.S. Forest Service Rocky Mountain Research Station, U.S. Forest Service regions 1 and 4, the U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, and Boise National Forest. We thank Matt Campbell at IDFG, and Mary Peacock, Veronica Kirchoff, and the Nevada Genomics Center at the University of Nevada for laboratory analyses.

References

Campbell M.R., Kozfkay, C. C., S.R. Narum, and N.R. Campbell. In Prep. Diagnostic Single Nucleotide Polymorphisms for assessing hatchery rainbow trout introgression in interior Columbia River redband trout populations

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html.

Meyer, K. A. 1999. Assessment of native salmonids above Hells Canyon Dam, Idaho. Idaho Department of Fish and Game, 99-15.

Monnot, L., J. B. Dunham, T. Hoem, and P. Koetsier. 2008. Influences of body size and environmental factors on autumn downstream migration of bull trout in the Boise river, Idaho. North American Journal of Fisheries Management 28:231–240.

Neville, H., J. Dunham, A. Rosenberger, J. Umek, and B. Nelson. 2009. Influences of wildfire, habitat size, and connectivity on trout in headwater streams revealed by patterns of genetic diversity. Transactions of the American Fisheries Society 138:000:000.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Table 1. F_{IS} values for the lower South Fork Boise River and adjacent Pierce, Granite and Dixie creeks. Significance was based on randomizations in FSTAT. Values significant at the 0.05 level are in bold, those significant at the table-wide value of 0.00104 are in bold italics.

Locus	SFB	PC	GC	DC
OM1295	0.014	-0.037	0.031	0.026
OMM1178	-0.003	-0.137	-0.164	-0.039
OMM1286	0.042	**0.236**	0.121	-0.109
OMM1272	0.015	-0.181	0.153	-0.028
OMM1173	0.061	-0.022	0.024	-0.003
OCH20	0.017	-0.037	-0.093	-0.032
OMM1220	***0.32***	***0.465***	**0.231**	0.168
Omm1235	-0.015	0.001	0.056	-0.115
Omm1236	***0.213***	***0.325***	**0.153**	***0.489***
OCH10	-0.055	-0.008	-0.061	-0.088
OCH9	0.018	-0.021	-0.063	0.042
OMM1234	0.065	0.001	-0.073	-0.102

Table 2. Number of individuals sampled (N), gene diversity (H_E), and allelic richness (R_S) standardized to a minimum sample size of 27 for the lower South Fork Boise River and adjacent Pierce, Granite and Dixie creeks.

	SFB	PC	GC	DC
N	185	32	33	32
HE	0.76	0.72	0.75	0.73
R_S	10.55	9.18	8.19	8.80

Figure 1. Map of the Boise River basin, including the location of samples from Neville et al (In Press) in the North and Middle Fork Boise Rivers (in black), and samples collected in 2008 from the SFB complex, including the sampled reach of the lower SFB river and adjacent tributaries (in red). Arrowrock and Anderson Ranch Reservoirs are noted. The North and Middle Fork Boise Rivers are connected to the lower SFB through Arrowrock Reservoir, but the lower SFB is isolated from Anderson Ranch Reservoir and the upper SFB by Anderson Ranch Dam.

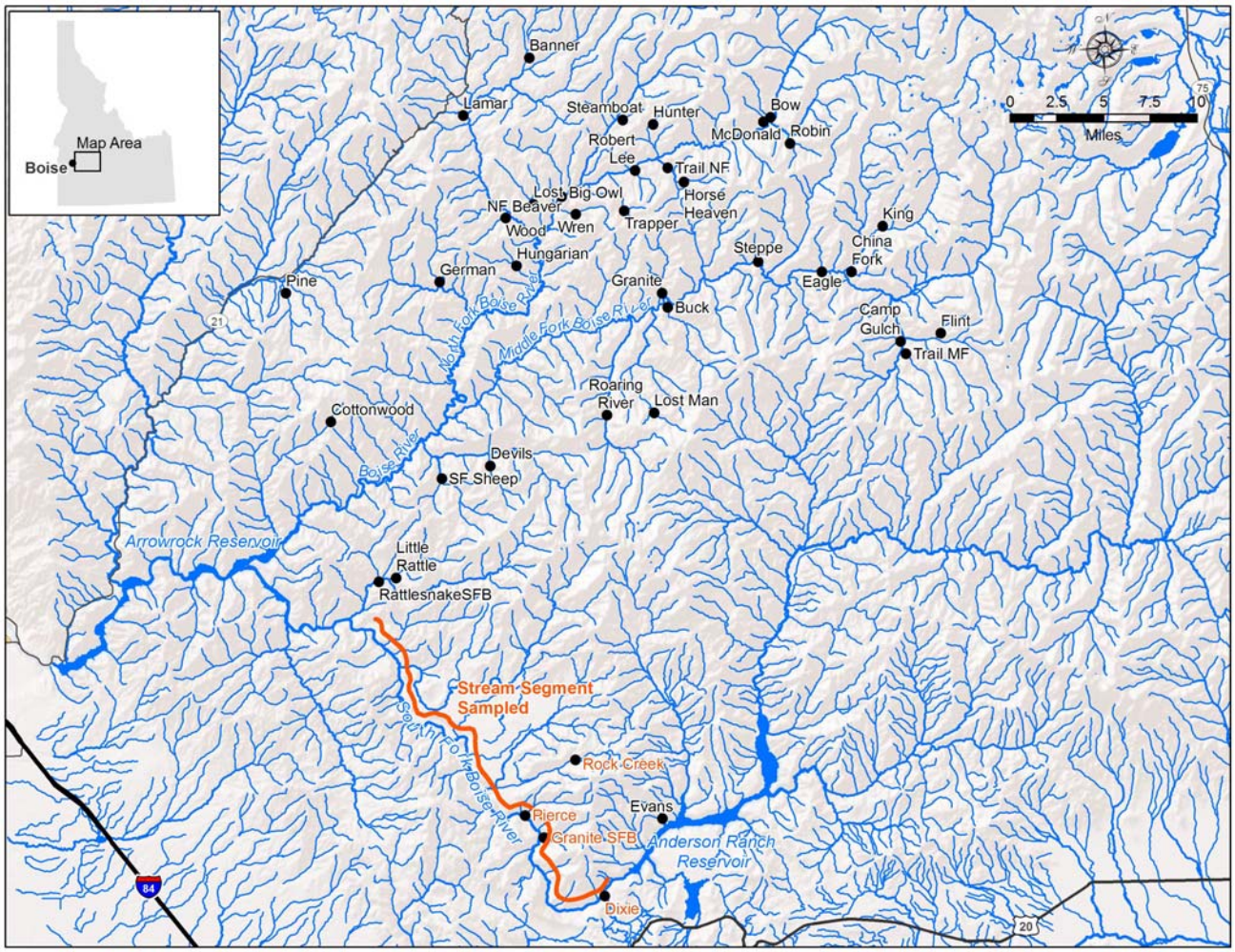
Figure 2. STRUCTURE diagram based on individuals sampled from the lower South Fork Boise River and adjacent Pierce (PC), Granite (GC) and Dixie (DC) creeks. Numbers on the x axis indicate sampling locations as I assigned them (i.e., by tributary or mainstem river; this information is for reference but was not used to define clusters); individuals are organized by river location, thus there are individuals from the mainstem river between the tributary samples, and seven collection locations. Each vertical bar represents an individual fish; colors represent the 6 different genetic clusters STRUCTURE identified, and colors within an individual indicate proportional ancestry in each cluster. Blue arrows identify 2 individuals caught in the mainstem SFB that were assigned clearly back to Pierce creek (based on ancestry to "blue" cluster found primarily in Pierce creek).

Figure 3. STRUCTURE diagram as explained in Figure 2 but including samples from 36 tributaries to the North and Middle Fork Boise rivers. Names on the x axis indicate sampling locations; this information is for reference but was not used to define clusters. Circles indicate streams where individuals were clearly related to samples from the SFB complex based on assignment to a common cluster. Stars indicate streams isolated by culvert barriers, with the solid colors for many of these streams illustrating loss of genetic diversity due to culvert isolation (see Neville et al, In Press).

Figure 4. Pie charts for each of the 29 clusters identified by STRUCTURE for the larger data set, showing the geographic sampling origin and number of individuals assigned to that cluster with ancestry >=0.7.

Figure 5. STRUCTURE results as described above, where the program was forced to identify 2 clusters. This approach picked up a previously-cryptic relationship between the SFB complex and Pine and Evans creeks, respectively.

Figure 1.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Lamar
Steamboat
Hunter
Bow
McDonald
Robin
Robert
Lee
Trail NF
Horse Heaven
Lost Big Owl
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Wood
Wren
Trapper
King
China Fork
Steppe
Eagle
Hungarian
German
Pine
Granite
Buck
Camp Gulch
Flint
Trail MF
North Fork Boise River
Middle Fork Boise River
Roaring River
Lost Man
Cottonwood
Boise River
Devils
SF Sheep
Arrowrock Reservoir
Little Rattle
RattlesnakeSFB
Stream Segment Sampled
South Fork Boise River
Rock Creek
Pierce
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Miles

Figure 2.

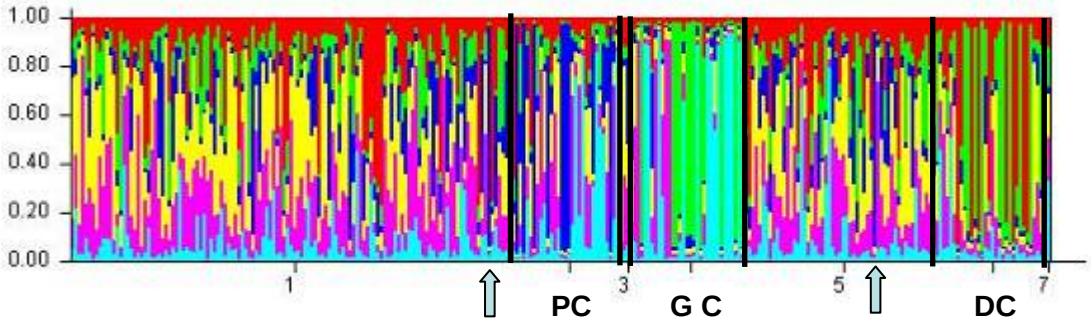
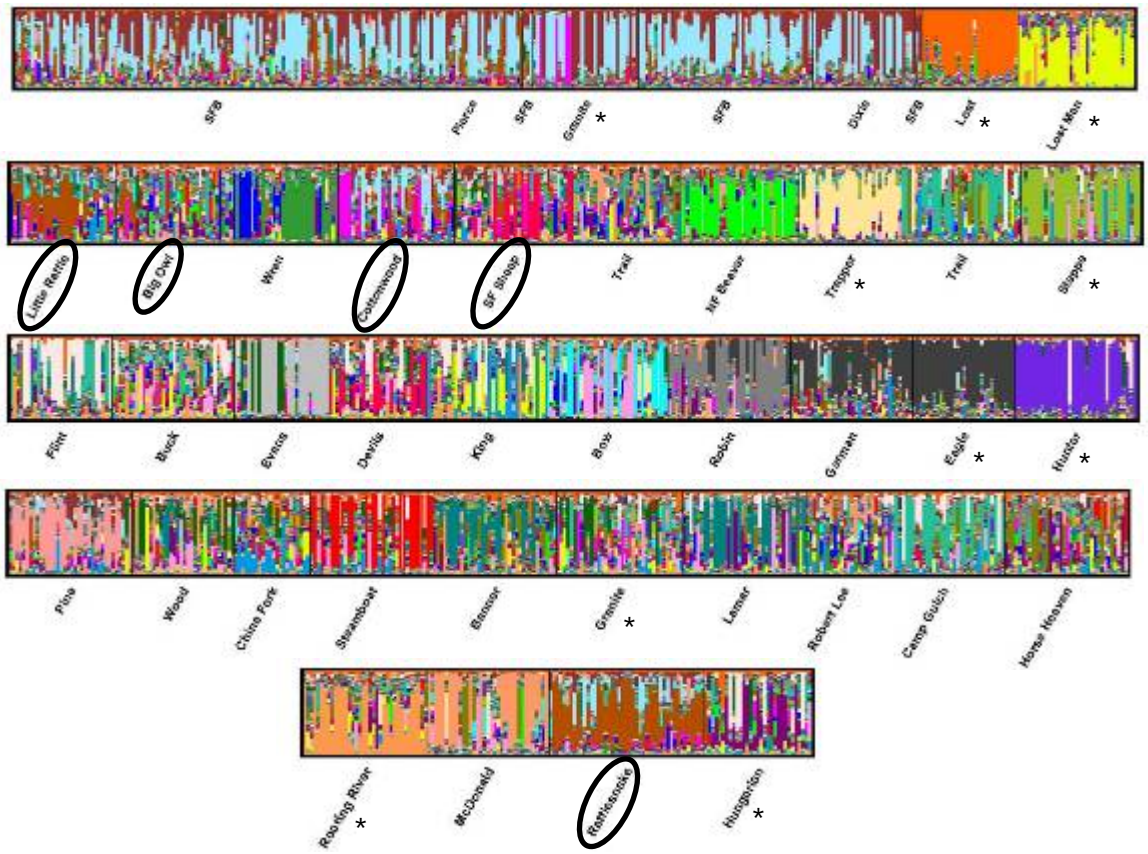


Figure 3.


SFB
Pierce
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Granite
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Lost
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Lost Man
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Little Rattle
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Cottonwood
SF Bluff
Trail
NF Beaver
Trapper
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Trail
Slippa
*
Flint
Buck
Evans
Devils
King
Bear
Robin
Gunnell
Eagle
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Hunter
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Pine
Wood
China Fork
Steamboat
Bisson
Granite
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Lamar
Robert Lee
Camp Gulch
Horse Heaven
Roaring River
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McDonald
Rattlesnake
Hungerford
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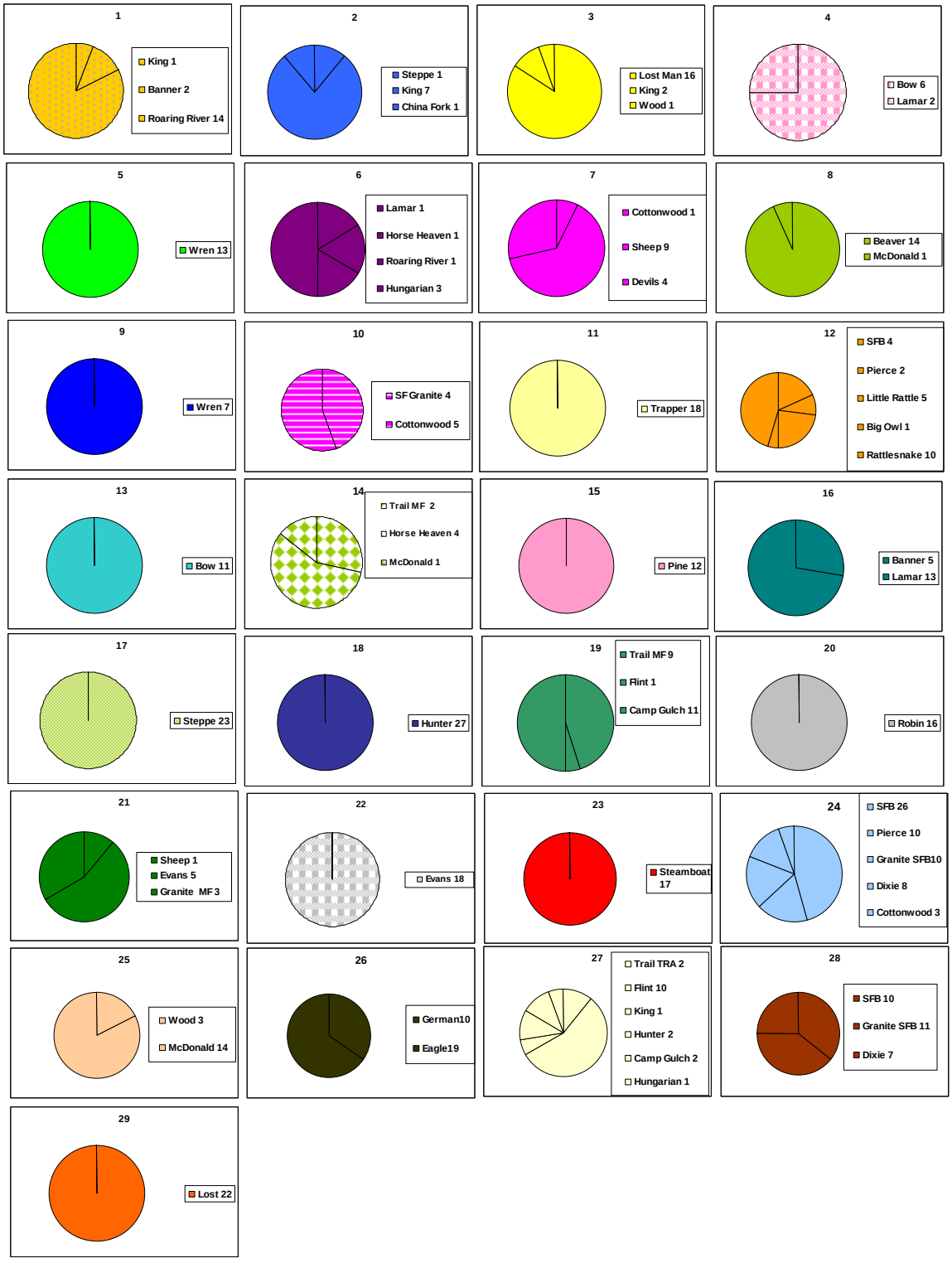
Figure 4.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Roaring River 14
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Steppe 1
King 7
China Fork 1
3
Lost Man 16
King 2
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Lamar 2
5
Wren 13
6
Lamar 1
Horse Heaven 1
Roaring River 1
Hungarian 3
7
Cottonwood 1
Sheep 9
Devils 4
8
Beaver 14
McDonald 1
9
Wren 7
10
SF Granite 4
Cottonwood 5
11
Trapper 18
12
SFB 4
Pierce 2
Little Rattle 5
Big Owl 1
Rattlesnake 10
13
Bow 11
14
Trail MF 2
Horse Heaven 4
McDonald 1
15
Pine 12
16
Banner 5
Lamar 13
17
Steppe 23
18
Hunter 27
19
Trail MF 9
Flint 1
Camp Gulch 11
20
Robin 16
21
Sheep 1
Evans 5
Granite MF 3
22
Evans 18
23
Steamboat 17
24
SFB 26
Pierce 10
Granite SFB10
Dixie 8
Cottonwood 3
25
Wood 3
McDonald 14
26
German10
Eagle19
27
Trail TRA 2
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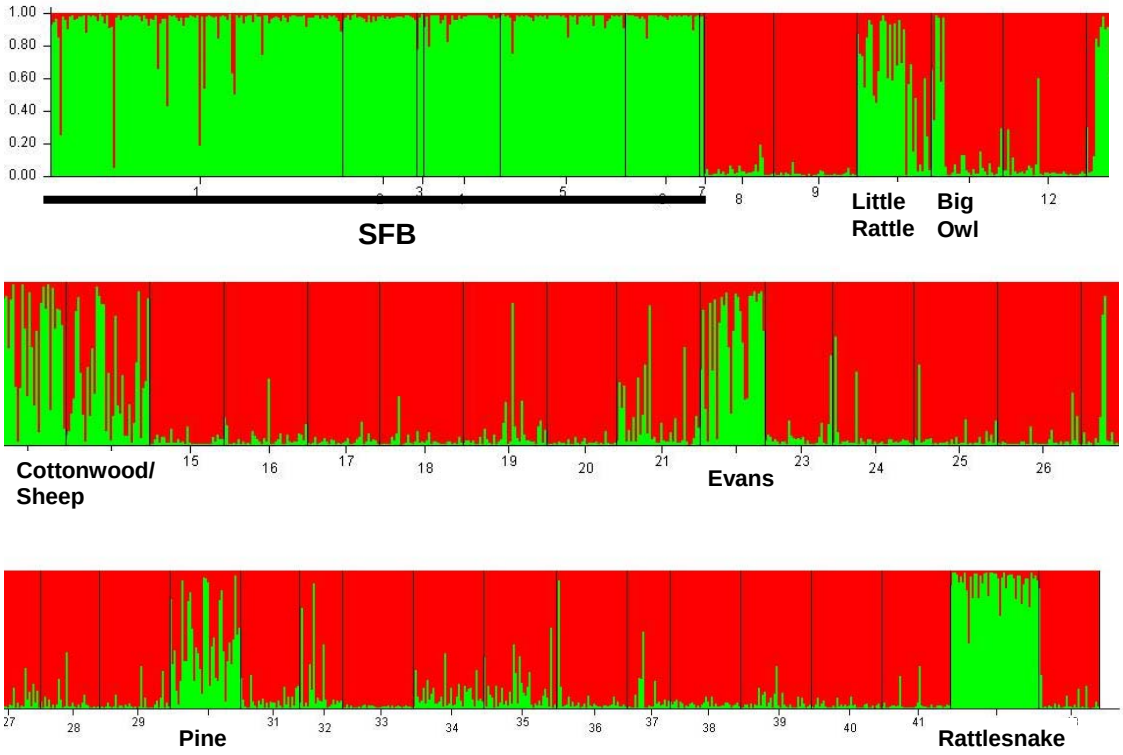
Figure 5.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SFB
Little Rattle
Big Owl
Cottonwood/ Sheep
Evans
Pine
Rattlesnake