Transactions of the American Fisheries Society 116:580–587, 1987

Genetic Divergence and Identification of Seven Cutthroat Trout Subspecies and Rainbow Trout

ROBB F. LEARY AND FRED W. ALLENDORF
Department of Zoology, University of Montana
Missoula, Montana 59812, USA

STEVAN R. PHELPS
Washington Department of Fish and Game, 115 General Administration Building
Olympia, Washington 98504, USA

KATHY L. KNUDSEN
Department of Zoology, University of Montana

Abstract.—We estimated the amount of genetic divergence among seven cutthroat trout *Salmo clarki* subspecies and rainbow trout *Salmo gairdneri* using electrophoretic data from 46 protein loci. There was little genetic divergence among the Colorado River, finespotted, greenback, and Yellowstone subspecies of cutthroat trout, but a large amount existed among the coastal, Lahontan, and westslope subspecies. These latter three subspecies were electrophoretically as similar to rainbow trout—or more so—as they were to the other four subspecies of cutthroat trout examined. Morphologically, in contrast, the cutthroat trout subspecies were all more similar to each other than to rainbow trout. The data, therefore, suggest that morphological and protein evolution have proceeded at different rates among some of these fishes. The presence of fixed or nearly fixed allele-frequency differences between the subspecies of cutthroat trout and rainbow trout and between many pairs of cutthroat trout subspecies provides a powerful means of identifying "genetically pure" populations of these taxa.

The cutthroat trout *Salmo clarki* is a polytypic species with a widespread geographic range native to the coastal and interior waters of western North America. R. J. Behnke (Colorado State University, unpublished) recognizes 14 allopatrically distributed subspecies. The natural range of a particular subspecies often consists of only one of the many small interior drainage basins in western North America. Despite intensive morphological and ecological investigation, the amount of genetic divergence among the various subspecies remains poorly understood because of the extreme morphological and ecological variation that exists within and among the subspecies. Furthermore, it is generally unknown how much genetic divergence a given degree of morphological or ecological differentiation represents.

Most local populations of cutthroat trout that existed at the beginning of this century in the interior of western North America no longer exist because of habitat alteration or introgression with introduced rainbow trout *Salmo gairdneri* or other subspecies of cutthroat trout (Behnke 1972; Allendorf and Phelps 1981; Busack and Gall 1981; Leary et al. 1984b; Campton and Utter 1985; Gyllensten et al. 1985). Many subspecies are now considered to be threatened or endangered (Behnke 1972; Behnke and Zarn 1976; Leary et al. 1984b) and preservation of the cutthroat trout is now the goal of many management programs. An accurate understanding of the amount of genetic divergence among the subspecies will aid in the identification of the remaining native populations, in their biologically sound management, and in their preservation.

Preservation of the cutthroat trout subspecies requires a reliable means of identification. The presence of many introgressed populations has greatly diminished the value of morphological characters to distinguish between introgressed and genetically pure populations. This is mainly because small amounts of genetic material (<10%) from another taxon in a population appear to have no detectable effect on the morphology of individuals (Leary et al. 1984b).

In this report, we examine the feasibility of using the electrophoretic examination of proteins to identify subspecies of cutthroat trout. To do this, we estimate the amount of genetic divergence among seven subspecies and the rainbow trout. The results indicate that there is little electrophoretic differentiation among four of the subspecies of cutthroat trout (Yellowstone *S. c. bouvieri*, Colorado River *S. c. pleuriticus*, greenback *S. c. stomias*, and finespotted *S. c.* ssp.),
tial divergence exists among th
lewisi, coastal *S. c. clarki*, and La
shawi subspecies. The latter thr
electrophoretically as similar to
more so, as they are to the othe
of cutthroat trout.

Methods

Samples of fish were obtained
ing sources (sample sizes are in p
orado River (N = 10) and gre
cutthroat trout from the U.S. I
Service's (USFWS) Fish Cultu
Center, Bozeman, Montana; fine
trout (N = 50) from the USFV
tional Fish Hatchery, Jackson, W
trout (N = 160) from the Monta
Fish, Wildlife and Parks' (MDF
State Trout Hatchery, Arlee, M
stone cutthroat trout (N = 50) fro
Yellowstone River State Trou
Timber, Montana; westslope cu
51) from O'Keefe Creek, Missou
tana; Lahontan cutthroat trout
Pyramid Lake Paiute Tribe's C
mana Hupa-Agai Hatchery,
coastal cutthroat trout (N = 4
Creek, Lane County, Oregon.

Although some of the sampl
this is not expected to have a l
qualitative interpretation of th
racy of estimates of electropho
more profoundly affected by th
examined than by the number c
1978; Gorman and Renzi 197
phoretic differences exist when
alleles at all or practically all loc
1974). In contrast, substantial e
ferences are generally associate
in which populations do not, o
same alleles at several loci (Av
amination of more than 30 loci
ple size of one individual per p
cally ensures that the data will re
of the above conditions exists.

Horizontal starch gel electro
al. 1974) was used to assay gene
loci encoding for proteins prese
or eye tissue from all specimen
buffers and staining methods w
dorf et al. (1977). We designate
three-letter italicized abbreviat
they encode, the first letter being

Seven
Trout

ation Building

cutthroat trout *Salmo*
data from 46 protein
otted, greenback, and
the coastal, Lahontan,
ally as similar to rain-
hroat trout examined.
similar to each other
rotein evolution have
l or nearly fixed allele-
ow trout and between
lentifying "genetically

n 1976; Leary et al. 1984b)
e cutthroat trout is now the
nent programs. An accurate
amount of genetic diver-
pecies will aid in the iden-
ning native populations, in
d management, and in their

cutthroat trout subspecies
eans of identification. The
trogressed populations has
ie value of morphological
sh between introgressed and
lations. This is mainly be-
of genetic material (<10%)
a population appear to have
n the morphology of indi-
984b).
camine the feasibility of us-
examination of proteins to
cutthroat trout. To do this,
ount of genetic divergence
cies and the rainbow trout.
at there is little electropho-
nong four of the subspecies
lowstone *S. c. bouvieri*, Col-
ıriticus, greenback *S. c. sto-*

---

mias, and finespotted *S. c.* ssp.), but that substantial divergence exists among the westslope *S. c. lewisi*, coastal *S. c. clarki*, and Lahontan *S. c. henshawi* subspecies. The latter three subspecies are electrophoretically as similar to rainbow trout, or more so, as they are to the other four subspecies of cutthroat trout.

Methods

Samples of fish were obtained from the following sources (sample sizes are in parentheses): Colorado River (N = 10) and greenback (N = 10) cutthroat trout from the U.S. Fish and Wildlife Service's (USFWS) Fish Cultural Development Center, Bozeman, Montana; finespotted cutthroat trout (N = 50) from the USFWS's Jackson National Fish Hatchery, Jackson, Wyoming; rainbow trout (N = 160) from the Montana Department of Fish, Wildlife and Parks' (MDFWP) Jocko River State Trout Hatchery, Arlee, Montana; Yellowstone cutthroat trout (N = 50) from the MDFWP's Yellowstone River State Trout Hatchery, Big Timber, Montana; westslope cutthroat trout (N = 51) from O'Keefe Creek, Missoula County, Montana; Lahontan cutthroat trout (N = 5) from the Pyramid Lake Paiute Tribe's Captain Dave Numana Hupa-Agai Hatchery, Nixon, Nevada; coastal cutthroat trout (N = 4) from Cummins Creek, Lane County, Oregon.

Although some of the sample sizes are small, this is not expected to have a large effect on the qualitative interpretation of the data. The accuracy of estimates of electrophoretic divergence is more profoundly affected by the number of loci examined than by the number of individuals (Nei 1978; Gorman and Renzi 1979). Small electrophoretic differences exist when populations share alleles at all or practically all loci examined (Avise 1974). In contrast, substantial electrophoretic differences are generally associated with conditions in which populations do not, or rarely, share the same alleles at several loci (Avise 1974). The examination of more than 30 loci, even with a sample size of one individual per population, practically ensures that the data will reliably reveal which of the above conditions exists.

Horizontal starch gel electrophoresis (Utter et al. 1974) was used to assay genetic variation at 46 loci encoding for proteins present in muscle, liver, or eye tissue from all specimens. Electrophoretic buffers and staining methods were those of Allendorf et al. (1977). We designate loci by a two- or three-letter italicized abbreviation of the enzyme they encode, the first letter being capitalized. When two or more loci encode the same enzyme, they are distinguished by Arabic numerals that denote increasing anodal mobility of the protein encoded by the most common allele at the loci. Thus, *Aat-1* and *Aat-2* designate two loci encoding for the enzyme aspartate aminotransferase. The protein encoded by the common allele at *Aat-2* migrates more anodally than the protein encoded by the common allele at *Aat-1*.

We label alleles at a locus according to the distance the protein they encode migrates from the origin. This distance is expressed as a percentage of the distance the protein encoded by a standard allele at the locus migrates. Standard alleles are designated *100* and, in this study, were the common alleles at the loci in the Arlee strain of rainbow trout. Thus, *Aat-1(200)* denotes an allele that encodes for a protein that migrates twice as far as the protein encoded by the standard allele *Aat-1(100)*. Alleles whose proteins migrate cathodally are designated by negative numerals. For example, *Aat-1(−75)* denotes an allele that encodes a protein that migrates 75% as far from the origin as the standard but in the cathodal direction. Protein mobilities were compared directly between all the taxa by running at least one individual from each taxon on the same gels.

We examined the following enzymes (loci and enzyme numbers of the IUBNC [1984] are in parentheses): adenylate kinase (*Adk-1,2*; 2.7.4.3), alcohol dehydrogenase (*Adh*; 1.1.1.1), aspartate aminotransferase (*Aat-1,2,3,4*; 2.6.1.1), creatine kinase (*Ck-1,2,3*; *Ckc-1,2*; 2.7.3.2), glucose-6-phosphate isomerase (*Gpi-1,2,3*; 5.3.1.9), glyceraldehyde-3-phosphate dehydrogenase (*Gap-3,4*; 1.2.1.12), glycerol-3-phosphate dehydrogenase (*G3p-1,2*; 1.1.1.8), glycyl-leucine dipeptidase (*Gl-1,2*; 3.4.13.-), isocitrate dehydrogenase (*Idh-1,2,3,4*; 1.1.1.42), lactate dehydrogenase (*Ldh-1,2,3,4,5*; 1.1.1.27), leucyl-glycyl-glycine tripeptidase (*Lgg*; 3.4.11.-), malate dehydrogenase (*Mdh-1,2,3,4*; 1.1.1.37), malic enzyme (*Me-1,2,3,4*; 1.1.1.40), phosphoglucomutase (*Pgm-1,2*; 5.4.2.5), 6-phosphogluconate dehydrogenase (*6Pg*; 1.1.1.44), sorbitol dehydrogenase (*Sdh-1,2*; 1.1.1.14), superoxide dismutase (*Sod-1*; 1.15.1.1), and xanthine dehydrogenase (*Xdh*; 1.1.1.204).

The two loci that constitute each of the following pairs of loci shared the same alleles in at least one of the samples: *Aat-3,4*, *Idh-3,4*, *Mdh-1,2*, *Mdh-3,4*, *Me-1,2*, *Me-3,4*, and *Sdh-1,2*. In these situations it is not possible to assign variation either within or between taxa to individual loci of each pair. Thus, we treated each of these pairs of duplicate loci as a single tetrasomic locus in all the samples in order to calculate allele frequencies and in the analysis of the data.

We examined the amount of electrophoretic differentiation among the taxa by two approaches: Nei's measure of standard genetic distance (Nei 1975) and principal components analysis (Pimentel 1979) of the arcsine transformation of the square root of the allele frequencies. The genetic distance estimates have heuristic value because they are available between populations of various taxonomic rank for a diversity of fishes. Comparison of our estimates with others allowed us to make qualitative judgments about the amount of differentiation observed among the subspecies of cutthroat trout. The principal components analysis provided a convenient means of pictorially representing the relative amount of allele-frequency variation among the taxa.

Results and Discussion

Electrophoretic Detection of Interbreeding between Taxa

Populations from taxa that do not, or only rarely, share allozymes at several loci, commonly referred to as diagnostic loci, can be identified by examining the genotypes of individuals at these loci (Ayala and Powell 1972). These loci can also be used to detect interbreeding between taxa. Samples obtained from "genetically pure" populations will contain alleles characteristic of only that taxon. In contrast, samples obtained from populations in which interbreeding has occurred or is occurring will contain at least some individuals that possess alleles characteristic of both taxa at diagnostic loci. When all such individuals identified are heterozygous for alleles characteristic of both taxa at all the diagnostic loci, this indicates the existence of first-generation hybrids. Matings between the parental types and hybrids, and subsequently between their progeny, will produce individuals homozygous at some diagnostic loci and heterozygous at others. In such introgressed populations, the multiple-locus genotype will be highly variable among individuals.

We did not detect any diagnostic loci among the Colorado River, finespotted, greenback, and Yellowstone cutthroat trout (Tables 1, 2), which precludes the use of electrophoresis as a reliable means of detecting interbreeding between these taxa. In contrast, we found two or more diagnostic loci between the coastal cutthroat, Lahontan cutthroat, westslope cutthroat, and rainbow trout populations, as well as between all these fishes and the other four subspecies of cutthroat trout analyzed (Tables 1, 2). Thus, electrophoresis can be used to detect interbreeding between all of the subspecies of cutthroat trout we analyzed and the rainbow trout, as well as between many pairs of cutthroat trout subspecies.

Detection of interbreeding between taxa has usually been based upon morphological comparisons. In such comparisons, it is presumed that first-generation hybrids will be intermediate morphologically between the parental taxa and that individuals from hybrid swarms will possess a diversity of morphologies ranging between the parental extremes. These assumptions, however, may not be valid. First-generation hybrids of salmonid fishes often are not morphologically intermediate between parental taxa (Leary et al. 1983, 1985;

TABLE 1.—Electrophoretic mobilities of proteins coded by alleles at loci that can be used to differentiate seven subspecies of cutthroat trout as well as rainbow trout. Protein mobilities are relative to that of the protein coded by the common allele of the homologous locus in rainbow trout. When a locus is variable within a sample, the protein of the most common allele is listed first.

		Cutthroat trout subspecies						
Locus	Rainbow trout	Westslope	Coastal	Lahontan	Yellow-stone	Finespotted	Greenback	Colorado
Aat-1	100	200	100	165,null	165	165	165	165
Ck-2	100	84	84	84	84	84	84	84
Ckc-1	100,38	100	100	100	38	38	38	38
Gl-1	100	100	115	115	101	101	101	101
Gpi-3	100	92,100	100	100	100	100	100	100
Idh-1	100	100	100	100	−75	−75	−75	−75
Idh-3,4	100,114,71,40	86,100,40	100,71	100,114	71,100	71,100	71,100	71,100
Lgg	100	100	100	111	135	135	135	135
Me-1,2	100,55	88,100	100	100	100	100	100	100
Me-3,4	100	100	110	100,110	110,90	110,90	110,100	110,100,90
Pgm-1	100	100,110	100,90	100,null	Null	Null	Null	Null
Sdh-1,2	100,200,40	40	100,200	100,200	100	100	100,0	100,0

TABLE 2.—Allele frequencies at lo
trout and seven subspecies of cutth

Locus	Alleles	Rainbow trout
Aat-1	*100*	1.000
	200	
	165	
	Null	
Aat-3,4	*100*	1.000
	110	
	90	
	77	
Adk-1	*100*	1.000
	367	
Ck-1	*100*	0.912
	76	0.088
Ckc-1	*100*	0.932
	38	0.068
Gap-4	*100*	1.000
	Null	
Gpi-1	*100*	1.000
	156	
Gpi-2	*100*	1.000
	131	
Gpi-3	*100*	1.000
	92	
G3p-1	*100*	0.991
	140	0.009
Idh-2	*100*	0.763
	140	0.237
Idh-3,4	*100*	0.705
	114	0.070
	86	
	71	0.034
	40	0.191
Ldh-4	*100*	0.981
	112	
	76	0.019
Mdh-1,2	*100*	1.000
	153	
Mdh-3,4	*100*	0.875
	95	
	83	0.125
Me-1,2	*100*	0.991
	88	
	55	0.009
Me-3,4	*100*	1.000
	110	
	90	
Pgm-1	*100*	1.000
	110	
	90	
	Null	
Pgm-2	*100*	0.959
	90	0.041
	85	
Sdh-1,2	*100*	0.965
	200	0.010
	40	0.025
	0	
Sod-1	*100*	0.772
	152	0.228

a all such individuals iden-
for alleles characteristic of
gnostic loci, this indicates
neration hybrids. Matings
pes and hybrids, and sub-
r progeny, will produce in-
at some diagnostic loci and
In such introgressed pop-
ocus genotype will be high-
viduals.
ny diagnostic loci among
nespotted, greenback, and
trout (Tables 1, 2), which
ectrophoresis as a reliable
terbreeding between these
and two or more diagnostic
l cutthroat, Lahontan cut-
hroat, and rainbow trout
between all these fishes and
ies of cutthroat trout ana-
us, electrophoresis can be
eding between all of the
trout we analyzed and the
as between many pairs of
ies.
eeding between taxa has
on morphological compar-
isons, it is presumed that
will be intermediate mor-
he parental taxa and that
d swarms will possess a di-
s ranging between the pa-
ssumptions, however, may
ration hybrids of salmonid
rphologically intermediate
(Leary et al. 1983, 1985;

be used to differentiate seven
e to that of the protein coded
variable within a sample, the

d	Greenback	Colorado
	165	165
	84	84
	38	38
	101	101
	100	100
	−75	−75
	71,100	71,100
	135	135
	100	100
	110,100	110,100,90
	Null	Null
	100,0	100,0

TABLE 2.—Allele frequencies at loci that show evidence of intraspecific genetic variation in samples from rainbow trout and seven subspecies of cutthroat trout.

			Cutthroat trout subspecies						
Locus	Alleles	Rainbow trout	Westslope	Coastal	Lahontan	Yellow-stone	Fine-spotted	Green-back	Colorado
Aat-1	*100*	1.000		1.000					
	200		1.000						
	165				0.553	1.000	1.000	1.000	1.000
	Null				0.447				
Aat-3,4	*100*	1.000	0.863	0.917	1.000	0.575	0.990	0.833	0.750
	110					0.200	0.010	0.167	
	90					0.225			0.250
	77		0.137	0.083					
Adk-1	*100*	1.000	1.000	1.000	1.000	1.000	0.610	1.000	1.000
	367						0.390		
Ck-1	*100*	0.912	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	76	0.088							
Ckc-1	*100*	0.932	1.000	1.000	1.000				
	38	0.068				1.000	1.000	1.000	1.000
Gap-4	*100*	1.000	0.961	1.000	1.000	1.000	1.000	1.000	1.000
	Null		0.039						
Gpi-1	*100*	1.000	0.931	1.000	1.000	1.000	1.000	1.000	1.000
	156		0.069						
Gpi-2	*100*	1.000	1.000	1.000	0.900	1.000	1.000	1.000	1.000
	131				0.100				
Gpi-3	*100*	1.000	0.010	1.000	1.000	1.000	1.000	1.000	1.000
	92		0.990						
G3p-1	*100*	0.991	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	140	0.009							
Idh-2	*100*	0.763	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	140	0.237							
Idh-3,4	*100*	0.705	0.417	0.625	0.900	0.500	0.500	0.500	0.625
	114	0.070			0.100				
	86		0.500						
	71	0.034		0.375		0.500	0.500	0.500	0.375
	40	0.191	0.083						
Ldh-4	*100*	0.981	1.000	0.833	1.000	1.000	1.000	1.000	1.000
	112			0.167					
	76	0.019							
Mdh-1,2	*100*	1.000	1.000	1.000	0.700	1.000	1.000	1.000	1.000
	153				0.300				
Mdh-3,4	*100*	0.875	1.000	0.833	1.000	1.000	1.000	1.000	1.000
	95			0.167					
	83	0.125							
Me-1,2	*100*	0.991	0.500	1.000	1.000	1.000	1.000	1.000	1.000
	88		0.500						
	55	0.009							
Me-3,4	*100*	1.000	1.000		0.500			0.500	0.375
	110			1.000	0.500	0.500	0.500	0.500	0.500
	90					0.500	0.500		0.125
Pgm-1	*100*	1.000	0.882	0.667	0.553				
	110		0.118						
	90			0.333					
	Null				0.447	1.000	1.000	1.000	1.000
Pgm-2	*100*	0.959	0.922	1.000	1.000	1.000	1.000	1.000	1.000
	90	0.041							
	85		0.078						
Sdh-1,2	*100*	0.965		0.500	0.500	1.000	1.000	0.500	0.700
	200	0.010		0.500	0.500				
	40	0.025	1.000						
	0							0.500	0.300
Sod-1	*100*	0.772	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	152	0.228							

Rinne et al. 1985). Furthermore, when only a small proportion of genes from another taxon occur in a population, individuals may be morphologically indistinguishable from the genetically predominant taxon (Busack and Gall 1981; Whitmore 1983; Leary et al. 1984b). This is especially true in introgressed populations where the genes from the parental taxa are distributed among many individuals. The existence of these problems reduces the ability of morphological characters to indicate reliably the genetic status of a population.

Many taxa of fishes have unique types of mitochondrial DNA, mtDNA (Avise et al. 1984; Berg and Ferris 1984; Bermingham and Avise 1986; Gyllensten and Wilson 1987). Certain properties of mtDNA, however, diminish the value of these differences as the sole criterion of the genetic status of a population. Because mtDNA is generally inherited maternally, an individual usually only contains one type of mtDNA. Thus, hybrids or individuals from hybrid swarms will not contain mtDNA genotypes different from the parental taxa (Avise et al. 1984; Gyllensten et al. 1985). Furthermore, if only males from a taxon participate in hybrid matings, the mtDNA of this taxon will be absent from hybrid swarms. The mtDNA of a taxon is also more likely to be lost from a hybrid swarm by genetic drift than is nuclear DNA because the effective population size for mtDNA is smaller than for nuclear DNA. Thus, when the proportional genetic contribution of a taxon to a hybrid swarm is small, genetic drift may restore the mitochondrial genome to genetic purity without appreciably altering the constitution of the nuclear genome. We conclude, therefore, that electrophoretic analysis of the proteins encoded by the loci in Table 1 currently provides the most reliable means available to identify genetically pure populations of many of the cutthroat trout subspecies we examined.

Genetic Divergence

Very little electrophoretic differentiation existed among the Colorado River, finespotted, greenback, and Yellowstone cutthroat trout. The genetic distance estimates between these subspecies (Table 3) are typical of those reported for conspecific populations in a diversity of freshwater and anadromous fishes (Avise 1974; Avise and Smith 1977; Buth and Burr 1978; Loudenslager and Gall 1980; Buth et al. 1984).

In contrast, substantial biochemical genetic differentiation existed between the coastal, Lahontan, and westslope cutthroat trout and between these fishes and the other four subspecies (Table 3). These genetic distances are truly exceptional for conspecific populations, being comparable to or larger than values observed between many species of fish (Johnson 1975; Avise and Ayala 1976; Buth and Burr 1978; Phelps and Allendorf 1983; Yates et al. 1984). Finally, the coastal, Lahontan, and westslope cutthroat trout were at least as similar to rainbow trout as they were to any of the other subspecies.

We used principal components analysis as another means of examining the relative amount of genetic differentiation among the taxa. In the analysis, we excluded those loci not represented in Table 1 because all the taxa share the same common allele at these loci. Thus, these loci would contribute very little to the overall variance in allele frequencies among the taxa relative to the large variance among them at the diagnostic loci. The allele common to the Yellowstone cutthroat trout at each diagnostic locus was also excluded from the analysis (*Idh-3,4*[*71*] and *Me-3,4*[*90*] were excluded in the two ambiguous cases; Table 2). Use of all alleles at a locus would generate linear dependencies in the data matrix because the frequency of a specific allele can be derived as the difference between 1.000 and the sum of the frequencies of the other alleles detected at the locus.

The plot of the first and second principal component scores provided a pictorial summary of the relative amount of variation in allele frequencies at the diagnostic loci among the taxa (Figure 1). The first axis, which accounted for 50% of the total variation in allele frequencies at the diagnostic loci, separated the taxa into three groups: westslope cutthroat and rainbow trout, coastal and Lahontan cutthroat trout, and the four other cutthroat subspecies. The second axis, which accounted for 25% of the variation, separated westslope cutthroat from rainbow trout and coastal and Lahontan cutthroat trout from all other taxa. The coastal, Lahontan, and westslope cutthroat trout were genetically at least as similar to rainbow trout as to the other subspecies.

Evolutionary Relationships

The subspecies of cutthroat trout we examined are morphologically more similar to each other than they are to the rainbow trout (Gold 1977; Leary et al. 1984a). Furthermore, limited karyotypic and mtDNA data also suggest that the cutthroat trout subspecies are more similar to each other than they are to rainbow trout (Gold et al. 1977; Loudenslager and Thorgaard 1979; Thor-

---

TABLE 3.—Nei's genetic distance

Cutthroat trout subspecies	Rainbow trout	W
Westslope	0.130	
Coastal	0.099	
Lahontan	0.138	
Yellowstone	0.246	
Finespotted	0.247	
Greenback	0.229	
Colorado	0.223	

gaard 1983; Gyllensten and
conventional view of the evo
ship of these taxa, therefore, is
are members of a single phylo
from rainbow trout, the subspe
of subsequent divergence withi
eage. Our finding that three
throat trout are electrophoret
more similar to rainbow trout
four other subspecies indicate
divergences of the protein lo
attributes have proceeded at di
these fishes. There are several
be invoked to account for this

It is possible that the conven
view is correct. This view is ten
that there has been accelerate
in the branch of the cutthroa
the Colorado River, finespott
Yellowstone subspecies. This
tion could be the result of one
at the time of or shortly after t
branch, resulting in the loss
by genetic drift. Bottleneck
pected to accelerate the rate o
tion (Lande 1984) and, there
count for the substantial kar
between the Yellowstone cutth
species that have been exami
straints upon the development
fishes (i.e., stabilizing selectio
imized the effect of genetic d
control their morphology. Th
those amenable to electropho
neck could have influenced pro
evolution but not morphologi

This view, however, is not
Variation in the mitochondri
lations is expected to be highl
tlenecks (Gyllensten and Wils
mitochondrial and protein dat
ilar patterns of differentiatio

ther four subspecies (Table
ances are truly exceptional
tions, being comparable to
; observed between many
on 1975; Avise and Ayala
1978; Phelps and Allendorf
4). Finally, the coastal, La-
cutthroat trout were at least
trout as they were to any of

components analysis as
nining the relative amount
on among the taxa. In the
those loci not represented
ll the taxa share the same
loci. Thus, these loci would
to the overall variance in
ong the taxa relative to the
them at the diagnostic loci.
the Yellowstone cutthroat
ic locus was also excluded
3,4[71] and *Me-3,4[90]* were
ambiguous cases; Table 2).
locus would generate linear
ata matrix because the fre-
llele can be derived as the
00 and the sum of the fre-
alleles detected at the locus.
and second principal com-
d a pictorial summary of the
riation in allele frequencies
among the taxa (Figure 1).
counted for 50% of the total
iencies at the diagnostic loci,
to three groups: westslope
v trout, coastal and Lahon-
nd the four other cutthroat
d axis, which accounted for
, separated westslope cut-
trout and coastal and La-
it from all other taxa. The
d westslope cutthroat trout
it as similar to rainbow trout
cies.

ships

utthroat trout we examined
more similar to each other
rainbow trout (Gold 1977;
Furthermore, limited karyo-
ta also suggest that the cut-
es are more similar to each
rainbow trout (Gold et al.
and Thorgaard 1979; Thor-

TABLE 3.—Nei's genetic distances between rainbow trout and seven subspecies of cutthroat trout.

Cutthroat trout subspecies	Rainbow trout	Cutthroat trout subspecies					
		Westslope	Coastal	Lahontan	Yellowstone	Finespotted	Greenback
Westslope	0.130						
Coastal	0.099	0.164					
Lahontan	0.138	0.175	0.077				
Yellowstone	0.246	0.295	0.191	0.164			
Finespotted	0.247	0.297	0.192	0.165	0.006		
Greenback	0.229	0.268	0.194	0.152	0.022	0.025	
Colorado	0.223	0.280	0.193	0.150	0.012	0.023	0.005

gaard 1983; Gyllensten and Wilson 1987). The conventional view of the evolutionary relationship of these taxa, therefore, is that cutthroat trout are members of a single phylogenetic divergence from rainbow trout, the subspecies being the result of subsequent divergence within the cutthroat lineage. Our finding that three subspecies of cutthroat trout are electrophoretically as similar or more similar to rainbow trout than they are to the four other subspecies indicates that evolutionary divergences of the protein loci and these other attributes have proceeded at different rates among these fishes. There are several hypotheses that can be invoked to account for this difference.

It is possible that the conventional phylogenetic view is correct. This view is tenable if one assumes that there has been accelerated protein evolution in the branch of the cutthroat lineage leading to the Colorado River, finespotted, greenback, and Yellowstone subspecies. This accelerated evolution could be the result of one or more bottlenecks at the time of or shortly after the formation of this branch, resulting in the loss of genetic variation by genetic drift. Bottleneck events are also expected to accelerate the rate of karyotypic evolution (Lande 1984) and, therefore, could also account for the substantial karyotypic differences between the Yellowstone cutthroat and other subspecies that have been examined. Selective constraints upon the developmental program of these fishes (i.e., stabilizing selection) could have minimized the effect of genetic drift on the loci that control their morphology. These loci need not be those amenable to electrophoresis, so the bottleneck could have influenced protein and karyotypic evolution but not morphological evolution.

This view, however, is not without a problem. Variation in the mitochondrial genome of populations is expected to be highly influenced by bottlenecks (Gyllensten and Wilson 1987). Thus, the mitochondrial and protein data should show similar patterns of differentiation, especially if it is considered that the mitochondrial genome evolves at a faster rate than the nuclear genome. This expectation is not supported by the available data.

Consideration of the geographical distribution of these fishes and the ability of many taxa in the genus *Salmo* native to western North America to produce fertile hybrid progeny (Busack and Gall 1981; Halliburton et al. 1983; Leary et al. 1984b; Campton and Utter 1985; Gyllensten et al. 1985) suggests another plausible explanation for supposed accelerated protein evolution. The westslope and coastal cutthroat trout are naturally sympatric with the rainbow trout (Allendorf et al. 1980; Campton and Utter 1985) and rainbow trout naturally exist syntopically with the Lahontan cutthroat trout in the Great Basin (McAfee 1966). Secondary contact between the rainbow trout and these subspecies could have resulted in temporary periods of limited introgression in the past. Camp-

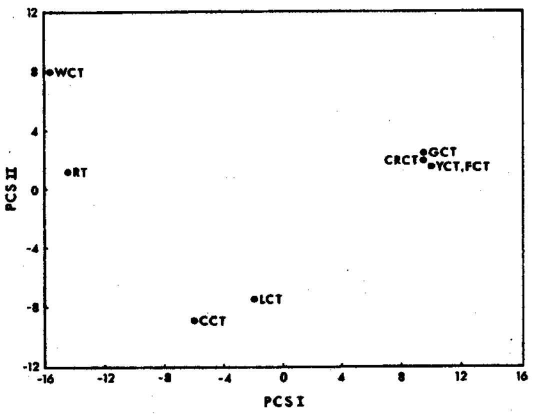

FIGURE 1.—Plot of first two principal component scores (PCS) derived from the matrix of alleles at the loci in Table 1 for seven subspecies of cutthroat and for rainbow trout. CCT = coastal cutthroat trout; CRCT = Colorado River cutthroat trout; FCT = finespotted cutthroat trout; GCT = greenback cutthroat trout; LCT = Lahontan cutthroat trout; WCT = westslope cutthroat trout; YCT = Yellowstone cutthroat trout; RT = rainbow trout.

ton and Utter (1985) suggested that there may have been continual, if infrequent, gene flow between coastal cutthroat trout and rainbow trout. As an average migration rate of only one individual per generation is sufficient to prevent populations from becoming completely divergent for selectively neutral alleles (Wright 1978), even a small amount of introgression could retard significantly the rate of protein divergence between these cutthroat and rainbow trout. This small amount of gene flow between the rainbow trout and these subspecies of cutthroat trout would not necessarily alter morphological and karyological evolution (Leary et al. 1984b) nor, for reasons previously discussed, evolution of mtDNA.

Another possibility is that the conventional phylogenetic view is incorrect. That is, the cutthroat and rainbow trout instead represent multiple phylogenetic divergences from a common ancestral lineage. Such a phenomenon can readily account for the exceptional amount of morphological, karyotypic, and biochemical variation among the taxa.

We feel it will be very difficult to demonstrate conclusively which, if any, of these evolutionary scenarios is correct. The present uncertainty about the phylogenetic relationship of the taxa we have examined, however, does not affect the application of our findings to the management of cutthroat trout. From a preservation perspective, managers should be concerned with maintaining existing levels of genetic diversity within and between populations regardless of their evolutionary origin.

The features revealed by our electrophoretic data that are important to managers are these: (1) electrophoresis can be used with a high degree of certainty to identify genetically pure populations of many cutthroat trout subspecies; and (2) the high amount of biochemical divergence among the subspecies suggests that the coastal, Lahontan, and westslope cutthroat trout should be accorded the same attention given to taxonomically recognized species.

Acknowledgments

We extend appreciation to Pat Dwyer and Larry Visscher of the U.S. Fish and Wildlife Service; Jack Boyce, Jim Crepeau, Thurston Dotson, and Don Peters of the Montana Department of Fish, Wildlife and Parks; Dave Galat and Steve Viggs; and the Pyramid Lake Paiute Tribe for making samples available to us. We are indebted to Don Buth, Dave Swofford, Don Campton, and an anonymous reviewer whose comments greatly improved the manuscript. H. McPherson stimulated our thinking.

References

Allendorf, F. W., D. M. Espeland, D. T. Scow, and S. Phelps. 1980. Coexistence of native and introduced rainbow trout in the Kootenai River drainage. Proceedings of the Montana Academy of Sciences 39:28–36.

Allendorf, F. W., N. Mitchell, N. Ryman, and G. Ståhl. 1977. Isozyme loci in brown trout (*Salmo trutta* L.): detection and interpretation from population data. Hereditas 86:179–190.

[redacted] Isozymes and the preservation of genetic variation in salmonid fishes. Ecological Bulletin (Stockholm) 34: 37–52.

Avise, J. C. 1974. Systematic value of electrophoretic data. Systematic Zoology 23:465–481.

Avise, J. C., and F. J. Ayala. 1976. Genetic differentiation in speciose and depauperate phylads: evidence from the California minnows. Evolution 30: 46–58.

Avise, J. C., E. Bermingham, L. G. Kessler, and N. C. Saunders. 1984. Characterization of mitochondrial DNA variability in a hybrid swarm between subspecies of bluegill sunfish (*Lepomis macrochirus*). Evolution 38:931–941.

Avise, J. C., and M. H. Smith. 1977. Gene frequency comparison between sunfish (Centrarchidae) populations at various stages of evolutionary divergence. Systematic Zoology 36:319–335.

Ayala, F. J., and J. R. Powell. 1972. Allozymes as diagnostic characters of sibling species of *Drosophila*. Proceedings of the National Academy of Sciences of the USA 69:1094–1096.

Behnke, R. J. 1972. The systematics of salmonid fishes of recently glaciated lakes. Journal of the Fisheries Research Board of Canada 29:539–571.

Behnke, R. J., and M. Zarn. 1976. Biology and management of threatened and endangered trouts. U.S. Forest Service General Technical Report RM 28.

Berg, W. J., and S. D. Ferris. 1984. Restriction endonuclease analysis of salmonid mitochondrial DNA. Canadian Journal of Fisheries and Aquatic Sciences 41:1041–1047.

Bermingham, E., and J. C. Avise. 1986. Molecular zoogeography of freshwater fishes in the southeastern United States. Genetics 113:939–965.

Busack, C. A., and G. A. E. Gall. 1981. Introgressive hybridization in populations of Paiute cutthroat trout (*Salmo clarki seleniris*). Canadian Journal of Fisheries and Aquatic Sciences 38:939–951.

Buth, D. G., and B. M. Burr. 1978. Isozyme variability in the cyprinid genus *Campostoma*. Copeia 1978: 298–311.

Buth, D. G., C. B. Crabtree, R. D. Orton, and W. J. Rainboth. 1984. Genetic differentiation between the freshwater subspecies of *Gasterosteus aculeatus*

in southern California. Bio
Ecology 12:423–432.

Campton, D. E., and F. M. U
bridization between steel
neri) and coastal cutthroa
two Puget Sound stream
Fisheries and Aquatic Sci

Gold, J. R. 1977. Systematic
ican trout (*Salmo*), with n
of Sheepheaven Creek, C
nal of Zoology 55:1858–1

Gold, J. R., J. C. Avise, and G.
mosome cytology in the cu
ki (Salmonidae). Cytologi

Gorman, G. C., and J. R. R
distance and heterozygos
phoretic studies: effects of
242–249.

Gyllensten, U., R. F. Leary, F.
Wilson. 1985. Introgre
throat trout subspecies wi
nuclear, and mitochond
Genetics 111:905–915.

Gyllensten, U., and A. C. Wils
DNA of salmonids: inte
ability detected with restri
317 *in* N. Ryman and F.
genetics and fishery ma
Washington Press, Seattle

Halliburton, R., R. E. Pipkin,
Reproductive success of a
en trout (*Salmo aguabo*
(*Salmo gairdneri*). Canac
and Aquatic Sciences 40:

IUBNC (International Union
clature Committee). 198
1984. Academic Press, O

Johnson, M. S. 1975. Bioch
Atherinid genus *Menidia*.

Lande, R. 1984. The expec
mosomal inversions. Evo

Leary, R. F., F. W. Allendorf, a
Consistently high meristic
between brook trout and
ology 32:369–376.

Leary, R. F., F. W. Allendorf, a
Major morphological eff
Pgm1-t in rainbow trout
Evolution 1:183–194.

Leary, R. F., F. W. Allendorf, a
Developmental instability
in interspecific hybrids o
tion 39:1318–1326.

Leary, R. F., F. W. Allendorf
Knudsen. 1984b. Introg

hose comments greatly im-
H. McPherson stimulated

'erences

Espeland, D. T. Scow, and S.
xistence of native and intro-
in the Kootenai River drain-
he Montana Academy of Sci-

hell, N. Ryman, and G. Ståhl.
in brown trout (*Salmo trutta*
nterpretation from population
79–190.
R. Phelps. 1981. Isozymes
n of genetic variation in sal-
gical Bulletin (Stockholm) 34:

ematic value of electrophoretic
logy 23:465–481.
yala. 1976. Genetic differen-
nd depauperate phylads: evi-
ornia minnows. Evolution 30:

nam, L. G. Kessler, and N. C.
Characterization of mitochon-
ty in a hybrid swarm between
ll sunfish (*Lepomis macrochi-*
31–941.
Smith. 1977. Gene frequency
sunfish (Centrarchidae) popu-
ges of evolutionary divergence.
36:319–335.
Powell. 1972. Allozymes as
s of sibling species of *Drosoph-*
the National Academy of Sci-
:1094–1096.
e systematics of salmonid fishes
lakes. Journal of the Fisheries
Canada 29:539–571.
arn. 1976. Biology and man-
ed and endangered trouts. U.S.
ral Technical Report RM 28.
Ferris. 1984. Restriction en-
s of salmonid mitochondrial
urnal of Fisheries and Aquatic
047.
. C. Avise. 1986. Molecular
shwater fishes in the southeast-
Genetics 113:939–965.
. E. Gall. 1981. Introgressive
ulations of Paiute cutthroat trout
ris). Canadian Journal of Fish-
ciences 38:939–951.
urr. 1978. Isozyme variability
us *Campostoma*. Copeia 1978:

btree, R. D. Orton, and W. J.
Genetic differentiation between
pecies of *Gasterosteus aculeatus*

in southern California. Biochemical Systematics and Ecology 12:423–432.

Campton, D. E., and F. M. Utter. 1985. Natural hybridization between steelhead trout (*Salmo gairdneri*) and coastal cutthroat trout (*Salmo clarki*) in two Puget Sound streams. Canadian Journal of Fisheries and Aquatic Sciences 42:110–119.

[redacted] Systematics of western North American trout (*Salmo*), with notes on the redband trout of Sheepheaven Creek, California. Canadian Journal of Zoology 55:1858–1873.

[redacted]. Chromosome cytology in the cutthroat series *Salmo clarki* (Salmonidae). Cytologia 42:377–382.

Gorman, G. C., and J. R. Renzi, Jr. 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. Copeia 1979: 242–249.

[redacted] Introgression between two cutthroat trout subspecies with substantial karyotypic, nuclear, and mitochondrial genomic divergence. Genetics 111:905–915.

Gyllensten, U., and A. C. Wilson. 1987. Mitochondrial DNA of salmonids: inter- and intraspecific variability detected with restriction enzymes. Pages 301–317 *in* N. Ryman and F. Utter, editors. Population genetics and fishery management. University of Washington Press, Seattle.

Halliburton, R., R. E. Pipkin, and G. A. E. Gall. 1983. Reproductive success of artificially hybridized golden trout (*Salmo aguabonita*) and rainbow trout (*Salmo gairdneri*). Canadian Journal of Fisheries and Aquatic Sciences 40:1264–1269.

IUBNC (International Union of Biochemistry, Nomenclature Committee). 1984. Enzyme nomenclature 1984. Academic Press, Orlando, Florida.

Johnson, M. S. 1975. Biochemical systematics of the Atherinid genus *Menidia*. Copeia 1975:662–691.

Lande, R. 1984. The expected fixation rate of chromosomal inversions. Evolution 38:743–752.

Leary, R. F., F. W. Allendorf, and K. L. Knudsen. 1983. Consistently high meristic counts in natural hybrids between brook trout and bull trout. Systematic Zoology 32:369–376.

Leary, R. F., F. W. Allendorf, and K. L. Knudsen. 1984a. Major morphological effects of a regulatory gene: *Pgm1-t* in rainbow trout. Molecular Biology and Evolution 1:183–194.

Leary, R. F., F. W. Allendorf, and K. L. Knudsen. 1985. Developmental instability and high meristic counts in interspecific hybrids of salmonid fishes. Evolution 39:1318–1326.

Leary, R. F., F. W. Allendorf, S. R. Phelps, and K. L. Knudsen. 1984b. Introgression between westslope cutthroat and rainbow trout in the Clark Fork River drainage, Montana. Proceedings of the Montana Academy of Sciences 43:1–18.

Loudenslager, E. J., and G. A. E. Gall. 1980. Geographic patterns of protein variation and subspeciation in the cutthroat trout. Systematic Zoology 29:27–42.

Loudenslager, E. J., and G. H. Thorgaard. 1979. Karyotypic and evolutionary relationships of the Yellowstone (*Salmo clarki bouvieri*) and west-slope (*S. c. lewisi*) cutthroat trout. Journal of the Fisheries Research Board of Canada 36:630–635.

McAfee, W. R. 1966. Eagle Lake rainbow trout. Pages 221–225 *in* A. Calhoun, editor. Inland fisheries management. California Department of Fish and Game, Sacramento.

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

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Phelps, S. R., and F. W. Allendorf. 1983. Genetic identity of pallid and shovelnose sturgeon (*Scaphirhynchus albus* and *S. platorynchus*). Copeia 1983:696–700.

Pimentel, R. A. 1979. Morphometrics: the multivariate analysis of biological data. Kendall/Hunt, Dubuque, Iowa.

Rinne, J. N., R. Sorensen, and S. C. Belfitt. 1985. An analysis of F_1 hybrids between Apache, (*Salmo apache*) and rainbow trout, (*Salmo gairdneri*). Journal of the Arizona–Nevada Academy of Science 20: 63–69.

Thorgaard, G. H. 1983. Chromosomal differences among rainbow trout populations. Copeia 1983:650–662.

Utter, F. M., H. O. Hodgins, and F. W. Allendorf. 1974. Biochemical genetic studies of fishes: potentialities and limitations. Pages 213–238 *in* D. C. Malins and J. R. Sargent, editors. Biochemical and biophysical perspectives in marine biology, volume 1. Academic Press, New York.

Whitmore, D. H. 1983. Introgressive hybridization of smallmouth bass (*Micropteris dolomieui*) and Guadalupe bass (*M. treculi*). Copeia 1983:672–679.

Wright, S. 1978. Evolution and the genetics of populations, volume 4. Variability within and among populations. University of Chicago Press, Chicago.

Yates, T. L., M. A. Lewis, and M. D. Hatch. 1984. Biochemical systematics of three species of catfish (Genus *Ictalurus*) in New Mexico. Copeia 1984:97–101.

Received December 24, 1985
Accepted August 25, 1987