GENE DUPLICATION WITHIN THE FAMILY SALMONIDAE: DISOMIC INHERITANCE OF TWO LOCI REPORTED TO BE TETRASOMIC IN RAINBOW TROUT

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ABSTRACT

We describe our studies of the genetics of allelic variation for NADP-dependent isocitrate dehydrogenase (IDH) in rainbow trout (*Salmo gairdneri*). Five populations of rainbow trout were studied to determine the phenotypic distribution of IDH; 453 progeny from a number of controlled matings were examined to determine the nature of inheritance of these alleles. The variation was found to be the result of four alleles producing protein subunits of differing electrophoretic mobilities. Progeny from crosses clearly demonstrated the presence of two disomic loci controlling the variation, rather than one tetrasomic locus as had been previously reported. These findings support our contention that the hypothesis of a tetraploid event in salmonid evolution should not be uncritically accepted.

A great deal has been published in the past few years concerning the extensive gene duplication that has been found in salmonid fishes (MASSARO and MARKERT 1968; BAILEY *et al*. 1970; ENGEL, OP'T HOF and WOLF 1970; WOLF, ENGEL and FAUST 1970; ALTUKHOV *et al*. 1972). Based on DNA content as well as chromosome arm number and morphology it has been postulated that this gene duplication has arisen through tetraploidization (OHNO, WOLF and ATKIN 1968; OHNO *et al*. 1969). This interpretation has been questioned by MORRISON (1970) who wrote, ". . . trout are most likely not recent tetraploids if they are tetraploids at all." He based this statement on data which showed that of approximately thirty loci investigated in trout, none follow a pattern of tetrasomic inheritance. Recently, however, three papers have been published reporting instances of tetrasomic inheritance in salmonids. Variants of isocitrate dehydrogenase (IDH) in rainbow trout (*Salmo gairdneri*) were concluded to reflect tetrasomic inheritance on the basis of the observed phenotypic distribution (WOLF, ENGEL and FAUST 1970). Two other papers (ENGEL, OP'T HOF and WOLF 1970; STEGEMAN

and GOLDBERG 1972) reporting examples in rainbow trout and brook trout (*Salvelinus fontinalis*) are discussed in the final section of our present paper.

This paper reports an examination of the nature of inheritance of IDH in rainbow trout through biochemical genetic analysis of populations and families of this species. A better understanding of this variation is needed if such variation is to find optimal use in evolutionary and population studies.

MATERIALS AND METHODS

Tissue extracts were prepared and horizontal starch gel electrophoresis was accomplished following the methods of UTTER and HODGINS (1970). The variation described in this paper was most clearly expressed in liver extracts and was presumed to represent the supernatant form of NADP-dependent IDH (HENDERSON 1965, 1968; WOLF, ENGEL and FAUST 1970). A phosphate buffer described by WOLF, ENGEL and FAUST was used. A potential of 125 volts was applied across the starch for 4 hours. Staining was accomplished by incubating the gels in 100 ml of a tris-citrate buffer (pH 7.0) containing the following components: 40 mg DL sodium isocitrate, 10 mg NADP, 10 mg nitro blue tetrazolium, 10 mg phenazine methyl sulfate, and 50 mg magnesium chloride.

Sexually mature anadromous rainbow trout were obtained from the South Tacoma Hatchery of the Washington State Department of Game and the intact carcasses transported to the laboratory. The sex products were then removed, placed in individual plastic bags, and stored at 5°C overnight. Tissue samples were taken from these fish and tested electrophoretically. On the basis of these results, selected matings were made the following day. Progeny testing was initiated when the fry reached sufficient size (approximately 3 cm) for their livers to be easily removed.

The fish used in the population studies were placed on ice and shipped to this laboratory. Upon arrival, the livers were removed and frozen individually at —20°C. All samples were tested within 30 days of arrival. Population sampling data are presented in Table 1. All of these populations represent the anadromous form of rainbow trout. Population 1 is from a Puget Sound area stream. Populations 2 to 5 are from the Columbia River drainage and range in location from 40 to 1,400 km upstream from the ocean, in ascending order.

RESULTS AND INTERPRETATIONS

IDH phenotypes: Examination of these rainbow trout stocks revealed a system indistinguishable from that described by WOLF, ENGEL and FAUST (1970). To lessen any possible confusion, their system of nomenclature has been adopted. Nine different phenotypes, ranging from a single band to a six-banded type (see Figures 1 and 2), were observed. These phenotypes can best be explained by assuming there are four alleles of differing mobilities with the active enzyme

TABLE 1

Population sampling data

| Stream of origin	Date collected	Cooperating agency	Stage of maturity
1. Chambers Creek, Wash.	Feb 72	Wash. State Department of Game	Mature
2. Big Creek, Ore.	Sep 72	Oregon Game Commission	Fingerling
3. Deschutes, Ore.	Sep 72	Oregon Game Commission	Fingerling
4. Snake River, Idaho	Aug 72	Idaho Fish and Game Department	Fingerling
5. Pahsimeroi River, Idaho	Aug 72	Idaho Fish and Game Department	Fingerling

Position of
homodimeric bands
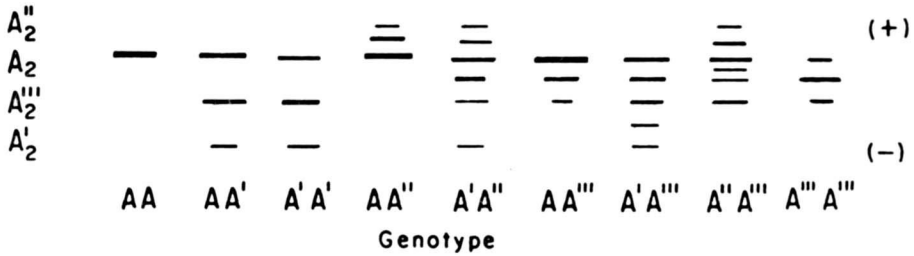
FIGURE 1.—Diagrammatic representation of the nine phenotypes of supernatant NADP-dependent IDH observed in rainbow trout. The genotypes listed below refer to the polymorphic locus, with the other locus assumed to be AA in all cases.

behaving as a dimeric molecule (DARNALL and KLOTZ 1972). Based on the number and relative intensity of bands, four cistrons coding for this enzyme were presumed to be present in each individual.

Population studies: Table 2 presents the distribution of IDH types found in the populations examined. The most striking feature of the data is the uniformity of allele frequencies (see Table 3). Examination of the same populations for other biochemical loci (α-glycerophosphate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, esterase, tetrazolium oxidase, and transferrin) revealed widely varying allele frequencies (ALLENDORF, unpublished data). Although the reason for this uniformity is not apparent, it is clear that the pattern of geographical variation seen for IDH is an exception to what we have observed for other loci.

Mating Experiments

Selected families from the previously described matings were examined to demonstrate the genetic relationship of the alleles involved. These results are presented in Table 4.
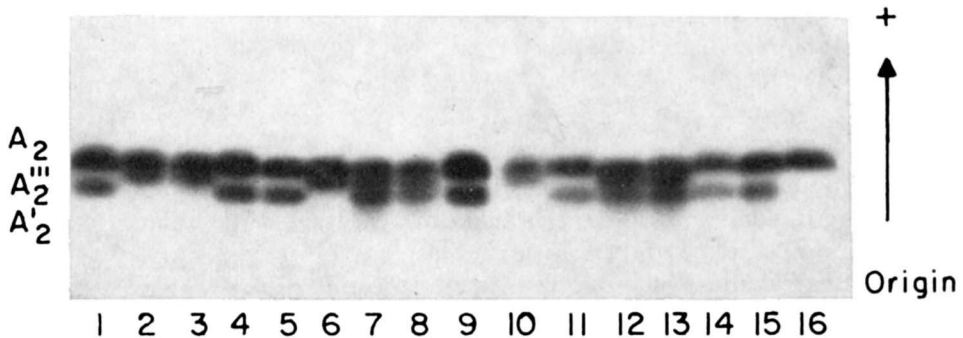

FIGURE 2.—Example of zymograms used in classifying progeny. These fish are from Lot #29 (AA' x AA'''). Four phenotypes representing the following genotypes are visible in the picture —AA (16); AA' (1,4,5,9,11,14,15); AA''' (2,3,6,10); A'A''' (7,8,12,13).

TABLE 2

Observed and expected (assuming Hardy-Weinberg distribution and disomic inheritance) phenotypic distribution in the populations examined

Population	Phenotype									
	AAAA	AAAA'	AAA'A'	AAAA'''	AAA'A'''	AAA''A'''	AAAA''	AAA'A''	AAA''A'''	AAA''A''
Chambers Creek	2	8	1	9	7	8	0	1	4	0
	(2.7)	(4.6)	(1.9)	(9.4)	(7.9)	(8.1)	(1.2)	(1.1)	(2.2)	(0.1)
Big Creek	2	8	2	15	6	5	0	1	1	0
	(4.6)	(6.5)	(2.3)	(10.9)	(7.7)	(6.4)	(0.5)	(0.4)	(0.6)	(0.0)
Deschutes River	0	7	2	2	7	2	0	5	2	0
	(0.8)	(3.9)	(4.8)	(2.6)	(6.4)	(2.1)	(1.2)	(2.9)	(2.0)	(0.5)
Snake River	2	5	0	5	16	10	0	0	0	0
	(1.2)	(3.8)	(3.0)	(7.4)	(11.5)	(11.1)	(0.0)	(0.0)	(0.0)	(0.0)
Pahsmeroi River	1	7	3	11	8	7	0	0	0	0
	(2.7)	(5.7)	(3.0)	(8.9)	(9.3)	(7.3)	(0.0)	(0.0)	(0.0)	(0.0)

TABLE 3

Allelic frequencies in the populations examined

Population	A	A'	A''	A'''
Chambers Creek	0.26	0.22	0.06	0.46
Big Creek	0.34	0.24	0.02	0.40
Deschutes	0.17	0.42	0.13	0.28
Snake River	0.18	0.28	0.00	0.54
Pahsimeroi River	0.27	0.28	0.00	0.44

At least three genetic models must be considered in interpreting the data. The simplest model to consider is one in which the variation is the result of a single disomic locus. This model must be rejected because of the five- and six-banded phenotypes which are only expected if more than two alleles are present in an individual.

The second model is one of gene duplication involving a single locus with the chromosomes carrying these alleles segregating in a tetrasomic manner. Under the predictions of this model, a fish of genotype AAA^xA^y would be expected to produce four kinds of gametes in the following ratio: *

$$AA \; : \; AA^x : AA^y : A^xA^y$$
$$1 \; : \; 2 \; : \; 2 \; : \; 1$$

Our results do not support this model (Table 4). The cross $AAAA \times AAA'''A'''$ (Lot #5) resulted in only one progeny phenotype rather than the three expected with this model. Additionally, Lots #10, 18, 19, and 24 showed analogous results, forcing us to reject this model.

The third model is one of gene duplication with two disomic loci. The predictions of this model agree with our data; the chi-square values and corresponding probabilities are presented in Table 4. Further examination reveals that all

* Assuming chromosome segregation (BURNHAM 1962).

TABLE 4

Observed and expected segregation of IDH variants

Progeny phenotypes: observed
(expected with disomic inheritance)
/expected with tetrasomic inheritance/

Lot no.	Female (genotype)	Male (genotype)	AA/AA	AA/AA'	AA/A'A'	AA/AA''	AA'/A''A''	AA/A''A''	AA''/A''A''	AA'/A'A'''	A'A'/A'A'''	χ²	Probability
5	AAAA (AA)	AAA'''A''' (A'''A''')	/5.7/			34 (34) /22.7/				/5.7/		: 17.20	: <.001
10	AAA''A''' (A''A''')	AAAA' (AA')		13 (12)		14 (12) /22.7/			11 (12) /8/	10 (12) /8/	/5.7/ /4/	: 0.83	: >.80
18	AAAA' (AA')	AAA''A'' (A''A'')	/4/			21 (22.8) /15.2/	25 (22.8) /15.2/		23 (22.8) /15.2/	22 (22.8) /15.2/	/8/ /4/	: 0.38 45.97	: >.90 <.001
19	AAA'A' (A'A')	AAA'A'' (A'A'')	/0.7/ /4.5/ /1.5/		13 (13.5) /6.8/	/1.5/		14 (13.5) /6.8/	/4.5/ /0.7/			: 0.04 26.67	: >.80 <.001
20	AAAA (AA)	AAAA' (AA')	26 (27.5) /27.5/	29 (27.5) /27.5/								: 0.16 0.16	: >.50 >.50
24	AAAA (AA)	AAA'A'' (A'A'')	1 (0) /20.2/	56 (60.5) /40.3/		64 (60.5) /40.3/						: 0.53 63.45	: >.30 <.001
29	AAAA' (AA')	AAAA''' (AA''')	16 (19.5) /19.5/	24 (19.5) /19.5/		19 (19.5) /19.5/	19 /19.5/					: 1.69 1.69	: >.50 >.50

* Genotype of the polymorphic locus; other locus presumed (AA).

of the variation seen appears to be located at one locus with the other locus being monomorphic. In every case in which two variant alleles are present in one parent, they segregate as forms of a single locus. In Lots #19 and 24, the alleles A' and A''' segregated in a 1:1 ratio, reflecting alternate states of a single locus. A similar relationship between the A'' and A''' alleles can be seen in Lots #10 and 18. BAILEY et al. (1970) described a similar situation for the B-form of malate dehydrogenase in rainbow trout.

An obvious exception to the expected results is seen in Lot #24. Only heterozygous progeny are expected to result from the cross $AA \times A'A'''$; however, 1 progeny out of 121 exhibited only a single, distinct band. The possibility that this fish represented contamination from another family was ruled out on the basis of the examination of several other biochemical loci. There are two possible genetic explanations for this individual that should be considered. One possibility is that this fish was the result of parthenogenic development and was haploid. This seems unlikely, since the fish was morphologically normal and we could not find a previous report of spontaneous parthenogenic development in salmonids.

The second explanation has some interesting genetic implications. If A' and A''' represent point mutations at different sites of the wild-type allele (A), then intragenic recombinations between these sites would result in the production of wild-type and double-mutant gametes in equal numbers. This possibility is strengthened by previous reports of a similar phenomenon at a lactate dehydrogenase locus in brook trout (*Salvelinus fontinalis*) by WRIGHT and ATHERTON (1968, 1970). In a mating analogous to ours, they found wild-type and double-mutant recombinants segregating at a frequency of approximately 3% in some families. These crossovers were only found to occur in certain males (never in females); evidence suggested that this high frequency of recombination was controlled by a segregating locus. The possible evolutionary role of such intragenic recombination was recently considered by WATT (1972).

<div align="center">DISCUSSION</div>

The results reported here clearly demonstrate the disomic inheritance of supernatant-IDH in the rainbow trout population we examined through family studies. The close fit of the observed and expected phenotypic distribution, assuming disomic inheritance in all populations examined (see Table 2), further supports a disomic model. Reasons for disagreement between our results and previous results reported by WOLF, ENGEL and FAUST (1970) should be considered. One explanation is that tetrasomic inheritance is extant in some rainbow trout populations whereas in other populations the alleles in question segregate in a disomic manner. A more likely explanation, however, lies in the method used by WOLF, ENGEL and FAUST (1970) to detect the presence of tetrasomic inheritance. We feel their assumption of tetrasomic inheritance based solely on the observed phenotypic distribution is founded upon weak evidence at best.

Only actual breeding experiments can conclusively verify the true mode of

inheritance. We believe that breeding studies of other rainbow trout populations will verify the existence of disomic inheritance for these loci. It is likely, however, that variation will be discovered at the other locus that we found to be monomorphic. Indeed, WOLF, ENGEL and FAUST (1970) did describe phenotypes which they classified as having three variant allelic doses, which would indicate that both loci were polymorphic.

Documenting the tetraploid nature of salmonids has become increasingly popular and in some instances has been based on weak data, erroneous conclusions, or both. Variants of sorbitol dehydrogenase in rainbow trout (ENGEL, OP'T HOF and WOLF 1970) were presumed to reflect tetrasomic inheritance on even weaker evidence than that presented for the tetrasomic inheritance of IDH; two of the three observed phenotypes could not be reliably distinguished and their data could be explained easily on the basis of a disomic model analogous to that presented here. A more recent paper has reported the tetrasomic inheritance of hexose 6-phosphate dehydrogenase in brook trout (STEGEMAN and GOLDBERG 1972). However, as with IDH and SDH, no breeding analysis was carried out. In addition, the phenotypic distribution was not presented for the population claimed to be segregating tetrasomically. These authors have not convincingly demonstrated the existence of tetrasomic inheritance rather than of two disomic loci. KINGSBURY and MASTERS (1972) described a simple two-allele single-locus esterase variant in rainbow trout. They then proceeded to state, ". . . the detection of these apparently duplicate genes for the expression of this esterase provides additional support for the hypothesis that salmonic (sic) fish are tetraploid." Somehow, these authors have confused the concept of polymorphism with the concept of gene duplication in an effort to support the tetraploid hypothesis. One other case of gene duplication reported in rainbow trout is based on extremely weak (only four fish) and, we believe, erroneous data (CEDERBAUM and YOSHIDA 1972). A sobering note in the documentation of tetraploidy in salmonids is a paper by UTTER and HODGINS (1972) in which they described polymorphisms at six loci in rainbow trout, only two of which exhibited evidence of duplicate genes.

We believe that although there is extensive evidence of gene duplication in salmonids, it has not been conclusively demonstrated that this has occurred through tetraploidy. Our data do not conflict with a hypothesis of tetraploidy in an ancestral salmonid species but rather emphasize the need for more careful consideration of some evidence that has been uncritically cited in support of this hypothesis. A recent paper (DAVISSON, WRIGHT and ATHERTON 1972) has provided evidence of an alternate means of gene duplication occurring in salmonids. This, along with the existence of many loci displaying no indication of gene duplication and the lack of a single conclusive report of tetrasomic inheritance, is sufficient cause to consider more critically the possibility of a tetraploid event in salmonid evolution.

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