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With 132 Figures

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# Biochemical Variants in Pacific Salmon and Rainbow Trout: Their Inheritance and Application in Population Studies

F. M. Utter, H. O. Hodgins, F. W. Allendorf, A. G. Johnson, and J. L. Mighell

## 1. Abstract

Data are presented supporting hypotheses of Mendelian inheritance for biochemical genetic variation in three species of Pacific salmon (*Oncorhynchus* spp.) and in rainbow trout (*Salmo gairdneri*). Variants studied included: chinook salmon (*O. tshawytscha*)--tetrazolium oxidase; sockeye salmon (*O. nerka*)--phosphoglucomutase; coho salmon (*O. kisutch*) --transferrin; rainbow trout--alpha glycerophosphate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, tetrazolium oxidase and transferrin. Variation in the frequencies of these polymorphisms among populations indicates a usefulness of these variants for the identification and characterization of populations.

## 2. Introduction

The use of biochemical genetic variants for characterizing populations of fishes has accelerated in recent years (see De Ligny, 1969, 1972). In most of these studies the allelic nature of particular variations had to be inferred because it was difficult or impossible to carry out direct breeding studies. In our investigations we have inferred a genetic basis for the variation that we have seen by lines of evidence including: 1. starch gel electrophoretic patterns for particular proteins that are consistent with genetic variants of the same proteins in other species, 2. repeatability of expression from duplicate samplings of a given individual, 3. stability of expression over long developmental periods, and 4. conformance of frequencies of phenotypes to a Hardy-Weinberg statistical distribution. While the above criteria cumulatively provide strong evidence for allelism, the strongest data are from breeding experiments.

This paper presents family data for biochemical genetic variants in three species of Pacific salmon (*Onchorhynchus* spp.) and in rainbow trout (*Salmo gairdneri*) and gives population data for these variants and similar variants in related species. Hypotheses of Mendelian inheritance are supported and differences within species for many of these variants are demonstrated and discussed.

## 3. Experimental Procedures

Parents of progeny used in this study were obtained as follows: coho salmon (*O. kisutch*)--Washington State Department of Fisheries; chinook (*O. tshawytscha*) and sockeye (*O. nerka*)--adult fish returning to the NMFS Northwest Fisheries Center Laboratory in Seattle; rainbow trout--adult fish reared at the Seattle Center; and anadromous rainbow trout (steelhead) from the Chambers Creek Hatchery of the Washington State Department of Game. Crosses were made after the parental phenotypes were determined from electrophoresis. The methods used for handling eggs and sperm were those reported by Poon and Johnson (1970). All

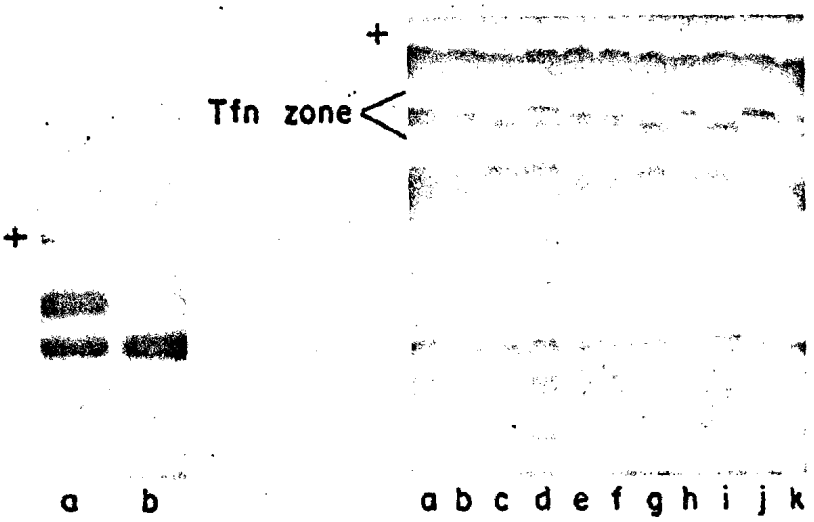


Fig. 1 Fig. 2

Fig. 1. PGM variation in sockeye salmon. a - heterozygote, b - common homozygote

Fig. 2. Transferrin phenotypes in coho salmon. a, b, e, f - BC; c, g, i - CC; d, h, k - AC; j - AA. The same phenotypes also occur in rainbow trout

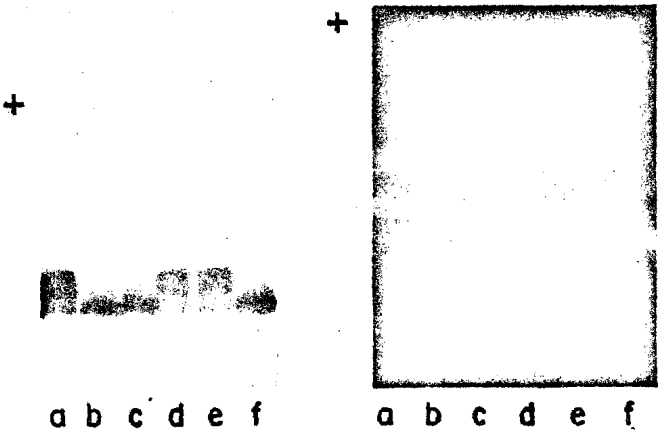


Fig. 3 Fig. 4

Fig. 3. AGPD variants in rainbow trout: a, d, e - heterozygotes; b, c, f - common homozygotes

Fig. 4. TO phenotypes in rainbow trout: a, b - AB; c, d, f - BB; e - AA

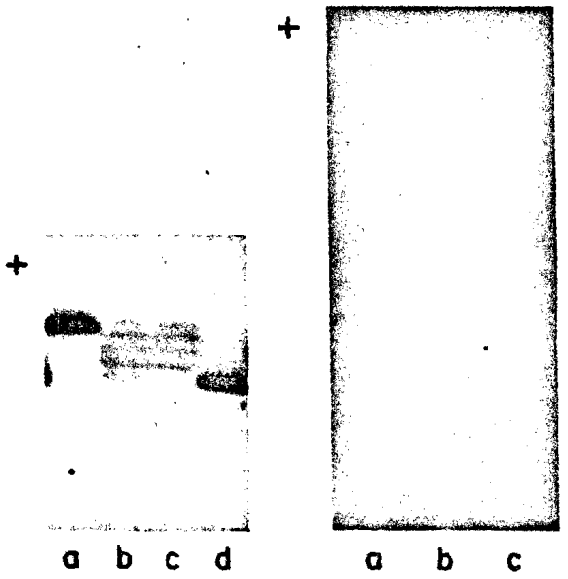


Fig. 5 Fig. 6

Fig. 5. LDH phenotypes of rainbow trout livers: a - $B^{2'}B^{2'}$; b, c - $B^{2'}B^{2''}$; d - $B^{2''}B^{2''}$

Fig. 6. MDH variants of rainbow trout: a, c - BB'; b - B'B'. Note asymmetry of heterozygous bands

progeny were hatched and reared under similar conditions at this Center. Chinook and sockeye salmon progeny were tested between 2 and 4 months after hatching. Coho salmon and rainbow trout were tested between 6 and 9 months after hatching; somewhat larger fish were required from these species because a blood sample (difficult to obtain from small fish) was needed to test for transferrin.

All biochemical systems but transferrin (Tfn) were found in skeletal muscle extracted as described by Utter and Hodgins (1970). Blood plasma for Tfn typing was obtained by withdrawing blood from the pericardial cavity of freshly killed fish with a capillary pipette. Approximately one drop of whole blood was expressed into two drops of Alsever's solution (a citrate-dextrose-saline anticoagulant) in 10 x 75-mm culture tubes. Each sample was centrifuged at 1 000 x g for 3 minutes before testing.

Details of electrophoresis were described by Utter and Hodgins (1969). Buffer systems for the respective biochemical variants were those described by Utter and Hodgins (1972). Specific staining methods for enzymes followed those described by Shaw and Prasad (1970). Tfn was detected by a non-specific protein staining method using a 0.1% nigrosin-buffalo black solution dissolved in a 5:4:1 water-methanol-acetic acid mixture. Destaining was carried out with the water-methanol-acetic acid solution.

## 4. Biochemical Variants

Descriptions of each of the biochemical systems studies in this report have been given elsewhere (Hodgins, Ames and Utter, 1969; Utter, Ames

Table 1. PGM phenotypes of parents and progeny in sockeye salmon matings[a]

| | Progeny phenotypes | | | Phenotypes of parents | |
|---|---|---|---|---|---|
| Lot | AA | AB | BB | Male | Female |
| 1 | 0(0) | 90(99.5) | 109(99.5) | AB | BB |
| 2 | 0(0) | 90(98.5) | 107(98.5) | BB | AB |
| 3 | 0(0) | 78(84) | 90(84) | BB | AB |
| Control | 0 | 0 | 100 | BB | BB |

[a]Parenthetical figures in Tables 1 through 8 represent expected numbers assuming Mendelian inheritance

Table 2. TO phenotypes of parents and progeny in chinook salmon matings

| | Progeny phenotypes[a] | | | Phenotypes of parents | |
|---|---|---|---|---|---|
| Lot | EE | EF | FF | Male | Female |
| 2-10 | 12(10.5) | 9(10.5) | 0(0) | EF | EE |
| 2-11 | 12(10) | 21(20) | 7(10) | EF | EF |
| 2-13 | 10(10) | 20(20) | 10(10) | EF | EF |
| 4-14 | 8(11) | 14(11) | 0(0) | EE | EF |
| 5-10 | 0(0) | 29(30) | 1(0) | EE | FF |
| 5-12 | 0(0) | 40(40) | 0(0) | FF | EE |
| 7-13 | 0(0) | 14(14.5) | 15(14.5) | FF | EF |
| Control | 100 | 0 | 0 | EE | EE |

[a]The designation of TO alleles conforms to that of Utter, Allendorf, and Hodgins (1973) and differs from that originally described by Utter (1971)

Table 3. Transferrin phenotypes of parents and progeny in coho salmon matings

| | Progeny phenotypes | | | | | | Phenotypes of parents | |
|---|---|---|---|---|---|---|---|---|
| Lot | AA | AC | CC | AB | BB | BC | Male | Female |
| 21 | 0 | 60(63.5) | 67(63.5) | 0 | 0 | 0 | CC | AC |
| 22 | 0 | 61(64.5) | 68(64.5) | 0 | 0 | 0 | CC | AC |
| 23 | 0 | 28(28) | 29(28) | 30(28) | 0 | 25(28) | BC | AC |
| 32 | 0 | 0 | 45(35.5) | 0 | 0 | 26(35.5) | CC | BC |
| 34 | 0 | 36(45.5) | 48(45.5) | 45(45.5) | 0 | 53(45.5) | AC | BC |
| 51 | 0 | 0 | 57(64) | 0 | 0 | 71(64) | BC | CC |
| 53 | 0 | 64(52.5) | 41(52.5) | 0 | 0 | 0 | AC | CC |
| Control | 0 | 0 | 20 | 0 | 0 | 0 | CC | CC |

Table 4. TO phenotypes of parents and progeny in rainbow trout matings

| | Progeny phenotypes | | | | | | Phenotypes of parents | |
|---|---|---|---|---|---|---|---|---|
| Lot | AA | AB | BB | AC | BC | CC | Male | Female |
| 1301 | 0(0) | 19(20) | 21(20) | 0(0) | 0(0) | 0(0) | BB | AB |
| A10 | 0(0) | 65(60) | 55(60) | 0(0) | 0(0) | 0(0) | BB | AB |
| A17 | 0(0) | 60(50) | 40(50) | 0(0) | 0(0) | 0(0) | BB | AB |
| SH2 | 0(0) | 20(21) | 22(21) | 0(0) | 0(0) | 0(0) | AB | BB |
| A23 | 0(0) | 105(104) | 103(104) | 0(0) | 0(0) | 0(0) | AB | BB |
| A24 | 0(0) | 67(65) | 63(65) | 0(0) | 0(0) | 0(0) | AB | BB |
| A29 | 0(0) | 38(44) | 50(44) | 0(0) | 0(0) | 0(0) | AB | BB |
| 4347 | 0(0) | 0(0) | 18(15) | 0(0) | 26(30) | 16(15) | BC | BC |
| Control | 0 | 0 | 200 | 0 | 0 | 0 | BB | BB |

Table 5. Tfn phenotypes of parents and progeny in rainbow trout matings

| | Progeny phenotypes | | | Phenotypes of parents | |
|---|---|---|---|---|---|
| Lot | AA | AC | CC | Male | Female |
| 1301 | 7(8.3) | 22(16.4) | 4(8.3) | AC | AC |
| 5641 | 32(30) | 28(30) | 0(0) | AC | AA |
| Control | 50 | 0 | 0 | AA | AA |

Table 6. AGPD phenotypes of parents and progeny in rainbow trout matings

| | Progeny phenotypes | | | Phenotypes of parents | |
|---|---|---|---|---|---|
| Lot | AA | AB | BB | Male | Female |
| SH2 | 0(0) | 22(21) | 20(21) | AB | BB |
| A24 | 0(0) | 62(64.5) | 67(64.5) | AB | BB |
| A28 | 0(0) | 10(10) | 10(10) | BB | AB |
| Control | 0 | 0 | 140 | BB | BB |

Table 7. LDH phenotypes of parent and progeny in rainbow trout matings

| | Progeny phenotypes | | | Phenotypes of parents | |
|---|---|---|---|---|---|
| Lot | $B^{2'}B^{2'}$ | $B^{2'}B^{2''}$ | $B^{2''}B^{2''}$ | Male | Female |
| SH8 | 0(0) | 45(45) | 0(0) | $B^{2''}B^{2''}$ | $B^{2'}B^{2'}$ |
| SH14 | 0(0) | 11(11) | 0(0) | $B^{2''}B^{2''}$ | $B^{2'}B^{2'}$ |
| A5 | 0(0) | 28(28) | 0(0) | $B^{2''}B^{2''}$ | $B^{2'}B^{2'}$ |
| A10 | 52(55) | 58(55) | 0(0) | $B^{2'}B^{2'}$ | $B^{2'}B^{2''}$ |
| A17 | 58(50) | 42(50) | 0(0) | $B^{2'}B^{2''}$ | $B^{2'}B^{2'}$ |
| A18 | 11(12) | 13(12) | 0(0) | $B^{2'}B^{2''}$ | $B^{2'}B^{2'}$ |
| A20 | 25(27) | 29(27) | 0(0) | $B^{2'}B^{2''}$ | $B^{2'}B^{2'}$ |
| A23 | 129(129) | 129(129) | 0(0) | $B^{2'}B^{2''}$ | $B^{2'}B^{2'}$ |
| A28 | 15(16.5) | 18(16.5) | 0(0) | $B^{2'}B^{2''}$ | $B^{2'}B^{2'}$ |
| A29 | 50(44) | 38(44) | 0(0) | $B^{2'}B^{2''}$ | $B^{2'}B^{2'}$ |
| A4 | 0(0) | 51(50) | 49(50) | $B^{2''}B^{2''}$ | $B^{2'}B^{2''}$ |
| Control | 200 | 0 | 0 | $B^{2'}B^{2'}$ | $B^{2'}B^{2'}$ |

Table 8. MDH phenotypes of parents and progeny in rainbow trout matings

| | Progeny phenotypes | | | Phenotypes of parents | |
|---|---|---|---|---|---|
| Lot | B'B' | BB' | BB | Male | Female |
| A4 | 43(50) | 57(50) | 0(0) | B'B' | BB' |
| A10 | 52(55) | 58(55) | 0(0) | BB' | B'B' |
| A17 | 54(50) | 46(50) | 0(0) | BB' | B'B' |
| A20 | 16(19.5) | 23(19.5) | 0(0) | BB' | B'B' |
| A23 | 103(109) | 115(109) | 0(0) | BB' | B'B' |
| A24 | 70(63.5) | 57(63.5) | 0(0) | BB' | B'B' |
| A16 | 14(20) | 45(40) | 21(20) | BB' | BB' |
| Control | 68 | 0 | 0 | B'B' | B'B' |

and Hodgins, 1970; Utter and Hodgins, 1970; Hodgins and Utter, 1971; Utter, 1971; Utter and Hodgins, 1972). These include lactate dehydrogenase (LDH) and phosphoglucomutase (PGM, Fig. 1) in sockeye salmon; tetrazolium oxidase (TO) in chinook salmon; Tfn (Fig. 2) in coho salmon and in rainbow trout; alpha glycerophosphate dehydrogenase (AGPD, Fig. 3), TO (Fig. 4), LDH (Fig. 5), and malate dehydrogenase (Fig. 6).

The phenotypes of most of the systems studied here are codominantly expressed on starch gel electrophoresis. Thus the presumed genotype can be interpreted directly from a given phenotype. The data for the fully codominant systems are given in Tables 1 through 8. In these systems, the observed phenotypes of the parents and progeny are presented and the expected numbers of progeny--assuming simple Mendelian inheritance--are shown in parentheses. In systems involving two alleles, all three possible phenotypes (the two homozygous phenotypes and the heterozygote) are listed, although some of these phenotypes may not be expected to occur from these matings. Similarly, all six possible genotypes are listed in systems involving three alleles. Controls are data from one or more matings where both parents have the same homozygous genotype.

Data from most crosses conform to expected Mendelian proportions based on parental phenotypes. The only qualitative exception to Mendelian inheritance in all of the matings is seen in Table 2 for TO phenotypes in chinook salmon progeny, lot 5-10. In the EE x FF cross, only heterozygous progeny are expected; however, one FF individual was found in this lot. It seems most likely that this individual is from another lot and had been placed in the wrong holding tank, because the remaining individuals were all of the expected phenotype. A quantitative deviation from expected ratios was observed in the cumulative totals of the sockeye salmon PGM crosses (Table 1) where an excess of homozygous progeny--though not significant in any single mating--became significant when the data were pooled ($X^2$ - 4.04, d.f. - 1, .05>P>.01), suggesting a selective factor favoring the BB phenotype.

A possible exception to the rule of codominance occurs in MDH variants of rainbow trout (Table 8). Bailey et al. (1970) observed that two loci of rainbow trout appear to code for MDH subunits (B') giving rise to active enzymes having the same electrophoretic mobility. One of these loci also coded for an allelic subunit (B). Because the B' sub-

unit was synthesized in all individuals, it was impossible to qualitatively differentiate between BB' heterozygotes and BB homozygotes. However, they were able to quantitatively differentiate these two genotypes on the basis of different intensities of staining of bands having the same mobilities. Our MDH phenotypes are similar to those described by Bailey et al. (1970) and support their hypothesis of duplicate loci.

The finding that data from each of our crosses are consistent with an assumption of Mendelian inheritance for the genes controlling the observed biochemical variants was expected and virtually eliminated the possibility that these variants could be artifacts reflecting different environmental stresses or exposures (although they may indirectly reflect environmental differences through processes of natural selection). The data also indicate that these variants do not vary between the juvenile life history stage and the time of spawning. Finally, the data give strong support for hypotheses of Mendelian inheritance for similar (probably homologous) variants of other salmonid species where family data have not yet been obtained.

## 5. Population Studies

Much of our effort has been directed towards studies of the variants described above (and their homologs in related species) in natural populations for use as genetic markers (see Utter et al., 1972). We summarize some of these findings below.

### Sockeye Salmon

We have found two-allele polymorphisms for LDH ($B^2$ locus) and for PGM in sockeye salmon (Hodgins, Ames and Utter, 1969; Hodgins and Utter, 1971; Utter and Hodgins, 1970). A distinct cline has been observed for both systems (Table 9). A virtual absence of the variant LDH allele ($B^2$) has been observed in fish taken from the Skeena River (B.C.) southward, while both alleles ($B^{2'}$ and $B^2$) have been regularly observed in samples taken from southeastern Alaska westward through the Kamchatka Peninsula, Siberia. The geographic variation in the distribution of the PGM alleles is more quantitative, but clear differences between regions are observed.

Table 9. Distribution of LDH and PGM variants in sockeye salmon

| Variant | Area | Frequency of less common allele | Number of fish |
|---|---|---|---|
| LDH ($B^2$ allele) | Kamchatka Pen. | .084 | 101 |
| | Bristol Bay | .120 | 750 |
| | Copper River | .117 | 115 |
| | Southeastern Alaska | .022 | 90 |
| | Skeena R., southward | .002 | 591 |
| PGM (A allele) | Bristol Bay | .292 | 406 |
| | Southeastern Alaska | .205 | 90 |
| | Puget Sound | .080 | 87 |

## Chinook Salmon

Polymorphisms have been reported for three systems in chinook salmon: TO (Utter, 1971), MDH (Bailey et al., 1970), and sorbitol dehydrogenase (Utter et al., 1972). We report here only on the TO variants because of the low frequencies of the variants in the other systems. A third TO allele (B) has been observed in chinook salmon (Utter et al., 1972); data for this allele have been pooled with E allele data because of its infrequent occurrence. Considerable heterogeneity is seen in the frequency of the E and F alleles in different areas (Table 10). Although no clear geographic patterns are evident, the lower TO allele frequencies of spring run fish suggests a possible relationship between TO alleles and ecological factors.

## Coho Salmon

A three-allele transferrin system was reported in coho salmon (Utter et al. 1970) that had a markedly different distribution in populations sampled from the Columbia River and Puget Sound. These differences have persisted in samples tested from additional areas and for different year classes (Table 11). The gene frequencies of the Puget Sound and Washington Coast samples are generally similar but are very different from those of the Columbia River. The B allele, having a frequency between 10% and 35% in other areas, is completely absent in Columbia River samples. The A allele is the sole or predominant gene in Columbia River fish, whereas the C allele is found most frequently in other areas.

It is difficult to explain these major differences between Columbia River fish and those taken from other areas on the basis of random factors alone. The Willipa, Nemah, and Chehalis Rivers are as close or closer to the Columbia River tributaries as they are to other coastal or Puget Sound streams, yet their gene frequencies are typical of the latter group. It may be that the A allele favorably or the B allele infavorably interacts with selective forces associated with the Columbia River system while such interactions are not operating in the other (much smaller) river systems. Regardless of cause, these differences of distribution of transferrin alleles appear to have much potential for identifying Columbia River coho salmon in areas of the Pacific Ocean where they mix with fish originating from other areas.

Table 10. Frequencies of the $TO^F$ allele in populations of chinook salmon

| Area | F allele frequency | Number of fish |
|---|---|---|
| Columbia River | | |
| Rapid River (spring run) | .088 | 98 |
| Wind River (spring run) | .156 | 80 |
| Kalama River (fall run) | .512 | 207 |
| Puget Sound | | |
| Skagit River (fall run) | .416 | 77 |
| Lake Washington (fall run) | .300 | 60 |
| Green River (fall run) | .274 | 42 |
| Skykomish River (fall run) | .198 | 58 |
| Alaska | | |
| Taku River (spring run) | .103 | 97 |

Table 11. Frequencies of transferrin alleles of coho salmon taken from three areas of Washington State

| Area | Gene Frequency A | B | C | Number of Fish |
|---|---|---|---|---|
| Puget Sound | | | | |
| Quilcene River | .200 | .125 | .675 | 60 |
| Issaquah Creek | .235 | .170 | .595 | 100 |
| Green River | .282 | .155 | .563 | 87 |
| Minter Creek | .357 | .140 | .503 | 84 |
| Skykomish River | .149 | .104 | .747 | 67 |
| Washington Coast | | | | |
| Dungeness River | .287 | .266 | .447 | 47 |
| Soleduck River | .190 | .220 | .590 | 50 |
| Chehalis River | .373 | .216 | .411 | 51 |
| Willipa River | .280 | .350 | .370 | 50 |
| Nemah River | .300 | .180 | .520 | 50 |
| Columbia River | | | | |
| Elokomin River | .786 | .000 | .214 | 98 |
| Toutle River | .980 | .000 | .020 | 100 |
| Speelyai Creek | 1.000 | .000 | .000 | 99 |
| Cowlitz River | 1.000 | .000 | .000 | 50 |

Rainbow Trout

Genetic variants at six loci were described for four populations of rainbow trout by Utter and Hodgins (1972); these observations are extended here (Table 12). A revised notation for transferrin alleles is introduced here because it was found that the steelhead (i.e. anadromous rainbow trout) had an allele (B) not found in other groups tested. It was previously assumed that the common transferrin allele of other rainbow trout populations studied (A) was fixed in steelhead.

Table 12. Frequencies of most common alleles[a] of various polymorphisms found in rainbow trout

| Area | LDH | TO | PGM | AGPD | MDH | Transferrin A | B | C | Number of fish |
|---|---|---|---|---|---|---|---|---|---|
| Chambers Cr., Wash., steelhead | .83 | .72 | 1.00 | .99 | .80 | 0 | 1.00 | 0 | 168 |
| Clearwater R., Idaho, steelhead | .29 | 1.00 | .99 | 1.00 | .97 | 0 | 1.00 | 0 | 72 |
| Entiat, Wash. | 1.00 | .36 | .96 | .79 | 1.00 | 1.00 | 0 | 0 | 45 |
| Quilcene, Wash. | 1.00 | .70 | .90 | .81 | .99 | 1.00 | 0 | 0 | 45 |
| Arlington, Wash. | 1.00 | .85 | .99 | 1.00 | .51 | .93 | 0 | .07 | 85 |
| Soap Lake, Wash. | .68 | .85 | 1.00 | 1.00 | .92 | .89 | 0 | .11 | 37 |

[a]Excepting the transferrin locus where frequencies of all alleles are given.

Biochemical genetic variants clearly have much potential for characterizing populations of rainbow trout. Each trout population may be distinguished from any other listed in Table 12 on the basis of a characteristic biochemical genetic profile. The two steelhead populations are the only ones having the Tfn B allele and are distinguishable from each other by very different LDH allelic frequencies. The Entiat and Quilcene fish are the only groups having a sizeable degree of AGPD polymorphism and differ from each other in distribution of TO alleles. The Arlington fish have a much greater amount of MDH variation than any other group and the Soap Lake fish are the only non-steelhead group having LDH polymorphism.

## 6. Summary

In summary, we have presented data demonstrating the Mendelian inheritance of biochemical genetic variants at six loci in four species of Pacific salmonid fishes and have presented data indicating that this variation appears to be useful for separating and characterizing salmonid populations.

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