Project F-73-R-14
Subproject VI, Study V

GENETIC ANALYSIS OF RAINBOW TROUT FROM THE BIG WOOD RIVER AND TRAIL CREEK, BLAINE COUNTY, IDAHO

by

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EXECUTIVE SUMMARY:

This report serves as the final report for our contract with Idaho Fish and Game in which we used genetic analyses to determine the genetic and taxonomic status of two naturally occurring trout populations in south central Idaho: Lower Big Wood River and Trail Creek, a tributary of the Big Wood River.

Analysis of protein and mitochondrial DNA (mtDNA) variation revealed the Lower Big Wood River population to be a rainbow trout (Oncorhynchus mykiss) population that was probably a native interior rainbow trout population that has undergone extensive introgression with hatchery rainbow trout of coastal origin. The genetic data for this population do not differ significantly from the genetic data for the Upper Big Wood River rainbow sample. It is likely that considerable gene flow occurs between the two samples, thus rainbow trout in the Big Wood River, as represented by these two samples, can be considered a single interbreeding population that has both non-native coastal and native interior genetic components.

Analysis of mtDNA variation among Trail Creek specimens also revealed both coastal and interior components, although most specimens (88%) exhibited typical interior rainbow trout haplotypes. Moreover, a unique and additional mtDNA haplotype was detected in half of the Trail Creek specimens. The second mtDNA type appears to be a smaller version of the native molecule (i.e., haplotype) that has undergone a major deletion of approximately 3,000 base pairs (roughly 20% of the 16,500 bp mtDNA molecule). Heteroplasmy, the occurrence of more than one mtDNA haplotype in a single individual, is uncommon and usually occurs as 2-300 bp deletions. Indeed, deletions as large as 3,000 bp have not been documented in the ichthyological literature

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We also examined genetic characteristics of the Hayspur Hatchery rainbow stock that has been planted into many southern Idaho drainages. The Hayspur population served as our reference for assessment of both taxonomic identity and genetic purity of the four sample populations. Finally, we compared genetic characteristics of the Lower Big Wood River and Trail Creek samples to other interior rainbow trout populations that we have previously analyzed for genetic purity and taxonomic status.

OBJECTIVES:

The objectives of this study were to (1) determine if either the Lower Big Wood River or Trail Creek populations could be identified as native interior rainbow trout, and (2) determine if genetic characteristics of hatchery rainbow trout (primarily of coastal rainbow origin), westslope cutthroat trout (O. clarki lewisi) or yellowstone cutthroat trout (O. c. bouvieri) were present in the sample populations.

METHODS:

Samples were obtained for specimens of rainbow trout from 2 populations: Lower Big Wood River (N = 25) and Trail Creek (N=25). Additionally, specimens from the Upper Big Wood River (N = 25) and the Hayspur Hatchery (N=30) were also examined. Specimens were collected by electroshocking or angling, placed on dry ice and transported to Boise State University where they were maintained at -20°C or -40°C until the time of analysis. All specimens from the Lower Big Wood River were examined for variation in allozymes, and 8 specimens were examined for variation in mtDNA.

summarize total variation among the data along orthogonal axes (the 'principal components'). PCA was used to graphically summarize the relative amount of allele-frequency variation among the populations. The relative positions of the Big Wood River populations in the PCA graphs (Figures 1 and 2) can be used to summarize their genetic purity by comparing their positions to the reference interior rainbow trout populations (King Hill, Doby George, and Columbia Creeks) and the reference rainbow trout hatchery populations (Hayspur, Cape Cod, Arlee).

Mitochondrial-DNA analysis. MtDNA was isolated using the phenol/chloroform separation and ethanol precipitation procedures of Lansman et al. (1981) and Sambrook et al. (1989). Frozen liver and heart were the primary tissue sources, and were augmented with skeletal muscle if volumes of the former tissues were insufficient. The isolated mtDNAs were digested using 9 Type II hexanucleotide restriction enzymes (BamH I, Bcl I, Bgl II, BstE II, EcoR I, EcoR V, Hind III, Nhe II, Pvu II) and two Type II pentanucleotide restriction enzymes (Ava I and Hinc II) according to the buffer system and incubation temperatures recommended by the manufacturer (Boehringer-Mannheim and Promega Corporation). DNA fragments were separated by agarose gel (0.8 - 1.2%) electrophoresis. DNA fragments were transferred to nylon membranes via capillary transfer and probed using the Southern blot procedures (Southern 1975).

Fragment patterns were visualized using the GENIUS$_{TM}$ nucleic acid labeling and detection kit (Boehringer-Mannheim) and compared to a one-kilobase molecular weight marker (Bethesda Research Laboratories) Fragments of questionable identity were rerun in adjacent lanes to minimize the chance of scoring two non-homologous fragments as identical. All unique

Table 2. Allele frequencies at the polymorphic loci for 10 populations of putative rainbow trout and the Hayspur hatchery population. All other loci in Table 1 were monomorphic for the same allele in each population.

		Sample populations and allele frequencies										
Locus	Alleles	Clover	King Hill	Little Jacks	Upper BigWood	Lower BigWood	Blue Jacket	Columbia	Doby George	Hayspur	Nickel	Emigrant
sAAT-1*	100	1.000	1.000	1.000	0.933	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	200	--	--	--	0.067	--	--	--	--	--	--	--
sAAT-3,4*	100	0.825	1.000	1.000	1.000	1.000	0.967	1.000	1.000	1.000	1.000	1.000
	110	--	--	--	--	--	0.033	--	--	--	--	--
	90	0.175	--	--	--	--	--	--	--	--	--	--
sAH-2*	100	1.000	0.806	1.000	1.000	1.000	1.000	0.385	0.625	1.000	0.960	0.950
	88	--	0.194	--	--	--	--	0.615	0.375	--	0.040	0.050
bGLUA*	100	0.472	0.972	0.906	0.933	0.940	1.000	1.000	0.925	0.783	0.900	1.000
	72	0.528	--	0.094	--	--	--	--	--	0.217	--	--
	117	--	0.028	--	--	--	--	--	0.025	--	--	--
	80	--	--	--	0.067	0.060	--	--	--	--	0.100	--

Table 1. Enzymes, enzyme commission number (EC; IBUNC 1984) and loci examined in rainbow trout samples. Tissues: E = eye, L = liver, M = muscle.

Enzyme	E.C.	Loci	Tissue	Buffer[a]
Acid phosphatatse	2.6.1.1	ACP-1*	L	MF
Aconitate hydratase	4.2.1.3	sAH-2*	L	AC
Alanine aminotransferase	2.6.1.2	ALAT-2*	M	AC+, MF
Alchohol dehydrogenase	1.1.1.1	ADH*	L	RW
Aspartate aminotransferase	2.6.1.1	sAAT-1*,sAAT-2*	L	AC, RW
		sAAT-3,4*	M	AC, RW
Beta-glucuronidase	3.2.1.31	bGLU*	L	RW
Creatine kinase	2.7.3.2	CK-A1*, CK-A2*	M	RW
		CK-B*	E	SR
		CK-C1*, CK-C2*	E	SR
Dipeptidase	3.4.-.-	PEPA-1*, PEPA-2*	E	SR
Glucose-6-phosphate	5.3.1.9	GPI-A*	E	SR
isomerase		GPI-B1*, GPI-B2*	M	RW
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	GAPDH-3*, GAPDH-4*	E	AC+
Isocitrate dehydrogenase	1.1.1.42	sIDHP-1,2*	L	AC
Lactate dehydrogenase	1.1.1.27	LDH-A1*, LDH-A2*	M	RW
		LDH-B1*, LDH-B2*	E	SR
		LDH-C*	E	SR
Malate dehydrogenase	1.1.1.37	sMDH-B1,2*	M	AC

TABLE 2. Continued.

Locus	Alleles	Clover	King Hill	Little Jacks	Upper BigWood	Lower BigWood	Blue Jacket	Columbia	Doby George	Hayspur	Nickel	Emigrant
PGM-2*	100	1.000	1.000	0.950	0.983	0.920	1.000	1.000	1.000	0.760	1.000	1.000
	90	--	--	0.050	0.017	0.080	--	--	--	0.240	--	--
	120	--	--	--	--	--	--	--	--	--	--	--
sSOD-1*	100	1.000	0.950	1.000	0.833	0.780	0.833	0.964	0.925	0.620	1.000	1.000
	152	--	--	--	0.167	0.220	--	--	--	0.380	--	--
	57	--	0.050	--	--	--	0.167	0.036	0.075	--	--	--
	90	--	--	--	--	--	--	--	--	--	--	--

Figure 1. Principal components analysis (PCA) of eight naturally occurring rainbow trout populations and three hatchery rainbow trout populations.

Figure 2. Principal components analysis (PCA) of ten naturally occurring rainbow trout populations and one hatchery rainbow trout population. Upper and Lower Big Wood River populations are noted in bold type.

mtDNA diversity and nearly all populations have either a single common mtDNA haplotype, or a common haplotype and a few closely-related haplotypes. In these latter populations, the mtDNA haplotypes typically differ from one another by less than 0.40% sequence divergence. Populations that have had multiple origins (e.g. many hatchery populations and hybridized populations) generally show a diversity of mtDNA haplotypes that may differ from one another by as much as 1.4% sequence divergence. Thus, intrapopulation sequence divergence greater than 1% most likely reflects recent hybridization with a non-native stock. Differences among haplotypes within the Lower Big Wood River and Trail Creek samples exceeded 1% sequence divergence.

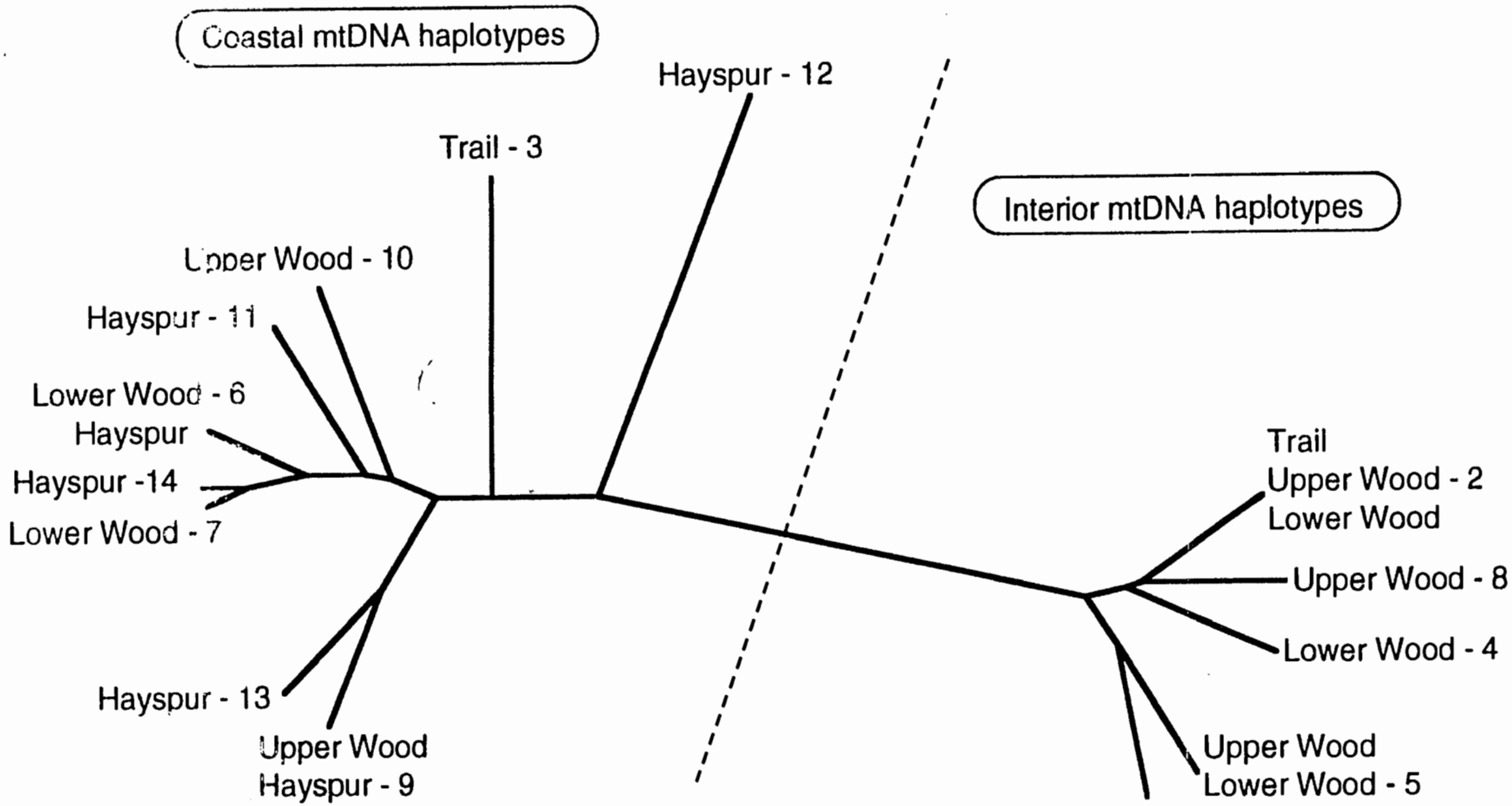
The King Hill Creek population sample was monomorphic for a single mtDNA haplotype that we have commonly observed in interior rainbow trout populations (Williams et al., in review, TAFS). We interpret this haplotype, as well as other closely-related haplotypes, as a mtDNA haplotype that was native to interior rainbow trout.

We observed intrapopulation variation in mtDNA haplotypes in the samples from the Lower and Upper Big Wood River, Trail Creek, and the Hayspur Hatchery stock (Table 3). In this report, we note only the haplotypes in the Hayspur sample that appear to be of coastal rainbow trout origin. Clustering of pairwise mtDNA haplotype divergences yielded a distance network of mtDNA relationships (Figure 3) that segregated the observed mtDNA haplotypes into an interior mtDNA cluster and a coastal cluster. The interior cluster contained the reference haplotype from King Hill Creek and four other closely related haplotypes that differed from one another by less than 0.45%. This cluster and the relatively low amount of within-group divergence is typical of interior populations.

Table 3 continued.

Location	Sample size	Haplotype designation	Composite mtDNA haplotype	Number of occurrences
Hayspur Hatchery	20	11	s r r s r r r s r q r	1
		12	s r r r r r r t r q r	1
		13	s r r t r r r t r q r	9
		6	s r r t r r r s r q r	2
		14	s r r t r r r s s q r	2

[a] + = Three of the specimens from Trail Creek exhibited common interior rainbow haplotypes that also contained a second mtDNA type that appears to be the common type minus a large deletion of approximately 3000 base pairs.



mtDNA network for Interior Rainbow Trout from the upper and lower Big Wood and Trail Creek in Idaho

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In contrast, the coastal cluster contained a greater number of haplotypes, as well as a larger amount of within-group divergence. Haplotypes in this cluster differed from one another by less than 0.75%. Divergence between the coastal and interior clusters was approximately 1.3%. The common haplotype of the Trail Creek sample (#2) occured in the interior cluster, whereas the other less common haplotype (#3) occurred in the coastal cluster. This same pattern was observed for both Big Wood River samples, where most of the sample specimens had interior haplotypes, but the population also contained individuals with coastal mtDNA haplotypes.

Genetic status of the populations

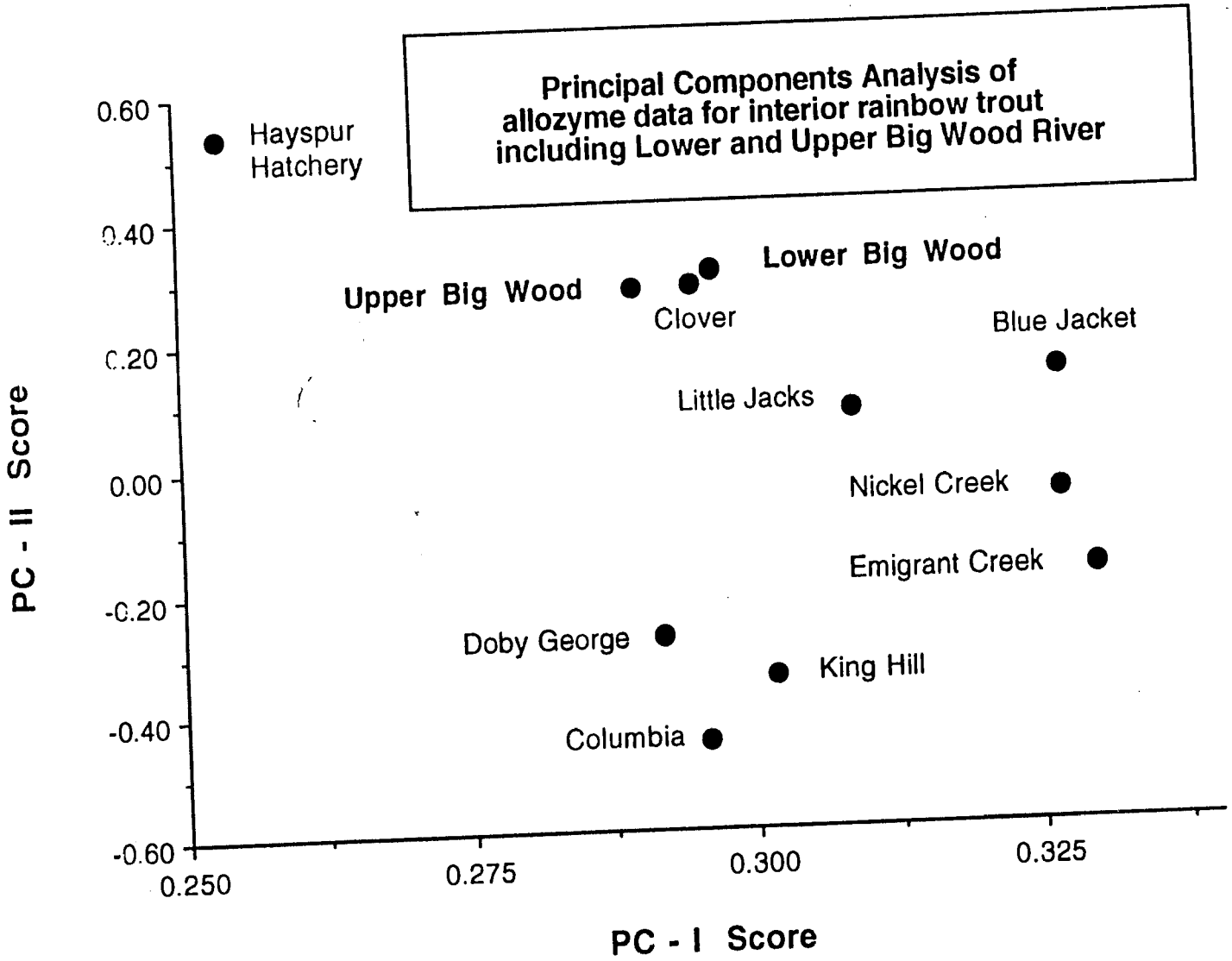
In summary, rainbow trout in the Big Wood River appear to be a native resident interior rainbow trout population that has become thoroughly introgressed with hatchery rainbow trout of coastal origin. Allozyme profiles were nearly typical of coastal rainbow trout. However, mtDNA characteristics of the populations contained a mixture of both interior and coastal types, with interior haplotypes predominating.

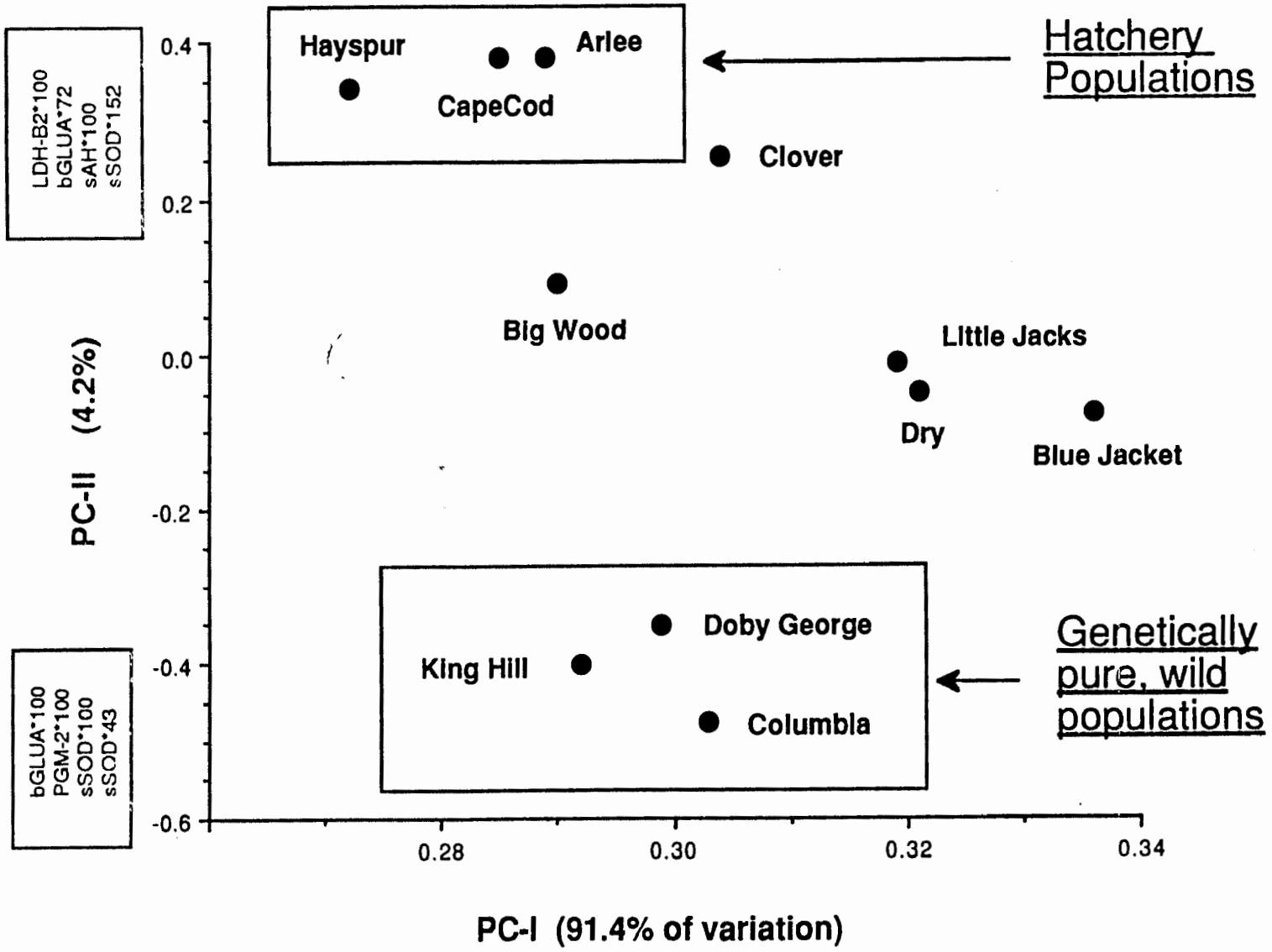
Trout in Trail Creek appeared to be a unique resident rainbow trout population that has also undergone introgression with hatchery rainbow trout, although probably to a lesser degree than the Big Wood River population. The Trail Creek population also contained individuals with a unique mtDNA haplotype that occurred in addition to the common mtDNA haplotype. The former mtDNA is unusual and is presently undergoing further analysis at Brigham Young University.

Figure 3. Fitch-Margoliash network for mtDNA haplotype relationships for 14 mtDNA haplotypes found among four naturally occurring rainbow trout populations in central Idaho and one hatchery rainbow trout population.

Table 3. List of mtDNA composite haplotypes from rainbow trout in Trail Creek and the Lower Big Wood River, number of times each haplotype occurred in a sample, and collection sites. Fragment patterns are denoted by numbers and represent patterns detected with the following restriction enzymes: Ava I, BamH I, Bcl I, Bgl II, BstE II, EcoR I, EcoR V, Hinc II, Hind III, Nhe II, Pvu II, respectively.

Location	Sample size	Haplotype designation	Composite mtDNA haplotype	Number of occurrences
Sample Populations:				
Trail Creek	6	2	r r r r r r r s r r r	2
		2+[a]	r r r r r r r s r r r	3
		3	r r t t r r r s r q r	1
Lower Big Wood	8	2	r r r r r r r s r r r	3
		4	r r r s r r r r r r r	2
		5	r r r s r r r s r r r	1
		6	r r r t r r r t r q r	1
		7	r r r t r r r s s q r	1
Reference Populations:				
King Hill Creek	8	1	r r r r r r r r r r r	8
Upper Big Wood	10	2	r r r r r r r s r r r	5
		5	r r r s r r r r r r r	2
		8	r r r s r r r s r r r	1
		9	r r r t r r r t r q r	1
		10	r r r t r r r s s q r	1


Principal Components Analysis of
allozyme data for interior rainbow trout
including Lower and Upper Big Wood River
Hayspur
Hatchery
Lower Big Wood
Upper Big Wood
Clover
Blue Jacket
Little Jacks
Nickel Creek
Emigrant Creek
Doby George
King Hill
Columbia
0.60
0.40
0.20
0.00
-0.20
-0.40
-0.60
0.250
0.275
0.300
0.325
PC - II Score
PC - I Score


LDH-B2*100
bGLUA*72
sAH*100
sSOD*152
bGLUA*100
PGM-2*100
sSOD*100
sSOD*43
Hayspur
Arlee
CapeCod
Clover
Big Wood
Little Jacks
Dry
Blue Jacket
King Hill
Doby George
Columbia
Hatchery Populations
Genetically pure, wild populations
PC-II (4.2%)
PC-I (91.4% of variation)
0.4
0.2
0.0
-0.2
-0.4
-0.6
0.28
0.30
0.32
0.34

in low frequency in the upper sample, but was absent from the lower river sample. Most differences in the dataset (Table 2) occurred between the hatchery population (Hayspur) and the previously identified genetically pure interior rainbow trout populations from King Hill, Doby George, and Columbia Creeks. These differences are graphically summarized in Figure 1, where hatchery populations are clearly segregated from the genetically pure interior rainbow trout populations. Populations that occupy intermediate positions in the graph, have intermediate allozyme profiles that are interpreted as the result of hybridization between native rainbow trout in these populations and introduced hatchery rainbow trout.

PCA of the allozyme dataset partitioned 92.4% of the total variation along the first principal component axis (PC-1) and 4.7% along the second (PC- 2) (Figure 2). Samples from the Big Wood River (both upper and lower river) are positioned in the graph much nearer the Hayspur Hatchery population than the genetically pure interior rainbow populations. This is not surprising, as both population have allozyme characteristics typical of coastal rainbow trout populations with respect to the two major diagnostic loci, sSOD* and LDH-B2*. Coastal rainbow trout typically show fixation of the LDH-B2*100 allele and elevated frequencies of the sSOD-1*152 allele, whereas interior rainbow populations are often highly polymorphic for LDH-B2 alleles and fixed for sSOD-1*100. Both Big Wood River samples had the sSOD*152 allele and were fixed for the LDH-B2*100 allele.

MTDNA ANALYSIS

Significant divergence among mtDNA haplotypes was found both within and among the sample populations (Table 3, Figure 3). At present, we have examined more than 35 natural populations of rainbow and cutthroat trout for

Enzyme	E.C.	Loci	Tissue	Buffer[a]
Malic enzyme	1.1.1.40	mMEP-2*, sMEP-1,2*	M	AC
		sMEP-1,2*	L	AC
N-Acetyl-beta-glucosaminidase	3.2.1.30	bGLUA*	L	RW
Phosphoglucomutase	5.4.2.2	PGM-1*, PGM-2*	M	AC+, RW
Phosphogluconate dehydrogenase	1.1.1.44	PGDH*	M	AC
Superoxide dismutase	1.15.1.1	sSOD-1*	L	RW
Tripeptide aminopeptidase	3.4.-.-	PEPB*	E	SR

[a] Buffer systems as follows:

AC = N-(3-aminopropyl)-morpholine and citric acid buffer of Clayton and Tretiak (1972). pH = 6.7 for liver and 6.5 for muscle.

AC+ = same as AC except 2 drops of 2-mercaptoethanol and 15mg beta-nicotinamide adenine dinucleotide are added for every 225 ml gel buffer. pH = 6.3 for liver, 6.3 for eye, and 6.9 for muscle.

MF = Tris-boric acid-ethylenediaminetetraacetic acid (EDTA) buffer of Markert and Faulhaber (1965).

RW = Tris-citric acid buffer of Ridgway et al. (1970).

SR = Tris-citric acid buffer of Gall and Bentley (1981).

TABLE 2. Continued.

Locus	Alleles	Clover	King Hill	Little Jacks	Upper BigWood	Lower BigWood	Blue Jacket	Columbia	Doby George	Hayspur	Nickel	Emigrant
GPI-B2*	100	1.000	1.000	1.000	0.839	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	152	--	--	--	0.161	--	--	--	--	--	--	--
sIDHP-1,2*	100	0.514	0.583	0.671	0.676	0.621	0.600	0.596	0.225	0.500	0.527	-------
	114	0.208	--	0.026	0.037	--	--	--	--	0.060	--	-------
	71	0.153	0.250	0.303	0.259	0.322	0.400	0.385	0.525	0.440	0.355	-------
	40	0.125	0.167	--	0.028	0.067	--	0.019	0.250	--	0.118	-------
LDH-B2*	100	1.000	0.225	0.875	1.000	1.000	1.000	0.324	0.579	1.000	0.840	0.650
	112	--	--	0.025	--	--	--	--	--	--	--	--
	76	--	0.775	0.100	--	--	--	0.676	0.421	--	0.160	0.350
MDH-B1,2*	100	0.700	0.825	0.950	0.800	0.850	0.983	1.000	1.000	0.850	0.790	-------
	125	--	0.050	--	--	--	0.017	--	--	--	--	-------
	83	--	0.025	0.050	--	--	--	--	--	0.150	0.040	-------
	74	0.300	0.100	--	0.200	0.150	--	--	--	--	0.070	-------
PEPA-1*	100	1.000	1.000	1.000	0.768	0.833	1.000	1.000	1.000	0.304	1.000	1.000
	115	--	--	--	0.232	0.167	--	--	--	0.696	--	--
	101	--	--	--	--	--	--	--	--	--	--	--

restriction fragment patterns (= haplotypes) for each enzyme were designated by a letter, and a composite mtDNA haplotype was produced for each specimen.

Mitochondrial DNA haplotypes were analyzed using RESTSITE (Nei and Miller 1990) which estimates the number of nucleotide substitutions per site (p) via the Nei (1987) method. The analysis produced a diagonal matrix of distances (p-values) between all pairs of mtDNA haplotypes. The number of nucleotide substitutions per site (p) has heuristic value as an estimate of genetic divergence among mtDNA haplotypes, since it is equivalent to the percent of DNA sequence divergence among haplotypes. Thus, if the common mtDNA haplotypes for two taxa differ by $p = 0.015$, then the two taxa differ from one another at 1.5% of their mitochondrial DNA. Sequence divergence estimates are becoming increasingly available for a diversity of fishes at various taxonomic rankings. Comparison of our estimates with others allowed us to make qualitative assessments of the amount of differentiation and hybridization between interior rainbow and hatchery rainbow populations.

Inferred phylogenetic trees were produced as a way of estimating evolutionary relationships among mtDNA haplotypes. The least-squares method of Fitch and Margoliash (1967) were employed the KITSCH program in Felsenstein's PHYLIP 3.3. KITSCH makes assumptions of additivity, independence and equal rates of evolutionary divergence among lineages.

RESULTS AND DISCUSSION:

ALLOZYME ANALYSIS

Among the sample populations, data were resolved for 11 polymorphic loci (Table 2). Samples from the lower and upper sites on the Big Wood River did not differ significantly, except at GPI-B2* where a fast allele was observed

A subset of the Trail Creek specimens (N = 6) were initially examined for mtDNA variation. Most (5 of 6) specimens had mtDNA typical of interior rainbow trout; however, 3 of these 5 specimens also exhibited a second mtDNA haplotype in addition to the interior haplotype. The occurrence of more than a single mtDNA haplotype in an individual specimen is called heteroplasmy and is uncommon. Indeed, it is noteworthy in the case of the Trail Creek fish because we believe the second mtDNA haplotype is the result of a 3000 base pair deletion in the native mtDNA haplotype. Deletions of this magnitude are unknown in the literature; they are usually in the range of 2-300 base pairs. Because of the uniqueness of the Trail Creek specimens due to the mtDNA polymorphism, all 25 specimens have been shipped to Brigham Young University where Dennis Shiozawa and Paul Evans are collaborating in additional studies. For these reasons, only mtDNA analysis was conducted on the Trail Creek specimens.

Allozyme analysis. Genetic differences among populations at the nuclear DNA level were determined by surveying electrophoretic variation in the proteins (= allozymes) coded for by 39 presumptive gene loci present in muscle, liver or eye tissue (Table 1). We used horizontal starch gel electrophoresis (Utter et al. 1974; 1987; Leary and Booke 1990) and followed buffer and staining methods of Allendorf et al. (1977). Nomenclature followed Shaklee et al. (1990) with allele designations labeled as relative mobilities with respect to the common allele at each locus for the Arlee Hatchery strain of rainbow trout.

Principal components analysis (PCA) was performed on the arcsine transformation of the square root of the allele frequencies which normalizes the allele frequency data. PCA is a multivariate procedure that reduces a large data set containing numerous variables to a smaller set of variables that

INTRODUCTION:

This report serves as the final report for our contract with the Idaho Fish and Game in which we used genetic analyses to determine the genetic and taxonomic status of two naturally occurring trout populations in the Lower Big Wood River and Trail Creek, a tributary of the Big Wood River. The native trout of these drainages should be interior rainbow trout (Oncorhynchus mykiss).

Considerable interest has developed recently in identifying and preserving genetic diversity in native trout populations throughout western North America (Behnke 1979, 1992; Trotter 1987). Many local populations of cutthroat trout (O. clarki) and inland rainbow trout (O. mykiss) no longer exist because of habitat alteration or introgression with introduced hatchery rainbow trout or nonnative cutthroat trout subspecies. Many extant populations are reduced in size and thus vulnerable to further reduction and possible extinction (Behnke 1979, 1988; Allendorf and Leary 1988). Consequently, identification and preservation of native trout is now a goal of many management programs.

Preservation of native trout populations requires a reliable means of identifying pure versus hybridized populations. Previous studies have demonstrated the utility of protein electrophoresis in detecting hybridization between various subspecies of cutthroat trout (Busack and Gall 1981; Gyllensten et al. 1985; Leary et al. 1987), between cutthroat and rainbow trout (Behnke 1979; Leary et al. 1984; Campton and Utter 1985), and between coastal and interior rainbow trout (Currens et al. 1990; Williams et al., in review). Therefore, we used allozyme and/or mitochondrial DNA (mtDNA) analyses to determine the genetic purity and taxonomic status of trout from the Lower Big Wood River and Trail Creek.