JOINT SEGREGATION OF BIOCHEMICAL LOCI IN SALMONIDAE. II. LINKAGE ASSOCIATIONS FROM A HYBRIDIZED SALVELINUS GENOME (*S. NAMAYCUSH* × *S. FONTINALIS*)*

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ABSTRACT

The results of more than 300 pairwise examinations of biochemical loci for joint segregation in brook trout (*Salvelinus fontinalis*) and in the hybridized genome of lake trout (*S. namaycush*) × brook trout are summarized. Nineteen loci have been assigned to the following eight linkage groupings on the basis of nonrandom assortment, including cases of both classical linkage and pseudolinkage: *ODH* with *PMI* with *PGI-3*, *PGI-2* with *SDH*, *ADA-1* with *AGP-2*, *AAT-(1,2)* with *AGP-1* with *MDH-1*, *MDH-3* with *MDH-4*, *LDH-3* with *LDH-4*, *IDH-3* with *ME-2* and *GUS* with *CPK-1*. Pseudolinkage (an excess of nonparental progeny types) was observed only for male testcross parents. The results suggest that this phenomenon involves homeologous chromosome arms as evidenced by the *de novo* association of presumed duplicate loci in each case. Classical linkage has not been found for the five pairs of duplicate loci examined in Salvelinus, suggesting that not all of the eight metacentrics in the haploid complement involve fusions of homeologous chromosomes. Females consistently showed a greater degree of recombination.

IT is accepted by most authors that the Salmonidae (trouts, salmons, chars, graylings and whitefishes) are the derivatives of a tetraploid lineage. It is clear from extensive inheritance and population studies of biochemical loci that (1) segregation is strictly disomic (BAILEY *et al.* 1970; ALLENDORF and UTTER 1973; ROPERS, ENGEL and WOLF 1973; MAY 1975; ALLENDORF 1975; WRIGHT, HECKMAN and ATHERTON 1975; MAY, STONEKING and WRIGHT 1979; MAY, WRIGHT and STONEKING 1979; STONEKING, MAY and WRIGHT 1979), and (2) there has been a substantial loss of duplicate gene activity (ALLENDORF, UTTER and MAY 1975; ALLENDORF 1978; MAY, WRIGHT and STONEKING 1979). The primary mechanism of diploidization in salmonids appears to be Robertsonian fusions of acrocentric chromosomes as evidenced by (1) intraspecific Robertsonian polymorphisms (OHNO *et al.* 1969; ROBERTS 1970; GOLD and GALL 1975; GOLD, AVISE and GALL 1977; THORGAARD 1976, 1977, 1978), and (2) variable 2n chromosome numbers with similar arm numbers in related salmonid species (SIMON 1963;

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OHNO *et al.* 1965; ROBERTS 1970; DAVISSON, WRIGHT and ATHERTON 1972, 1973; CHIARELLI and CAPANNA 1973; GOLD and GALL 1975; ZENZES and VOICULESCU 1975).

This particular process of diploidization and the coincidental loss of duplicate gene expression raise three interesting and approachable questions. Was the loss of duplicate gene activity a random single-locus event, or did it involve the loss of whole linkage groups; *i.e.*, are single loci linked more often to other single loci or to members of duplicate loci? Did the fusion of acrocentric chromosomes involve homeologous (formerly homologous in the tetraploid set) or nonhomeologous chromosomes; *i.e.*, are duplicated pairs of loci linked? Finally, did specific fusions take place prior or subsequent to the formation of genera or of species; *i.e.*, are the same linkage relationships shared by related species?

An unusual genetic phenomenon, termed "pseudolinkage," has been observed only in salmonids. This phenomenon is characterized by the occurrence of an excess of nonparental progeny types in the backcrosses of males heterozygous for certain loci; similarly heterozygous females exhibit random assortment (MORRISON 1970; DAVISSON, WRIGHT and ATHERTON 1973; WRIGHT, HECKMAN and ATHERTON 1975; MAY, WRIGHT and STONEKING 1979). To date, pseudolinkage has been observed in heterozygous males composed of apparently divergent genomes. The small size and large 2n numbers of salmonid chromosomes have prevented the visualization of a definitive meiotic mechanism that would produce such results. A fourth question is raised: How widespread is this phenomenon and, furthermore, are all cases of pseudolinkage produced by a single meiotic event?

An extensive examination was initiated of joint segregation of biochemical loci in brook trout (*Salvelinus fontinalis*) and in a hybridized Salvelinus genome—lake trout (*S. namaycush*) × brook trout—in order to examine these questions. The results of these inheritance studies are reported here.

MATERIALS AND METHODS

Single-pair matings involving brook, lake, splake (lake × brook) and Benner Spring Research Synthetic (BSRS) trout were made in the Fall of 1978 (Table 1). The BSRS strain was derived from crosses involving different inbred brook trout lines, splakes and splake backcross progeny. Ripe fish were killed, the gametes stored at 5° for 12 to 24 hr and the tissues typed electrophoretically to determine the appropriate parents for desired crosses. The progeny were reared at the Pennsylvania Fish Commission's Upper Spring Creek Hatchery and were typed electrophoretically in the Spring of 1979.

The horizontal starch gel electrophoretic techniques used were reported in detail in MAY, WRIGHT and STONEKING (1979). The enzymes studied, the abbreviations, loci and alleles, tissue specificity and appropriate buffer systems are listed in Table 2. Staining following the general methods of ALLENDORF *et al.* (1977), with the following exceptions: AAT (MAY, WRIGHT and STONEKING 1979), ODH (octanol-2 as substrate; BREWER 1970) and MUP (5 mg 4-methylumbelliferyl phosphate in 10 ml RSL buffer and observed under fluorescent light; UTTER, personal communication).

Genic nomenclature and statistical treatment of segregation data follow that of MAY, WRIGHT and STONEKING (1979). The terminology *XYZ-1,2* is used when duplicated *XYZ* loci share electrophoretically identical alleles and when one cannot identify which locus is variable (*e.g.*, *AAT-1,2* or *MDH-3,4*). *XYZ-(1,2,3)* is used when referring to more than one *XYZ* locus.

TABLE 1

Sources of the two parents of each family

Family	Source of female parent	Source of male parent
Y-21	Splake *A**	F_2 of ST O† × ST MC‡
Y-23	BSRS§	ST 3
Y-25	BSRS	ST 3
Y-26	BSRS	ST 3
Y-40	Unknown ST‖	Splake *A*
Y-41	Unknown ST	Splake
Y-42	Unknown ST	Splake *A*
Y-43	Splake *A*	ST 22
Y-45	ST Albino¶	Splake *A*
Y-50	Unknown ST	ST (3×22) × Belle Open**
Y-61	BSRS	ST 15
Y-77	LT††	Splake *B*‡‡
Y-79	N.Y.S.V.§§	Splake *B*
Y-80	Belle Open	Splake *B*
Y-81	LT	Splake *B*
Y-94	N.Y.S.V.	BSRS
Y-98	Belle Open	BSRS

* F_1 cross of *S. namaycush* (female) from L. Cayuga, N.Y. × *S. fontinalis* (male) from hatchery at Edray, WV.
† Single numbers reflect inbred lines of *S. fontinalis* maintained by brother-sister matings for 8 to 12 generations.
‡ Wild male from Marsh Creek, Pa.
§ A mixed population of fish derived from multiple crosses of lake, splake, and first backcross fish with intercrossed fish of *S. fontinalis* inbred lines (see Table 2).
‖ Either an ST 2 or Belle Open.
¶ Albino color mutant stock with two generations of full-sib inbreeding.
** An open bred population of *S. fontinalis* from the Bellefonte, Pa. hatchery population.
†† *S. namaycush* from Lake Manitou, Ontario.
‡‡ F_1 cross of *S. namaycush* (female) from L. Manitou × *S. fontinalis* (male) from Maple, Ontario.
§§ An open bred population of *S. fontinalis* derived from New York Synthetic V, Rome, N.Y.

RESULTS

Enzyme systems: The following isozyme systems have not been described previously in Salvelinus. Descriptions of the variation for other isozymes listed in Table 2 can be found in MAY, WRIGHT and STONEKING (1979) and STONEKING, MAY and WRIGHT (1979).

AAT: Three loci have previously been reported in salmonid species. The two muscle loci, *AAT-(1,2)*, have been reported to be variable in *Salvelinus* (MAY, WRIGHT and STONEKING 1979), Oncorhynchus (MAY 1975; MAY, UTTER and ALLENDORF 1975) and cutthroat trout (*Salmo clarki*) (ALLENDORF and UTTER 1976). A single eye-specific locus, *AAT-3*, has been described in Oncorhynchus spp. (MAY 1975) and brook trout (STONEKING, WAGNER and HILDEBRAND 1980). We now report a liver-specific locus, *AAT-4*, with three alleles. Heterodimeric bands are observed following electrophoresis of liver extracts that result from combinations of the protein products of *AAT-4* and *AAT-3*, or the

TABLE 2

Enzymes, loci, tissues and buffers used during this investigation of joint segregation of biochemical loci in Salvelinus*

Enzyme	E.C. no.	Loci	Alleles†	Tissue	Buffer
Aspartate aminotransferase	2.6.1.1	*AAT-1,2*‡	100 118	Muscle	RSL§
		AAT-4	100 133 170	Liver	MF‖
Adenosine deaminase	3.5.4.4	*ADA-1*	100 114	Liver	MF
Alcohol dehydrogenase	1.1.1.1	*ADH*	100 205	Liver	RSL
Alphaglycerophosphate dehydrogenase	1.1.1.8	*AGP-1*	0 78 100	Muscle	AHZV¶
		AGP-2	100 111	Muscle	CT**
Creatine phosphokinase	2.7.3.2	*CPK-1*	80 100	Muscle	RSL
Diaphorase	1.6.4.3	*DIA*	85 100	Muscle, Liver	CT
Glutamate-pyruvate transaminase	2.6.1.2	*GPT*	68 100	Liver	MF
B-Glucuronidase	3.2.1.31	*GUS*	100 106	Liver	MF
Isocitrate dehydrogenase	1.1.1.42	*IDH-3*	100 120 140	Eye	AHZV
Lactate dehydrogenase	1.1.1.27	*LDH-3*	72 86 100	Eye	MF
		LDH-4	100 240	Liver	RSL
Malate dehydrogenase	1.1.1.37	*MDH-1*	100 140	Eye	CT
		MDH-3,4	74 100 120	Muscle	CT
Malic enzyme	1.1.1.40	*ME-1*	0 100	Muscle	CT
		ME-2	60 100	Muscle	CT
4-Methylumbelliferyl phosphatase		*MUP*	Fast Slow	Liver, Muscle	CT
Octanol dehydrogenase	1.1.1.73	*ODH*	100 116	Liver	MF

TABLE 2—Continued

Enzyme	E.C. no.	Loci	Alleles†	Tissue	Buffer
Phosphoglucoisomerase	5.3.1.9	*PGI-1*	100 150	Muscle	RSL
		PGI-2	39 100	Muscle	RSL
		PGI-3	100 106	Muscle	RSL
Phosphoglucomutase	2.7.5.1	*PGM-2*	0 100	Muscle	CT
Phosphomannoisomerase	5.3.1.8	*PMI*	100 104	Eye	MF
Sorbitol dehydrogenase	1.1.1.14	*SDH*	66 100	Liver	RSL
Superoxide dismutase	1.15.1.1	*SOD*	100 187	Liver	RSL

* Enzyme names follow those commonly used in the salmonid literature.
† Based on relative mobilities of homomeric protein products (see MAY, WRIGHT and STONEKING 1979).
‡ Both loci share the same alleles.
§ RIDGWAY, SHERBURNE and LEWIS (1970), 250V (<74 mA).
‖ MARKERT and FAULHABER (1965), 275 V (<75 mA), electrode buffer diluted 1:5 for gel.
¶ AYALA *et al.* 1973 (Type C), modified to pH 6.1 with Tris HCl replacing Tris Base.
** Electrode buffer-0.04 M citric acid adjusted to pH 6.1 with N-(3-aminopropyl)-morpholine; diluted 1:10 for gel buffer <90 mA (CLAYTON and TRETIAK 1972).

products of *AAT-4* and another undescribed *AAT* locus. The enzyme products of *AAT-4* have a less anodal mobility in these two Salvelinus spp. than that of the products of *AAT-(1,2,3)*. Designating this liver locus as *AAT-4* is a departure from the standard genic nomenclature of numbering loci strictly from the origin (ALLENDORF and UTTER 1979). However, historically *AAT-3* refers to the eye form and *ATT-(1,2)* to the muscle form in salmonids; therefore, renaming the loci in this case is not justified.

ADA: Two anodal zones of activity were noted for this apparently monomeric enzyme. The less anodal zone differed between brook and lake trout, with the protein product of the lake allele (114) being the more anodal. We designate the single locus that codes for this variability as *ADA-1*. The monomeric nature of this enzyme precludes knowledge concerning the similarity of subunits between zones through random aggregation. The absence of variability in the more anodal zone prevents a determination of the number of loci involved. However, the closeness of the two zones on the gel would suggest their duplicate status if the upper zone is found to be coded by a single locus.

GPT: A single locus expressed in the liver showed a species-specific difference between brook and lake trout. This apparently dimeric enzyme stained rapidly, although accurate determination of genotypes was possible only with fresh, unfrozen samples. Additional zones of more anodal activity were noted, but could not be resolved.

GUS: Electrophoresis of liver extracts produced a broad, blurred banding region. A species-specific difference was noted in which extracts of lake trout produced activity slightly more anodal than extracts of brook trout. Backcrosses to brook trout segregated in expected 1:1 proportions; therefore, this difference was ascribed to a single locus. We were unable to resolve the total number of loci involved in the expression, or the quaternary structure, of this enzyme.

MUP: Two zones of activity were apparent following electrophoresis of muscle and liver extracts. Staining with standard acid or alkaline phosphatase procedures (BREWER 1970) did not result in any corresponding activity, thus justifying a different designation based on the particular substrate, 4-methylumbelliferyl phosphate. Extracts of lake trout from L. Manitou, Ontario, differed in electrophoretic mobility of the more anodal zone from both that in lake trout of L. Cayuga, New York, and from that in all brook trout individuals examined; the mobilities of the protein products were identical in the latter two. Inheritance results indicated a single locus controlling expression of the faster zone of this presumably dimeric enzyme; the absence of variability precluded determining the number of loci controlling expression of the slower zone.

ODH: The activity of a single locus with two alleles was observed for this dimeric enzyme. Inheritance results indicated the possibility of a null allele segregating at this locus; further studies are needed to substantiate this possibility.

PGM: Three zones of activity were noted for this monomeric enzyme on the CT buffer (see Table 2). The most cathodal zone (zone 1) is not expressed in salmonids with the RSL buffer routinely used. Variation in this zone observed in a natural brook trout population supports a single-locus model coding for a monomeric enzyme (STONEKING, WAGNER and HILDEBRAND 1980). Zone 3, the most anodal, corresponds to *PGM-2* described by ALLENDORF *et al.* (1977). Banding patterns lead one to suspect that zone 3 represents a duplicated locus. Zone 2 corresponds to the traditional *PGM* locus, which has been found to be polymorphic in many salmonid species (UTTER and HODGINS 1970, 1972; MAY 1975). We refer to this traditional locus as *PGM-2*, the most cathodal as *PGM-1* and the most anodal as *PGM-(3,4)*.

Single-locus segregation: Although aberrant single-locus segregation does not affect joint segregation unless aberrancies occur at both loci being tested, a substantial increase in the number of aberrations for individuals with hybridized genomes over that in pure brook trout individuals was observed. Five aberrations in 52 examinations (multiple families) in pure brook trout, 11 of 59 in BSRS trout and 33 of 134 in F_1 splake were noted, giving frequencies of aberrant single-locus segregation of 0.096 in pure brook trout, 0.186 in BSRS trout and 0.246 in splakes. Males showed a higher level of nonrandom single-locus segregation than females (0.248 *vs.* 0.129). The only locus that showed a tendency to segregate one species-specific allele more frequently than the other in F_1 splake individuals was *SDH*. An excess of the lake allele among the progeny occurred in nine of 10 families; this excess was significant in five families.

Joint segregation: A summary of our joint segregation studies is presented in Table 3. Each block constitutes a pairwise combination of loci, and the value in

TABLE 3

Number of families involved in pairwise examinations (individual blocks) of joint segregation of biochemical loci in brook trout (Salvelinus fontinalis) *and in a hybridized Salvelinus genome—lake trout* (S. namaycush) × *brook trout.*

SEGREGATION IN MALES

SEGREGATION IN FEMALES

	AAT-1,2	AAT-4	ADA-1	ADH	AGP-1	AGP-2	CPK-1	DIA.	GPT	GUS	IDH-3	LDH-3	LDH-4	MDH-1	MDH-3,4	ME-1	ME-2	MUP	ODH	PGI-1	PGI-2	PGI-3	PGM-2	PMI	SDH	SOD
AAT-1,2		1	3	5	1	6	1	4	4	3	-	6	2	4	4	-	-	3	4	-	-	1	3	1	1	1	4	4
AAT-4	1		-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ADA-1	2	-		6	2	5	3	5	5	4	-	10	1	5	5	1	-	5	6	1	2	2	1	1	2	-	5	5
ADH	1	-	2		1	8	4	8	8	7	-	8	2	8	8	1	-	7	9	1	2	1	1	2	3	1	8	8
AGP-1	4	-	2	1		-	-	1	-	1	-	2	-	1	1	-	-	1	1	-	-	1	1	-	-	-	1	1
AGP-2	3	-	5	4	2		3	6	6	5	-	7	2	6	6	1	-	5	7	-	1	1	1	2	3	1	6	6
CPK-1	1	-	-	1	2	1		3	2	3	-	4	1	3	3	-	-	2	3	-	-	-	1	2	1	1	3	3
DIA.	-	-	1	2	-	3	-		8	7	1	6	1	8	8	-	-	7	8	1	-	-	-	1	1	-	8	8
GPT	-	-	1	2	-	1	-	1		7	-	6	1	8	8	-	-	7	8	1	1	-	-	1	1	-	8	8
GUS	1	-	1	3	1	2	1	1	1		-	5	1	7	7	-	-	6	7	1	2	-	1	1	1	-	8	8
IDH-3	-	-	1	1	-	2	-	2	1	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LDH-3	3	-	6	5	4	6	2	2	2	3	1		2	6	6	1	-	6	7	1	2	2	2	2	3	1	6	6
LDH-4	1	-	2	2	1	2	1	1	1	2	1	3		1	1	-	-	1	1	-	-	-	1	1	2	1	1	1
MDH-1	1	-	1	4	1	4	1	2	1	2	1	4	1		8	-	-	7	8	1	1	-	-	1	1	-	8	8
MDH-3,4	-	-	-	2	-	2	-	2	1	1	1	2	-	2		-	-	7	8	1	1	-	-	1	1	-	8	8
ME-1	1	-	3	5	1	5	1	3	2	2	2	5	3	4	2		8	-	1	-	1	1	-	-	1	-	-	-
ME-2	-	-	1	3	-	3	-	2	1	-	1	3	-	3	2	3		-	-	-	-	-	-	-	-	-	-	-
MUP	3	-	1	3	3	4	2	2	1	2	1	4	1	3	2	3	2		7	1	1	-	-	1	1	-	7	7
ODH	1	-	1	4	1	3	1	2	2	3	1	4	2	3	2	4	2	3		1	2	1	-	1	2	-	8	8
PGI-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		1	-	1	-	-	-	1	1
PGI-2	1	-	1	2	1	1	1	1	1	2	-	2	2	1	-	2	-	1	2	1		1	1	-	1	-	1	1
PGI-3	1	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		1	-	1	-	-	-
PGM-2	2	-	-	1	2	1	2	-	-	1	-	2	1	1	-	1	-	2	1	1	1	-		1	-	1	-	-
PMI	-	-	1	1	-	-	-	-	1	1	-	1	1	-	-	1	-	1	-	1	-	-	-		1	1	1	1
SDH	1	1	2	-	2	1	1	-	-	-	-	1	-	-	-	1	-	1	-	-	-	-	-	-		-	-	-
SOD	-	-	2	3	-	4	-	3	1	1	1	4	1	3	2	4	3	2	2	-	-	-	-	-	-		1	2
																												9

Duplicate loci are listed *XYZ-N,N+1* (*e.g., AAT-1,2*) when the specific variant locus is unknown. In such cases, the left hand (or upper) block represents examinations where at least one locus was variable and the right hand (or lower) block represents examinations of the other locus when both loci were variable in the same individual. Results from heterozygous males are above the diagonal and those from heterozygous females are below the diagonal. Linkage associations are indicated by shaded blocks.

the block is the number of families examined. Segregation in males is above the diagonal and segregation in females is below the diagonal. The data are presented in this particular format to show both the extent of the study and to reveal particular associations in Salvelinus that other investigators may wish to pursue. The family data for the 11 pairwise examinations that are considered to represent clear linkage associations (shaded blocks) are presented in Tables 4 through 8. Where the parental linkage phase was known from species specificity, the linkage association was either classical linkage or pseudolinkage (an excess of nonparental types; to date observed only in males). The smallest sum of classes has been assumed to be recombinant in those instances where the linkage phase was unknown. Therefore, we refer to three classes of linkage associations: (1) classical linkage; (2) pseudolinkage; or (3) nonrandom assortment, linkage phase unknown.

TABLE 4

Joint segregation of CPK-1 *with* GUS, LDH-3 *with* LDH-4, PGI-2 *with* SDH, ODH *with* PMI *and* PGI-3 *with* PMI *in Salvelinus*

Family	Parents			Progeny				P†	r‡	N§
	Sex*	*A* Locus	*B* Locus	*AA BB*	*AA' BB*	*AA BB'*	*AA' BB'*			
		CPK-1	*GUS*							
Y-25	*F*	*AA'*‖	*BB'*	22	4	8	33	<0.001	0.180	67
	M	*AA*	*BB*							
Y-43	*F*	*AA'*	*BB'*	29	9	8	32	<0.001	0.218	78
	M	*AA*	*BB*							
Y-79	F	*AA*	*BB*	25	0	6	37	<0.001	0.088	68
	M	*AA'*	*BB'*							
		LDH-3	*LDH-4*							
Y-23	*F*	*AA'*	*BB'*	38	40	33	46	>0.70	0.535	157
	M	*AA*	*BB*							
Y-42	F	*AA*	*BB*	68	13	6	72	<0.001	0.880	159
	M	*AA'*	*BB'*							
		PGI-2	*SDH*							
Y-23	*F*	*AA'*	*BB'*	22	48	68	17	<0.001	0.252	155
	M	*AA*	*BB*							
		ODH	*PMI*							
Y-26	*F*	*AA'*	*BB'*	25	4	9	31	<0.001	0.188	69
	M	*AA*	*BB*							
		PGI-3	*PMI*							
Y-26	*F*	*AA'*	*BB'*	8	23	31	15	<0.001	0.299	77
	M	*AA*	*BB*							
Y-94	F	*AA*	*BB*	74	0	0	85	<0.001	0.000	159
	M	*AA'*	*BB'*							

* The informative parent is italicized.
† P of χ^2 test of joint segregation.
‡ Nonparental fraction assuming smallest classes to be nonparentals.
§ Informative number of progeny.
‖ Information on specific alleles involved may be obtained from authors.

All three types of linkage associations are evident in the results of the five pairwise comparisons involving nine loci shown in Table 4. *CPK-1* and *GUS* are shown to be classically linked in the three families studied (with two females and one male as the informative parents). The females showed a higher recombination value (mean of 0.200) than the male (0.088). One examination in a male and one in a female for *LDH-3* and *LDH-4* confirmed earlier conclusions regarding these two loci—pseudolinkage in the male, but random assortment in the female (MORRISON 1970). *PGI-2* and *SDH* assorted nonrandomly, though the linkage phase was unknown in the single female examined.

Two linkage associations are also shown (Table 4) that involve a common locus, therefore combining into a linkage grouping (as opposed to a true linkage group since the linkage phase is unknown). *ODH* and *PMI* assorted nonrandomly in the single female examined (family Y-26; $r = 0.188$). *PGI-3* and *PMI* were examined for joint segregation in one male (family Y-94) and one female (family Y-26). The male revealed no recombination, while the female showed a recombination frequency of 0.299. On the other hand, the female in family Y-26 revealed random assortment for *ODH* and *PGI-3* (see Table 3); therefore, the map order of *PGI-3–PMI–ODH* is suggested.

The results from joint segregation analysis of *ADA-1* and *AGP-2* in four males and one female are presented in Table 5. Three males showed no apparent recombination. The single female showed a recombination fraction of 0.154. The r value of 0.125 for one male (family Y-45) suggests the presence of a chromosomal rearrangement, possibly an inversion, since the two loci are still associated.

Random assortment of *AGP-1* and *MDH-1* (Table 6) was revealed in the two females examined (families Y-21 and Y-43). However, all males showed nonrandom assortment. The linkage phase of the splakes from Maple, Ontario, (males of families Y-79 and Y-81) could be determined from species specificity. They produced an excess of nonparental progeny types. Therefore the non-random

TABLE 5

Joint segregation of ADA-1 *and* AGP-2 *in Salvelinus*

Family	Parents			Progeny						P	r	N
	Sex	*A* Locus	*B* Locus	*AA BB*	*AA' BB*	*AA BB'*	*AA' BB'*	*AA B'B'*	*AA' B'B'*			
		ADA-1	*AGP-2*									
Y-23	*F*	*AA'*	*BB'*	12	72	60	12	—	—	<0.001	0.154	156
	M	*AA*	*BB*									
Y-40	F	*AA*	*BB'*	0	19	18	20	26	0	<0.001	0.000	45
	M	*AA'*	*BB'*									
Y-41	F	*AA*	*BB*	0	63	82	0	—	—	<0.001	0.000	145
	M	*AA'*	*BB'*									
Y-45	F	*AA*	*BB'*	4	21	20	36	28	3	<0.001	0.125	56
	M	*AA'*	*BB'*									
Y-94	F	*AA*	*BB*	0	91	60	0	—	—	<0.001	0.000	151
	M	*AA'*	*BB'*									

TABLE 6

Joint segregation of AGP-1 *with* MDH-1 *and* IDH-3 *with* ME-2 *in Salvelinus*

Family	Parents			Progeny						P	r	N
	Sex	*A* Locus	*B* Locus	*AA BB*	*AA' BB*	*AA BB'*	*AA' BB'*	*A'A' BB*	*A'A' BB'*			
		AGP-1	*MDH-1*									
Y-21	*F*	*AA'*	*BB'*	23	16	15	24	—	—	>0.05	0.397	78
	M	*AA*	*BB*									
Y-40	F	*AA'*	*BB*	11	40	26	40	31	12	<0.001	0.712	80
	M	*AA'*	*BB'*									
Y-41	F	*AA*	*BB*	22	69	49	17	—	—	<0.001	0.752	157
	M	*AA'*	*BB'*									
Y-42	F	*AA*	*BB*	24	75	42	18	—	—	<0.001	0.736	159
	M	*AA'*	*BB'*									
Y-43	*F*	*AA'*	*BB'*	44	38	45	32	—	—	>0.50	0.478	159
	M	*AA*	*BB*									
Y-45	F	*AA*	*BB*	25	54	51	29	—	—	<0.001	0.660	159
	M	*AA'*	*BB'*									
Y-79	F	*AA'*	*BB*	11	31	25	46	36	11	<0.001	0.735	83
	M	*AA'*	*BB'*									
Y-81	F	*AA*	*BB*	9	50	17	2	—	—	<0.001	0.859	78
	M	*AA'*	*BB'*									
		IDH-3	*ME-2*									
Y-21	*F*	*AA'*	*BB'*	5	27	40	6	—	—	<0.001	0.141	78
	M	*AA*	*BB*									
Y-23	*F*	*AA'*	*BB'*	61	3	5	81	—	—	<0.001	0.053	150
	M	*AA*	*BB*									
Y-26	*F*	*AA'*	*BB'*	38	5	2	28	—	—	<0.001	0.096	73
	M	*AA*	*BB*									
Y-40	F	*AA*	*BB*	91	1	2	65	—	—	<0.001	0.019	159
	M	*AA'*	*BB'*									
Y-41	F	*AA*	*BB*	59	4	2	84	—	—	<0.001	0.040	149
	M	*AA'*	*BB'*									
Y-42	F	*AA'*	*BB*	38	35	1	43	2	38	<0.001	0.038	79
	M	*AA'*	*BB'*									
Y-43	*F*	*AA'*	*BB'*	72	4	7	71	—	—	<0.001	0.071	154
	M	*AA*	*BB*									
Y-79	F	*AA*	*BB*	63	8	7	69	—	—	<0.001	0.102	147
	M	*AA'*	*BB'*									
Y-80	F	*AA*	*BB*	33	4	1	37	—	—	<0.001	0.067	75
	M	*AA'*	*BB'*									
Y-81	F	*AA*	*BB*	42	0	1	35	—	—	<0.001	0.013	78
	M	*AA'*	*BB'*									
Y-98	F	*AA*	*BB*	79	3	1	70	—	—	<0.001	0.026	153
	M	*AA'*	*BB'*									

assortment is attributed to pseudolinkage and all r values for the other males are calculated on the assumption that the larger classes are nonparentals.

Also shown in Table 6 are the results of examinations for joint segregation of *IDH-3* and *ME-2* in four female individuals and seven male individuals. The females showed a greater average recombination than males (0.086 *vs.* 0.048). *IDH-3* and *ME-2* were reported to be completely linked in a single male splake by STONEKING, MAY and WRIGHT (1979). The evidence of variable recombination from the seven males (r of 0.013 to 0.102) and four females (r of 0.053 to 0.141) examined in this study further point up the need to do multiple crosses when possible.

The results of analyses of joint segregation of the duplicated *AAT-(1,2)* loci with *AGP-1* and *MDH-1* (Table 7) are not straightforward because it is impossible to tell *a priori* which *AAT* locus is variable in a given family. All males showed nonrandom assortment in both comparisons; however, there seem to be two modes of recombination values—one relatively high ($r > 0.20$), the other relatively low ($r < 0.15$). All three females tested for joint segregation of *AAT-1,2* with *AGP-1* showed random assortment; no females were involved in tests of *AAT-1,2 with MDH-1*. It has been previously found (WRIGHT *et al.* 1980) that *AAT-1* and *AAT-2* do not assort randomly in pure brook trout males from intercrosses of inbred lines, while they do assort randomly in females. Moreover, in the same families producing the inferred pseudolinkage of the two *AAT* loci, *AGP-1* was linked with one *AAT* locus with an r value of 0.09, but associated with the other *AAT* locus with an$r$ value of 0.70. Combining the previous data with those of the present investigation leads to the following model: *ATT-1* is linked to *MDH-1*, while *AAT-2* is linked to *AGP-1*, and the two linkage groups are pseudolinked to each other (the two *AAT* loci are arbitrarily defined here, based on their classical linkage to either *AGP-1* or *MDH-1*).

Therefore, the "recombination values" (frequency of nonparentals) for the four families, Y-42, Y-45, Y-79 and Y-81, from Tables 6 and 7 are presented in Table 8 to reffect the results predicted from the above model. Whereas the r values presented in Table 7 were based on the assumption that the smallest two classes of progeny were recombinants (since the linkage phase for *AAT-1,2* was unknown), they were recalculated in some instances to reflect pseudolinkage according to the above model (Table 8). Thus, *AAT-2* in the male of family Y-45 reflected classical linkage with *AGP-1*, but pseudolinkage with *MDH-1*. On the other hand, *AAT-1* in males of families Y-42, Y-79 and Y-81 had classical linkage with *MDH-1*, but pseudolinkage with *AGP-1*. The male of Y-81 exhibited less recombination for all associations in the linkage grouping *AAT-(1,2)* with *AGP-1* with *MDH-1*. Similar results were reported involving *IDH-3* with *ME-2* (Table 6) for this male.

The linkage phase was known in every doubly heterozygous parent listed in Table 9, where the joint segregation analyses of the duplicated loci *MDH-3* and *MDH-4* are shown. All eight males exhibited pseudolinkage (reported previously for a single male splake by MAY, WRIGHT and STONEKING 1979), while three females showed random assortment.

TABLE 7

Joint segregation of AAT-1,2 *with* AGP-1 *and* AAT-1,2 *with* MDH-1 *in Salvelinus*

Family	Parents Sex	*A* Loci	*B* Locus	Progeny *AA AA BB*	*AA AA BB'*	*AA AA B'B'*	*AA AA' BB*	*AA AA' BB'*	*AA AA' B'B'*	*AA A'A' BB*	*AA A'A' BB'*	*AA A'A' B'B'*	*AA' A'A' BB*	*AA' A'A' BB'*	*P*	*r*	*N*
		AAT-1,2	*AGP-1*														
Y-23	*F*	*AA, AA'*	*BB'*	—	—	—	22	20	—	38	40	—	21	19	>0.95	0.500	82
	M	*AA', A'A'*	*BB*														
Y-25	F	*AA, AA*	*BB*	5	34	—	36	38	—	44	3	—	—	—	<0.001	0.093	86
	M	*AA', AA'*	*BB'*														
Y-41	*F*	*AA, AA'*	*BB'*	42	32	—	42	41	—	—	—	—	—	—	>0.30	0.471	157
	M	*AA, AA*	*BB*														
Y-42	F	*AA, A'A'*	*BB*	—	—	—	43	18	—	23	74	—	—	—	<0.001	0.260	158
	M	*AA, AA'*	*BB'*														
Y-45	F	*AA', A'A'*	*BB*	—	—	—	43	2	—	23	41	—	6	38	<0.001	0.090	89
	M	*AA, AA'*	*BB'*														
Y-50	*F*	*AA', AA*	*BB'*	14	18	—	24	21	—	18	11	—	4	7	>0.70	0.488	43
	M	*AA', AA'*	*BB*														
Y-79	F	*AA, AA*	*BB'*	27	43	8	9	34	39	—	—	—	—	—	<0.001	0.205	83
	M	*AA, AA'*	*BB'*														
Y-81	F	*AA, A'A'*	*BB*	—	—	—	16	2	—	9	50	—	—	—	<0.001	0.143	77
	M	*AA, AA'*	*BB'*														
Y-94	F	*AA, AA'*	*BB*	38	2	—	30	28	—	9	20	—	6	20	<0.001	0.121	66
	M	*AA', AA'*	*BB'*														
		AAT-1,2	*MDH-1*														
Y-42	F	*AA, A'A'*	*BB*	—	—	—	2	59	—	96	1	—	—	—	<0.001	0.019	158
	M	*AA, AA'*	*BB'*														
Y-45	F	*AA', A'A'*	*BB*	—	—	—	11	35	—	36	28	—	29	15	<0.001	0.289	90
	M	*AA, AA'*	*BB'*														
Y-79	F	*AA, AA*	*BB*	5	73	—	73	9	—	—	—	—	—	—	<0.001	0.088	160
	M	*AA, AA'*	*BB'*														
Y-81	F	*AA, A'A'*	*BB*	—	—	—	0	18	—	59	0	—	—	—	<0.001	0.000	77
	M	*AA, AA'*	*BB'*														

TABLE 8

Joint segregation of AGP-1 *with* AAT-(1,2) *with* MDH-1 *in four male splake trout backcross families*

Family (*AAT*)	Pairwise examinations		
	AGP-1/MDH-1	*AAT-1,2/AGP-1*	*AAT-1,2/MDH-1*
Y-42 (2)†	0.736*	0.740	0.019
Y-45 (1)	0.660	0.090	0.711
Y-79 (2)	0.735	0.795	0.088
Y-81 (2)	0.859	0.857	0.000

* Fraction of assumed nonparental progeny types.
† Designated *AAT* locus, which is variable in each family based on classical linkage to *AGP-1* or *MDH-1* (see text).

TABLE 9

Joint segregation of MDH-3 *with* MDH-4 *in Salvelinus*

Family	Parents			Progeny						
	Sex	*A* Locus	*B* Locus	*AA BB*	*AA' BB*	+ *AA BB'*	*AA' BB'*	*P*	*r*	*N*
		MDH-3	*MDH-4*							
Y-21	*F*	*AA'*	*BB'*	23	36*	21		>0.30	0.450	80
	M	*AA*	*BB*							
Y-40	F	*AA*	*BB*	6	146	8		<0.001	0.912	160
	M	*AA'*	*BB'*							
Y-41	F	*AA*	*BB*	10	134	15		<0.001	0.843	159
	M	*AA'*	*BB'*							
Y-42	F	*AA*	*BB*	6	147	6		<0.001	0.925	159
	M	*AA'*	*BB'*							
Y-43	*F*	*AA'*	*BB'*	49	78	33		>0.70	0.488	160
	M	*AA*	*BB*							
Y-45	F	*AA*	*BB*	14	137	9		<0.001	0.856	160
	M	*AA'*	*BB'*							
Y-61	*F*	*AA'*	*BB'*	21	37	22		>0.50	0.463	80
	M	*AA*	*BB*							
Y-77	F	*AA*	*BB*	6	68	4		<0.001	0.872	78
	M	*AA'*	*BB'*							
Y-79	F	*AA*	*BB*	1	152	5		<0.001	0.962	158
	M	*AA'*	*BB'*							
Y-80	F	*AA*	*BB*	7	67	3		<0.001	0.870	77
	M	*AA'*	*BB'*							
Y-81	F	*AA*	*BB*	7	68	3		<0.001	0.871	78
	M	*AA'*	*BB'*							

* Electrophoretic identity of alleles at both loci precludes separation of these two progeny classes.

TABLE 10

Specific progeny classes from six families of male splakes all of genotype [MDH-(3,4) (AA′, AA′), LDH-4 (BB′)] *backcrossed to homozygous female brook trout, where* A = *allele 100*, A′ = *74*, B = *100, and* B′ = *240* (AA, AA′ *and* AA′, AA *progeny excluded*)

Progeny genotypes		Distribution of progeny among families						
MDH-(3,4)	*LDH-4*	Y-40	Y-41	Y-42	Y-45	Y-79	Y-80	Total
AA, AA	*BB*	2	5	3	10	1	4	25
AA, AA	*BB′*	4	5	3	4	0	3	19
AA′, AA′	*BB*	2	7	2	6	4	2	23
AA′, AA′	*BB′*	6	8	4	3	1	1	23

χ^2 for joint segregation = 0.400, $p > 0.50$, for *MDH-3* and/or *MDH-4* with *LDH-4* comparing the sums of totals for lines 1 and 4 versus lines 2 and 3.

DAVISSON, WRIGHT and ATHERTON (1973) suggested a fusion of two acrocentrics to explain the pseudolinkage of *LDH-3* and *LDH-4*. Limited data were available to test for the relationship of the pseudolinkage of the *LDH* loci to the pseudolinkage of *MDH-3* and *MDH-4*. The data from the progeny distribution for *MDH-(3,4)* and *LDH-4* in six splake males backcrossed to brook trout females are presented in Table 10. The data were summed to increase the sample size since the phase was known to be the same in each family. This is a test of whether or not *MDH-3* and/or *MDH-4* are jointly assorting with *LDH-4*. The results indicate random assortment, suggesting no linkage relationship between *LDH-4* and either of these *MDH* loci. Although one BSRS female (family Y-61) demonstrated nonrandom joint segregation of *MDH-(3,4)* with *LDH-4* ($N = 43$, $p \simeq 0.01$), two other females showed random assortment. It was also possible to examine joint segregation of *MDH-(3,4)* with *LDH-3*. One splake male (family Y-42) revealed an excess of nonparental to parental progeny types (10 to 2); although the sample size is small, the suggestion of pseudolinkage is intriguing. *LDH-3* and *MDH-3,4* were found to assort randomly in three females (families Y-23, Y-25 and Y-26). However, only a single *MDH* locus was variable in each family and the use of females is insufficient to establish the absence of linkage.

DISCUSSION

The hybridized genome of lake × brook trout was exploited using F_1 splakes and splake backcrosses (BSRS) in order to maximize the number of pairs of biochemical loci that could be examined. This was done to expand the initial mapping process in Salvelius and to identify those linkage associations that should be examined in other salmonid species. Although there is an increase of aberrant single locus segregation in F_1 splake over splake backcrosses (BSRS) over pure brook trout, this is not viewed as an indication of major disparity between the two genomes. In fact, backcrosses and further generations of splake intercrosses are quite fertile. An examination of ten F_8 splakes from Maple, Ontario, for 11 loci possessing species-specific alleles revealed that all loci were still variable;

indeed, a total of 113 brook alleles to 107 lake alleles were found in the expected 1:2:1 ratio (our unpublished data).

We have assigned 19 of 28 variable loci to eight linkage groupings: *LDH-3* with *LDH-4*, *ODH* with *PMI* with *PGI-3*, *MDH-3* with *MDH-4*, *AAT-1* with *MDH-1* with *AGP-1* with *AAT-2*,*ADA-1* with *AGP-2*, *CPK-1* with *GUS*, *IDH-3* with *ME-2* and *PGI-2* with *SDH*. "Linkage grouping" connotes a collection of linkage associations related by one or more common loci, as contrasted with the conventional "linkage group" consisting of loci known to be classically linked. The particular loci involved in these linkage associations and groupings lead to the following conclusions and expectations about the historical restructuring and present state of the Salvelinus genome.

IDH-3 and *ME-2* have been shown to be classically linked. The companion duplicates *IDH-4* and *ME-1* should also be linked if (1) their duplicate status has been correctly interpreted, (2) *IDH-3* and *ME-2* are located on a single chromosome arm, and (3) no chromosomal rerrangement has occurred to separate them. *ME-1* and *ME-2* have been shown to assort randomly (STONEKING, MAY and WRIGHT 1979). Is this also true for *IDH-3* and *IDH-4*? If duplicate loci are shown to be linked, one can propose with some confidence that they reside on a metacentric chromosome derived from a Robertsonian fusion.

Salvelinus fontinalis and *S. namaycush* have lost activity of the companion duplicates for *AGP-1* and *SDH*. The linkage of *AGP-1* to one member of the duplicate pair of loci *AAT-(1,2)* and *SDH* to *PGI-2* of the pair *PGI-(1,2)* support the hypothesis that whole linkage groups are not shut off or physically lost as the primary mechanism for the loss of duplicate gene activity. This hypothesis may initially seem less tenable in light of the linkage of *PGI-3* with *PMI* with *ODH*, all single loci. However, since we presently estimate that there has been a 60% loss of duplicate gene expression (unpublished data from this laboratory), one expects to find some linkage combination of single loci. The case, assumed by most authors, that loss of duplicate gene activity is a random, individual locus phenomenon (BAILEY, POULTER and STOCKWELL 1978; ALLENDORF 1979; FERRIS and WHITT 1979; KIMURA and KING 1979; TAKAHATA and MARUYAMA 1979) is made more plausible with this evidence.

No case of classical linkage has been documented among the six sets of duplicate loci that have been examined in salmonids to date: *AAT-(1,2)* (*S. clarki*, ALLENDORF and UTTER 1976; *S. fontinalis*, WRIGHT *et al.* 1980); *LDH-(3,4)* (splake trout, MORRISON 1970; *S. fontinalis*, DAVISSON, WRIGHT and ATHERTON 1973; *S. gairdneri*, WRIGHT, HECKMAN and ATHERTON 1975); *MDH-(3,4)* (*O. gorbuscha*, ASPINWALL 1974; *S. gairdneri*, ALLENDORF 1975); splake trout, MAY, WRIGHT and STONEKING 1979); *MDH-(1,2)* (*S. trutta*), MAY, STONEKING and WRIGHT 1979); *ME-(1,2)* (splake trout, STONEKING, MAY and WRIGHT 1979); and *PGI-(1,2)* (*S. fontinalis*, this paper). Even though the phase was not known in several of the above studies, it appears that in Salvelinus (n = 42, 8 metacentrics) at least, fusions do not seem to have involved formerly homeologous chromosomes. However, this situation may not be the same in other salmonids such as *S. gairdneri* (n = 29–30, 22–23 metacentrics) which have gone through

more fusion events (that is, have more metacentric than acrocentric chromosomes).

ALLENDORF (1975) showed nonrandom assortment of *AGP-1* and *IDH-3* in two *S. gairdneri* males, but not in two females. MAY, WRIGHT and STONEKING 1979 reported a similar finding, with one *S. fontinalis* male assorting nonrandomly, while two males and one female assorted randomly. The present study reveals random assortment for these loci in two BSRS females and two BSRS males, in two F_1 splake females and three F_1 splake males, and in two *S. fontinalis* females. However, nonrandom assortment was found for two F_1 splake males. It is not possible to distinguish classical linkage from pseudolinkage since the linkage phase was not known in any of these studies and the assumed r value approached 0.5. The absence of association in females could be explained either by the greater amount of recombination in females if classical linkage were responsible, or by the fact that females do not demonstrate pseudolinkage. Any association of *AGP-1* and *IDH-3* is puzzling since both loci are involved in other linkage groupings [*i.e.*, *AAT-(1,2)* with *AGP-1* with *MDH-1* and *IDH-3* with *ME-2*]. It is possible that pseudolinkage is far more prevalent than imagined in salmonids and that a linear linkage model is not appropriate. The variable numbers of meiotic multivalents that have been reported for salmonids (OHNO *et al.* 1965; DAVISSON, WRIGHT and ATHERTON 1973; GOLD and GALL 1975; unpublished results of G. Lee, this laboratory) undoubtedly play a substantial role in the variable linkage associations found from sex to sex or individual to individual.

MAY, WRIGHT and STONEKING (1979) reported a circuitous relationship for *SOD* with *DIA* with *CPK-1* based on one splake male. This study does not support those earlier findings, but rather points to the need to examine multiple families and not be misled by nonrandom assortment at low levels of statistical significance.

The nature of the chi-square test is such that one expects to find occasionally (one in 20) instances of statistically significant nonrandom joint segregation for which there is no real genetic basis. Most of the cases where $0.01 < p < 0.05$ are probably of this nature, since they usually occurred in only one family (data available upon request). However, a few of these individual families where low significance cases were observed and specifically those cases where $p < 0.001$, such as *ADA-1* with *ME-1*, *GPT* with *IDH-3*, *GPT* with *LDH-4* and *GPT* with *ME-2*, may reflect chromosomal polymorphisms. The chromosome methodology for Salmonidae has not been refined enough to identify chromosomal polymorphisms other than the occurrence of Robertsonian fissions and fusions. It is not possible to identify the particular chromosomes involved (DAVISSON, WRIGHT and ATHERTON 1972; GOLD 1979) with the exception of the male sex chromosome in rainbow trout and sockeye salmon (THORGAARD 1977, 1978). Translocations or any other chromosome structural changes and their concordant linkage rearrangements will trouble other investigations until general linkage maps are established for each salmonid species. Linkage groups must be confined to single arms so that it will be possible to discuss fusions of particular arms that have taken place within different genera and species. For this reason, it seems more beneficial to begin

linkage maps with species possessing few metacentrics in the haploid complement such as Salvelinus spp. with 8, *S. trutta* with 11, or *O. keta* with 13 metacentrics.

Unlike other research organisms, few distinct lines of salmonid species exist that can be drawn on for genetic variability; the artificially maintained populations that do exist (hatchery strains) tend to be very openbred rather than inbred. Therefore, it will be necessary to take advantage of the great number of variants that have been uncovered in native salmonid populations (UTTER *et al.* 1978) to develop the specific or generic genome maps. An expedient approach toward this objective would be to examine the literature for populations possessing variants at two loci that have either been shown to be linked in one species or have not been previously examined in any species, and to use males from such populations for inheritance studies. Our own studies are now focusing on *S. gairdeneri* and *S. trutta*. Such studies will answer the third question raised above: How conserved among salmonids are the linkage relationships that we have uncovered in Salvelinus?

We are still left with the problem of trying to explain the phenomenon that has been termed "pseudolinkage." The evidence to date is that (1) it occurs only in males, (2) it results in an excess of nonparental progeny types, (3) it apparently involves homeologous chromosome arms as reflected by the association of duplicate loci, (4) it is usually observed in divergent genomes and (5) the pseudolinkage event is retained to varying degrees in the male progeny of the male splake crossed to a female brook trout (*i.e.*, a variable amount of classical linkage as well as nonrandom assortment is observed in subsequent generations; DAVISSON, WRIGHT and ATHERTON 1973).

Are all pseudolinkage events due to only a single meiotic phenomenon? DAVISSON, WRIGHT and ATHERTON (1973) found that pseudolinkage of *LDH-3* and *LDH-4* in male splakes was accompanied by an intraindividual polymorphism for one extra metacentric chromosome (and two fewer acrocentrics). Our present data showing pseudolinkage of *MDH-3* and *MDH-4* coupled with the above would suggest that these two duplicated pairs of loci should be linked if a single Robertsonian fusion of two acrocentrics is responsible for both pseudolinkage events. The much tighter association of the *MDH* loci ($\bar{r} = 0.893$) than the LDH loci ($\bar{r} = 0.788$; from MORRISON 1970) suggests that if the two pairs are linked, the *MDH* loci should lie closer to their respective centromeres. No linkage between one of the *MDH* loci and either *LDH-3* or *LDH-4* was observed in a female (Y-23). However, because of the absence of pseudolinkage in females, the greater level of recombination and the need to test both *MDH* loci at the same time, it would be more desirable to make the tests in males. The lack of association between either *MDH-3* or *MDH-4* and *LDH-4* (Table 10) in male splake does not support the expectation that these two pairs of duplicated loci are associated. It is also necessary to test both *MDH* loci against *LDH-3*. This test was possible only in the family Y-42 male. Although the sample size was far too small, there is reason to suspect some association between *LDH-3* and one of the *MDH* loci. The association, if real, would be another case of pseudolinkage since the phase is known. This latter possible association coupled with the lack of association between *LDH-4* and

the *MDH* loci would not support a simple Robertsonian fusion model to explain pseudolinkage.

The lack of association between the *AGP-1* or the *MDH-1* loci and the *LDH-(3,4)* or *MDH-(3,4)* loci lends further evidence to the present conclusion that pseudolinkage is not merely a simple Robertsonian fusion event, as suggested by DAVISSON, WRIGHT and ATHERTON 1973. WRIGHT *et al.* (1980) proposed that observed limited multivalent homeologous pairing of two acrocentric and one arm of centrically fused metacentric chromosomes could account for pseudolinkage in males. A breakthrough in salmonid chromosome methodology is clearly required to understand this fascinating genetic phenomenon. Solving the mechanics of pseudolinkage may guide us in understanding the process of diploidization of a genome following an ancestral tetraploid event.

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