CZES Coastal Zone and Estuarine Studies

Electrophoretic Survey of Protein Variation in Eight Strains of Rainbow Trout *(Salmo gairdneri)* from the U.S. Fish and Wildlife Service Genetic Laboratory

by
George B. Milner, David J. Teel
and Fred M. Utter

December 1979

ELECTROPHORETIC SURVEY OF PROTEIN VARIATION IN EIGHT STRAINS OF RAINBOW TROUT (*Salmo gairdneri*) FROM THE U.S. FISH AND WILDLIFE SERVICE FISH GENETIC LABORATORY

by

George B. Milner, David J. Teel

and

Fred M. Utter

Report of Research (FY 79)

Financed by
U.S. Fish and Wildlife Service

(Cooperative Agreement No. 14-16-0009-79-911)

National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northwest and Alaska Fisheries Center
Coastal Zone and Estuarine Studies Division
2725 Montlake Boulevard East
Seattle, WA 98112

December 1979

ABSTRACT

Protein variation was used to genetically characterize and measure the genetic variation of rainbow trout from the U.S. Fish and Wildlife Service's Fish Genetics Laboratory. Estimates are given for the levels of variation occuring both within the strains and among the strains. A comparison of these values and estimates of the amount of genetic variation within and among steelhead populations of the Columbia River drainage indicated that (1) the within strain variation is approximately the same for the two groups but that (2) the among strain variation of the Fish Genetics Laboratory strains is lower than that of the Columbia River steelhead. Genetic similarities were measured and are in the range expected for conspecific strains. The lowest similarities were exhibited by pairs that included New Zealand, the most divergent strain. Consideration is given to possible causes of the excessive number of genotype distributions that departed from Hardy-Weinberg proportions. Potential breeding and management applications of electrophoretically detected protein variations are discussed.

Continuation of this project will increase the value of the present data by providing a more complete picture of the overall genetic structure and relationships in cultured rainbow trout strains.

TABLE OF CONTENTS

INTRODUCTION

Numerous strains of rainbow trout (RBT), Salmo gairdneri, are being reared by hatchery and research facilities of the U.S. Fish and Wildlife Service (USFWS) in conjunction with their mandate for perpetuation, maintenance, and stock improvement of this species. A current mission of the USFWS Fish Genetics Laboratory (FGL) is to evaluate these strains to better understand their respective relationships and adaptive capabilities. A necessary portion of this mission is to examine the biochemical genetic characteristics of these strains to identify and preserve the total amount of genetic variation existing within the species, and to apply this knowledge to stock management. This report summarizes the biochemical genetic data from eight of these strains. The significance of these data is outlined in conjunction with the requirements for further investigation.

ELECTROPHORESIS

FGL provided eight strains of RBT (Table 1) for electrophoretic examination of protein variation. Utter et al. (1974) and May (1975) describe the methods of tissue extraction and electrophoresis. The use of eye (vitreous fluid), liver, white muscle, and heart tissues allowed the study of 32 loci controlling variation in 17 enzymes (Table 2).

POLYMORPHIC LOCI AND ALLELIC FREQUENCIES

Eleven of the 32 loci were polymorphic, five being polymorphic in all eight strains. The average proportion of polymorphic loci per strain was 22.6%. The number of alleles identified at an individual locus ranged from one to five. The list below gives the number of alleles identified at each polymorphic locus.

Locus	Number of alleles identified
ALB-1,2	2
GPD-1	2
ICD-3,4	4
LDH-4	2
LDH-5	2
MDH-3,4	5
ME-2	2
PEP-1	2
PEP-3	2
PGM	2
SOD	3

Allelic frequencies, with their 95% confidence intervals are listed in the Appendix. The distribution of genotype frequencies was tested for Hardy-Weinberg (HW) proportions using the chi-square goodness of fit test at $\alpha = 0.05$. Seven significant departures from HW were observed among 32 genotype distributions. This level of deviation was 4.4 times that expected by chance. The departures occurred among five loci and five

Table 1 -- Description of eight RBT Strains
(Information provided by FWS)

Strain	FGL strain number	Fish type	Description
Fish Lake (FL)	10	Wild	From Fish Lake, Utah, 1973. Sample fish are two generations from wild stock.
New Zealand (NZ)	13	Semi-wild	Imported from New Zealand wild stock to McNenny NFH 1960; FGL obtained the strain from egg samples in 1964, 1965, and 1966. It has been maintained at FGL since this time. This group is approximately eight generations removed from wild fish.
Desmet (D)	20	Wild	Taken from eggs of wild fish captured by Wyoming Fish and Game; Desmet Lake, Wyoming, April, 1974. Sample is from fish one generation removed from wild fish. Wyoming uses Desmet Lake as a brood lake and does not plant fish back into the lake.
Winter (WS) Standard	16	Hatchery	Developed at FGL by inclusion of Donaldson, New Zealand, and Sand Creek strain fish during 1969, 1970, and 1971. The strain has been maintained by random mating and has never been selected for any trait. Four generations have passed since initial hybridization.
Sand Creek (SC)	14	Semi-wild	Obtained from McKenny NFH as egg samples in 1964, 1965, and 1966, originally from the Sand Creek drainage in northeastern Wyoming. Fish have been maintained at FGL as a random mating pool since 1966.
Formalin 1/ Selected (FS)	26	Hatchery	Developed at FGL by selection for resistance to Formalin, and is a mixture of the Donaldson, New Zealand, and Sand Creek strains. During the past 5 years (two generations), no selection pressure has been applied.

1/ Reference to trade names does not imply endorsement by the National Marine Fisheries.

Table 1 - Continued

Strain	FGL strain number	Fish type	Description
Kamloops (K)	27	Hatchery	Introduced to FGL in 1978 from Spring Creek Trout Hatchery, Lewistown, Montana. Has been maintained as hatchery stock for an unknown time.
Alaska (A)	19	Wild	From wild fish Iliamna Lake drainage (Alaska), May 1974. FGL introduced them as yearling in May 1975. Sample fish are one generation from wild stock.

Table 2.--List of proteins, their abbreviations, and the number of loci surveyed.

Abbreviation	Protein	Number of loci[a]
ADH	Alcohol dehydrogenase	1
ALB	Para-albumin	2
CK	Creatine kinase	2
EST	Esterase	1
GOT	Glutamate-oxaloacetate transaminase	3
GPD	Glycerol-3-phosphate dehydrogenase	2
GPI	Glucose phosphate isomerase	3
ICD	Isocitrate deydrogenase	2
LDH	Lactate dehydrogenase	5
MDH	Malate dehydrogenase	4
ME	Malic enzyme	1
MPI	Mannose phosphate isomerase	1
PEP	Peptidase	6
PGD	Phosphogluconate dehydrogenase	1
PGM	Phosphoglucomutase	1
SOD	Superoxide dismutase	1
XDH	Xanthine dehydrogenase	1

[a] Five protein systems (ALB-1,2; GOT-1,2; ICD-3,4; MDH-1,2 and MDH-3,4) include duplicated loci that cannot be electrophoretically distinguished.

strains. The directions of the departures were about evenly split between excesses and deficits of heterozygotes as shown below.

Strain	Locus	Excess (+) or Deficit (-) of heterozygotes
Fish Lake	GPD-1	+
Sand Creek	PGM	-
Alaska	ME-2	+
	SOD	-
Formalin Selected	GPD-1	+
	LDH-5	-
Kamloops	SOD	+

The excessive departures from HW proportions might result from the following:

(1) Incorrect scoring of phenotypes due to errors of interpretation or to the use of inappropriate genetic model, e.g., a model ignoring null alleles or the effects of regulatory genes.

(2) Broodstock management procedures, e.g.: (a) procedures allowing the overlapping of generations with a strain that is the result of two or more introductions having different allelic frequencies or (b) non-random mating.

(3) Non-representative samples, e.g., samples taken from the progeny of a small number of fish.

(4) Some form of selection.

(5) Chi-square test inaccuracies resulting from the use of small class expectations.

(6) Chance events.

Since similar observations have been made in other studies (Milner, unpublished; Busack et al. 1979), it is unlikely that the excessive number of

departures are merely due to chance (6). Examination of brood stock management methods and the sampling procedures of this study could determine the plausibility of causes (2) and (3). Inaccuracies of chi-square tests (5) resulting from small class expectations could be remedied by combining small class expectations before using the test. However, since this procedure is not always satisfactory, it might be better to use the likelihood ratio test (Sokal and Rohlf 1969). To investigate selection (4) as a cause, would require experiments designed to measure the relative long term reproductive success of each genotype. Errors in reading phenotypes (1) are not a likely cause since observations were verified, in most cases, on independent gels and by independent scorers. The effects of null alleles and regulatory genes (1) on the apparent distribution of genotypes are difficult to detect and cannot be ruled out as possible causes of the possible departures from HW proportions.

GENETIC VARIATION

Genetic variation was analyzed by methods described by Nei (1973 and 1975). The magnitude of genetic variation detected by this sampling of loci for all strains was 0.065. The within and among strain components of this variation were 0.061 and 0.004, respectively. The among strain component accounted for only 6.2% of the total variation.

The average genetic variation per locus and its confidence interval (95%) for each strain are given below.

Strain	Genetic variation	Confidence interval
Fish Lake	0.072	0.02-0.12
New Zealand	0.073	0.01-0.13
Sand Creek	0.049	0.00-0.10
Winter Standard	0.055	0.01-0.11
Alaska	0.053	0.00-0.10
Desmet	0.053	0.00-0.11
Formalin Selected	0.070	0.01-0.13
Kamloops	0.061	0.01-0.11

The confidence intervals were so large, due to the relatively small number of loci sampled, that no strain differences could be detected.

The structure and magnitudes of genetic variations of FGL strains are compared below with those of Columbia-Snake River strains (wild and hatchery) of anadromous RBT.

	Genetic variation	
Source of variation	This study	Columbia-Snake River RBT
Within strain	0.061	0.068
Among strain	0.004	0.016
Total	0.065	0.084

The within-strain genetic variation of the FGL strains is approximately the same as that of the Columbia-Snake River strains. But, the total and among strain variations of the FGL strains are lower than those of the Columbia-Snake River strains. This difference likely reflects the inclusion of coastal and inland population groups (Allendorf 1975; Behnke 1979) in the Columbia-Snake River samples but not in the FGL populations. (The Kamloops strain of the FGL is nominally an inland population; however, the allelic frequencies of this study strongly indicate an undefined coastal ancestry for this strain). The comparison of the two structures suggest that the among strain and total genetic variation of the FGL populations could be increased by the addition of appropriate strains (possibly from the inland population group).

Genetic similarities (Nei 1972) for all pair wise comparisons were greater than 0.988 except for pairs that included the New Zealand strain (Table 3). Genetic similarities for New Zealand pairs ranged from 0.970 to 0.980, and are a reflection of this strain's divergent allelic frequencies especially at SOD, PGM, and MDH-3,4 loci -- see Appendix. The results of cluster analysis (Sneath and Sokal 1973), based on the genetic similarities in Table 3, are shown in Figure 1.

Table 3.--Matrix of genetic similarities for eight strains of FGL rainbow trout (Nei, 1972)

	FL	NZ	SC	WS	A	D	FS	K
FL	1.000							
NZ	0.975	1.000						
SC	0.997	0.971	1.000					
WS	0.995	0.972	0.999	1.000				
A	0.994	0.970	0.992	0.989	1.000			
D	0.995	0.975	0.996	0.993	0.998	0.1000		
FS	0.997	0.979	0.998	0.997	0.988	0.993	1.000	
K	0.998	0.980	0.997	0.996	0.995	0.997	0.997	1.000

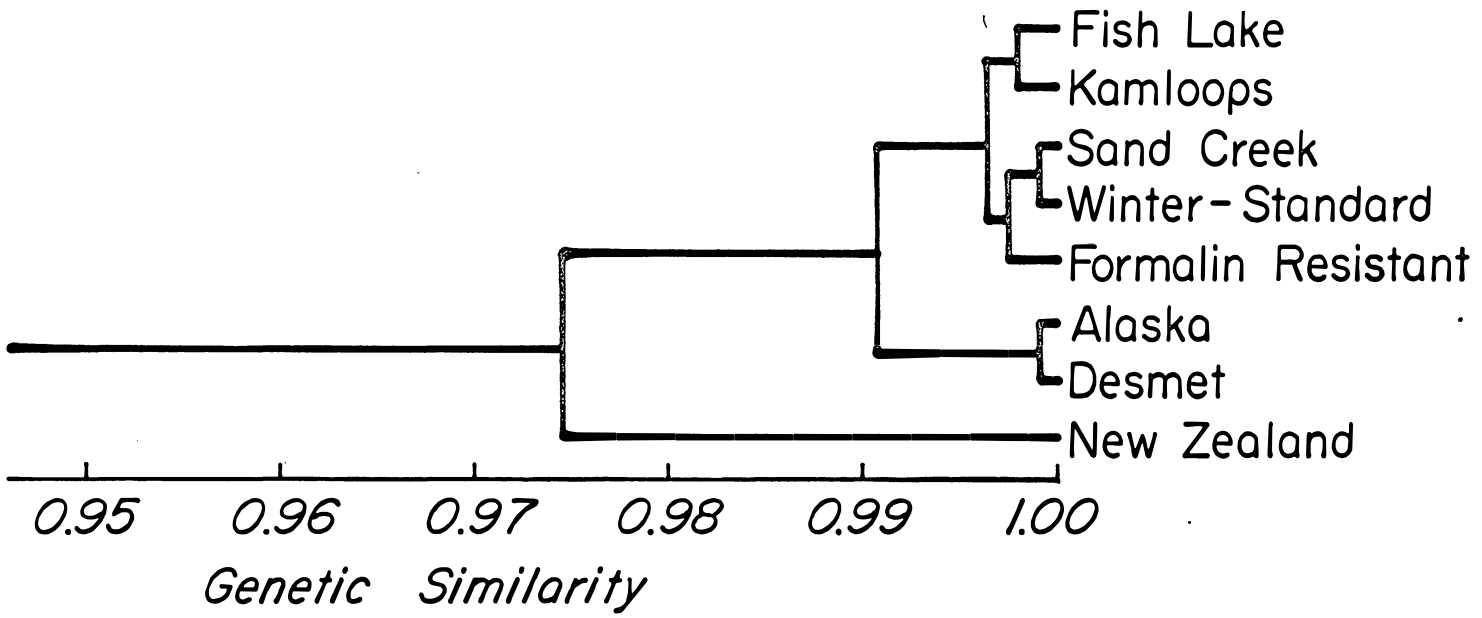

Figure 1.--Dendrogram of genetic similarities.

The clustering of Formalin Selected and Winter Standard strains with Sand Creek is not surprising since the Sand Creek strain was included in the derivation of the other two groups. However, the New Zealand strain, which also contributed to the derivation of the Formalin Selected and Winter Standard groups, was markedly different from all other strains. These data suggest that the Formalin Selected and Winter Standard strains presently have a minimal influence of New Zealand ancestry perhaps reflecting a minor proportion of original New Zealand genes in their derivation.

APPLICATIONS

Important variables in strain improvement programs are within strain, among strain, and total genetic variation. The breeder has a means of estimating these variables and of using the estimates in evaluating and selecting strains best suited for his breeding objectives if protein variation is representative of the total variation of the genome. Negative correlation between genetic similarities of strains reported here and the results of FGL's performance tests of crosses involving the same strains would support this relationship. For example, when using strain crosses to obtain hybrid vigor, genetic theory predicts that the highest probability of success will be attained by using genetically divergent strains (Falconer 1960). The program's efficiency and probability of success would be expected to benefit by using this means to select genetically diverse strains if genetic variation detected by electrophoresis is representative of the total genome. Thus, protein variation may be useful both for monitoring the effects of mating systems on the level of genetic variation and for application in selection programs.

The following applications of protein variation can improve fishery management practices:

(1) Identification of breeding units to better understand the structure of natural populations (Allendorf 1975; Milner and Teel 1979; Ryman et al. 1979; Utter et al. In Press 1979).

(2) Selection of non-indigenous strains with high genetic variability (which correlates with adaptability) for supplementing threatened populations or for developing new fisheries.

(3) Estimation of strain contribution to fisheries of mixed strains (Milner 1977; Seeb and Wishard 1977; Grant 1977).

(4) Determination of the success of hatchery plants or non-native introductions and their effects on native gene pools (Crawford et al. 1978, 1979).

Where appropriate, the effectiveness of these last two applications can be increased by directed alteration of allelic frequencies, thus amplifying electrophoretic differences between strains (Utter et al 1976; Reisenbichler and McIntyre 1977; Seeb and Wishard 1977).

In conclusion, continuation of this project will extend the value of the present analyses in addition to providing new data. Electrophoretic examination of the unsampled FGL strains will allow a more complete delineation of the levels and structure of the genetic variation. In addition, a complete set of data will be available to examine the relationships of genetic similarity estimates and performance results of FGL strain crosses. A complementary effort would include investigating the applicability of methods for estimating individual strain contribution to strains of mixed origin or to fisheries of mixed strains.

LITERATURE CITED

Allendorf, F. W.
1975. Genetic variability in a species possessing extensive gene duplication: Genetic interpretation of duplicate loci and examination of genetic variation in populations of rainbow trout. PH.D. Thesis, Univ. of Wash. 98 pp.

Behnke, R. J.
1979. Monograph of the native trouts of the genus salmo of western North America, 102 pp. Unpublished manuscript.

Busack, C., A. R. Halliburton, and G. A. E. Gall
1979. Electrophoretic variation and differentiation in four strains strains of domesticated rainbow trout (Salmo gairdneri). Can. J. Genet. Cytol, 21: 81-94.

Crawford, B. A., S. A. Leider, J. M. Tipping and M. W. Chilcote
1978. Kalama River steelhead investigations. Wash. State Game Dept., Fish. Res. Rept 111 pp.

Crawford, B. A., S. A. Leider and M. W. Chilcote
1979. Kalama steelhead investigations. Wash. State Game Dept., Fish. Res. Rept 100 pp.

Falconer, D. S.
1960. Introduction to quantitative genetics, New York. The Ronald Press Company, 365 pp.

Grant, S.
1977. Biochemical genetic variation in sockeye salmon: The use of biochemical markers for stock identification in Bristol Bay and Cook Inlet, Alaska. Final Rept., Contract 3131. State of Alaska, Dept. of Fish and Game, Div. of Commercial Fisheries, 38 pp.

May, B.
1975. Electrophoretic variation in the genus Oncorhynchus: The methodology, genetic basis, and practical application to fisheries research and management. M. S. Thesis, Univ. of Wash. 95 pp.

Milner, G. B.
1977. Biochemical genetic variation: Its use in mixed fishery analysis. In: Genetic implications of Steelhead Management, Special Report 77-1, Calif. Coop. Fish. Res. Unit, pp 10-15, T. J. Hassler and R. R. Van Kirk (eds.).

Milner, G. B., and D. J. Teel
1979. (Oct.) Columbia River Stock Identification Study, National Oceanic and Atmospheric Administration, Natl. Mar. Fish. Serv., Northwest & Alaska Fisheries Center, Coastal Zone & Estuarine Studies Div.

Nei, M.
1972. Genetic distance between populations. Amer. Nat. 106:283-292.

Nei, M.
1973. Analysis of gene diversity in subdivided populations. Proc. Nat. Acad. Scie. 70: 3321-3323.

Nei, M.
1975. Molecular population genetics and evolution. New York American Elsevier.

Reisenbichler, R. R. and J. D. McIntyre
1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, Salmo gairdneri. J. Fish. Res. Board, Canada. 34:1223-128.

Ryman, N., F. W. Allendorf and G. Stahl
1979. Reproductive isolation with little genetic divergence in sympatric populations of Brown trout (Salmo trutta). Genetics 92: 247-262.

Seeb, J. and L. N. Wishard
1977. The use of biochemical genetics in the management of Pacific salmon stocks: Genetic marking and mixed fishery analysis. Final Report, Wash. State Dept. of Fish. Service Contract 792.

Sneath, P. H. and R. R. Sokal
1973. Numerical Taxonomy. San Francisco, W. H. Freeman & Company.

Sokal, R. R. and F. J. Rohlf
1969. Biometics, San Francisco, W. H. Freeman, 776 pp.

Utter, F. M., F. W. Allendorf, and B. May
1976. The use of protein variation in the management of salmonid populations. Trans. 41st North American Wildlife and Natural Res. Conference: 373-384. Washington, D.C., Management Inst.

Utter, F. M., D. Campton, S. Grant, G. Milner, J. Seeb and L. Wishard
In press. Population structures of indigenous salmonid species of the Pacific Northwest: I. A within and between species examination of natural populations based on genetic variation of proteins. In: Proc. of Symposium "Salmonid ecosystems of the North Pacific". Oregon State University Press.

Utter, F. M., H. O. Hodgins and F. W. Allendorf
1974. Biochemical genetic studies of fishes: Potentialities and limitations. 213-238. In: D. Malins (Ed.) Biochemical and Biophysical Perspectives in Marine Biology. Vol. 1, New York, Academic Press.

APPENDIX

Allelic Frequencies and Confidence Intervals

Strain 1/	Locus	Number of alleles sampled	Allele2/	Allelic3/ frequency	Confidence4/ Interval
Fish Lake	ADH	168	100	1.00	0.98-1.00
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00
Fish Lake	ALB	264	100	0.61	0.52-0.69
			102	0.39	0.31-0.48
New Zealand		412	100	0.69	0.63-0.75
			102	0.31	0.25-0.37
Sand Creek		380	100	0.44	0.37-0.51
			102	0.56	0.49-0.63
Winter Standard		396	100	0.46	0.39-0.53
			102	0.54	0.47-0.61
Alaska		376	100	0.60	0.52-0.66
			102	0.40	0.34-0.48
Desmet		364	100	0.62	0.55-0.69
			102	0.38	0.31-0.45
Formalin Selected		396	100	0.47	0.40-0.54
			102	0.53	0.46-0.60
Kamloops		392	100	0.57	0.50-0.64
			102	0.43	0.36-0.50

Strain 1/	Locus	Number of alleles sampled	Allele2/	Allelic3/ frequency	Confidence4/ Interval
Fish Lake	CK-1	168	100	1.00	0.98-1.00
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00
Fish Lake	CK-2	168	100	1.00	0.98-1.00
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00

Strain 1/	Locus	Number of alleles sampled	Allele2/	Allelic3/ frequency	Confidence4/ Interval
Fish Lake	EST	168	100	1.00	0.98-1.00
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00
Fish Lake	GOT-1, 2	312	100	1.00	0.99-1.00
New Zealand		368	100	1.00	0.99-1.00
Sand Creek		400	100	1.00	0.99-1.00
Winter Standard		364	100	1.00	0.99-1.00
Alaska		400	100	1.00	0.99-1.00
Desmet		400	100	1.00	0.99-1.00
Formalin Selected		400	100	1.00	0.99-1.00
Kamloops		400	100	1.00	0.99-1.00

Strain 1/	Locus	Number of alleles sampled	Allele2/	Allelic3/ frequency	Confidence4/ Interval
Fish Lake	GOT-3	148	100	1.00	0.98-1.00
New Zealand		186	100	1.00	0.98-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		184	100	1.00	0.98-1.00
Alaska		188	100	1.00	0.98-1.00
Desmet		168	100	1.00	0.98-1.00
Formalin Selected		100	100	1.00	0.97-1.00
Kamloops		180	100	1.00	0.98-1.00
			100	0.78	0.71-0.84
Fish Lake*	GPD-1	168	270	0.22	0.16-0.29
			100	0.98	0.95-0.99
New Zealand		210	270	0.02	0.01-0.05
			100	0.93	0.89-0.96
Sand Creek		200	270	0.07	0.04-0.11
			100	0.98	0.96-0.99
Winter Standard		200	270	0.02	0.01-0.04
Alaska		200	100	1.00	0.99-1.00
			100	0.99	0.97-1.00
Desmet		200	270	0.01	0.00-0.03
			100	0.81	0.75-0.86
Formalin Selected*		200	270	0.19	0.14-0.25
			100	0.87	0.82-0.91
Kamloops		200	270	0.13	0.09-0.18

Strain 1/	Locus	Number of alleles sampled	Allele2/	Allelic3/ frequency	Confidence4/ Interval
Fish Lake	GPD-2	168	100	1.00	0.98-1.00
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00
Fish Lake	GPI-1	168	100	1.00	0.98-1.00
New Zealand		208	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		190	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00

Strain 1/	Locus	Number of alleles sampled	Allele2/	Allelic3/ frequency	Confidence4/ Interval
Fish Lake	GPI-2	168	100	1.00	0.98-1.00
New Zealand		208	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		190	100	1.00	0.98-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00
Fish Lake	GPI-3	168	100	1.00	0.98-1.00
New Zealand		208	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		190	100	1.00	0.98-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00

Strain 1/	Locus	Number of alleles sampled	Allele2/	Allelic3/ frequency	Confidence4/ Interval
			100	0.74	0.67-0.81
			40	0.17	0.12-0.24
			120	0.01	0.00-0.05
Fish Lake	ICD-3, 4	312	71	0.07	0.04-0.12
			100	0.66	0.59-0.72
			40	0.15	0.11-0.21
			120	0.04	0.02-0.07
New Zealand		384	71	0.16	0.11-0.21
			100	0.66	0.59-0.72
			40	0.19	0.14-0.25
			120	0.08	0.05-0.12
Sand Creek		380	71	0.08	0.05-0.12
			100	0.66	0.59-0.73
			40	0.14	0.09-0.20
			120	0.11	0.07-0.17
Winter Standard		312	71	0.09	0.05-0.14
			100	0.83	0.76-0.88
			40	0.15	0.10-0.22
Alaska		288	71	0.02	0.01-0.06
			100	0.68	0.61-0.75
			40	0.20	0.14-0.26
			120	0.09	0.06-0.14
Desmet		352	71	0.03	0.01-0.07
			100	0.63	0.54-0.70
			40	0.17	0.12-0.24
			120	0.12	0.07-0.18
Formalin Selected		264	71	0.09	0.05-0.15
			100	0.83	0.77-0.88
			40	0.08	0.05-0.13
Kamloops		336	71	0.09	0.05-0.14

Strain 1/	Locus	Number of alleles sampled	Allele2/	Allelic[3]/ frequency	Confidence[4]/ Interval
Fish Lake	LDH-1	168	100	1.00	0.98-1.00
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00
Fish Lake	LDH-2	168	100	1.00	0.98-1.00
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00

Strain 1/	Locus	Number of alleles sampled	Allele2/	Allelic3/ frequency	Confidence4/ Interval
Fish Lake	LDH-3	168	100	1.00	0.98-1.00
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00
Fish Lake	LDH-4	168	100	1.00	0.98-1.00
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	0.99	0.96-1.00
			76	0.01	0.00-0.04
Winter Standard		200	100	0.85	0.79-0.89
			76	0.15	0.11-0.21
Alaska		200	100	0.99	0.96-1.00
			76	0.01	0.00-0.04
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00

Strain 1/	Locus	Number of Alleles Sampled	Allele2/	Allele3/ Frequency	Confidence4/ Interval
Fish Lake	LDH-5	168	100	1.00	0.98-1.00
New Zealand		194	100	1.00	0.98-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	0.99	0.97-1.00
			92	0.01	0.00-0.03
Desmet		200	100	1.00	0.99-1.00
Formalin Selected*		190	100	0.96	0.92-0.98
			92	0.04	0.02-0.08
Kamloops		200	100	1.00	0.99-1.00
Fish Lake	MDH-1, 2	168	100	1.00	0.98-1.00
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00

Strain 1/	Locus	Number of alleles sampled	Allele2/	Allelic3/ frequency	Confidence4/ Interval
			100	0.87	0.81-0.91
			122	0.06	0.04-0.11
Fish Lake	MDH-3, 4	324	85	0.06	0.04-0.11
			100	0.60	0.53-0.67
			77	0.36	0.30-0.43
New Zealand		392	91	0.04	0.02-0.08
			100	0.94	0.90-0.97
			85	0.05	0.03-0.09
Sand Creek		388	91	0.01	0.00-0.03
			100	0.89	0.84-0.93
			77	0.05	0.03-0.09
			85	0.05	0.03-0.09
Winter Standard		400	91	0.01	0.00-0.03
			100	0.99	0.97-1.00
			77	0.00	0.00-0.02
Alaska		400	85	0.01	0.00-0.03
			100	0.91	0.86-0.94
Desmet		376	40	0.09	0.06-0.14
			100	0.87	0.81-0.91
			77	0.00	0.00-0.02
			85	0.10	0.07-0.15
Formalin Selected		400	91	0.03	0.01-0.06
			100	0.92	0.89-0.95
			85	0.07	0.05-0.12
Kamloops		400	91	0.01	0.00-0.03

Strain 1/	Locus	Number of alleles sampled	Allele2/	Allelic3/ frequency	Confidence4/ Interval
			100	0.87	0.81-0.91
Fish Lake	ME-2	164	87	0.13	0.09-0.19
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
			100	0.81	0.75-0.86
Alaska*		194	87	0.19	0.14-0.25
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00
Fish Lake	MPI	168	100	1.00	0.98-1.00
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00

Strain 1/	Locus	Number of alleles sampled	Allele2/	Allelic3/ frequency	Confidence4/ Interval
			100	0.94	0.89-0.97
Fish Lake	PEP-1	168	111	0.06	0.03-0.11
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00
Fish Lake	PEP-2	168	100	1.00	0.98-1.00
New Zealand		184	100	1.00	
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00

Strain 1/	Locus	Number of alleles sampled	Allele2/	Allelic3/ frequency	Confidence4/ Interval
Fish Lake	PEP-3	168	100	1.00	0.98-1.00
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	0.99	0.97-1.00
			129	0.01	0.00-0.03
Kamloops		200	100	1.00	0.99-1.00
Fish Lake	PEP-4	168	100	1.00	0.98-1.00
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00

Strain 1/	Locus	Number of alleles sampled	Allele2/	Allelic3/ frequency	Confidence4/ Interval
Fish Lake	PEP-5	168	100	1.00	0.98-1.00
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00
Fish Lake	PEP-6	136	100	1.00	0.97-1.00
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00

Strain 1/	Locus	Number of alleles sampled	Allele2/	Allelic3/ frequency	Confidence4/ Interval
Fish Lake	PGD	168	100	1.00	0.98-1.00
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00
			100	0.89	0.83-0.93
Fish Lake	PGM	168	90	0.11	0.07-0.17
			100	0.19	0.14-0.25
New Zealand		204	90	0.81	0.75-0.86
			100	0.95	0.91-0.97
Sand Creek*		200	90	0.05	0.03-0.09
			100	0.90	0.89-0.94
Winter Standard		200	90	0.10	0.06-0.14
			100	0.99	0.96-1.00
Alaska		200	90	0.01	0.00-0.04
			100	0.98	0.95-0.99
Desmet		198	90	0.02	0.01-0.05
			100	0.77	0.70-0.82
Formalin Selected		180	90	0.23	0.18-0.30
			100	0.80	0.74-0.85
Kamloops		200	90	0.20	0.15-0.26

Strain 1/	Locus	Number of alleles sampled	Allele2/	Allelic3/ frequency	Confidence4/ Interval
			100	0.83	0.77-0.88
Fish Lake	SOD	168	152	0.17	0.12-0.23
			100	0.64	0.58-0.71
New Zealand		200	152	0.36	0.29-0.42
			100	0.91	0.86-0.94
Sand Creek		200	152	0.09	0.06-0.14
			100	0.98	0.96-0.99
Winter Standard		200	152	0.02	0.01-0.04
			100	0.53	0.46-0.60
Alaska*		200	152	0.47	0.40-0.54
			100	0.61	0.55-0.68
			152	0.38	0.32-0.45
Desmet		200	38	0.01	0.00-0.03
			100	0.93	0.89-0.96
Formalin Selected		196	152	0.07	0.04-0.11
			100	0.74	0.68-0.80
			152	0.21	0.16-0.27
Kamloops*		200	38	0.05	0.03-0.09
Fish Lake	XDH	168	100	1.00	0.98-1.00
New Zealand		210	100	1.00	0.99-1.00
Sand Creek		200	100	1.00	0.99-1.00
Winter Standard		200	100	1.00	0.99-1.00
Alaska		200	100	1.00	0.99-1.00
Desmet		200	100	1.00	0.99-1.00
Formalin Selected		200	100	1.00	0.99-1.00
Kamloops		200	100	1.00	0.99-1.00

1/ An asterisk indicates that the distribution of genotypes significantly ($\alpha = 0.05$) departed from Hardy-Weinberg proportions.

2/ Alleles are designated by their mobility relative to the typically most common allele (100) at each locus.

3/ Two loci that could not be electropheoretically distinguished (ALB, GOT, ICD, and MDH) were considered one locus with four independent alleles to simplify the calculation of allelic frequencies.

4/ 95 percent chi-square confidence interval.