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Joint Segregation of Biochemical Loci in Salmonidae. III. Linkage Associations in Salmonidae Including Data from Rainbow Trout (*Salmo gairdneri*)

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The results of 107 pairwise examinations of joint segregation of biochemical and skin color loci in rainbow trout, Salmo gairdneri, *are presented. Three examinations revealed significant nonrandom assortment: Idh-3 with Me-2, Ada-1 with G3p-3, and Mdh-3 with Mdh-4. We believe the first two instances to be cases of classical linkage and the latter instance to reflect pseudolinkage based on similar findings in* Salvelinus. *All known salmonid linkage associations are reviewed. The results of this summary indicate a high degree of genome conservatism among genera within the Salmonidae, which would seem to be in contrast to the highly plastic genome of this family based on karyotypic data. Data are presented which negate the view that Robertsonian fusion of homologous acrocentric chromosome arms was the preferred mode of metacentric formation in salmonid evolution.*

KEY WORDS: Salmonidae; isozymes; comparative gene mapping; linkage associations.

INTRODUCTION

With the advent of the electrophoretic methodology in the late 1960s it became possible to map loci with known gene products. The role of these structural genes is obvious in cellular metabolism, whereas the precise

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function of most morphological mutants is not obvious. Linkage maps of structural genes and their concomitant regulatory loci may lead to a better understanding of the arrangement and regulation of genomes. Comparative gene mapping of such loci among taxa offers direct insight into evolution (Lundin, 1979).

While comparative gene mapping of diploid organisms may provide insights into microevolution, the study of rearrangements in polyploid derivative genomes may reveal more profound knowledge about macroevolution, those instances of great evolutionary change. What happens to duplicated structural and regulatory loci following a polyploid event? How is their loss or retention governed following diploidization? What types of chromosomal rearrangements are possible in a polyploid derivative lineage?

Comparative gene mapping in polyploid derivative lineages involves two types of homology among loci, orthologous and paralogous (Fitch, 1976). Orthologous genes are ancestrally related genes separated by speciation alone, whereas paralogous genes are those which result from an ancestral duplication event.

The salmonid fishes are ideal organisms for studying the ongoing process of evolution by polyploidy. They obviously constitute a tetraploid derivative genome based on their having relatively double (1) the DNA content per cell, (2) the chromosome arm number, and (3) the number of electrophoretically detected biochemical loci at 40% of the genes examined (Ohno *et al.*, 1969; Engel *et al.*, 1975; May, 1980) compared to their diploid counterparts. Further, inheritance results for biochemical loci for these fish indicate that they have not completed the diploidization process (May *et al.*, 1979b, 1980; Wright *et al.*, 1980).

One very important manifestation of the continued evolution of the salmonid genome is the genetic phenomenon of pseudolinkage. Pseudolinkage was the term given to the production of an excess of nonparental progeny types from certain doubly heterozygous male individuals for Ldh-3 and Ldh-4 in *Salvelinus,* while females showed independent assortment (Morrison, 1970; Davisson *et al.*, 1973). Pseudolinkage has now been shown to be more than a single isolated phenomenon, involving associations of nonduplicated with duplicated loci as well as associations of duplicated loci (May *et al.*, 1979b, 1980; Wright *et al.*, 1980). None of these associations is exhibited in females, and the phenomenon appears to be related to a form of residual tetrasomy permitted by homeologous pairing in males.

We have recently reported an extensive number of linkage associations, both classical linkage and pseudolinkage, in *Salvelinus* (Stoneking *et al.*, 1979; May *et al.*, 1979b, 1980; Wright *et al.*, 1980; May, 1980). We now present results of conservation of some of these linkage associations in the genus *Salmo* (*S. gairdneri*) and summarize the results of all linkage studies on Salmonidae.

MATERIALS AND METHODS

Horizonal starch gel electrophoretic methods followed those detailed by May *et al.* (1979b). Staining followed the procedures of Allendorf *et al.* (1977) and May (1980). The loci examined and their abbreviations are listed in Table I. Domestic stocks of *S. gairdneri* maintained in hatcheries in Pennsylvania, West Virginia, and Montana served as the parents of the 26 families examined. Methods of matings, genic nomenclature, and statistical treatment of the data were detailed by May *et al.* (1979b) with modifications described by May (1980).

RESULTS

Significant departures from expected Mendelian ratios for single-locus segregations were found in 28 of 199 examinations. However, most of the marginally significant chi-square values occurred in only one of several families for each single locus tested and, therefore, are probably due to chance alone.

Table I. Enzymes, Loci, Alleles, Tissues, and Buffer Systems Used During this Investigation of Joint Segregation in *Salmo gairdneri*

Enzyme	Locus	Alleles[a]	Tissue	Buffer[b]
Adenosine deaminase	Ada-1	92	K	MF
Albino color	Alb	A, a	—	—
Creatine kinase	Ck-1	76	M	RSL
Fructose diphosphatase	Fdp	77	K	MF
Golden color	Gol	G, g	—	—
Glycerol-3-phosphate dehydrogenase	G3p-1	140	M	AHZV
Glycerol-3-phosphate dehydrogenase	G3p-3	87	M	CT
Isocitrate dehydrogenase	Idh-2	83	M	AHZV
Isocitrate dehydrogenase	Idh-3	67, 138, 170	L, E	CT, AHZV
Lactate dehydrogenase	Ldh-3	∅	E	R, AHZV
Lactate dehydrogenase	Ldh-4	76	L, E	R
Lactate dehydrogenase	Ldh-5	97	E	AHZV, RSL
Malate dehydrogenase	Mdh-3, 4	67, 84, 118	M	CT
Malic enzyme	Me-2	s	M, L	CT
Phosphoglycerate kinase	Pgk	s, m, f	M, L	CT
Phosphoglucomutase	Pgm-2	90	M, L	CT
Phosphoglucomutase regulatory	PgmR-1	−, +	L	CT
Sorbitol dehydrogenase	Sdh-2	s	L	RSL
Superoxide dismutase	Sod	152	L	RSL

[a]Excluding common allele 100. aa, albino; Gg, palamino; gg, golden; s, slow; m, medium; f, fast; −, lack of expression (common allele); ∅, null.
[b]See May *et al.* (1980) for details of these buffers.

The pairwise combinations of loci examined are presented in Fig. 1. The number in each block represents the number of families examined for that particular pairwise comparison (no informative N less than 40). Examinations in males are above the diagonal and those in females are below the diagonal. As in previous studies on *Salvelinus* (May *et al.*, 1979b, 1980; Stoneking *et al.*, 1979), a number of statistically significant chi-square values for joint segregation were recorded which were not considered biologically significant since they usually occurred in only a single family of several families tested. (Extended data on the preceding results will be provided on request.)

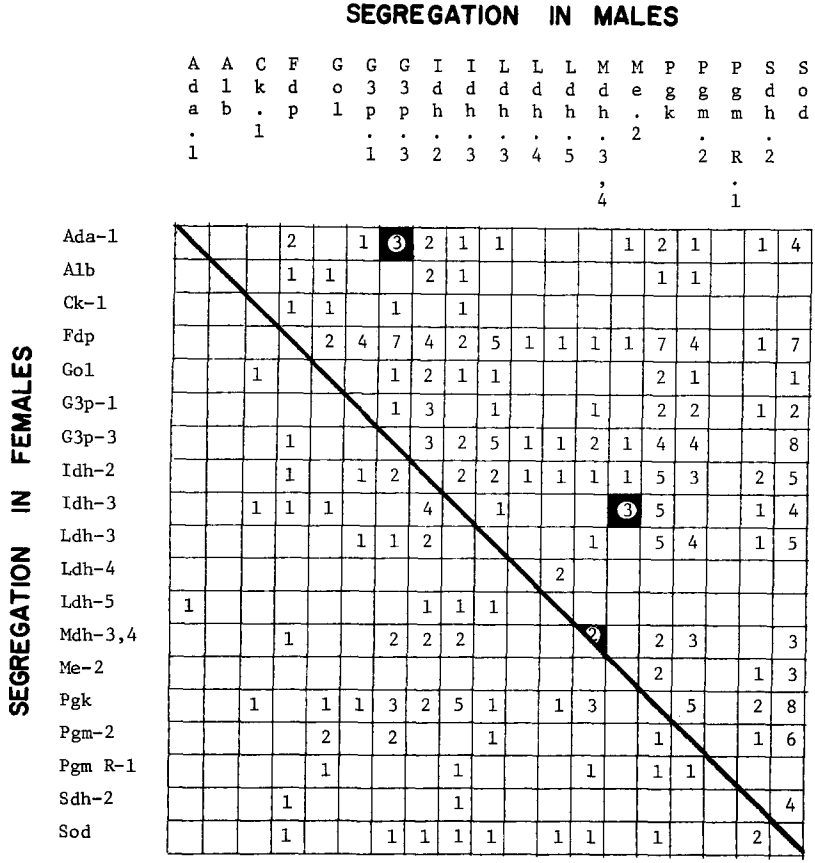

Fig. 1. Pairwise examinations of joint segregation of biochemical loci in *Salmo gairdneri*. The numbers in the blocks are the number of families examined, with examinations in males above the diagonal and examinations in females below the diagonal. Significant linkage associations are indicated by shaded blocks. All Mdh-3,4 comparisons with other loci involved only one of the MDH loci. The single exception was the males of families Z-58 and Z-83, where both loci were variable. It was not possible to compare these two loci to any other loci in those two families because of the Mdh-(3,4) segregation results.

Table II. Joint Segregation of Idh-3 with Me-2 in *Salmo gairdneri*

Family	Parents			Progeny						
	Sex	A locus[a]	B locus[b]	AA BB	AA BB′	AA′ BB	AA′ BB	P^c	r^d	N^e
Z-7	F	AA	BB	35	1	1	32	<0.001	0.029	69
	M	AA′	BB′							
Z-8	F	AA	BB	31	0	0	36	<0.001	0.000	67
	M	AA′	BB′							
Z-72	F	AA	BB	25	0	3	47	<0.001	0.040	75
	M	AA′	BB′							

[a]A = allele 138 and A′ = allele 100 for Idh-3 (A′ = allele 67 in Z-7).
[b]B = allele 100 and B′ = allele "slow" for Me-2 (the homotetramer coded by the slow allele was not visible).
[c]Probability of chi-square test of joint segregation.
[d]Nonparental fraction assuming smallest classes to be parentals.
[e]Informative number of progeny.

Three cases of nonrandom assortment, which reflect linkage associations, were found and are shaded in Fig. 1: Idh-3 with Me-2 (specific results in Table II), Ada-1 with G3p-3 (Table III), and the paralogous duplicated loci Mdh-3 with Mdh-4 (Table IV). Both Idh-3 and Me-2 are members of duplicate pairs. Idh-3 is the IDH locus which has been reported to be variable in *S. gairdneri* by other authors (see May, 1980). No variation has previously been described for Me-(1,2) in *S. gairdneri*. These loci are designated Idh-3 and Me-2 to agree with the association found in *Salvelinus* (Stoneking *et al.*, 1979; May *et al.*, 1980); however, it is entirely possible that this association in *S. gairdneri* actually represents Idh-4 with Me-1 since Idh-3 and Idh-4 share common alleles, as do Me-1 and Me-2. This distinction will not be easily determined without homology studies or the use of other marker loci.

Table III. Joint Segregation of Ada-1 with G3p-3 in *Salmo gairdneri*

Family	Parents			Progeny								
	Sex	A locus[a]	B locus[b]	AA BB	AA′ BB	AA BB′	AA′ BB′	AA B′B′	AA′ B′B′	P	r	N
Z-6	F	AA	BB	5	23	26	0	—	—	<0.001	0.093	54
	M	AA′	BB′									
Z-10	F	AA	BB	0	35	27	1	—	—	<0.001	0.016	63
	M	AA′	BB′									
Z-73	F	AA	BB′	18	0	18	17	1	21	<0.001	0.025	40
	M	AA′	BB′									

[a]A = allele 100 and A′ = allele 92 for Ada-1.
[b]B = allele 100 and B′ = allele 87 for G3p-3.

Table IV. Segregation of Mdh-3 and Mdh-4 in Male and Female *Salmo gairdneri*, Where A = Allele 100 and A′ = Allele 67 (Z-1, 5, 18, 32, 58, and 83), 118 (Z-17), and 85 (Z-71, 74, 79, and 101)[a]

Parents			Progeny				
Family	Female	Male	AAAA	AAAA′	AAA′A′	P^b	N
Z-1	AAAA	AAAA′	112	128	—	>0.1	240
Z-5	AAAA′	AAAA	32	46	—	>0.1	78
Z-17	AAAA′	AAAA	42	38	—	>0.5	80
Z-18	AAAA′	AAAA	104	120	—	>0.1	224
Z-32	AAAA	AAAA′	51	63	—	>0.1	114
Z-58	AAAA	AAA′A′[c]	5	63	12	<0.001	80
Z-71	AAAA	AAAA′	26	38	—	>0.1	64
Z-74	AAAA′	AAAA	40	38	—	>0.5	78
Z-79	AAAA′	AAAA	45	35	—	>0.1	80
Z-83	AAAA	AAA′A′[c]	4	75	1	<0.001	80
Z-101	AAAA′	AAAA	178	156	—	>0.1	334

[a]One cannot tell which MDH locus is variable in AAAA′ individuals.
[b]Probability of chi-square test of 1:1 or 1:2:1 segregation.
[c]Both loci are assumed to be heterozygous, $P < 0.001$.

Ada-1 with G3p-3 (Table III) would appear to be the same linkage association as in *Salvelinus* (May *et al.*, 1980) since (1) both genera possess only a single locus for G3p-3, the presumptive G3p-4 locus having been lost, and (2) the ADA variation is the least anodal ADA activity in both cases. This association was assumed to be a case of classical linkage in *Salvelinus* since females and males showed nonrandom assortment. The three males of *S. gairdneri* have an average recombination value (r) of 0.045. The results in *Salvelinus* show one female where $r = 0.154$, three males where $r = 0.0$, and one male where $r = 0.125$. May *et al.* (1980) suggested that the latter male was a case of a chromosomal inversion. Alternately, it is possible that one of the two distinct recombination values represents the association between the structural loci whereas the other value is actually an instance of mapping loci which modify ADA or G3P activity (Finnerty and Johnson, 1979).

The results of segregation for the duplicate genes Mdh-(3,4) are presented in Table IV. Those families where only one variant allele was possessed by the male or female parent produced progeny in a 1:1 Mendelian ratio as expected for a disomic locus. Additionally, no exceptional progeny types due to double reduction gametes were observed among the progeny (AAA′A′ individuals) of any family even though large numbers of progeny were examined in four families (Z-1, 18, 32, and 101). The results for two families (Z-58 and Z-83) where the two male parents possessed two copies of allele A and two of A′ are also shown in Table IV. The significant nonrandom assortment shown is probably not a case of classical linkage since Allendorf (1975) found three such *S. gairdneri* males to segregate at the 1:2:1 ratios

expected of random assortment. The orthologous counterparts of these paralogous loci have been shown to be pseudolinked in *Salvelinus* (May *et al.*, 1979b, 1980).

No pairs of loci were found to assort randomly in *S. gairdneri* which had been found to assort nonrandomly in *Salvelinus,* or vice versa (see Fig. 1).

DISCUSSION

The case seems quite clear that the salmonid genome has largely achieved diploidization based on the single-locus inheritance of biochemical genes (May *et al.*, 1979b, 1980). On the other hand, the progeny data from family Z-58 (see also Z-83) for Mdh-3 and Mdh-4 (Table IV) exemplify the difficulties inherent in interpreting inheritance patterns of duplicate loci for an organism still undergoing diploidization. This cross for Mdh-(3,4) in *S. gairdneri* was AAAA × AAA′A′ (male heterozygous), where allele 100 = A and allele 67 = A′. The progeny distribution was 5 AAAA, 63 AAAA′, and 12 AAA′A′. Phenotypes for all other loci confirmed that these fish were from this one family only. There are two possibilities to account for these results. The first possibility is that of classical linkage. This possibility would imply that two disomic loci are involved, each heterozygous for alleles A and A′. Further, the linkage phase in the male parent must have been A_1A_2' and $A_1'A_2$ (where the subscripts refer to the two loci). This situation would result in the excess of AAAA′ progeny observed. However, classical linkage is not supported by the results of Allendorf (1975) where three males of genotype AAA′A′, when mated to AAAA females, produced AAAA, AAAA′, and AAA′A′ progeny in the expected 1:2:1 ratios for two disomic loci. It is apparent that the two loci must have both been heterozygous in each of those males and are unlinked.

The second possibility is that the genotype of the male was AA at one locus and A′A′ at the other locus and that homeologous pairing followed by chromatid segregation resulted in the production of AA and A′A′ gametes. As an example, the AA locus might reside on a metacentric chromosome and the A′A′ locus on an acrocentric. Homeologous pairing of the homologous regions of the acrocentric and metacentric chromosome arms bearing the Mdh-(3,4) alleles coupled with crossovers in the interstitial region and directed disjunction would produce the AA and A′A′ gametes [see Wright *et al.* (1980) for this model].

Further evidence for pseudolinkage (residual tetrasomy) of Mdh-3 and Mdh-4 in *S. gairdneri* comes from Clayton *et al.* (1975). Three progeny occurred within three families with a common male parent which would be unexpected from disomic inheritance and exclusively bivalent pairing. It is entirely possible that these three progeny resulted from crossovers between homeologously paired chromosomes.

Table V. Linkage Associations in Salmonidae

Linkage association	Species	Type of association[a]	Reference
Aat-4[b] with Gam-1	*Salvelinus* hybrid[c]	Phase unknown	May (1980)
Odh with Mpi	*Salvelinus* hybrid[c]	Probably classical	May *et al.* (1980)
Mpi with Gpi-3	*Salvelinus* hybrid[c]	Probably classical	May *et al.* (1980)
Sdh with Gpi-2	*Salvelinus* hybrid[c]	Probably classical	May *et al.* (1980)
Aat-1 with Mdh-1	*Salvelinus* hybrid[c]	Probably classical	May *et al.* (1980)
Aat-2 with Mdh-1	*Salvelinus* hybrid[c]	Probably pseudolinkage	May *et al.* (1980)
Gus with Ck-1	*Salvelinus* hybrid[c]	Classical linkage	May *et al.* (1980)
Aat-2 with G3p-1	*Salvelinus* hybrid[c]	Probably classical	May *et al.* (1980)
Aat-2 with G3p-1	*Salvelinus fontinalis*	Probably classical	May *et al.* (1980)
Aat-2 with G3p-1	*Salvelinus fontinalis*	Classical linkage	Wright *et al.* (1980)
Aat-1 with G3p-1	*Salvelinus* hybrid	Probably pseudolinkage	May *et al.* (1980)
G3p-1 with Mdh-1	*Salvelinus* hybrid	Probably pseudolinkage	May *et al.* (1980)
Aat-1 with Aat-2	*Salvelinus* hybrid	Probably pseudolinkage	May *et al.* (1980)
Aat-1 with Aat-2	*Salvelinus fontinalis*	Pseudolinkage	Wright *et al.* (1980)
Aat-1 with Aat-2	*Salmo clarki*	Probably pseudolinkage	Allendorf and Utter (1976)
Idh-3 with Me-2	*Salvelinus* hybrid	Classical linkage	Stoneking *et al.* (1979)
Idh-3 with Me-2	*Salvelinus* hybrid	Classical linkage	May *et al.* (1980)

Idh-3 with Me-2	*Salmo gairdneri*	Probably classical	This paper
Ada-1 with G3p-3	*Salvelinus hybrid*	Probably classical	May *et al.* (1980)
Ada-1 with G3p-3	*Salmo gairdneri*	Probably classical	This paper
Mdh-3 with Mdh-4	*Salvelinus* hybrid	Pseudolinkage	May *et al.* (1979b, 1980)
Mdh-3 with Mdh-4	*Salmo gairdneri*	Probably pseudolinkage	This paper
Mdh-3 with Mdh-4	*Oncorhynchus gorbuscha*	Probably pseudolinkage	Aspinwall (1974)
Ldh-3 with Ldh-4	*Salvelinus* hybrid	Pseudolinkage	Morrison (1970)
Ldh-3 with Ldh-4	*Salvelinus* hybrid	Pseudolinkage	Davisson *et al.* (1973)
Ldh-3 with Ldh-4	*Salvelinus* hybrid	Pseudolinkage	May *et al.* (1980)
Ldh-3 with Ldh-4	*Salvelinus fontinalis*	Pseudolinkage	Davisson *et al.* (1973)
Ldh-3 with Ldh-4	*Salmo gairdneri*	Pseudolinkage	Wright *et al.* (1975)

Linkage groupings	Reference
Odh probably classical Mpi probably classical Gpi-3	May *et al.* (1980)
(Aat-2 classical linkage G3p-1) pseudolinkage (Aat-1 probably classical Mdh-1)	May *et al.* (1980)

[a] Designations as probably classical linkage or probably pseudolinkage are based on similar findings in *Salvelinus* and/or characteristics of pseudolinkage associations even though the linkage phase was unknown.

[b] Abbreviations not in Table I: AAT, aspartate aminotransferase; GAM, 4-methylumbelliferyl aminidase; GPI, glucosephosphate isomerase; ODH, octanol dehydrogenase.

[c] From F_1 and backcrosses involving *Salvelinus fontinalis* and *Salvelinus namaycush*.

Chromosomal evidence supports the view that homeologous pairing occurs in *S. gairdneri* males. Whereas females show bivalent pairing only, meiotic cell counts from a single male reveal a constant number of metacentrics and acrocentrics arranged in variable configurations. For example, at metaphase I two rings of two metacentrics each that are seen in one cell are seen as a ring of four metacentrics in another, or a rod of two metacentrics and two acrocentrics observed in one cell will appear as two bivalents in another cell (Wright, Delany, and Lee, in preparation). The work of Gold and Gall (1975) on the closely related *S. aguabonita* also reveals variable meiotic configurations in male individuals.

Thus, Mdh-(3,4) in rainbow trout is (are) similar to Aat-(1,2) in brook trout (Wright *et al.*, 1980). In general, the four gene doses are partitioned into two discrete loci. These two loci segregate disomicly when only bivalent pairing occurs. When homeologous pairing occurs, inheritance approaches that for a single tetrasomic locus, and the alleles at one locus in the parent can be transferred to the other locus in the progeny by recombination.

In Table V is presented a review of the known salmonid linkage associations, including cases of classical linkage, pseudolinkage, or nonrandom assortment where linkage phase is unknown. We have designated most of these latter associations "probably classical linkage" or "probably pseudolinkage" based on similar findings in *Salvelinus* and/or the aforementioned characteristics of pseudolinkage. The conservatism among salmonid genomes illustrated in Table V might be expected for cases of classical linkage; but why should this be true for pseudolinkage events which involve residual homology as reflected by homeologous arm pairing? The diploidization process (increasing bivalent pairing) should result in a decrease of shared pseudolinkage associations among genera. This latter finding of conservatism of pseudolinkage associations is even more puzzling in light of the apparent great plasticity of the salmonid genome as evidenced by variable karyotypes, variable 2N numbers, but relatively constant arm numbers among species (Gold *et al.*, 1980; May, 1980).

Ohno *et al.* (1969) have argued that Robertsonian fusions of ancestral homologues were the means of achieving rapid and nearly complete diploidy of the salmonid genomes. Such a process would be revealed by the classical linkage of duplicate loci residing on metacentrics. Yet the results of linkage studies to date do not support such a conclusion. The results of joint segregation studies of duplicate loci in Salmonidae are summarized in Table VI. No case of classical linkage has been proven for any of these examinations. This result plus the frequent finding of pseudolinkage for duplicate loci lead to the conclusion that most Robertsonian fusions probably involved nonhomologous arms in the ancestral tetraploid set which provided the opportunity for the present homeologous pairing observed in males (Lee and Wright, 1981; Wright, Delany, and Lee, in preparation). It is thus apparent

Table VI. Examination of Joint Segregation of Duplicate Loci in Salmonidae

Duplicate loci	Type of association	Reference
Salvelinus		
Aat-1 with Aat-2	Pseudolinkage	May *et al.* (1980), Wright *et al.* (1980)
Gpi-1 with Gpi-2	None	May *et al.* (1980)
Ldh-3 with Ldh-4	Pseudolinkage	Morrison (1970), Davisson *et al.* (1973), May *et al.* (1980)
Mdh-3 with Mdh-4	Pseudolinkage	May *et al.* (1979b, 1980)
Me-1 with Me-2	None	Stoneking *et al.* (1979), May *et al.* (1980)
Salmo gairdneri		
Ldh-3 with Ldh-4	Pseudolinkage	Wright *et al.* (1975)
Mdh-3 with Mdh-4	Probably pseudolinkage	Allendorf *et al.* (1975), this paper
Salmo clarki		
Aat-1 with Aat-2	Probably pseudolinkage	Allendorf and Utter (1976)
Salmo trutta		
Mdh-1 with Mdh-2	None	May *et al.* (1979a)
Coregonus clupeaformis		
G3p-1 with G3p-2	None	Clayton *et al.* (1973)
Oncorhynchus gorbuscha		
Mdh-3 with Mdh-4	Probably pseudolinkage	Aspinwall (1974)
Oncorhynchus keta		
Aat-1 with Aat-2	None	May *et al.* (1975)

that Robertsonian fusions alone need not lead to diploidization of duplicate loci. Rather, intraarm rearrangements are a more plausible method of restructuring the four homologous arms into two nonhomologous sets.

To date, inheritance studies have focused on the closely related genera of *Oncorhynchus, Salmo,* and *Salvelinus.* Inheritance studies of more distantly related salmonid genera, such as *Coregonus* or *Thymallus,* may produce modes of inheritance more nearly disomic or tetrasomic for those loci listed in Tables V and VI. Two salmonid species deserve special attention because of their great difference in chromosome arm number from those in all other salmonids (May, 1980); *Salmo salar* (with 72–74 arms) and *Thymallus thymallus* (140+ arms).

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