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Pat;

We have completed the analysis of the fin clips from sculpin collected from the mainstem Madison River (Norris section, 12T 0454918 UTM 5048375, 4/17/12, N=20; Valley Garden section, 12T 0444743 UTM 5024024, 4/18/12, N=20) and three of its tributaries: South Fork Madison River (12T 0484695 UTM 4947863, 4/17/12, N=20), Ruby Creek above falls (12T 0444170 UTM 4989783, 4/19/12, N=20), and Cherry Creek at Wylie Bridge (12T 0462145 UTM 5048725, 5/17/12, N=20). Each fish's genotype was determined at 11 microsatellite loci (Table 1). Your primary questions were how much genetic divergence exists among the samples and do any appear to not be an appropriate source of fish to potentially introduce sculpin back into Cherry Creek in the lower portion of the drainage.

We used a variety of methods to examine amounts of genetic divergence among the samples. First, the log likelihood G test of Goudet et al. (1996) in GENEPOP version 4.0 (Rousset 2008) was used to determine if there was evidence of allele frequency differences between pairs of samples. Since multiple comparisons were performed between samples, we accounted for the possibility that a significant difference may simply represent a chance departure from homogeneity by combining probability (P) values among loci using Fisher's method. When significant differences existed between samples at one or more loci and the overall P value was significant, allele frequency differences were determined to exist between the samples. Next, we computed the proportion of the total genetic variation detected between two samples due to allele frequency differences between them (F_{ST}) using the procedure of Weir and Cockerham (1984) in GENEPOP version 4.0. The program GenAlEx6 (Peakall and Smouse 2006) was used to estimate Nei's unbiased genetic distance (D, Nei 1975) between all possible pairs of samples and the matrix of the estimates was subjected to un-weighted pair group arithmetic average cluster analysis to produce a dendrogram summarizing the pair wise comparisons. We then used the assignment test of Paetkau et al. (2004) in GenAlEx6 to determine how well individuals could be placed back to their sample of origin.

Among the samples, evidence of genetic variation was detected at all of the loci analyzed (Table 1). The allele frequencies significantly differed between pairs of samples at three to ten loci. Furthermore, the overall P value was highly significant ($P<0.001$) between all pairs of samples. Thus, there was good evidence that genetic differences existed between all the samples so they were kept separate in the following analyses.

Based on F_{ST}, in general there was relatively little genetic divergence among the samples except that the Ruby Creek fish were highly divergent from the others (Table 2). This was also very evident in the dendrogram produced by un-weighted pair group arithmetic average cluster analysis of –ln of Nei's unbiased genetic distance estimates (Table 2, Figure 1). Ruby Creek was also the only sample in which all individuals were assigned back to it (Table 3). All the other samples had at least two individuals mis-assigned to one or more of the other samples except Ruby Creek.

We estimated levels of genetic variation in the samples using average expected heterozygosity and the average number of alleles per locus. These values were very similar among the two mainstem Madison River, South Fork Madison, and Cherry Creek samples (Table 1). In contrast, the Ruby Creek sample possessed only about half the amount of genetic variation detected in the other samples (Table 1). Ruby Creek possessed only three variants at low frequency not observed in one or more of the other samples (private alleles, Table 1). The Ruby Creek sample mainly possessed only a subset of alleles detected in the other samples often at unusually high frequency (Table 1). Low frequency private alleles contribute little to estimates of genetic divergence. Thus, the main factors responsible for the high divergence of the Ruby Creek sculpins is the greatly reduced amount of genetic variation that they possess and the unusually high frequency of many variant alleles compared to those observed in the other samples.

Almost undoubtedly the main factor responsible for the low genetic diversity in the Ruby Creek population is its isolation above a waterfall. This fall may have been created as recently as 1959 by the earthquake that formed Quake Lake (Pat Clancey, Montana Fish, Wildlife & Parks, personal communication). It is possible the fish above the fall may have experienced a severe population decline during this event and lost an appreciable amount of genetic variation. Whether this occurred or not is unknown but, once the fall was formed the fish above it obviously became isolated. The resulting lack of gene flow and reduced available habitat to the population certainly resulted in a reduction in effective population size compared to its historic level. Thus, over time there has probably been substantial genetic drift in the Ruby Creek population resulting in an increased rate in the loss of genetic variation. These two explanations for the low amount of genetic variation observed in Ruby Creek are not mutually exclusive.

Regardless of the cause, because of their high divergence and low levels of genetic variation we do not recommend using Ruby Creek as a source of fish for sculpin re-introductions in the Madison River drainage. The other four samples have similar levels of genetic diversity and there is relatively little divergence among them. From a genetics perspective, therefore, all should be considered suitable sources of fish for potential sculpin re-introductions in the Madison River drainage.

Robb Leary

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Literature Cited

Goudet, J., M. Raymond, T. deMeeus, and F. Rousset. 1996. Testing differentiation in diploid populations. Genetics 144:1933-1940.

Nei, M. 1975. Molecular population genetics and evolution. Elsevier-North Holland, New York.

Paetkau, D., R. Slade, M. Burden, and A. Estoup. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation based exploration of accuracy and power. Molecular Ecology 13:55-65.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288-295.

Rousset, F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8:103-106.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

Table 1

Allele frequencies at the loci showing evidence of genetic variation in samples of sculpin collected from the Madison River Norris (Norris) and Valley Garden (VG) sections, Cherry Creek, South Fork Madison River (SFM), and Ruby Creek. H_e=average expected heterozygosity. N=average number of alleles per locus.

		Sample and allele frequencies				
		Madison River				
Locus	Alleles	Norris	VG	Cherry	SFM	Ruby
Cott100	*185*	0.275	0.325	0.650	0.425	0.400
	187	0.475	0.175	0.200	0.325	0.000
	189	0.200	0.475	0.150	0.225	0.600
	191	0.025	0.025	0.000	0.025	0.000
	195	0.025	0.000	0.000	0.000	0.000
Cott687	*152*	0.000	0.000	0.000	0.050	0.000
	161	0.000	0.000	0.000	0.000	0.025
	165	0.025	0.000	0.025	0.000	0.000
	167	0.000	0.000	0.075	0.025	0.000
	169	0.050	0.025	0.025	0.075	0.000
	171	0.075	0.075	0.025	0.000	0.000
	173	0.000	0.225	0.125	0.025	0.050
	175	0.000	0.050	0.150	0.075	0.000
	177	0.300	0.125	0.150	0.275	0.625
	179	0.050	0.100	0.100	0.150	0.075
	181	0.050	0.000	0.075	0.200	0.000
	183	0.025	0.100	0.075	0.000	0.225
	185	0.125	0.125	0.050	0.050	0.000
	187	0.125	0.150	0.000	0.025	0.000
	188	0.000	0.000	0.050	0.000	0.000
	189	0.100	0.025	0.025	0.000	0.000
	191	0.000	0.000	0.000	0.050	0.000
	193	0.050	0.000	0.050	0.000	0.000
	195	0.025	0.000	0.000	0.000	0.000
Cott130	*148*	1.000	0.975	1.000	1.000	1.000
	150	0.000	0.025	0.000	0.000	0.000
Cgo33	*147*	0.026	0.150	0.000	0.000	0.000
	153	0.132	0.075	0.400	0.350	0.875
	155	0.342	0.300	0.350	0.300	0.125
	157	0.026	0.025	0.000	0.000	0.000
	161	0.211	0.100	0.150	0.175	0.000
	163	0.263	0.350	0.075	0.125	0.000
	165	0.000	0.000	0.025	0.000	0.000
	167	0.000	0.000	0.000	0.050	0.000

Table 1-continued

		Sample and allele frequencies				
		Madison River				
Locus	Alleles	Norris	VG	Cherry	SFM	Ruby
Cott255	*163*	0.000	0.000	0.000	0.025	0.000
	181	0.000	0.000	0.000	0.050	0.000
	183	0.000	0.025	0.000	0.000	0.000
	187	0.000	0.000	0.025	0.025	0.000
	189	0.475	0.425	0.550	0.500	0.000
	191	0.225	0.200	0.175	0.175	0.000
	193	0.025	0.075	0.100	0.175	1.000
	195	0.050	0.100	0.025	0.025	0.000
	197	0.175	0.150	0.000	0.025	0.000
	199	0.025	0.025	0.125	0.000	0.000
	201	0.025	0.000	0.000	0.000	0.000
CottES19	*135*	0.025	0.000	0.000	0.000	0.000
	137	0.900	0.800	0.875	0.900	0.025
	139	0.075	0.200	0.000	0.100	0.975
	141	0.000	0.000	0.125	0.000	0.000
Cott113	*129*	0.000	0.000	0.025	0.000	0.000
	135	0.625	0.750	0.800	0.550	1.000
	137	0.375	0.250	0.175	0.450	0.000
Cgo1114	*121*	0.175	0.200	0.000	0.150	0.925
	125	0.750	0.500	0.775	0.825	0.075
	127	0.025	0.025	0.200	0.025	0.000
	131	0.050	0.275	0.025	0.000	0.000
CottES10	*169*	0.000	0.025	0.000	0.000	0.000
	171	0.225	0.100	0.125	0.125	0.625
	173	0.650	0.750	0.275	0.625	0.375
	179	0.000	0.000	0.100	0.000	0.000
	181	0.000	0.000	0.300	0.000	0.000
	183	0.125	0.125	0.150	0.250	0.000
	185	0.000	0.000	0.050	0.000	0.000

Table 1-continued

		Sample and allele frequencies				
		Madison River				
Locus	Alleles	Norris	VG	Cherry	SFM	Ruby
Cgo310	*194*	0.000	0.000	0.000	0.000	0.025
	240	0.026	0.025	0.000	0.000	0.000
	246	0.000	0.025	0.000	0.000	0.000
	248	0.000	0.000	0.000	0.000	0.150
	250	0.079	0.375	0.000	0.025	0.000
	252	0.605	0.325	0.575	0.375	0.000
	254	0.158	0.025	0.150	0.200	0.425
	256	0.000	0.050	0.000	0.050	0.225
	258	0.000	0.050	0.000	0.150	0.050
	260	0.079	0.075	0.125	0.000	0.125
	262	0.000	0.050	0.000	0.025	0.000
	266	0.026	0.000	0.025	0.100	0.000
	276	0.026	0.000	0.125	0.075	0.000
Cott127	*185*	0.725	0.350	0.625	0.625	0.000
	187	0.225	0.650	0.375	0.250	1.000
	189	0.050	0.000	0.000	0.125	0.000
H_e		0.503	0.543	0.502	0.522	0.239
A		4.818	4.727	4.545	4.545	2.273

Table 2

F_{ST} (above diaganol) and -ln Nei's unbiased genetic distance (below diaganol) between samples of sculpin collected from the Norris and Valley Garden (VG) reaches of the Madison River, Cherry Creek, South Fork Madison River (SFM), and Ruby Creek.

	Norris	VG	Cherry	SFM	Ruby
Norris		0.059	0.056	0.010	0.462
VG	0.075		0.096	0.070	0.378
Cherry	0.065	0.131		0.039	0.444
SFM	0.012	0.095	0.046		0.424
Ruby	0.720	0.488	0.647	0.609	

Table 3

Results of assignment test among samples of sculpin collected from the Norris and Valley Garden (VG) reaches of the Madison River, Cherry Creek, South Fork Madison River (SFM), and Ruby Creek.

	Norris	VG	Cherry	SFM	Ruby
Norris	12	2	1	5	
VG	4	16			
Cherry			18	2	
SFM	6		1	13	
Ruby					20

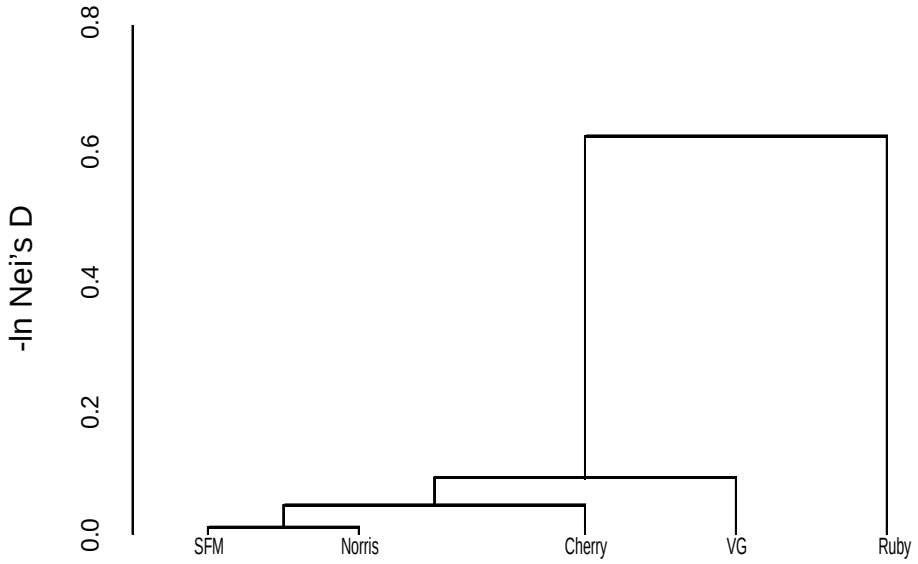

Figure 1. Dendrogram produced by un-weighted pair group arithmetic average cluster analysis of –ln Nei's unbiased genetic distance estimate between five samples of sculpin collected from the Madison River drainage. SFM=South Fork Madison River. Norris=Norris section of Madison River. Cherry=Cherry Creek. VG=Valley Garden section of Madison River. Ruby=Ruby Creek.