CONSERVATION GENETICS OF BULL TROUT IN THE COLUMBIA AND KLAMATH RIVER DRAINAGES

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Abstract: We used electrophoretic analysis of proteins encoded by 51 loci to determine the population genetic structure of bull trout (Salvelinus confluentus) in the Columbia and Klamath River drainages. The sampled populations have little genetic variation within them and substantial genetic differences among them. Preserving the genetic diversity of bull trout will require the continued existence of many populations throughout this region. Bull trout from the Columbia and Klamath drainages are reproductively isolated and are evolutionarily distinct. These two groups of bull trout therefore would qualify as separate "species" under the United States Endangered Species Act according to criteria established for anadromous salmonid fishes.

Genotype frequencies at the four variable loci in a group of bull trout used for artificial supplementation indicate an extremely small number of effective parents. The release of such fish into the wild could have harmful effects on native fish populations.

Non-native brook trout (Salvelinus fontinalis) have been introduced throughout the range of bull trout, and hybridization between these species is increasingly reported. Protein and mitochondrial DNA genotypes collected from one stream over an eight year period indicate the displacement of bull trout by brook trout. This displacement was rapid and accompanied by extensive production of nearly sterile interspecific hybrids by both reciprocal crosses.

INTRODUCTION

Bull trout (Salvelinus confluentus) are a recently recognized species with an extensive distribution in northwestern North America (Cavender 1978; 1980). Historically, they existed in the upper Sacramento River drainage in California northwards to the upper Yukon and upper MacKenzie drainages in Canada. Below the 49th parallel bull trout are largely restricted to waters west of the Continental Divide. Above this point they exist on both sides of the Divide.

Little is known about the biology of bull trout. Four forms are distinguishable by life history characteristics. Bull trout are, or were, anadromous in some coastal river systems, but to a much lesser degree than the closely related Dolly Varden (Salvelinus malma). The lacustrine form matures in lakes and spawns in tributaries where young reside for one to three years (Fraley and Shepard 1989). Fluvial bull trout have a similar life history except they move between main rivers and tributaries. Individuals of these three forms can make extensive spawning migrations, usually do not attain sexual maturity until age five or six, and can reach a size exceeding 10 kg (Fraley and Shepard 1989; Holton 1990). Resident bull trout spend their entire lives in small streams. Although these fish now constitute the majority of bull trout in some areas, practically nothing is known of their biology.

Bull trout are now thought to be extinct in California and are considered a species of special concern throughout most of their remaining distribution (Hesseldenz 1985; Johnson 1987; Williams et al. 1989). Numerous, interrelated factors are thought to be responsible for the dramatic decline in abundance of bull trout. Its piscivorous nature (e.g., Boag 1987) led

commercial and sports fishermen, as well as fisheries managers, to the erroneous conclusion that its presence led to depleted numbers of more "desirable" fishes such as Pacific salmon (Oncorhynchus spp), rainbow trout (Oncorhynchus mykiss), and cutthroat trout (Oncorhynchus clarki). In some areas, a bounty was placed on bull trout to aid eradication efforts. Dam construction blocked spawning migrations in many situations. Clear cutting, road construction, mining, and grazing have destroyed spawning habitat through sedimentation and made some waters unsuitable for bull trout.

Introduced brook trout (Salvelinus fontinalis), brown trout (Salmo trutta), and rainbow trout are also thought to have displaced many bull trout populations. Displacement may have occurred in conjunction with extensive hybridization with brook trout (e.g., Leary et al. 1983). We have shown previously that hybridization can be frequent when brook and bull trout occur together, and that F_1 hybrids are nearly completely sterile.

Conservation of bull trout is an objective of many state, provincial, and federal management agencies. A knowledge of species population genetic structure is essential in order for this to be accomplished effectively (Allendorf and Leary 1988; Meffe and Vrijenhoek 1988; Quattro and Vrijenhoek 1989). That is, it is necessary to have some idea of how the genetic variation of the species is partitioned into genetic differences among populations and genetic variation within populations. When only slight genetic differences exist among populations, then most of the species genetic diversity can be conserved by ensuring continued existence of relatively few populations. When substantial genetic divergence exists among populations, conserving genetic diversity will require preservation of many populations throughout the range of a species.

In this paper, we examine the population genetic structure of bull trout in the Columbia and Klamath River drainages using protein electrophoresis. We also describe a series of samples over eight years from one stream where hybridization between brook and bull trout has occurred.

MATERIALS AND METHODS

Samples

Fish were collected by electroshocking or angling from 18 locations within the Columbia River and three locations within the upper Klamath River drainage (Table 1 and Fig. 1). Only four fish were collected from two tributaries of the Malheur River; these two samples were combined in all of the data analysis.

Protein Electrophoresis

Horizontal starch gel electrophoresis (Leary and Booke 1990) was used to determine the genotype of each fish at 45 loci coding for proteins present in muscle, liver, or eye (Table 2). In samples collected in 1990, we also analyzed the products of six additional loci (Table 2). None of these additional loci, however, were variable; they were, therefore, excluded from the data analyses.

Nomenclature of loci and alleles follows recommendations of Shaklee et al. (1990). Relative mobilities of electromorphs encoded by alleles detected at variable loci are as follows: sAAT-3,4 *1=75, *2=86; GPI-A *1=108, *2=111; GPI-B2 *1=108, *2=135; IDDH *1=100, *2=120; LDH-B1 *1=135, *2= 95; mIDHP-1 *1=300, *2=650; mMEP-2 *1=200, *2=145; sMEP-2 *1=93, *2=87; PEPA-1 *1=111, *2=115; PGM-2 *1=100, *2=119, *3=90. These mobilities are relative to the

electromorph encoded by the common allele at the homologous (orthologous) locus in Arlee rainbow trout maintained by the Montana Department of Fish, Wildlife and Parks.

Mitochondrial DNA

MtDNA restriction fragments were either visualized in agarose gels by ethidium bromide staining, or detected by Southern blot hybridization, as previously described (Forbes and Allendorf, 1991). Lambda phage DNA digested with HindIII or a 1-Kb DNA size standard ladder (Bethesda Research Laboratories) provided a fragment size standard.

RESULTS

Clark Fork River Samples

Samples from the Clark Fork River (13) and Gold Creek (14), a tributary of the Clark Fork River, were from hatchery fish being raised for supplementation of the wild populations. The Clark Fork River sample contained progeny of at most three females and two males taken from the river (Joe Chapman, Idaho Department of Fish and Game, personal communication). The Gold Creek sample contained progeny from at most eight females and an unknown number of males taken from Gold Creek (Joe Chapman, personal communication).

An excess of heterozygotes from random mating proportions is expected when individuals from a small number of families comprise a sample (Rasmussen 1979). There is an excess of heterozygotes at all polymorphic loci in the Clark Fork sample; this excess is statistically significant at two loci (Table 3). The exceptionally high proportion of heterozygotes (0.88) at mIDHP-1 suggests that most fish came from a single pair mating between individuals

homozygous for the two different alleles at this locus; all progeny from such a mating will be heterozygous at the locus. Allele frequencies at the four polymorphic loci also support the inference that most of these fish resulted from a single pair mating (Table 3). The only allele frequencies possible in a full-sib family are 0.00, 0.25, 0.50, 0.75, and 1.00 because two parents possess four copies of each gene; the allele frequencies at all four loci are very near these values (Table 3).

Allele frequencies estimated from this sample are not a reliable estimator of allele frequencies in the Clark Fork River population because of the small number of parents that produced this sample (Allendorf and Phelps 1981; Waples and Teel 1990). This sample, therefore, was eliminated from the analysis of genetic divergence among populations.

There is also an excess of heterozygotes at all three polymorphic loci in the Gold Creek sample. However, none of these deviations are statistically significant (Table 3). This and the larger number of parents used this group suggests that allele frequencies in the sample probably constitute a reasonable estimate of those in the population. This sample was, therefore, used in the analysis of genetic divergence among populations.

Genetic Variation within Samples

Genetic variation was detected at 10 of 51 loci examined (Table 4). Only one of ten polymorphic loci, PGM-2, had more than two alleles. There was no indication of deviations from expected Hardy-Weinberg proportions at these loci in any of the population samples.

All populations appear to contain rather low levels of genetic variation (Table 4). The proportion of polymorphic loci in each sample ranges from zero

to 0.098 and average expected heterozygosity from zero to 0.032. No genetic variation was detected in two samples from the John Day River (3 and 4) and all three samples from the Klamath drainage (19-21). In addition, no variation was found in the four fish from the two tributaries of the Malheur River (6). All of these samples that lacked variation were examined at 51 gene loci.

Genetic Divergence among Populations

The allele frequencies differ significantly among samples at nine of ten variable loci (contingency chi-square analysis, $P<0.05$). The only exception is sAAT-3,4 for which only a single heterozygous individual was detected.

We partitioned the total amount of genetic variation among samples into hierarchial geographic components (Table 5) using the methods described by Chakraborty and Leimar (1987). Values presented in Table 5 and the G_{ST} values presented in Table 4 are the unbiased estimators of Nei and Chesser (1983).

This analysis reveals a number of noteworthy features. First, compared to other salmonid fishes for which protein data are available these bull trout populations contain a small amount of genetic variation (Allendorf and Leary 1988). Furthermore, only approximately 40% of the total genetic variation detected is attributable to genetic variation within populations (Table 5). Thus, bull trout, like many other salmonid fishes inhabiting interior waters, are characterized by a low amount of genetic variation within and a large amount of genetic divergence among populations (Allendorf and Leary 1988).

Genetic differences between Columbia and Klamath bull trout account for an appreciable amount of the total genetic variation in our samples. These differences mainly result from complete genetic divergence between fish in the

two drainages detected at GPI-B2 (Table 4). The existence of genetic variation at nine loci in Columbia bull trout and absence of variation at these loci in Klamath bull trout account for the remainder of this genetic divergence. Principal component analysis of allele frequencies at the 10 variable loci clearly separates the Columbia and Klamath River samples into two distinct clusters (Fig. 2a).

A substantial amount of the total genetic variation within the Columbia River drainage is also due to genetic differences among samples (40.0%). Most of this genetic divergence reflects highly variable frequencies at GPI-A (1.000-0.318) and mIDHP-1 (1.00-0.000) (Table 4). Additional divergence mainly reflects variant alleles at appreciable frequency (>0.150) at LDH-B1, PEPA-1, PGM-2, and sMEP-2 in only one sample and their absence, or near absence, from all others (Table 4).

Genetic distances (Nei 1972) between populations within the Columbia River drainage do not correspond closely to geographical distances ($r=0.014$, $P>0.90$; Figure 3). Principal component analysis of allele frequencies at the ten polymorphic loci also indicates that the degree of genetic divergence among Columbia River populations is not strongly associated with geographic proximity (Fig. 2b). Populations have little tendency to group by geographic proximity on the basis of the first two principal components, which account for 96% of the total variation in allele frequencies. For example, samples 16 and 18, which are both tributaries of the North Fork of the Flathead River, are nearly at opposite extremes on the first principal component axis.

Hybridization with Brook Trout

We have previously provided evidence for extensive hybridization between

brook trout and bull trout in South Fork Lolo Creek, Missoula County, Montana (Leary et al. 1983, 1985a). We now have information on temporal changes in the proportions of these two species and their hybrid from five samples collected in a single section of this creek between 1982 and 1990.

Brook trout and bull trout rarely possess the same allele at 10 of 45 loci analyzed (Leary et al. 1983). The genotype of individuals at these 10 diagnostic loci (Ayala and Powell 1972) was used to determine whether they were brook trout, bull trout, or hybrids. First-generation hybrids between species are expected to be heterozygous at all diagnostic loci. Later generation hybrids, or back-crosses between hybrids and the parental species, should have various genotypic combinations depending upon the particular cross and results of Mendelian segregation (Allendorf and Leary 1988).

Only one out of some 50 naturally occurring hybrids that we have detected with these 10 diagnostic loci was not a first-generation hybrid (Table 6 and unpublished data). This fish was collected from the South Fork of Lolo Creek in 1983 and was homozygous for brook trout alleles at three diagnostic loci and heterozygous at the other seven. This fish, therefore, was apparently a backcross progeny between a hybrid and a brook trout. Thus, these hybrids are nearly completely sterile. In some cases, hybrids between fish species reproduce asexually so that the first-generation hybrid genotype is passed on generation after generation (Dawley 1989). However, this cannot be the case with bull and brook trout hybrids since almost all hybrids are male (Leary et al. 1983).

The frequency of brook trout has increased dramatically (contingency chi-square analysis, $P<0.02$) in the South Fork of Lolo Creek (Table 6; Fig. 4). Bull trout were the predominant fish in early samples, but brook trout

were predominant in later samples. Sample sizes in the later samples are small; nevertheless, contingency table chi-square analysis of the proportions of all three types indicates significant differences among samples ($P = 0.05$).

MtDNA is inherited maternally and, therefore, allows determination of the direction of hybridization between species. We digested DNA from two bull trout and four brook trout, identified by allozyme analysis, with fifteen restriction endonucleases having six-basepair recognition sequences (AvaI, BamHI, BstEII, BclI, BglI, BglII, DraI, EcoRI, EcoRV, HindII, HindIII, PstI, PvuII, ScaI, StuI). The fifteen enzymes revealed two brook trout mtDNA haplotypes that differ at a HindIII site and a PstI site. Twelve of these enzymes showed different fragment patterns between the two species. The thirteen unique-sequence enzymes give a proportion of shared fragments of $F = 42/93 = 0.452$, for an estimated sequence divergence of $P = 0.047$ (Nei and Li 1979).

We used one to three of the enzymes that distinguished bull trout and brook trout mtDNAs to analyze 21 of the fish collected from the South Fork of Lolo Creek, including six of seven hybrids collected in 1987 and 1990. Four hybrids had bull trout mtDNA, and two had brook trout mtDNA. Thus, both reciprocal hybrid crosses (female bull trout X male brook trout; male bull trout X female brook trout) have occurred in this stream.

DISCUSSION

Genetic Population Structure

The population genetic structure of bull trout in the Columbia and Klamath drainages is typical of salmonid fishes inhabiting interior waters. There is relatively little genetic variation within populations, but

substantial genetic differences among populations.

The observed genetic differences mainly arise from highly variable allele frequencies at two loci that are polymorphic throughout the Columbia drainage and variant alleles at five other loci with much narrower geographic distributions. These narrowly distributed alleles, however, often exist at appreciable frequency where they occur. Preservation of the genetic variation of the bull trout in this portion of its range will require continued existence of many populations throughout the area.

In contrast to salmonids in interior drainages, those from anadromous populations generally exhibit substantial genetic variation within populations and relatively little genetic divergence among populations (Ryman 1983). We believe the simplest explanation for this difference in population genetic structure is a difference in the amount of gene flow among populations. Anadromous fishes travel greater distances and have more opportunity for exchange among populations (gene flow) due to straying. This increased gene flow will hinder accumulation of genetic differences among populations through natural selection and genetic drift, but will maintain high levels of variation within populations (Gyllensten 1985).

The observed genetic divergence among bull trout populations probably mainly reflects the operation of stochastic forces such as bottlenecks, founder effects, and genetic drift on selectively neutral genetic variation. This does not mean, however, that important adaptive differences do not exist among populations. Protein electrophoresis allows one to examine levels of genetic variation at only a small proportion of all genes. As gene flow decreases, the probability that populations will acquire adaptations to their local environment increases. Thus, it is likely that important adaptive

differences exist among some of the populations at unexamined genes.

Hybridization with Brook Trout

The samples from South Fork of Lolo Creek indicate that hybridization between bull and brook trout can be quite frequent. These data further indicate that hybridization accompanied displacement of bull trout by brook trout. In the early 1980's bull trout were the predominant Salvelinus in the area; now brook trout are predominant. We expect that this trend will continue until bull trout are displaced from the area, and brook trout will continue to invade further upstream. This is likely to continue until bull trout are extirpated from the creek or brook trout meet an upstream dispersal barrier.

We do not know how widespread hybridization with brook trout is throughout the range of bull trout. In our experience, hybridization is widespread and common between resident bull trout and brook trout in western Montana. Hybridization also has been reported in Alberta (page 218, Scott and Crossman 1973) and in the Klamath River drainage (Behnke 1980). Hybridization is a contributing factor to the general decline of bull trout populations in Oregon (Philip Howell, Oregon Department of Fish and Wildlife, personal communication).

The frequent production of sterile interspecific hybrids is an unstable situation that should lead to loss of one of the two parental types. Conceptually, this is similar to heterozygous disadvantage, or underdominance, at a single gene. In the simplest model, the hybrids have a fitness near zero and the two parental species have relative fitnesses near one. The more numerous species will have an advantage because less of their total

reproductive effort will be wasted in hybrid production.

For example, assume that species A is twice as numerous as species B, and mating is at random. The following proportion of matings will result:

A x A	A x B	B x B
0.44	0.44	0.11

In this example, only one-third of the reproductive effort of individuals from the more common species A produces sterile hybrid progeny, while two-thirds of the reproductive effort of individuals from species B produces sterile hybrids.

Life history differences between bull and brook trout will tend to favor the brook trout in this situation. Brook trout become sexually mature at age 2 or 3, are relatively short-lived, and tend to "overpopulate" small streams (page 211, Scott and Crossman). In contrast, bull trout do not reach sexual maturity until 3-6 years and are long-lived (page 217, Scott and Crossman 1973).

Conservation of Genetic Diversity

Our analysis indicates that groups of fish being raised for release into the Clark Fork River in a supplementation program had a very small number of contributing parents. Such supplementation programs may be harmful even if the artificially raised fish are released into the same waters from which the parents were collected.

Ryman and Laikre (1991) have shown that release of artificially reared progeny may severely reduce the effective population size of local populations

because of greater reproductive success of those adults used to provide hatchery progeny. Survival in the hatchery from egg to release may exceed 75%, while survival in the wild over similar life history stages is likely to be less than 10%. The reduced effective population size will result in a loss of genetic variation which can eventually reduce productivity (e.g., Leary et al. 1985b; Quattro and Vrijenhoek 1989).

Adverse genetic effects of supplementation are increased if fish are released into waters other than where the parents were collected. The large amount of genetic divergence we found among populations indicates reproductive isolation that would allow evolution of local adaptations. Stocking and subsequent reproduction of hatchery fish in the wild may lead to loss of these local adaptations (Marnell 1986; Ferguson 1990; Philipp 1991). Such adverse effects will not be restricted to supplemented populations. Hatchery fish are likely to "stray" more than native wild fish. This straying could increase gene flow among local populations and cause a loss of local adaptations even in unsupplemented populations.

Hatchery supplementation of salmonids is often seen as an attractive, albeit expensive, solution to compensate for loss of habitat. Hatchery supplementation, however, has not compensated for habitat loss of anadromous salmonid populations throughout the western United States (Goodman 1990). Despite continuing introductions many anadromous salmonid populations have been lost and many are threatened with extinction (Nehlsen et al. 1991).

Hatchery supplementation has been proposed to compensate for an estimated 250,000 young bull trout lost by construction of Hungry Horse Dam on the South Fork of the Flathead River in Montana (Anonymous 1991). We believe that any hatchery supplementation with bull trout should proceed with extreme

caution. It would be more prudent initially to attempt to increase population size through habitat improvement and restrictive regulations rather than hatchery introductions. Hatcheries can play an essential role in recovery programs if appropriate genetic guidelines are followed. However, too often hatcheries have been a way of treating the symptoms (reduction in numbers of fish) while ignoring the causes of decline (e.g., degradation or loss of habitat).

The decline in bull trout populations has prompted interest in petitioning to have them listed under the United States Endangered Species Act (ESA). The fragmented population structure of salmonid species has led to individual local populations being listed as "species" under the ESA. Sacramento River winter-run chinook salmon (Oncorhynchus tshawytscha) were determined to be a "species" under the ESA in 1987 (52 Fed. Reg. 6041, 6042, 6047). Sockeye salmon (Oncorhynchus nerka) from the Snake River and two separate groups of chinook salmon from the Snake River (fall run and spring/summer run) have been proposed to be listed as species under the ESA (56 Fed. Reg. 29546, 29549).

Under the ESA, a "species" is defined to include "any distinct population segment of any species of vertebrate fish or wildlife which interbreeds when mature" (Public Law 95-632 (1978), 92 Stat. 3751). The National Marine Fisheries Service (NMFS) has published a position paper to interpret the definition of "species" under the ESA as it applies to anadromous salmonids (Waples 1991). According to NMFS, a population, or group, of populations will be considered "distinct" and, therefore, a "species" if it represents an evolutionary significant unit (ESU). There are two criteria to satisfy to be considered an ESU (Waples 1991):

(1) It must be reproductively isolated from other conspecific population units.

(2) It must represent an important component in the evolutionary legacy of the species.

Klamath River and Columbia River bull trout would qualify as separate ESU's under these criteria. These two major drainages have been separated at least since the last major glaciation in this area over 10,000 years ago. There is no current opportunity for natural genetic exchange between bull trout in these two drainages, and our data indicate that substantial genetic divergence has accumulated between them. Second, Klamath River bull trout contribute substantially to the ecological and genetic diversity of the species as a whole, and thus constitute an important component in the evolutionary legacy of bull trout.

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TABLE 1. Sample locations, collection date, and sample size (N) of bull trout collected from Idaho (ID), Washington (WA), Oregon (OR), Montana (MT), and British Columbia (BC).

Location	Date	N
Columbia River Drainage		
A. Lewis River, WA		
1. Rush Creek	Sep 90	26
B. Metolius River, OR		
2. Jack Creek	Oct 90	30
C. John Day River, OR		
3. Deardorff Creek	Nov 90	13
4. Granite Boulder Creek	Nov 90	16
D. Grande Ronde River, OR		
5. South Fork Catherine Creek	Sep 90	26
E. Malheur River, OR		
6. Big Creek	Nov 90	3
7. Little Crane Creek	Nov 90	1
F. Methow River, WA		
8. Early Winters Creek	Sep 90	25
G. Columbia River, BC		
9. Lower Arrow Lake	Oct 90	6
H. Kootenay River, BC		
10. Kootenay Lake	Spring 82	11
I. Kootenai River, MT		
11. Kootenai River	Spring 82	20
12. West Fork Quartz Creek	Jun 82	11
J. Clark Fork River, ID		
13. Clark Fork River	Sep 90	25
14. Gold Creek	Jul 90	25
K. Bitterroot River, MT		
15. South Fork Lolo Creek	Jul 82, Aug 83	34
L. North Fork Flathead River, MT		
16. Dry Fork	Summer 85	10
17. Upper Kintla Lake	Fall 84, Aug 86	35
18. Whale Creek	Summer 85	6
Upper Klamath River Drainage		
M. South Fork Sprague River, OR		
19. Brownsworth Creek	Sep 90	10
20. Demming Creek	Sep 90	9
21. Leonard Creek	Sep 90	10

Table 2. Enzymes, number (EC, IUBNC 1984), and loci examined in bull trout samples. Tissues: E=eye, L=liver, M=muscle. Buffer indicates the buffer system that gave the best resolution for each enzyme.

Enzyme	EC	Loci	Tissue	Buffer
Adenylate kinase	2.7.4.3	AK-1, AK-2	M	AC
Alcohol 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
Creatine kinase	2.7.3.2	CK-A1, CK-A2	M	RW
		CK-B,CK-C1,CK-C2	E	SR
Dipeptidase	3.4.-.-	PEPA-1,PEPA-2	E	SR
Glucose-6-phosphate isomerase	5.3.1.9	GPI-A	E	SR
		GPI-B1,GPI-B2	M	RW
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	GAPDH-3,4	E	AC+
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH-1,G3PDH-2	L	AC
Isocitrate dehydrogenase	1.1.1.42	mIDHP-1,mIDHP-2	M	AC+
		sIDHP-1,sIDHP-2	E	AC+
L-Iditol dehydrogenase	1.1.1.14	IDDH	L	RW
L-Lactate dehydrogenase	1.1.1.27	LDH-A1,LDH-A2	M	RW
		LDH-B1,LDH-B2,LDH-C	E	SR
Malate dehydrogenase	1.1.1.37	sMDH-A1,2	L	AC
		sMDH-B1,2	M	AC+
Malic enzyme	1.1.1.40	mMEP-1,mMEP-2,sMEP-1	M	AC
		sMEP-2	L	AC
Phosphoglucomutase	5.4.2.2	PGM-1,PGM-2	M	AC
Phosphogluconate dehydrogenase	1.1.1.44	PGDH	M	AC

Table 2 - continued

Enzyme	EC	Loci	Tissue	Buffer
Superoxide dismutase	1.15.1.1	sSOD	L	RW
Tripeptide aminopeptidase	3.4.-.-	PEPB	E	SR
Xanthine dehydrogenase-like	-.-.-.-	XDHℓ	L	RW
Additional enzymes analyzed only in 1990 samples:				
Acid phosphatase	3.1.3.2	ACP-2	L	AC
beta-Glucuronidase	3.2.1.31	bGUS	L	RW
N-Acetyl-beta-glucosaminidase	3.2.1.30	bGLUA	L	RW
Phosphoglycerate kinase	2.7.2.3	PGK-2	M	AC+
Pyruvate kinase	2.7.1.40	PK-3,PK-4	E	AC+

AC = N-(3-aminopropyl)-morpholine and citric acid buffer of Clayton and Tretiak (1972).

AC+ = Same as AC except 2 drops of 2-mercaptoethanol and 15 mg beta-nicotinamide adenine dinucleotide are added for every 200 ml of gel buffer.

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

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

Table 3. Genotypic proportions observed in the progeny from bull trout collected from the Clark Fork River and Gold Creek. p(1) is the frequency of the *1 allele. F_{is} is the proportional excess of heterozygotes.

		Genotype				
Sample	Locus	11	12	22	p(1)	F_{is}
Clark Fork	GPI-A	10	15	0	0.700	-0.429*
	IDDH	24	1	0	0.980	-0.002
	mIDHP-1	1	22	2	0.480	-0.763***
	sIDHP-1	12	13	0	0.740	-0.351
Gold Creek	GPI-A	19	6	0	0.880	-0.136
	IDDH	16	9	0	0.820	-0.220
	mIDHP-1	0	11	14	0.220	-0.282

* = P<0.05; *** = P<0.001.

Table 4. Allele frequencies in bull trout from the Columbia and Klamath River drainages. Only the frequency of the *1 allele is given at the loci with two alleles. Het is the average expected heterozygosity at all 45 loci. G_{ST} is a measure of differentiation among samples at each locus (Chakraborty and Leimar 1987). Larger values indicate increasing divergence, with a maximum of one.

Sample	sAAT-3,4	GPI-A	GPI-B2	IDDH	LDH-B1	mIDHP-1	mMEP-2	sMEP-2	PEPA-1	PGM-2 *1	PGM-2 *2	PGM-2 *3	Het
1. Rush Creek	1.000	1.000	1.000	1.000	1.000	0.288	1.000	1.000	1.000	1.000	---	---	0.010
2. Jack Creek	1.000	1.000	1.000	0.967	1.000	0.300	1.000	1.000	1.000	1.000	---	---	0.012
3,4. John Day (2)	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	---	---	0.000
5. Catherine Cr.	1.000	1.000	1.000	0.942	1.000	1.000	1.000	1.000	1.000	0.846	0.154	---	0.009
6. Malheur R.	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	---	---	0.000
8. Early Winters	1.000	0.860	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	---	---	0.006
9. Arrow Lake	1.000	0.667	1.000	1.000	1.000	0.500	1.000	1.000	1.000	1.000	---	---	0.023
10. Kootenay Lake	1.000	0.636	1.000	1.000	0.955	0.364	1.000	1.000	1.000	1.000	---	---	0.025
11. Kootenai River	1.000	0.675	1.000	1.000	1.000	0.725	1.000	0.975	1.000	1.000	---	---	0.023
12. Quartz Creek	1.000	0.318	1.000	1.000	0.733	1.000	0.955	1.000	0.733	1.000	---	---	0.032
14. Gold Creek	1.000	0.880	1.000	0.820	1.000	0.220	1.000	1.000	1.000	1.000	---	---	0.015
15. Lolo Creek	1.000	1.000	1.000	1.000	1.000	0.853	1.000	1.000	1.000	0.971	---	0.029	0.010
16. Dry Fork	1.000	1.000	1.000	1.000	1.000	0.900	1.000	1.000	1.000	1.000	---	---	0.004
17. Kintla Lake	0.992	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	---	---	0.007
18. Whale Creek	1.000	1.000	1.000	0.917	1.000	---	1.000	1.000	1.000	1.000	---	---	0.004
19-21. Klamath (3)	1.000	1.000	---	1.000	1.000	1.000	1.000	1.000	1.000	1.000	---	---	0.000
G_{ST}	0.0000	0.3376	1.0000	0.0698	0.1896	0.5712	0.0000	0.0991	0.2245	0.1154			

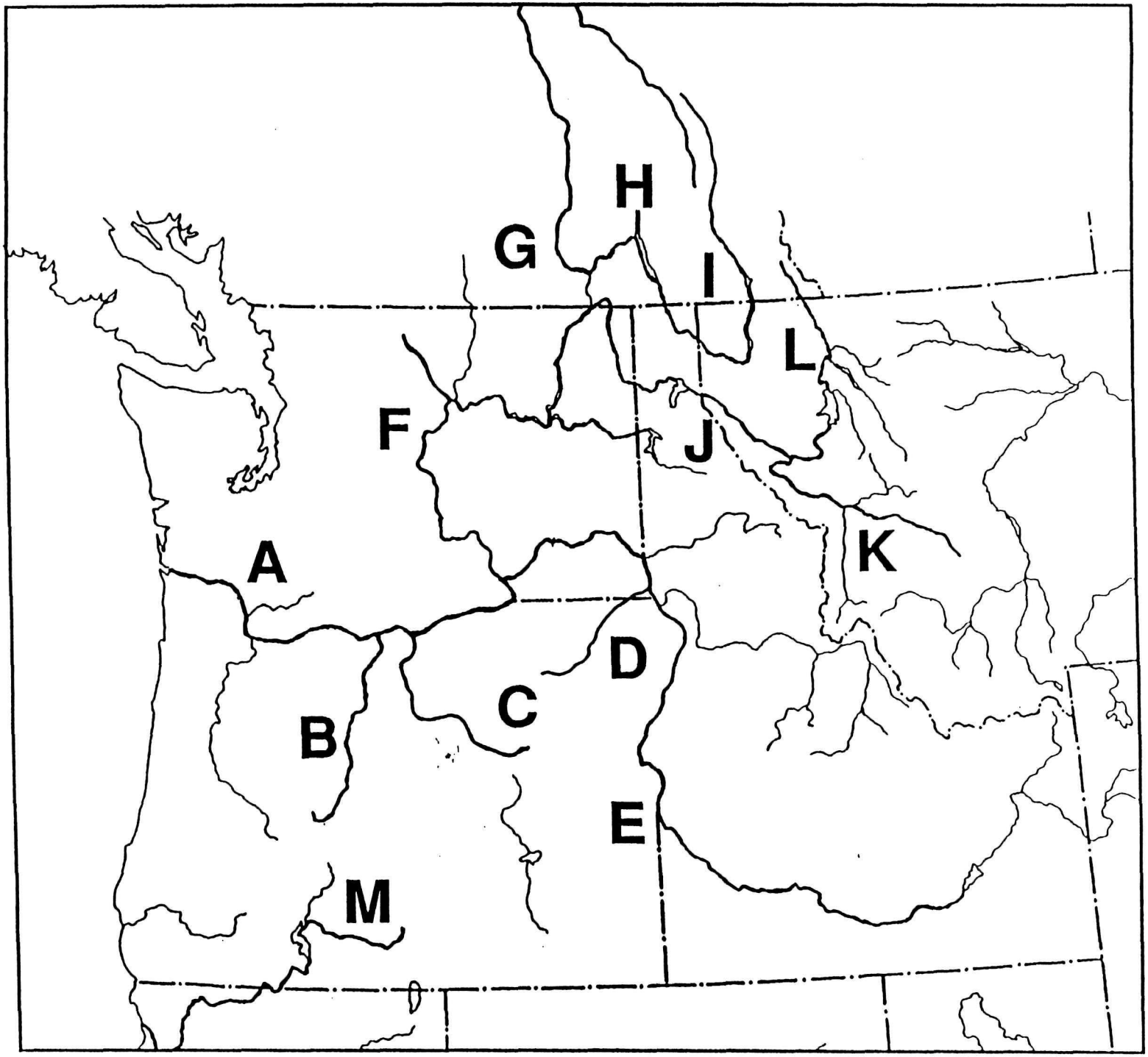
Table 5. Amount and distribution of genetic variation in bull trout from the Columbia and Klamath River drainages using the methods of Chakraborty and Leimar (1987). H_T is the total heterozygosity, H_s is the average heterozygosity within each sample, and G_{ST} is a measure of differentiation among samples.

Region	No. Samples	H_T	H_s	G_{ST}	Distribution of variation	
					Between Populations	Within Populations
Columbia	16	0.0178	0.0107	0.4004	40.0%	60.0%
Klamath	3	0.0000	0.0000	---	---	---
Total	19	0.0216	0.0090	0.5817	58.2%	41.8%

Table 6. Numbers of brook trout, bull trout, and first generation hybrids in five samples collected from South Fork Lolo Creek, Montana. Percentage of sample represented by each type is given in parentheses.

	Number of fish			
Sample year	Brook Trout	Hybrid	Bull Trout	N
1982	8 (20.5)	14 (35.9)	17 (43.6)	39
1983	10 (29.4)	7 (20.6)	17 (50.0)	34
1986	11 (40.7)	5 (18.5)	11 (40.7)	27
1987	7 (46.7)	5 (33.3)	3 (20.0)	15
1990	11 (64.7)	2 (11.8)	4 (23.5)	17

Figure 1. Approximate sample locations of populations of bull trout in the Columbia River and upper Klamath River drainages. Letters correspond to those in Table 1.


H
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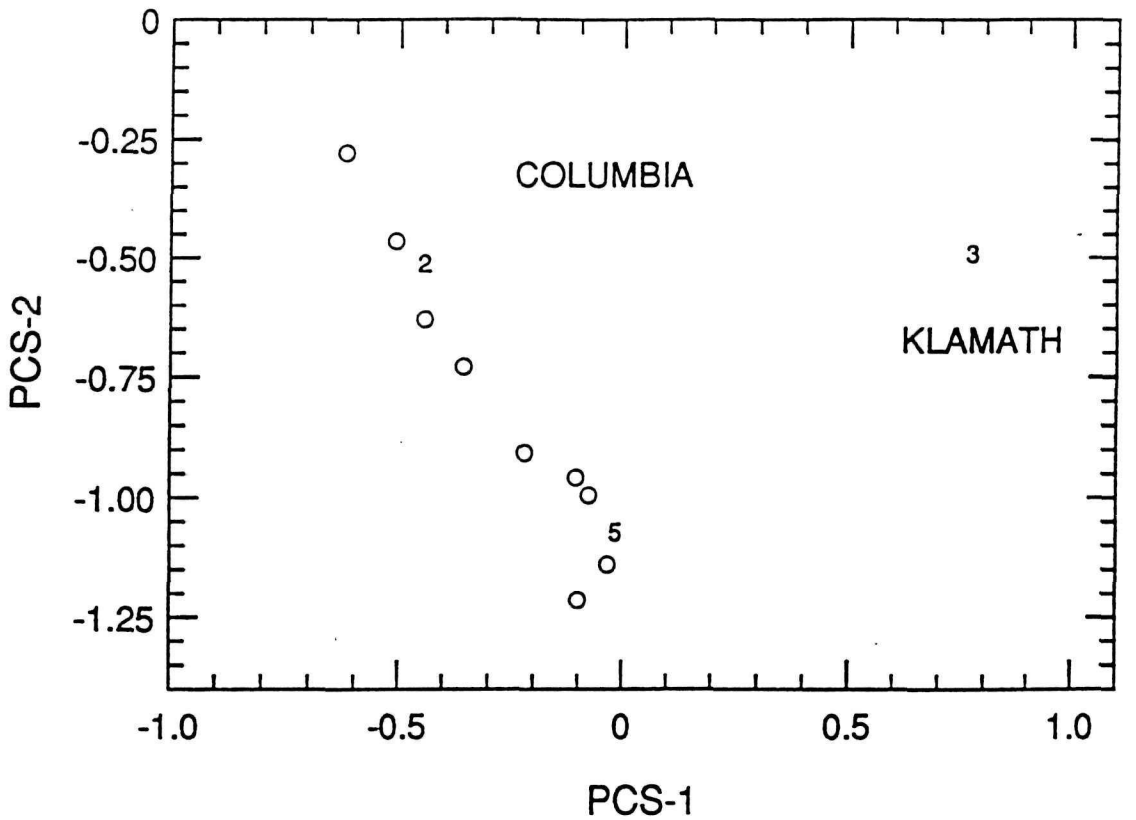

Figure 2 (a). Plot of first two principal component scores derived from allele frequencies in Table 3 for all population samples. Open circles represent individual populations; numbers indicate populations located at the same values.

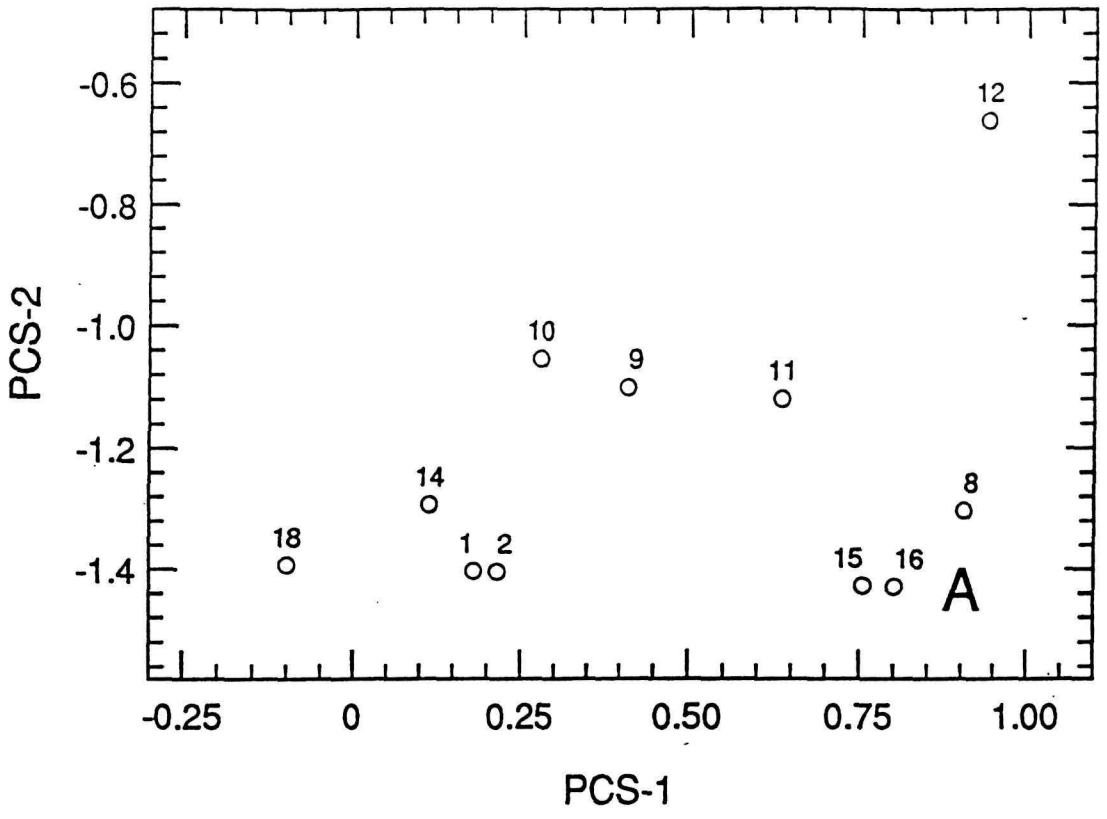

Figure 2 (b). Plot of first two principal component scores derived from allele frequencies in Table 3 for samples from the Columbia River drainage. Numbers correspond to those in Table 1; samples 3-6 and 17 are located at point A.

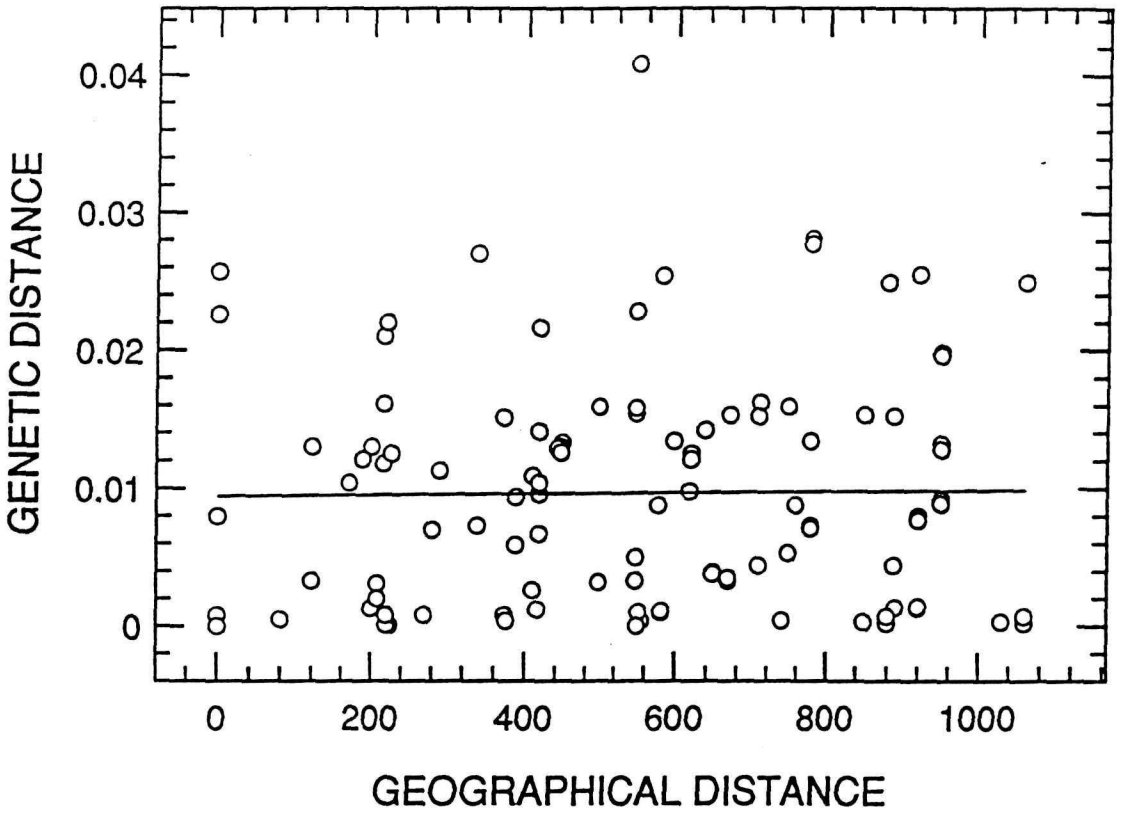

Figure 3. Plot of genetic distance versus geographical distance (river kilometers) for 16 bull trout samples from the Columbia River drainage. The straight line is the principal axis of the correlation.

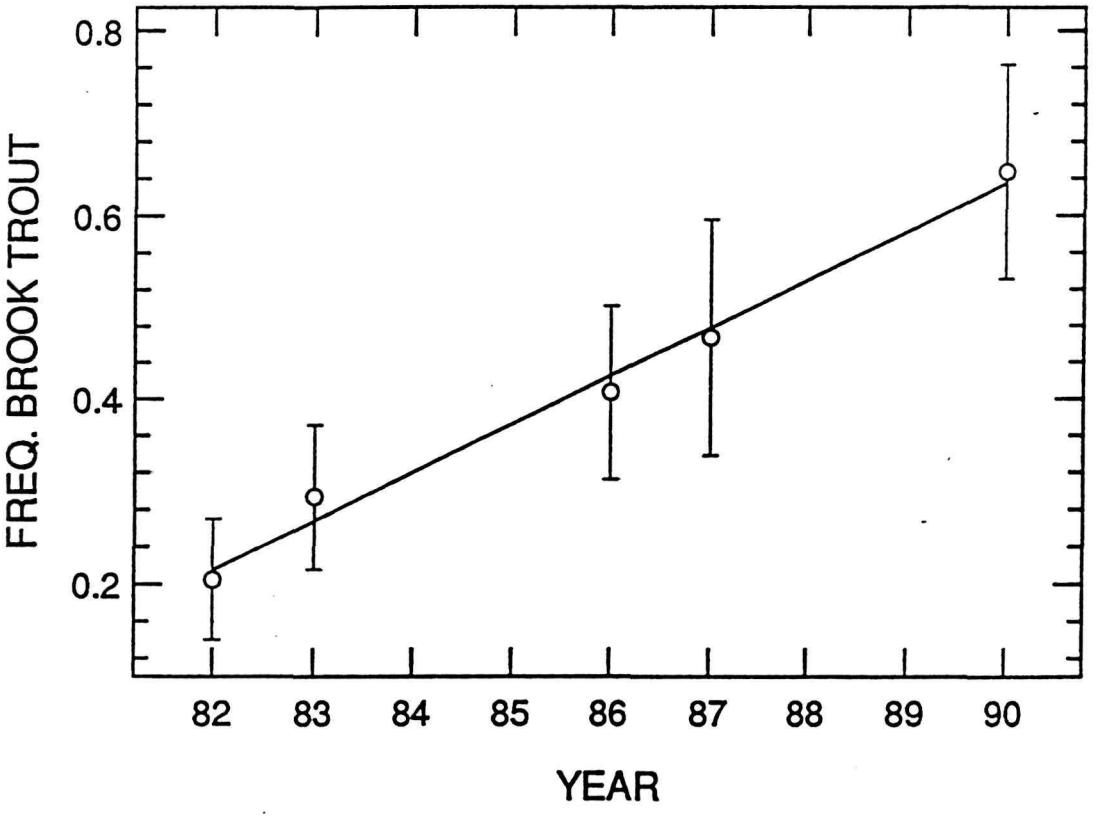

Figure 4. Regression of the frequency of brook trout over time in samples of brook trout, bull trout, and their hybrids from the South Fork of Lolo Creek, Montana. Error bars show one standard deviation.