Inheritance of duplicated fumarase and phosphoglucosmutase loci in lake trout (*Salvelinus namaycush*)

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Two polymorphic, duplicated loci (fumarase-1,2 and phosphoglucosmutase-3,4), previously undescribed in salmonids, were examined in lake trout to determine their electrophoretic expression, mode of inheritance, and linkage associations. Both loci appear to be located on chromosomes which show residual tetrasomy. Neither locus pair shows joint segregation with other loci examined or with each other.

INTRODUCTION

Electrophoretic data are widely used for obtaining genetic information about fish populations. Such information has been used in stock identification, genetic tagging, measures of inbreeding in hatchery broodstocks, and the detection of triploids (e.g., Allendorf and Utter, 1979; Grant *et al.*, 1980; Shaklee, 1983; Purdom *et al.*, 1976).

Electrophoretic analyses of lake trout (*Salvelinus namaycush*) populations present three problems: (a) this species has lower genetic variability at isozyme loci relative to other salmonid species, (Allendorf and Utter, 1979; Dehring *et al.*, 1981), (b) the electrophoretic banding patterns are complicated due to the tetraploid ancestry of the salmonids (May, 1980), which is reflected in an increased number of loci coding for metabolic enzymes relative to related diploid Salmoniformes, and (c) residual associations between homeologous chromosomes can result in exchange of alleles between loci (i.e., isoloci) located distal to the centromere and in the apparent linkage of non-syntenic disomic loci (pseudolinkage; Davisson *et al.*, 1973; Wright *et al.*, 1983).

In order to identify additional genetic markers for population studies on lake trout, an extensive survey of isozyme loci was performed in our laboratory (Krueger *et al.*, in prep.). This survey

revealed polymorphisms for fumarase (FUM) and a new phosphoglucosmutase (PGM) zone of activity which have not been described in salmonids. The present study describes the electrophoretic expression and mode of inheritance of these loci.

METHODS

Superior (Marquette) and Seneca Lake strains of lake trout were obtained from the Tunison Nutrition Fish Laboratory (TNFL) in Cortland, NY, and from the Allegany National Fish Hatchery near Warren, PA. Crosses were made on 24 October and 1 November 1984 at the National Fishery Research and Development Laboratory (NFRDL) near Wellsboro, PA, and on 12 November 1984 at TNFL. All crosses were produced by stripping and storing gametes from individual fish, which were then sacrificed and sampled for muscle, liver, kidney, heart, and eye tissue. These tissues were analysed electrophoretically within hours of tissue and gamete collection. Based on identification of individual genotypes, parental mating pairs were selected and gametes were combined within 12 hours of collection. All test lots were incubated and raised to the fingerling stage at NFRDL. Each test lot (family) was labelled using

numbers to designate the female parent and letters to designate the male parent. Fry were sacrificed after they reached a minimum length of approximately 6.0 cm. Eighty individuals were sampled from each family, except crosses 10-A ($N=40$), 13-D ($N=72$), and 99-HH ($N=170$), and were frozen whole at -80°C . Prior to electrophoresis, fry were sampled for muscle, kidney and eye tissue. Electrophoretic procedures, staining recipes, interpretation of banding patterns, and nomenclature followed May *et al.* (1979b) and Allendorf *et al.* (1977). Alleles were designated by numbers, with the most common allele being '1'. Arrangement of alleles at duplicated loci, when it was known, was designated in the format 11/22, where the slash separates alleles from different homeologous pairs. Mobilities of allelic products at Aat-1,2, Mdh-3,4, and Me-1,2 coincided with those found in Lake Superior lake trout by Dehring *et al.* (1981), and allelic products at Pep-pap-1,2 appeared to coincide with those found by Hollister *et al.* (1984). For ease of data comparisons between laboratories, we have used the mobilities given by these authors (table 1). Resolution of FUM and PGM was markedly improved by using an agar overlay in addition to the standard stain recipes; FUM could only be scored consistently using the agar

overlay. The parental strains had previously been screened for over 90 loci (Krueger *et al.*, in prep); therefore, potential parents were examined only at those loci which were variable in these strains. Fry were examined electrophoretically at those loci which were polymorphic in the parents and which provided information on single locus and/or joint segregation (table 1).

Data were analysed using a linkage analysis program modified from Suiter *et al.* (1983). Crosses were analysed for single locus segregation and joint segregation as described in May *et al.* (1979b), except as noted in text.

RESULTS

Enzyme systems

Salmonid isozyme banding patterns have been described in detail by May (1980) and Johnson (1984) for all polymorphic loci used in this study except for those coding for FUM and PGM, which are described below.

Fum-1,2: Two identical anodal zones of fumarase activity were found for these loci in muscle tissue (fig. 1). Since the two zones showed parallel patterns in all phenotypes observed, we presumed

Table 1 Enzyme systems used in this study, with the tissues and buffer systems on which they were best resolved

Enzyme	LOCI	E.C. #	Allele Mobility	No.	Tissue	Buffer ^a
Aspartate aminotransferase	Aat-1,2	2.6.1.1	100	2	Liver	R
			85	1		
Fumarase	Fum-1,2	4.2.1.2	100	1	Muscle	C +, 4
			90	2		
Malate dehydrogenase	Mdh-3,4	1.1.1.37	100	1	Eye	A
			72	2		
Malic enzyme	Me-1,2	1.1.1.40	100	1	Muscle	C
			115	2		
Peptidase with phenylalanyl-proline	Pep-pap-1,2	3.4.11-13	100	3	Kidney	C
			82	2		
			73	1		
Phosphoglucumutase	Pgm-2	2.7.5.1	100	1	Eye	A
			67	2		
	Pgm-3,4	2.7.5.1	100	1	Eye	A
			94	2		
			91	3		
Superoxide dismutase	Sod-2	1.15.1.1	100	1	Muscle	R
			85	2		

^a Buffer systems used;

R, Ridgway *et al.*, 1970

C, CT of May *et al.*, 1979b

C +, C (above) with the pH raised to 7.0 with NaOH

A, AZM of May *et al.*, 1979b

4, continuous tris-citrate type I of Selander *et al.*, 1971

Figure 1 Photos and diagrams of electrophoretic banding patterns of fumarase-1,2 and phosphoglucumutase-3,4.

one of the two zones was an artifact; we consistently read the lower zone. Band density ratios conformed to those expected for a tetrameric enzyme encoded by four gene doses with two alleles.

Pgm-3,4: Five zones of activity were noted in eye tissue, one on the cathodal side of the origin and four on the anodal side. The cathodal zone corresponded to Pgm-1, and the slowest of the anodal zones was Pgm-2. The two central anodal zones are probably not PGM activity. The fastest of the anodal zones showed banding patterns which conformed to those expected for a monomeric enzyme coded by duplicate loci (see fig. 1). This zone was Pgm-3,4, described in muscle tissue by May *et al.* (1980). Three alleles were noted for Pgm-3,4, plus a null allele class. Null alleles ($\emptyset$) were recognised on the basis of band intensity ratios; thus an individual with two copies of the 1 allele and one copy each of the 2 and $\emptyset$ allele would show bands at the 1 and 2 allelic product positions in a 2:1 ratio. An individual with more than one copy of a null allele would not be identifiable as having a null allele by visual examination of the phenotype, because band intensity ratios would conform to those expected for genotypes without null alleles. Null alleles have also been detected in salmonids at Mdh-1 (May *et al.*, 1979a), Aat-1,2 (Stoneking *et al.*, 1981a), Idh-4, Me-2, and Pgm-1 (Stoneking *et al.*, 1981b). Stoneking *et al.* (1981a) discuss in detail the problems involved with interpreting gel banding patterns at a locus with a null allele.

Single locus segregation

In order to examine the inheritance mode of duplicated loci, it is necessary to determine that individual alleles segregate in a Mendelian fashion. All crosses which had a single copy of any allele at either Fum-1,2 or Pgm-3,4 were tested for non-random distribution of the allele in the progeny. In the absence of meiotic drive or differential survival of progeny, each allele should appear in 50 per cent of the progeny. Abberant single locus segregation was noted for Pgm-3,4 in one individual, the female in cross 10-A ($p = 0.011$; see table 2), but due to the number of simultaneous tests performed (23 crosses), this deviation was not considered significant (Cooper, 1968). Progeny from crosses with a single copy of any allele at Fum-1,2 or Pgm-3,4 were also examined for evidence of double reduction, which would result in an individual carrying two doses of the rare allele (Burnham, 1962). This was not noted for either of the duplicated loci.

Fum-1,2: only three crosses (9-B, 9-E, and 99-HH) had genotypes appropriate for determining whether inheritance of Fum-1,2 is other than disomic, i.e., a homozygote (1111 or 2222) or asymmetrical heterozygote (1112 or 1222) crossed with a symmetrical heterozygote (1122) (see table 2). The first two crosses used the same symmetrically heterozygous (1122) female (#9), and in both crosses the progeny could have only been produced with all heterozygous (12) gametes coming from

Table 2 Single locus segregation of fumarase and phosphoglucumutase in lake trout. Where it was possible to test for more than one allele or combination of alleles in a family, the alleles being tested are underlined; offspring categories which were grouped together for analysis are shown with single, double or no underlining. An asterisk indicates significance differences from expected ratios at or below the 0.05 level

Cross	Parents	N	Progeny genotypes					X ²	p		
	Female/male		1111	1112	1122						
<i>Fum-1,2</i>											
3- <i>F</i>	1112/1112	80	27	40	13		4.90 ^b	0.086			
3- <i>D</i>	1112/1112	80	20	38	22		0.30 ^b	0.86			
13- <i>D</i>	1111/1112	72	30	42	—		2.00 ^a	0.16			
13- <i>F</i>	1111/1112	80	35	45	—		1.25 ^a	0.26			
11- <i>MM</i>	1111/1112	40	20	20	—		0.00 ^a	1.00			
55- <i>DD</i>	1111/1112	80	40	40	—		0.00 ^a	1.00			
9- <i>B</i>	1122/1112	79	—	33	46		2.13 ^a	0.14			
9- <i>E</i>	1122/1111	80	—	80	—		—	—			
99- <i>HH</i>	1111/1122	170	29	108	33		12.60 ^b	0.002*			
<i>Pgm-3,4</i>											
		N	<u>1111</u>	<u>1112</u>	<u>1113</u>	<u>1123</u>	<u>1122</u>	<u>1223</u>	<u>1222</u>	X ²	p
3- <i>F</i>	1112/2223	80	—	—	—	24	19	<u>17</u>	<u>20</u>	0.45 ^a	<0.50
3- <i>F</i>	1112/2223	80	—	—	—	<u>24</u>	19	<u>17</u>	<u>20</u>	0.05 ^a	<0.90
3- <i>D</i>	1112/1123	80	8	22	<u>11</u>	<u>24</u>	5	<u>10</u>	—	1.25 ^a	<0.50
3- <i>D</i>	1112/1123	80	8	<u>22</u>	<u>11</u>	<u>24</u>	5	10	—	0.73 ^c	<0.90
10- <i>A</i>	1223/1112	40	—	6	3	<u>4</u>	15	5	7	6.40 ^a	<0.025*
10- <i>A</i>	1223/1112	40	—	<u>6</u>	3	4	<u>15</u>	<u>5</u>	7	4.93 ^a	<0.25
11- <i>UU</i>	1112/1122	80	5	33	—	—	12	—	30	3.20 ^c	<0.50
11- <i>MM</i>	1112/1112	40	10	23	—	—	7	—	—	1.35 ^b	<0.75
13- <i>D</i>	1122/1123	72	—	17	—	<u>17</u>	25	<u>13</u>	—	2.00 ^a	<0.25
13- <i>D</i>	1122/1123	<u>72</u>	—	17	—	<u>17</u>	<u>25</u>	13	—	2.44 ^a	<0.50
13- <i>F</i>	1122/2223	80	—	—	—	—	—	40	40	0.00	1.00
44- <i>SS</i>	1112/1122	80	7	41	—	29	—	—	3	9.87 ^c	<0.025*
55- <i>DD</i>	1111/1122	76	15	43	—	18	—	—	—	1.55 ^b	<0.50
55- <i>RR</i>	1111/1122	79	4	69	—	6	—	—	—	44.16 ^b	<0.005*
			<u>1110</u>	<u>1112</u>	<u>1120</u>	<u>1122</u>	<u>1220</u>	<u>1222</u>	<u>2222</u> + <u>2220</u>		
9- <i>B</i> ^d	1220/1122	80	2	9	11	31	8	16	3		

^a tested against an expected 1:1 ratio with 1 d.f.

^b tested against an expected 1:2:1 ratio with 2 d.f.

^c tested against an expected 1:3:3:1 ratio with 3 d.f.

^d see text for explanation of this family.

the female parent. This implied that the female parent was homobivalent (11/22—i.e., the bivalent chromosome pairs were homozygous for different alleles, so that a 1 allele could only segregate with a 2 allele in meiosis). In cross 99-HH the male was symmetrically heterozygous and produced progeny that could only have been derived from three types of gametes: 11, 12, and 22. Since he produced heterozygous gametes in excess of a 1:2:1 ratio, he formed either random bivalents or heterobivalent tetravalents with crossing-over.

Pgm-3,4: Nine parents used in nine crosses had symmetrically heterozygous genotypes (see table 2). Of the three symmetrically heterozygous females, progeny phenotypes indicated that one was homobivalent, yielding only heterozygous gametes (in crosses 13-D and 13-F) while the other two females were heterobivalent (crosses 10-A and 9-B). Three of the symmetrically heterozygous

males produced an excess of heterozygous gametes, based on progeny phenotypes (crosses 9-B, 44-SS and 55-RR). The remaining three males produced a 1:2:1 ratio of 11:12:22 gametes (crosses 3-D, 13-D, 55-DD, and 11-UU).

Cross 9-B needs a detailed discussion, as it is the only cross which apparently included a null allele (table 2). The parental genotypes were 1220 for the female and 1122 for the male. If both parents were heterobivalent, 8 progeny classes would be produced (1112, 1122, 1222, 2222, 1110, 1120, 1220, and 2220 in a 1:3:3:1:1:3:3:1 ratio); however, only two 1110 or 1111 and four 2220 or 2222 progeny were produced, whereas the expected numbers were 5 and 10, respectively. (Note that 1110 and 2220 phenotypes are indistinguishable from homozygotes.) This suggests that the male was homobivalent (11/22), and only produced 11 and 22 gametes through occasional

tetravalent pairing and crossing-over. If this was the case, 1112 progeny should have been produced in small numbers and the remaining progeny classes (1120, 1220, 1122 and 1220) would be expected in a 1:1:1:1 ratio. However, these genotypes are not reliably separable, as a 2:1 band ratio cannot always be readily distinguished from a 2:2 or 3:1 ratio. If the female was also homobivalent (10; 22) she could only have produced 12 and 20 gametes. The presence of 1110 progeny and high numbers of 1222 and 1120 progeny indicate that she also produced 10 and 22 gametes. Therefore she must have been heterobivalent (12/20).

Joint segregation

All pairwise combinations of loci which yielded data on joint segregation are shown in table 3 (informative $N \geq 40$). Detailed data will be provided upon request. Fumarase was tested for linkage with five other loci, including Pgm-3,4, in males and one other locus in females. Pgm-3,4 was also tested for linkage with four other loci, including Fum-1,2, in males, and two other loci in females. In one family (13-D) it was possible to test for linkage between Pgm-3 and Pgm-4. There were no families in which it was possible to test for linkage between the two fumarase loci. Five tests showed significant deviation from independent segregation: Aat-1,2 with Me-1,2 in cross 3-F ($r = 0.363$); Pep-pap-1,2 with Me-1,2 in cross 9-E ($r = 0.385$); Fum-1,2 with Pgm-3,4 in cross 13-D ($r = 0.389$); Me-1,2 with Pgm-3,4 in cross 3-F ($r = 0.372$); and Fum-1,2 with Pgm-3,4 in cross 3-F ($r = 0.325$). However, only one of these r values fits the criteria

of $r \leq 0.35$ established by May *et al.* (1979b) for detection of non-random assortment, and each of these pairs of loci are shown to be unlinked in other crosses. Given the large chromosome arm number in this species ($NF = 100$; Davisson, 1969) compared with the low number of pairs available for testing, the probability of finding linkage is quite low.

DISCUSSION

In order to determine the mode of inheritance of salmonid loci for prediction of progeny genotype ratios, it is first necessary to understand the structure of the salmonid genome—that is, the types of chromosome pairing and segregation which occur. In female salmonids only bivalent pairing of chromosomes is thought to occur in meiosis (Wright *et al.*, 1983; Allendorf and Thorgaard, 1984; however, see Rex, 1984). Evidence indicates that these are homologous bivalents. If random bivalents formed (homologous and homeologous), it is highly likely that tetravalents would occur, since they are seen to occur in males. A symmetrically heterozygous female at a given locus will either be homobivalent (11/22), producing only 12 gametes, or heterobivalent (12/12), producing a 1:2:1 ratio of 11, 12, and 22 gametes respectively. In contrast, males exhibit some residual multivalent associations in which crossing-over is inhibited near the centromere, as evidenced by duplicated loci known to be involved in multivalents which are fixed for alternate alleles (e.g., Ldh-3,4; Wright *et al.*, 1983). Where crossing-over is not inhibited, that is, for genes distal to the centromere, apparently exactly one cross-over

Table 3 Tests for joint segregation of loci in lake trout. The numbers refer to the number of crosses which were diagnostic for joint segregation for a given pair of loci. Examinations in males are above the diagonal and in females are below the diagonal

	Aat-1,2	Mdh-3,4	Pep-pap-1,2	Fum-1,2	Me-1,2	Pgm-3,4	Sod-2
Aat-1,2			1	2		1 ^a	
Mdh-3,4				2		3	2
Pep-pap-1,2				2	1		
Fum-1,2	2				4 ^b	2	
Me-1,2	1		1				
Pgm-3,4	1					1	2
Sod-2			1			1	

^a a single examination was made in which one of the Aat loci was tested against both of the Pgm loci in a single male (cross 13-D).

^b a single examination was made in which one of the Fum loci was tested against both of the Pgm loci in a single male (cross 13-D).

occurs per chromosome arm pair (Allendorf *et al.*, in prep., Thorgaard *et al.*, 1983). In addition, of the three possible types of chromosome segregation in a tetravalent (Burnham, 1962), primarily alternate segregation occurs in salmonids. Double reduction progeny, which result from adjacent II pairing, are seen very rarely (Wright *et al.*, 1983). If no crossing-over takes place, a homobivalent male who forms tetravalents will produce only heterozygous (12) gametes (see table 4). If single crossovers always occur between each chromosome pair, he will produce 11, 12, and 22 gametes in a 1:2:1 ratio. The chromosomes in a heterobivalent individual can take either of two arrangements in a tetravalent, with the like alleles being either paired or unpaired (see fig. 2). If no cross-overs occur, the former arrangement will produce only 12 gametes and the latter will produce 11 and 22 gametes in equal ratios; since both arrangements are formed randomly and therefore with equal frequency, 11, 12, and 22 gametes will be formed in a total ratio of 1:2:1. If cross-overs do occur, when like alleles are paired only 12 gametes will be produced; when unlike alleles are paired, 11, 12, and 22 gametes will be produced in a 1:2:1 ratio. The expected total gamete ratio will be 1:6:1. Thus a locus which is close to the centromere (no gene-centromere crossing-over) on

Table 4 Expected ratios of 11, 12 and 22 gametes from a symmetrically heterozygous individual given various types of chromosome configurations, pairing modes, and crossing-over frequencies, and only alternate segregation. A = like alleles paired, B = unlike alleles paired (see fig. 2). Adapted from Wright *et al.*, 1983.

	bivalent	Type of chromosome pairing tetravalent	
		no x-over	single x-over
homobivalent (11/22)	0:1:0	0:1:0	1:2:1
heterobivalent (12/12)	1:2:1	1:2:1 A) 0:1:0 B) 1:0:1	1:6:1 0:1:0 1:2:1

a chromosome which pairs tetravalently is functionally equivalent to a locus on a chromosome which only pairs bivalently. A locus distal to the centromere will occasionally be identifiable in homobivalent individuals by the production of double reduction gametes (11 and 22) via chromatid non-disjunction (Burnham, 1962; Wright *et al.*, 1983). Such a locus may also be identifiable in heterobivalent individuals, due to the production of an excess of heterozygous gametes; however, mixed ratios of bivalent and

Figure 2 Possible arrangements of alleles in a tetravalent in a symmetrically heterozygous individual. The two homologous chromosome pairs (denoted by solid or open-circle centromeres) which form the tetravalents can be homobivalent (both pairs homozygous for different alleles) or heterobivalent (both pairs heterozygous).

tetravalent pairing in an individual forming homobivalents will also produce an excess of heterozygous gametes.

The results of this study indicated that the fumarase loci are probably located distal to the centromere on chromosomes which still show residual tetravalent pairing. The only female which was symmetrically heterozygous (used in crosses 9-*E* and 9-*B*) was homobivalent; the only symmetrically heterozygous male (cross 99-*HH*) produced an excess of heterozygous gametes, indicating either that he was heterobivalent with crossing-over occurring between duplicate loci, or that he was homobivalent with mixed bivalent and tetravalent pairing.

Of the three females which were symmetrically heterozygous at Pgm-3,4 one was homobivalent and two were heterobivalent (segregating in a 1:2:1 ratio). Of the six symmetrically heterozygous males, three produced 11, 12, and 22 gametes in a 1:2:1 ratio, and three produced an excess of asymmetrically heterozygous gametes (table 3). These ratios indicate that Pgm-3,4 must also be located on chromosome arms with tetravalent (homeologous) pairing of distally homologous regions. The variance in progeny ratios between families suggests that there were both homobivalent and heterobivalent males in the crosses, producing 1:2:1 and 1:6:1 gamete ratios, respectively. The low number of homozygous gametes produced by the male in cross 55-*RR* suggests that he was homobivalent; both tetravalent formation and crossing-over would be required to produce homozygous gametes from a homobivalent. Unpublished data from May and Wright indicates that gene-centromere distances for the fumarase and phosphoglucumutase systems are greater than 45 centimorgans in *Salvelinus* hybrids.

Additional information on these loci was provided by an electrophoretic analysis of 13 lake trout populations performed in our laboratory (in prep.). With respect to only the 1 and 2 alleles at Pgm-3,4, all five of the possible genotypes (1111, 1112, 1122, 1222, and 2222) were seen in high numbers. This provides additional evidence that the two loci exchange alleles via homeologous pairing and crossing-over, since it is highly unlikely that two loci would carry the same alleles unless they had diverged recently. The low frequency of allele 2 at Fum-1,2 reduced the probability of finding individuals with more than two doses; however, a single population with a high frequency of this allele contained two 1222 individuals and one 2222 individual, which suggests that the loci do share alleles.

In summary, this study describes the inheritance of two previously undescribed polymorphic, duplicate loci—Fum-1,2 and Pgm-3,4. Data from these loci will be useful for future population and inheritance studies of lake trout and other salmonids. Data interpretation from inheritance studies must consider that exchange of alleles occurs within both locus pairs. The presence of a null allele class at Pgm-3,4 complicates the interpretation of gel banding patterns, as discussed by Stoneking *et al.* (1981).

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