CHANGES IN THE GENETIC COMPOSITION OF THE LAKE KOOCANUSA TROUT FISHERY

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ABSTRACT

The genetic composition of the Lake Koocanusa trout fishery has changed dramatically between 1979 and 1983. In 1979, the fishery was composed mainly, if not entirely, of coastal rainbow trout. Electrophoretic analysis of fish collected from the reservoir in 1983 and 1986 and four of its tributaries in 1987 indicates that the reservoir fishery now represents an introgressed fishery containing coastal rainbow, inland rainbow and westslope cutthroat trout genes. We postulate that this change is largely due to the invasion of Lake Koocanusa by inland rainbow trout during the mid 1970s. Subsequently, these fish have hybridized extensively with the trout in the reservoir and its tributaries.

INTRODUCTION

Lake Koocanusa was created in 1972 when the Kootenai River was impounded by Libby Dam. The Montana Department of Fish, Wildlife and Parks originally planned to manage the reservoir as a westslope cutthroat trout, *Salmo clarki lewisi*, fishery. This policy was initially successful, but by 1979 coastal rainbow trout, *S. gairdneri*, had become the predominant trout in the reservoir (Phelps and Allendorf 1980). The origin of these fish is unknown.

Since 1979, the composition of the salmonid fishery in Lake Koocanusa has again changed dramatically. Kokanee salmon, *Oncorhynchus nerka*, successfully invaded the reservoir in 1980 and now have become the predominant salmonid in terms of biomass and numbers (Joe Huston, personal communication). Furthermore, as we will demonstrate in this report, the genetic characteristics of the trout in the reservoir have been altered markedly.

METHODS

Fish for electrophoretic analysis were collected by Montana Department of Fish, Wildlife and Parks personnel. Two gillnet samples were obtained from Lake Koocanusa. The first sample (N=23) was collected near the mouth of the Kootenay River in the spring of 1983 (Fig. 1). The other sample (N=60) was collected from the Rexford area in the spring of 1986 (Fig. 2). These samples will be referred to as the upper and middle Lake Koocanusa samples, respectively. Electrofishing was used to obtain samples from the following four tributaries to Lake Koocanusa (Figs. 1 and 2): Big Creek (June 19, 1987; N=20), Pinkham Creek (June 18, 1987; N=20), Sinclair Creek

(June 18, 1987; N=20) and Warland Creek (June 17, 1987; N=21). The fish were collected at random. They should, therefore, provide a reliable indicator of the genetic characteristics of the fish in these areas at the time of sampling.

We determined the genotype of each fish at 45 loci coding for enzymes in muscle, liver, or eye tissue using horizontal starch gel electrophoresis. The following enzymes encoded by the loci in parentheses were analyzed: adenylate kinase (Adk1,2), alcohol dehydrogenase (Adh), aspartate aminotransferase (Aat1,2,3,4), creatine kinase (Ck1,2,3; Ckc1,2), glucose-6-phosphate isomerase (Gpi1,2,3), glyceraldehyde-3-phosphate dehydrogenase (Gap3,4), glycerol-3-phosphate dehydrogenase (G3p1,2), glycyl-leucine dipeptidase (Gl1,2), isocitrate dehydrogenase (Idh1,2,3,4), lactate dehydrogenase (Ldh1,2,3,4,5), leucyl-glycyl-glycine tripeptidase (Lgg), malate dehydrogenase (Mdh1,2,3,4), malic enzyme (Me1,2,3,4), phosphoglucomutase (Pgm1,2), 6-phosphogluconate dehydrogenase (6Pg), sorbitol dehydrogenase (Sdh), superoxide dismutase (Sod), and xanthine dehydrogenase (Xdh).

Genetic differences between cutthroat and rainbow trout

Populations from taxa that do not, or rarely, share electrophoretically detectable alleles at several loci can be identified by examining the genotypes of individuals at these loci. Because of this attribute, such loci are commonly termed diagnostic loci (Ayala and Powell 1972). Diagnostic loci can also be used to detect interbreeding between taxa. Individuals in samples obtained from 'genetically pure' populations will possess alleles and genotypes at all diagnostic loci characteristic of only this taxon. In contrast, samples collected from populations in which interbreeding has occurred or is occurring will contain at least some individuals that possess alleles characteristic of both of the parental taxa at some diagnostic loci. When all such individuals identified are heterozygous for alleles characteristic of both parental taxa at all diagnostic loci, this indicates the existence of first-generation hybrids (e.g. Leary et al. 1983). Matings between the parental types and hybrids, and subsequently between their progeny, will produce individuals homozygous at some diagnostic loci and heterozygous at others. The multiple locus genotype will be highly variable among the individuals collected from such introgressed populations. When the alleles characteristic of the parental taxa are randomly distributed among the diagnostic loci and individuals, this indicates that the population has been introgressed for an appreciable length of time (5 or more generations). In such situations, it is unlikely that the population contains genetically pure representatives of the parental taxa.

A variable number of diagnostic loci between the rainbow trout and all subspecies of cutthroat trout examined and between many pairs of cutthroat trout subspecies are known (Leary et al. in press, Table 1). The alleles and genotypes at these loci possessed by the individuals in the samples were used to determine the genetic status of the populations from which the fish were obtained. That is, whether the population is a genetically pure representative of one of the taxa in Table 1 or is an introgressed population containing genes from two or more of the taxa.

Genetic differences between coastal and interior rainbow trout

Populations of rainbow trout that are native to waters west of the Cascade crest are electrophoretically distinguishable from those spawning in waters east of the divide (Allendorf and Utter 1979; Allendorf et al. 1980). The former, or coastal, populations usually have a low frequency (less than 0.10) of the Ldh4(76) allele and a high frequency (greater than 0.10) of the Sod(152) allele. The reverse situation characterizes populations of the latter or inland rainbow trout. The allele frequencies at these two loci were used to determine what types of rainbow trout have contributed genes to the sampled populations.

RESULTS

Introgressed populations

We detected alleles characteristic of both the rainbow and westslope cutthroat trout at all, or practically all, the diagnostic loci between these fishes in every sample (Table 2). Furthermore, each sample contained individuals whose multiple locus genotype at the diagnostic loci is indicative of introgression. Thus, all the samples came from rainbow-westslope cutthroat trout introgressed populations.

The alleles characteristic of the rainbow and westslope cutthroat trout, however, do not appear to be randomly distributed among the diagnostic loci and individuals in each sample. There is a significant excess of individuals that have genotypes characteristic of westslope cutthroat trout at all the diagnostic loci in the samples from Big and Sinclair Creeks (Table 3). The Pinkham Creek and upper Lake Koocanusa samples contain an excess of individuals whose multiple locus genotype is characteristic of rainbow trout (Table 3). The middle Lake Koocanusa and Warland Creek samples contain an excess of individuals whose multiple locus genotype is characteristic of westslope cutthroat trout and others whose multiple locus genotype is characteristic of rainbow trout. Although these populations are all introgressed, the available data suggest that they probably still contain some genetically pure individuals of one or both of the parental taxa.

Rainbow trout genetic contributions

The Ldh4(76) and Sod(152) allele frequencies can be used to determine whether inland or coastal rainbow trout have contributed genes to the sampled populations. Because the samples all came from introgressed populations, however, we must correct the Ldh4 and Sod allele frequencies for the presence of westslope cutthroat trout genes in order to determine the types of rainbow trout that have contributed genes to the populations. Fortunately, this can be done accurately. We have electrophoretic data from over 100 populations of westslope cutthroat trout. We have never observed the Ldh4(76) allele in any of these samples of 20 or more fish and very rarely have we observed the Sod(152) allele. Thus, westslope cutthroat trout will essentially contribute only the Ldh4(100) and Sod(100) alleles to an introgressed population. The frequency of the Ldh4(76) and Sod(152) alleles in the rainbow trout contributing genes to the populations, therefore, can be obtained by dividing the observed frequency of these alleles in the sample by the mean proportion of rainbow trout genes at the diagnostic loci. These frequencies will be referred to as 'adjusted allele frequencies'.

The adjusted allele frequencies (Table 4) reveal three different situations. First, the Ldh4 and Sod allele frequencies in the rainbow trout contributing genes to the Big Creek population are similar to those usually observed in coastal rainbow trout populations, including that inhabiting

Lake Koocanusa in 1979. It appears, therefore, that inland rainbow trout have had no, or a very small, genetic contribution to this population. In contrast, the upper lake Koocanusa, Pinkham Creek, and Warland Creek samples possess adjusted allele frequencies characteristic of inland rainbow trout indicating that this fish has contributed the majority of rainbow trout genes to these populations. Finally, the Ldh4 allele frequencies in the Sinclair Creek and middle Lake Koocanusa samples indicate an inland rainbow trout contribution and the Sod allele frequencies a coastal rainbow trout contribution. Both types of rainbow trout, therefore, have apparently had a relatively substantial input to these populations.

DISCUSSION

The results of our electrophoretic analyses indicate that the genetic composition of the trout in Lake Koocanusa has changed dramatically since 1979 in two important ways. First, while the trout in the reservoir appeared to be mainly genetically pure coastal rainbow trout in 1979, they now contain on the average a substantial proportion of westslope cutthroat trout genes. Furthermore, there is strong evidence that they also contain an appreciable amount of inland rainbow trout genes. Since we do not know the Ldh4 and Sod allele frequencies in the inland rainbow trout that have invaded the reservoir, it is not possible to estimate their relative genetic contribution. The trout in Lake Koocanusa now constitute an introgressed fishery containing coastal rainbow, inland rainbow and westslope cutthroat trout genes. The nonrandom distribution of the rainbow and westslope cutthroat trout genes among the individuals in the samples, however, indicates that some genetically pure dividuals of these species still exist in the fishery, albeit often in low proportions (Table 3).

There are a number of factors, none of which are mutually exclusive, that can account for the nonrandom distribution of rainbow and westslope cutthroat trout genes among the fish in the samples. This phenomenon is expected in populations that have only recently become introgressed, in samples that contain individuals from two or more spawning populations that have different proportions of rainbow and westslope cutthroat trout genes, and when genetically pure representatives of one or both of the parental species constitute continual or sporadic migrants to an introgressed population.

We believe that all of the above factors are partially responsible for the nonrandom distribution of rainbow and westslope cutthroat trout genes among the fish in Lake Koocanusa. There was no evidence of a significant amount of inland rainbow trout genes in the reservoir's trout in 1979. These fish, therefore, must be recent invaders strongly supporting recent introgression as a reasonable explanation. The upper Lake Koocanusa sample was obtained from a spawning run. These fish have a significantly higher (x^2; $P < 0.001$) average proportion of rainbow trout genes than the middle Lake Koocanusa sample which was not collected from a spawning run. Thus, it appears that the trout fishery in Lake Koocanusa is composed of individuals from genetically divergent populations that may spawn in different tributaries or at different times. This is not an unusual situation for trout fisheries in large inland lakes (e.g. Ryman 1983; Crozier and Ferguson 1986). The Montana Department of Fish, Wildlife and Parks has stocked Lake Koocanusa and some of its tributaries with a hatchery strain of westslope cutthroat trout from 1970 to 1976 and 1981 to the present (Joe Huston, personal communication). These fish could certainly constitute migrants to some of the reservoir spawning populations.

We believe that the radical change in the genetic composition of the Lake Koocanusa trout fishery since 1979 is largely due to the invasion of inland rainbow trout. In 1979, the reservoir fishery apparently was composed of mainly coastal rainbow trout despite the presence of native and introduced westslope cutthroat trout in reservoir tributaries. This suggests that interbreeding between these fishes before 1979 was uncommon. The apparent absence of coastal rainbow trout genes in two introgressed populations inhabiting tributaries and in the upper Lake Koocanusa sample also supports this contention. Furthermore, this means that inland rainbow trout must have invaded this system at least two generations ago. This is the minimum time required for them to hybridize with the prior residents and the progeny of these matings to reproduce. We do not know the generation time of inland rainbow trout in the Lake Koocanusa system but suspect it is at least three years. Thus, inland rainbow trout must have successfully invaded this system in the mid 1970s and subsequently hybridized extensively with the populations present prior to this time.

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TABLE 1. -- Electrophoretic mobilities of alleles at loci that can be used to differentiate seven subspecies of cutthroat trout and rainbow trout. Allelic mobilities are relative to the common allele at the homologous locus in rainbow trout. When a locus is variable within a sample, the most common allele is listed first.

		Cutthroat trout subspecies						
Locus	Rainbow	Westslope	Coastal	Lahontan	Yellowstone	Finespotted	Greenback	Colorado
Aat1	100	200	100	165,null	165	165	165	165
Ck2	100	84	84	84	84	84	84	84
CkC1	100,38	100	100	100	38	38	38	38
Gl1	100	100	115	115	101	101	101	101
Gpi3	100	92,100	100	100	100	100	100	100
Idh1	100	100	100	100	-75	-75	-75	-75
Idh3,4	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
Me1,2	100,55	88,100	100	100	100	100	100	100
Me3,4	100	100	110	100,110	110,90	110,90	110,100	110,100,90
Pgm1	100	100,110	100,90	100,null	null	null	null	null
Sdh1,2	100,200,40	40	100,200	100,200	100	100	100,0	100,0

TABLE 2

Allele frequencies at the diagnostic loci between the westslope cutthroat and rainbow trout in six introgressed populations of these fishes in the Kootenai River drainage. The allele characteristic of westslope cutthroat trout at each locus is listed first.

		Samples and allele frequencies					
Locus	Alleles	Lake Koocanusa (upper)	Lake Koocanusa (middle)	Big Creek	Pinkham Creek	Sinclair Creek	Warland Creek
Aatl	200	0.095	0.486	0.850	0.200	0.850	0.500
	100	0.905	0.514	0.150	0.800	0.150	0.500
Ck2	84	0.065	0.525	0.800	0.200	0.900	0.548
	100	0.935	0.475	0.200	0.800	0.100	0.452
Gpi3	92	0.065	0.350	0.850	0.125	0.875	0.524
	100	0.935	0.650	0.150	0.875	0.125	0.476
Idh3,4	86	0.011	0.163	0.388	0.113	0.413	0.250
	114	0.076	0.021	-	0.088	0.013	0.012
	100	0.620	0.558	0.350	0.650	0.525	0.476
	71	0.174	0.188	0.050	-	0.025	0.095
	40	0.120	0.071	0.213	0.150	0.025	0.167
Mel	88	-	0.367	0.900	0.125	0.900	0.643
	100	1.000	0.633	0.100	0.875	0.100	0.357
Sdh	40	0.065	0.275	0.775	0.200	0.850	0.500
	100	0.935	0.725	0.225	0.800	0.150	0.500
Average westslope		0.052	0.388	0.825	0.179	0.867	0.536
Average rainbow		0.948	0.612	0.175	0.821	0.133	0.464

Note: The frequency of the Idh3,4(86) allele is usually 0.500 in populations of westslope cutthroat trout. The proportion of westslope cutthroat trout genes at these loci, therefore, is estimated to be twice the frequency of the Idh3,4(86) allele.

TABLE 3

Numbers of fish having multiple locus genotypes at the diagnostic loci in Table 2 characteristic of the parental taxa or of hybrid origin (hybrids). Probability refers to the chi-square statistic comparing the observed distribution of fish to that expected assuming the alleles are randomly distributed among individuals and diagnostic loci.

Sample	Westslope	Hybrids	Rainbow	Probability
Lake Koocanusa				
middle	3	51	6	< 0.001
upper	0	6	15	< 0.050
Big Creek	8	13	0	< 0.050
Pinkham Creek	0	14	6	< 0.001
Sinclair Creek	16	4	0	< 0.001
Warland Creek	6	12	3	< 0.001

TABLE 4
(See Page 6 for Explanation)

Adjusted allele frequencies at the Ldh4 and Sod loci in six rainbow-westslope cutthroat trout introgressed populations and in the rainbow trout collected from Lake Koocanusa in 1979.

	Alleles and their frequency			
Population	Ldh4(100)	Ldh4(76)	Sod(100)	Sod(152)
Lake Koocanusa				
1979	0.970	0.030	0.810	0.190
middle	0.850	0.150	0.891	0.109
upper	0.744	0.256	1.000	-
Big Creek	1.000	-	0.857	0.143
Pinkham Creek	0.787	0.213	0.970	0.030
Sinclair Creek	0.812	0.188	0.436	0.564
Warland Creek	0.744	0.256	0.949	0.051

Figure 1. Approximate location of samples collected from upper Lake Koocanusa and its tributaries. 1) = Sinclair Creek, 2) = upper Lake Koocanusa, 3) = Pinkham Creek.

Figure 2. Approximate location of samples collected from middle Lake Koocanusa and its tributaries. 4) = Big Creek, 5) = middle Lake Koocanusa, 6) = Warland Creek.

Figure 1

Figure 2