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INTROGRESSION BETWEEN WESTSLOPE CUTTHROAT AND RAINBOW TROUT IN THE CLARK FORK RIVER DRAINAGE, MONTANA

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Abstract--Using horizontal starch gel electrophoresis we determined the genetic composition of six natural *Salmo* populations in the Clark Fork River drainage in Montana. Blanchard Creek, the South Fork of Lolo Creek, Stoney Creek, and Tin Cup Creek were found to contain introgressed populations of westslope cutthroat trout and rainbow trout. Granite Creek and O'Keefe Creek, however, contain pure native westslope cutthroat trout. The high proportion of introgressed populations in the samples indicates that relatively few pure populations of westslope cutthroat trout now exist in the drainage.

We compared the distributions of eight meristic characters between the six Clark Fork River drainage samples and two hatchery rainbow trout samples. The meristic data alone conclusively identified only the Blanchard Creek population as not being pure westslope cutthroat trout. The reduced power of meristic comparisons compared to electrophoresis in identifying pure and introgressed populations is due to the large amount of intraspecific morphological variability in these fishes, and the large amount of morphological similarity between westslope cutthroat and rainbow trout.

INTRODUCTION

Most local populations of native *Salmo* present at the beginning of this century in the interior of western North America no longer exist (6). Mining, logging, grazing, irrigation, and road and dam construction have reduced the amount of suitable habitat, and are believed to have favored the displacement of the native trout by introduced brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) (8). The most important factor for the loss of the native trout populations, however, has been the introduction of rainbow trout (*Salmo gairdneri*) and subspecies of cutthroat trout (*Salmo clarki*) into waters outside their natural range. These introductions are believed to have resulted in widespread hybridization and introgression between the native and introduced trouts resulting in the loss of the native trout gene pools (6, 12).

The preservation of the remaining native rainbow trout, westslope cutthroat trout (*Salmo clarki lewisi*), and Yellowstone cutthroat trout (*Salmo clarki bouvieri*) populations in Montana is a goal of the Montana Department of Fish, Wildlife and Parks (MDFWP). In order to preserve these populations, however, they must first be identified.

The identification of pure and introgressed populations of these fishes has usually relied upon morphological comparisons between populations. This procedure is based on the generally untested assumption that hybridized and introgressed populations are morphologically intermediate to the parental species (16). Morphological comparisons, however, are not likely to detect reliably all introgressed populations of these fishes since morphological characters do not differ much between them (7, 20). Furthermore, hybrid and introgressed fishes are not always morphologically intermediate to the parental species (9, 11, 14, 15, 16, 21, 23, 24, 28).

The rainbow trout, westslope cutthroat trout, and Yellowstone cutthroat trout are all electrophoretically distinct taxa (17, 19). The electrophoretic differences between these taxa are due to genetic differences at individual genes controlling the structure of enzymatic proteins. These differences are inherited as discrete units; introgressed populations can be electrophoretically identified because they will possess proteins from more than one taxon. With adequate sample sizes, therefore, electrophoresis will identify virtually all pure and introgressed populations of these taxa.

The objective of this study was to determine the extent to which introgression has occurred between introduced trouts and native westslope cutthroat trout in the Clark Fork River drainage in Montana. We collected electrophoretic and meristic data from all the natural populations sampled and from two hatchery rainbow trout populations. This enabled us to determine how accurately the meristic data reflected the genetic composition of the natural populations as determined by electrophoresis. The results indicate that 1) relatively few pure populations of native westslope cutthroat trout remain in the Clark Fork River drainage and 2) the meristic data we collected could not detect all the introgressed populations we sampled.

METHODS

Samples

Samples of natural *Salmo* populations were obtained from the following six creeks in the Clark Fork River drainage during the summer of 1982: Blanchard Creek (T 14N, R 14W, S 4; N=47); Granite Creek (T 11N, R 23W, S 7; N=29) O'Keefe Creek (T 14N, R 20W, S 16; N=51); the South Fork of Lolo Creek (T 12N, R 21W, S 29; N=32); Stoney Creek (T 7N, R 16W, S 21; N=23); and Tin Cup Creek (T 3N, R 21W, S 17; N=50). The westslope cutthroat trout is the only *Salmo* species native to this drainage in Montana (10). A high proportion of introgressed populations in the samples, therefore, would indicate that relatively few pure native westslope cutthroat trout populations remain in this drainage.

Samples of hatchery rainbow trout were obtained from the Arlee (N=50) and Shasta (N=67) strains. The Arlee strain is maintained by the MDFWP at the Jocko River State Trout Hatchery, Arlee, Montana. The Shasta strain is maintained by the United States Fish and Wildlife Service (USFWS) at the Ennis National Fish Hatchery, Ennis, Montana.

Electrophoresis

The protein products of 43 loci (Table 1) were analyzed in all of the fish using horizontal starch gel electrophoresis (26). Electrophoretic buffers and staining methods were after Allendorf et al. (1). The designation of loci and alleles follows the procedures outlined by Allendorf and Utter (4). The designation of all alleles is relative to the common allele at the particular locus in rainbow trout.

There are 12 loci that differentiate rainbow trout, westslope cutthroat trout, and Yellowstone cutthroat trout (17, 19, Table 2; Fig. 1). These are referred to as diagnostic loci (5). The genetic composition of each natural population was determined by comparing the alleles present at these diagnostic loci to those in previously identified pure populations of these three trout. The proportion of the genetic material in introgressed populations from the native and introduced trouts was quantified by averaging the respective percentages over all diagnostic loci.

Meristics

The counts of seven meristic characters were taken from all the fish in the two hatchery rainbow trout and the six Clark Fork River drainage samples: rays in the anal fin, rays in the dorsal fin, rays in the pelvic fins, rays in the pectoral fins, gillrakers on the upper first branchial arches,

Table 1. Enzymes and loci examined (E = eye, L = liver, M = muscle).

Enzyme	Loci	Tissue
Adenylate kinase (AK)	Ak	M
Alcohol dehydrogenase (ADH)	Adh	L
Aspartate aminotransferase (AAT)	Aat-1,2	L
	Aat-(3,4)	M
Creatine kinase (CK)	Ck-1,2	M
	Ck-3	E
Glucose phosphate isomerase (GPI)	Gpi-1,2,3	M
Glyceraldehyde-3-phosphate dehydrogenase (GAP)	Gap-3,4	E
Glycerol-3-phosphate dehydrogenase (G3P)	G3p-1,2	L
Glycyl-leucine peptidase (GL)	Gl-1,2	E
Isocitrate dehydrogenase (IDH)	Idh-1,2	M
	Idh-(3,4)	L
Lactate dehydrogenase (LDH)	Ldh-1,2	M
	Ldh-3,4,5	E
Leucyl-glycyl-glycine peptidase (LGG)	Lgg	E
Malate dehydrogenase (MDH)	Mdh-(1,2)	L
	Mdh-(3,4)	M
Malic enzyme (ME)	Me-(1,2),3	M
	Me-4	L
Phosphoglucomutase (PGM)	Pgm-1,2	M
	Pgm-1-t	L
6-phosphogluconate dehydrogenase (6PG)	6Pg	E
Sorbitol dehydrogenase (SDH)	Sdh	L
Superoxide dismutase (SOD)	Sod	L
Xanthine dehydrogenase (XDH)	Xdh	L

The protein products of the pairs of loci in () are electrophoretically identical. Therefore, they are considered to be single tetrasomic loci in all analyses.

Table 2. Loci that differentiate rainbow trout, westslope cutthroat trout, and Yellowstone cutthroat trout. Alleles are designated as the proportional migration distance in the gel relative to the distance traveled by the common allele in rainbow trout which is given mobility of 100.

	Alleles		
Loci	Rainbow	Westslope	Yellowstone
Aat-1	100	200	165
Ck-2	100	84	84
Gl-1	100	100	101
Gpi-3	100	92	100
Idh-1	100	100	-75
Idh-3,4	100, 114, 71, 40	100, 86, 71, 40	100, 71
Lgg	100	100	135
Me-1,2	100, 57	88	100
Me-3	100	100	84
Me-4	100, 75	100	110
Pgm-1	100, null	100, null	null
Sdh	100, 200, 40	40	100

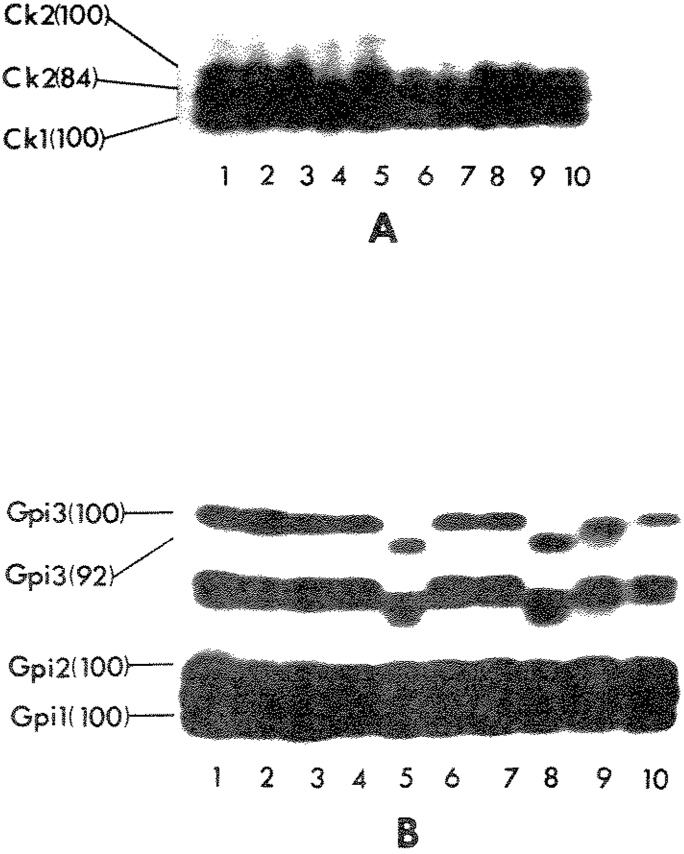

Figure 1. Diagnostic loci that differentiate rainbow trout, westslope cutthroat trout and Yellowstone cutthroat trout: A. Ck-2: 1-3, 5, 8-10 = rainbow trout phenotype; 4 and 7 = westslope or Yellowstone cutthroat trout phenotype; 6 = rainbow trout and cutthroat genetic material in the same individual. B. Gpi-3: 1-4, 6-7, 10 = rainbow or Yellowstone cutthroat trout phenotype; 5 and 8 = westslope cutthroat trout phenotype, 9 = westslope cutthroat and rainbow trout genetic material in the same individual.

gillrakers on the lower first branchial arches, and mandibular pores. Fin ray counts include all the rays, and all counts followed standard ichthyological procedures (13). The counts of the five bilateral characters were taken on the left and right side of each fish. It was not uncommon to find that the counts differed on the two sides of an individual for the same character. This phenomenon is referred to as asymmetry (27). As an eighth meristic character, we used the number of bilateral characters for which an individual was asymmetric.

The distributions of each meristic character were compared for homogeneity for all possible pair-wise combinations between the populations with the Wilcoxon two-sample test (25). We used this nonparametric analysis because it is not affected by deviations from the assumptions of normally distributed data sets with equal variances as is the t-test. All of the meristic characters were simultaneously compared among the samples with discriminant analysis (18). This multivariate analysis separates populations as far apart as possible in two dimensional space using the synergistic interaction between all the characters. Subsequently one can use the analysis to place individuals into the population to which they are morphologically the most similar. The classification of many individuals to the wrong population indicates a high degree of overall morphological similarity. The total count (left + right) of the bilateral characters was used in these analyses.

RESULTS

Electrophoretic Comparisons

The Blanchard Creek, the South Fork of Lolo Creek, Stoney Creek, and Tin Cup Creek samples contained alleles from both rainbow trout and westslope cutthroat trout at all six diagnostic loci between these fishes (Table 3). Thus, these are introgressed populations of rainbow trout and westslope cutthroat trout. There was no evidence of Yellowstone cutthroat trout genes in any of these introgressed populations.

The genotypic distributions at all the variable loci in each population conformed to expected random mating proportions, except in Stoney Creek. The deviation from random mating proportions in Stoney Creek is most likely due to the movement of fish from genetically different populations in Rock Creek into the lower reach of Stoney Creek. Thus, these populations appear to be randomly mating "hybrid swarms."

There is no evidence of rainbow trout or Yellowstone cutthroat trout genes in the O'Keefe Creek and Granite Creek

Table 3. Allele frequencies at the loci differentiating rainbow trout and westslope cutthroat trout in four introgressed populations of these fishes in the Clark Fork River drainage, Montana.

		Population and allele frequencies			
Locus	Allele	Blanchard Creek	South Fork Lolo Creek	Tin Cup Creek	Stoney Creek
Aat-1	100	0.840	0.016	0.050	0.196
	200	0.160	0.984	0.950	0.804
Ck-2	100	0.883	0.016	0.040	0.174
	84	0.117	0.984	0.960	0.826
Gpi-3	100	0.840	0.063	0.050	0.196
	92	0.160	0.937	0.950	0.804
Idh-3,4	100	0.723	0.290	0.174	0.217
	114	0.021	--	--	--
	86	0.043	0.484	0.480	0.402
	71	0.106	--	0.010	0.011
	40	0.106	0.226	0.337	0.370
Me-1,2	100	0.894	0.008	0.020	0.196
	88	0.106	0.992	0.980	0.804
Sdh	100	0.809	0.031	0.080	0.239
	40	0.191	0.969	0.920	0.761
Avg. proportion rainbow genes		0.853	0.027	0.048	0.200
Avg. proportion westslope genes		0.147	0.973	0.952	0.800

samples. There is a possibility that genes from rainbow or Yellowstone cutthroat trout are present in these populations at a low frequency and that by chance we have not detected them. With these sample sizes, however, the probabilities of not detecting as little as one percent rainbow trout genes in these populations are 0.002 and 0.030, respectively. The probabilities for not detecting one percent Yellowstone cutthroat trout genes are 0.00003 and 0.003, respectively. Thus, the O'Keefe Creek and Granite Creek populations are almost certainly pure westslope cutthroat trout.

Meristic Comparisons

The amount of morphological variability among these populations is so great that no two populations have the same meristic distribution for all of the characters (Fig. 2). Pair-wise comparisons indicate that all of the populations have significantly different distributions for at least three characters, except for the Blanchard Creek and Stoney Creek samples that differ only for the number of rays in the pelvic fins.

The rainbow and westslope cutthroat trout samples had consistently different meristic distributions only for the number of rays in the pelvic fins (Fig. 2B) and gillrakers on the upper first branchial arches (Fig. 2C). These characters, therefore, are the only two that we counted that might be useful in detecting introgressed populations.

Instead of being intermediate for these characters, however, the introgressed samples tend to resemble the parental species which contributed the most genetic material to the population (Table 3). The Blanchard Creek sample does not have a significantly different distribution of the number of rays in the pelvic fins than either of the rainbow trout samples. The other introgressed samples do not have a significantly different distribution of this character from either of the westslope cutthroat trout samples. Based on these data, therefore, one could not be certain that Blanchard Creek does not contain a pure rainbow trout population and the others pure westslope cutthroat trout.

The regions occupied by the westslope cutthroat trout, rainbow trout, and introgressed samples in the two dimensional space produced by simultaneously comparing all the meristic characters with discriminant analysis (see Methods) all overlap (Fig. 3). The overlap is so great that only 64% of the fish are classified to the correct sample. These three genetically unique groups of fishes, therefore, are not meristically unique for the eight characters we counted.

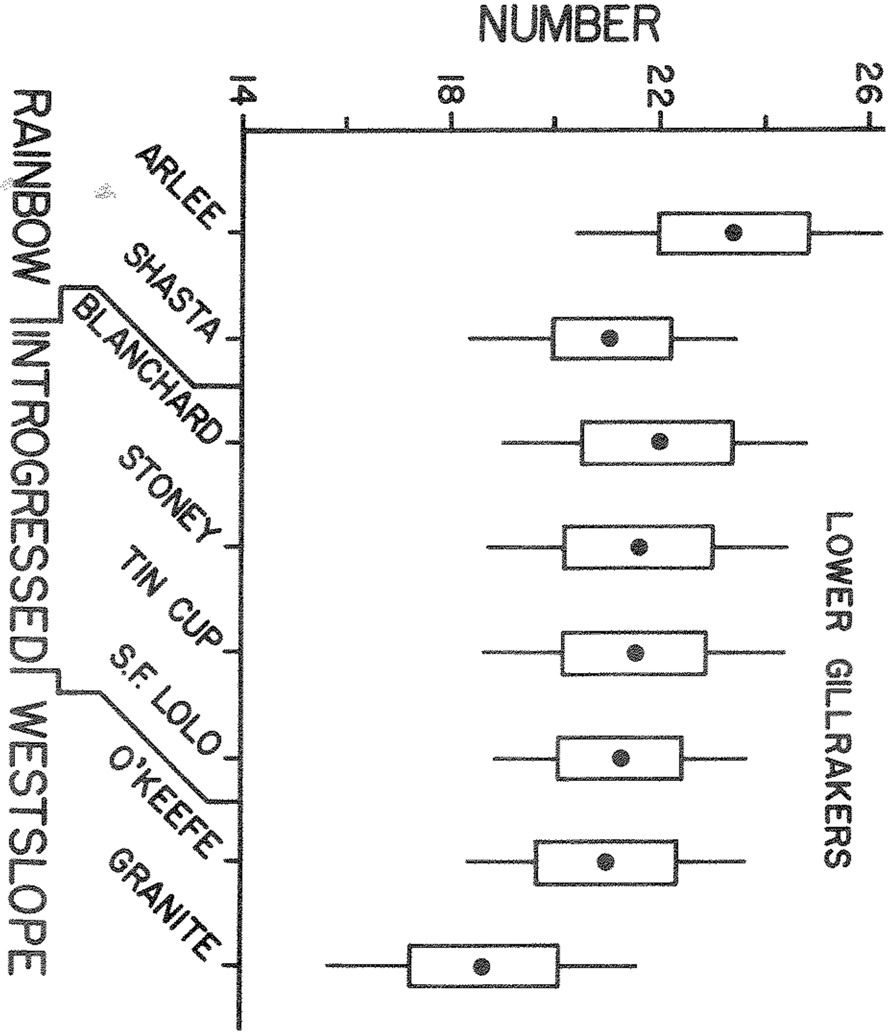

Figure 2A. Means +/- two standard deviations of number of gillrakers on the lower first branchial arches from six Clark Fork River drainage populations and two hatchery rainbow trout strains.

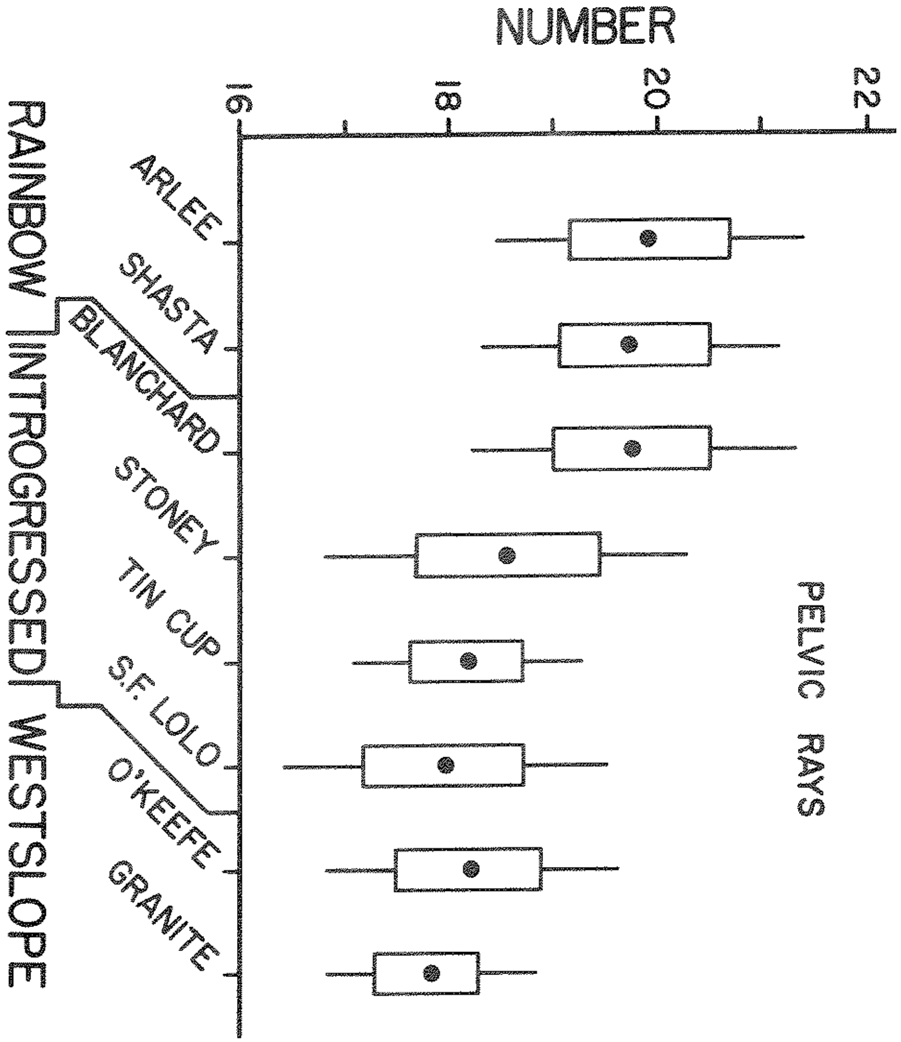

Figure 2B. Means +/- two standard deviations of number of rays in the pelvic fins from six Clark Fork River drainage populations and two hatchery rainbow trout strains.

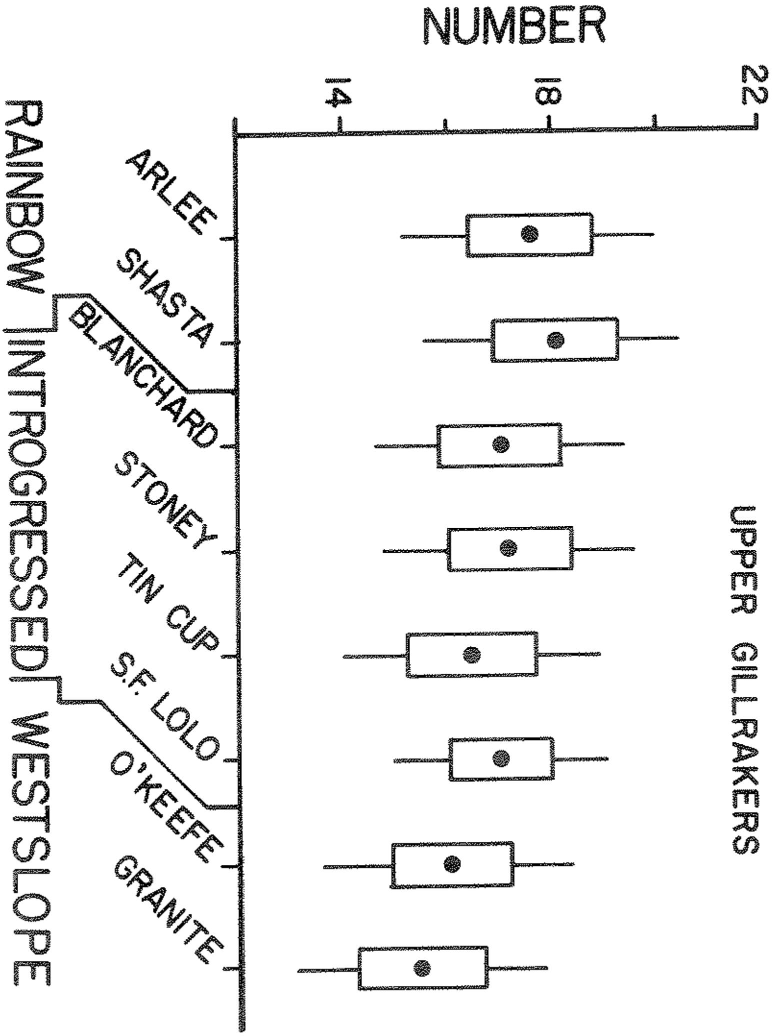

Figure 2C. Means +/- two standard deviations of gillrakers on the upper first branchial arches from six Clark Fork River drainage populations and two hatchery rainbow trout strains.

DISCUSSION

Detection of Introgressed Populations

The electrophoretic and meristic data we have presented demonstrate that the meristic comparisons are not as powerful as electrophoresis in detecting pure and introgressed populations of rainbow trout and westslope cutthroat trout. The meristic data conclusively identify only the Blanchard Creek sample as not being pure westslope cutthroat trout. The reduced power of the meristic comparisons is due to the large amount of intraspecific variability among and similarity between these species for the characters we analyzed. Because of this condition introgressed and pure populations of these species are not meristically unique, although genetically they are unique. Furthermore, the assumption that hybridized and introgressed populations are morphologically intermediate to the parental species is not supported by these data or our data from brook trout X bull trout, *Salvelinus confluentus*, hybrids (15). We conclude that reliance on morphological comparisons will markedly underestimate the number of introgressed populations in the samples, as these characters are not likely to reflect less than 10% foreign genes in a population.

Extent of Introgression in the Clark Fork River Drainage

The creeks we sampled were chosen because they were classified by the MDFWP to contain pure westslope cutthroat trout. In determining the extent of introgression in the Clark Fork River drainage we have to eliminate the O'Keefe Creek sample from the data. This creek was sampled because it was known to have been isolated from the Clark Fork River for many years because of irrigation. Thus, it is not therefore comparable to the other samples that were collected without prior knowledge of possible natural or man-induced isolation from other waters in the drainage.

Four of the remaining five samples are introgressed. In addition, Allendorf and Phelps (3) have reported that Mill Creek (T 14N, R 21W, S 21) contains an introgressed population of westslope cutthroat and rainbow trout. Although not many creeks have been sampled, the results from those that have been sampled are clear: not many pure native westslope cutthroat trout populations now exist in the Clark Fork River drainage.

We have found introgressed populations of westslope cutthroat and rainbow trout, westslope and Yellowstone cutthroat trout, and all three taxa in other drainages in

Montana. The available data indicate that this phenomenon is likely to be widespread in any drainage where it has been identified.

Management Implications

Once a population has become introgressed, the process of Mendelian segregation of chromosomes and recombination make it impossible for the population to revert to the original pure native type. Strong natural selection against the introduced genes may produce fish that are morphologically similar to the original native fish. However, these fish will still retain some genes from the introduced fish.

Strong selection against the genes being introduced from the rainbow trout should be electrophoretically detectable. Such selection should result in either a deviation from random genotypic proportions or to differences in allele frequencies between diagnostic loci. However, there are no statistical differences in the allele frequencies between the diagnostic loci in any of the introgressed samples. Furthermore, only the Stoney Creek sample showed any evidence of a deviation from random genotypic proportions. This deviation is more likely due to individuals from two or more genetically different populations being included in the sample than to selection. Our data thus provide no evidence of selection against rainbow trout chromosomes or genes in the introgressed populations.

The westslope cutthroat trout is in danger of extinction in Montana. Introgression with introduced rainbow trout is apparently widespread and there is no evidence that natural selection is acting to reduce the frequency of genetic material from rainbow trout in introgressed populations. Hatcheries can serve as a valuable reservoir of the genetic resources represented by the remaining westslope cutthroat trout populations. Domestication, however, will result in some genetic changes because of adaptation to hatchery conditions. Genetic drift may also cause genetic changes unless large numbers of fish are used in founding and maintaining hatchery populations (2, 22). It is more important, therefore, that the remaining natural populations of westslope cutthroat trout be identified and preserved.

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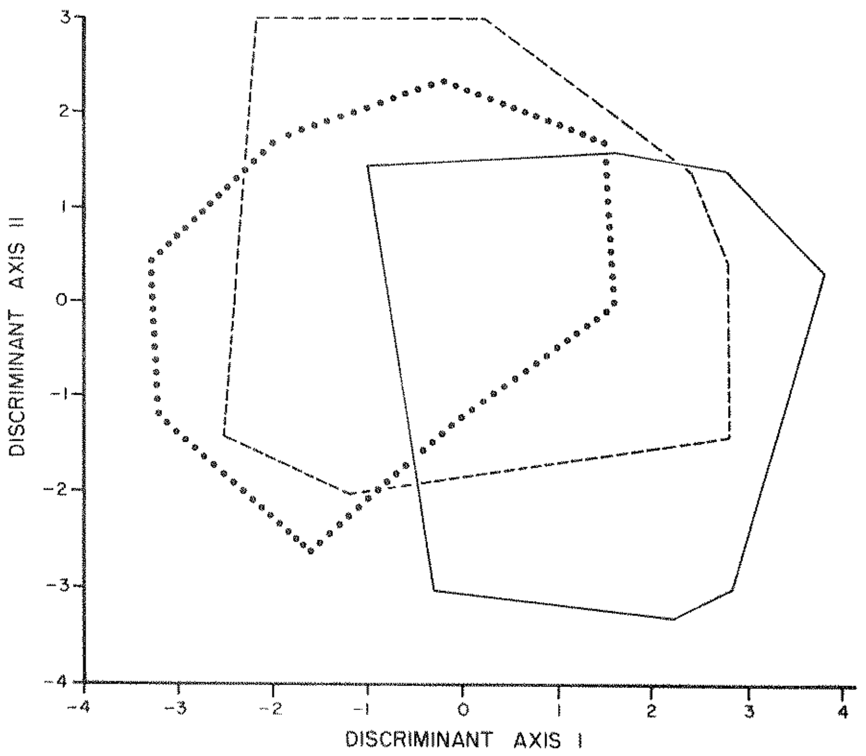

Figure 3. Areas occupied in two dimensional space by rainbow trout (———), westslope cutthroat trout (·····), and introgressed populations (-----) simultaneously comparing all eight meristic characters among them with discriminant analysis.

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LITERATURE CITED

1. Allendorf, F. W., N. Mitchell, N. Ryman, and G. Stahl. 1977. Isozyme loci in brown trout (*Salmo trutta* L.). Detection and interpretation from population data. Hereditas 86:179-190.
2. Allendorf, F. W., and S. R. Phelps. 1980. Loss of genetic variation in a hatchery stock of cutthroat trout. Transactions of the American Fisheries Society 109:537-543.
3. Allendorf, F. W., and S. R. Phelps. 1981. Isozymes and the preservation of genetic variation in salmonid fishes. In: Fish gene pools, ed. N. Ryman, Ecological Bulletins (Stockholm) 34:37-52.
4. Allendorf, F. W., and F. M. Utter. 1979. Population genetics. In: Fish physiology, ed. W. S. Hoar, D. J. Randall, and J. R. Brett, Vol. 8, 407-454. New York: Academic Press.
5. Ayala, F. J., and J. R. Powell. 1972. Allozymes as diagnostic characters of sibling species of *Drosophila*. Proceedings National Academy of Sciences USA 69:1094-1096.
6. Behnke, R. J. 1972. The systematics of salmonid fishes of recently glaciated lakes. Journal of Fisheries Research Board of Canada 29:539-571.
7. Behnke, R. J. 1979. Monograph of the native trouts of the genus *Salmo* of western North America, USDA Forest Service, and Bureau of Land Management. (Unpublished report).
8. Behnke, R. J. and M. Zarn. 1976. Biology and management of threatened and endangered trouts. USDA Forest Service General Technical Report Rm-28. Fort Collins, CO: Rocky Mountain Forest and Range Experimental Station. 45 pp.
9. Berry, P. Y., and M. P. Low. 1970. Comparative studies on some aspects of the morphology and histology of *Ctenopharyngodon idellus*, *Aristichthys nobilis* and their hybrid (Cyprinidae). Copiea 1970:708-726.
10. Brown, C. J. D. 1971. Fishes of Montana. Bozeman: Big Sky Books, Montana State University. 207 pp.

11. 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.
12. Hanzel, D. A. 1959. The distribution of cutthroat trout *(Salmo clarki)* in Montana. Proceedings Montana Academy of Sciences 19:32-71.
13. Hubbs, Carl L., and K. F. Lagler. 1970. Fishes of the Great Lakes Region. Ann Arbor: University of Michigan Press. 213 pp.
14. Hubbs, Clark L., and K. Strawn. 1957. Relative variability of hybrids between the darters, *Etheostoma spectabile* and *Percina caprodes.* Evolution 11:1-10.
15. 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.
16. Neff, N. A., and G. R. Smith. 1979. Multivariate analysis of hybrid fishes. Systematic Zoology 28:176-196.
17. Phelps, S. R. and F. W. Allendorf. 1982. Genetic comparison of Upper Missouri cutthroat trout to other *Salmo clarki lewisi* populations. Proceedings Montana Academy of Sciences 41:14-22.
18. Pimentel, R. A. 1979. Morphometrics. Dubuque, IA: Kendall/Hunt Pub. Co. 276 pp.
19. Reinitz, G. L. 1977. Electrophoretic distinction of rainbow trout *(Salmo gairdneri)*, westslope cutthroat trout (*Salmo clarki*) and their hybrids. Journal of Fisheries Research Board of Canada 34:1236-1239.
20. Roscoe, J. W. 1974. Systematics of westslope cutthroat trout. M.S. Thesis Colorado State University, Fort Collins. 74 pp.
21. Ross, M. R., and T. M. Cavender. 1981. Morphological analyses of four experimental intergeneric hybrid crosses. Copeia 1981:377-387.
22. Ryman, N. and G. Stohl. 1980. Genetic changes in hatchery stocks of brown trout *(Salmo trutta).* Canadian Journal of Fisheries and Aquatic Sciences 37:82-87.
23. Simon, R. C. and R. E. Noble. 1968. Hybridization in *Oncorhynchus* (Salmonidae). I. Viability and inheritance in artificial crosses of chum and pink salmon. Transactions of the American Fisheries Society 97:109-118.

24. Smitherman, R. O., and F. E. Hester. 1962. Artificial propagation of sunfishes, with meristic comparisons of three species of *Lepomis* and five of their hybrids. Transactions of the American Fisheries Society 91:333-341.
25. Sokal, R. R., and F. J. Rohlf. 1981. Biometry. San Francisco: W. H. Freeman Pub. Co. 859 pp.
26. Utter, F. M., H. O. Hodgins, and F. W. Allendorf. 1974. Biochemical genetic studies of fishes: potentialities and limitations. In: Biochemical and biophysical perspectives in marine biology, ed. D. C. Malin, and J. R. Sargent, Vol. 1, 213-238. New York: Academic Press.
27. Van Valen, L. 1962. A study of fluctuating asymmetry. Evolution 16:125-142.
28. West, J. L. and F. E. Hester. 1964. Intergeneric hybridization of centrarchids. Transactions of the American Fisheries Society 93:280-288.