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Sex linkage of two enzyme loci in rainbow trout

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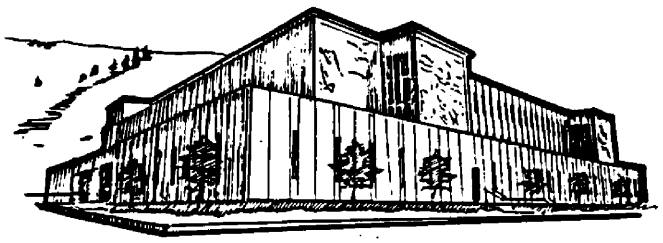

Sex Linkage of Two Enzyme Loci in Rainbow Trout

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The objective of this study was to detect sex-linked enzyme loci in the rainbow trout (Oncorhynchus mykiss). Previous cytological and breeding experiments have demonstrated an XX/XY sex determining system in this and other salmonid species. In spite of a large amount of linkage information from this species, no known cases of sex-linkage of any loci had been reported before this study.

I initially examined the joint segregation of nine enzyme encoding loci and sex in the Arlee hatchery strain of rainbow trout. Two loci encoding the enzymes hexosaminidase (HEX-2) and superoxide dismutase (sSOD-1) demonstrated statistically significant non-random associations in progeny from heterozygous fathers. A series of more extensive matings were then performed to test for the linkage of these two loci and sex.

Linkage information from fathers indicates that the average distance from HEX-2 to SEX is 8.1 map units (i.e., 8.1% recombination). The average distance from HEX-2 to sSOD-1 in fathers is 23.6 map units. No evidence of non-random segregation of HEX-2 and sSOD-1 was found in mothers. This contrast between recombination rates in males and females is in agreement with previous linkage studies with rainbow trout and other salmonid species.

These results indicate that both HEX-2 and sSOD-1 are on a chromosome that also carries a region involved in primary sex determination (the SEX locus). However, unlike the extreme XX/XY heterogamety in mammalian species, functional alleles for these loci are found on both the X and Y-chromosomes. Previous studies have reported that these same enzyme loci are linked to each other in salmonid fishes from the genus Salvelinus; however, these loci are not linked to SEX in Salvelinus. The sex-linkage of these loci in rainbow trout is apparently the result of a centric fusion between the autosome bearing HEX-2 and sSOD-1 and the sex chromosome in the rainbow trout lineage after divergence from a common ancestor with the Salvelinus species. Previous gene-centromere mapping data via gynogenesis combined with the data from this study suggest a gene order of (SEX)-centromere-(HEX-2)-(sSOD-1).

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Finally, I would like to thank my parents, Richard and Katherine, who always encouraged me. This thesis is dedicated to them.

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INTRODUCTION

Muller (1914) proposed that the evolution of heteromorphic sex chromosomes involved the suppression of crossing over between a proto-X and proto-Y. Crossover suppression can occur through the action of modifier loci that reduce recombination between the sex-determining regions on the X and Y and syntenic loci with opposing selective forces in males and females (Nei 1969). As a result, the Y chromosome may degenerate due to an accumulation of lethal mutations that are shielded from homozygosity by their counterparts on the X chromosome, without the remedying effects of X-Y recombination (Charlesworth 1978). Complete crossover suppression between sex chromosomes, however, is not advantageous because some pairing is required for precise disjunction during male meiosis (Koller and Darlington 1934). This necessity for X-Y pairing accounts for the presence of a homologous segment between the human X and Y that derives its name from a mode of inheritance caused by obligate X/Y exchange - the "pseudoautosomal" region (Burgoyne 1982, 1986).

The extreme X-Y divergence in humans and other mammals appears to be the exception among vertebrates (Bull 1983). Both male and female heterogamety is common in fishes (reviewed in Gold 1979; Bull 1983; Price 1986). In some instances both forms, male (XX/XY) and female (ZW/ZZ), exist in the same species (Bull 1983). Amphibians possess a similar diversity of sex determining mechanisms, with widespread occurrence of both male and female heterogamety (Bull 1983). Reptiles differ somewhat in that a large proportion of lizards, turtles, and crocodilians determine sex by incubation temperature during embryogenesis (Bull 1980, 1983).

Conservation of a single two-factor sex determining system occurs only among birds (female heterogametic) and mammals (male heterogametic).

In fishes of the family Salmonidae, cytological differences indicate that males are the heterogametic sex (Thorgaard 1977, 1978, 1983; Phillips and Ihssen 1985). These observations are supported by gynogenetically produced progeny and sex-reversal experiments (Johnstone et al. 1979; Okada et al. 1979; Donaldson and Hunter 1982; Refstie et al. 1982). However, there is little information on sex linkage in these fish. X-linked loci are hemizygous in mammals because the degenerate Y chromosome lacks functional alleles; this is likely not the case in salmonids because of the viability of YY individuals produced in sex-reversal studies. No loci have been reported in salmonids that show differential gene copy number in males and females.

The purpose of this investigation was to detect loci that are linked to the sex-determining factor in rainbow trout (Oncorhynchus mykiss). The genetics of sex-linked loci in salmonid fishes is especially interesting because of their polyploid ancestry (see Allendorf and Thorgaard 1984). Muller (1925) proposed that heterogamety is a major deterrent to the evolution of polyploidy among vertebrates because the asymmetry of sex determining factors in an aneuploid genome frequently causes sterility or reduced viability. In this paper, I describe the inheritance in rainbow trout of two enzyme loci, HEX-2 and sSOD-1, that are linked to a region that contains the major sex determining locus.

METHODS

Sampling and electrophoresis

Fish used in this study are from the Arlee strain of rainbow trout, maintained at the Jocko River State Trout Hatchery, Arlee, Montana. The history of the strain is presented by Leary et al. (1983). Fish used as parents had their gametes removed at the hatchery and their tissues immediately sampled and electrophoresed in the lab to determine their genotypes at several enzyme loci.

Progeny were reared until an age when sex could be determined by examining the gonads under low power magnification (Lincoln and Scott 1983; Malison et al. 1986). This was possible six months after hatching, when the fish were more than 100 mm in length. Fish were stored frozen at -80 C until dissection.

Horizontal starch gel electrophoresis was used to identify the protein products for all gene loci. Gel preparation, buffers, and staining procedures are those of Harris and Hopkinson (1976) and Allendorf et al. (1977). I have adopted the genetic nomenclature recently described for the identification of isozymes and isozyme loci in fishes (Shaklee et al. 1990).

Four families initially were examined to detect the presence of a sex-linked locus. The male parent of each family was chosen to be heterozygous for as many enzyme loci as possible. Since males are the heterogametic sex, we expected to detect sex-linkage only when the male parent is heterozygous.

During the initial screening two isozyme loci, HEX-2 and sSOD-1, out of nine surveyed, showed statistically significant associations with phenotypic sex. The following loci, with enzyme name and number in parentheses, were also surveyed during the preliminary screening: EST-1 (esterase, 3.1.1.-); sIDDH (L-iditol dehydrogenase, 1.1.1.14); mIDHP-2 and sIDHP-1 (isocitrate dehydrogenase, 1.1.1.42); LDH-B2 (L-lactate dehydrogenase, 1.1.1.27); sMDH-B1,2 (malate dehydrogenase, 1.1.1.37); PGM-1r (regulatory locus for phosphoglucomutase (Allendorf et al. 1983), 5.4.2.2).

Hexosaminidase

Hexosaminidase (HEX) has been intensively studied in humans because individuals with Tay-Sachs disease lack activity for one form of this enzyme. Multiple loci code for different forms of HEX (EC 3.2.1.52) in humans (Mahuran et al. 1985).

I used two buffer systems to detect genetic variation at loci encoding HEX in rainbow trout: an amine-citrate buffer (pH 6.1) described by Clayton and Tretiak (1972), and the pH 8.5 buffer of Ridgway et al. (1970). I used the positive, glucose-specific HEX stain described by Harris and Hopkinson (1976) with one alteration: a small amount of dimethyl sulfoxide (less than 1 ml) was used as the substrate solvent rather than ethyl alcohol. HEX activity was detected with both glucose-derived and galactose-derived staining substrates. This is indicative of beta-N-acetylhexosaminidase activity (EC 3.2.1.52; Calvo et al. 1978).

Superoxide Dismutase

Genetic variation for cytosolic superoxide dismutase (EC 1.15.1.1; sSOD-1) was first described in rainbow trout by Utter (1971). Cytosolic sSOD-1 activity predominates in liver tissue, although activity is present in several other tissues (Allendorf et al. 1977). The products of a second locus (sSOD-2) for this enzyme is present in homogenate from eye. Two common sSOD-1 electromorphs exist in rainbow trout and are present in the Arlee population (100 and 150). The inheritance of this variation in rainbow trout was first described by Utter et al. (1973).

RESULTS

Hexosaminidase

Results indicate that two loci encode HEX in rainbow trout. Evidence for the products of only a single locus (HEX-2) was found in fish large enough to be classified as male or female (greater than 100 mm). The strongest activity for HEX-2 occurs in liver tissue, although activity for the HEX-2 isozyme was also observed in tissue homogenate from gill, fin, and kidney. Little or no HEX-2 activity was found in the other tissues that were examined (eye, muscle, heart, stomach, and brain).

Another more cathodal zone of HEX activity was detected in homogenate from whole fry that had not yet absorbed their yolk sac (R. Danzmann, unpublished results). All individuals examined had a single band of activity for this form of the enzyme regardless of their genotype at HEX-2; thus, this zone is apparently encoded by a distinct locus (HEX-1). No activity for the enzyme produced by this locus was detected in fish after absorption of the yolk sac.

Three HEX-2 alleles were found in the Arlee population. Heterozygotes at this locus show a three-banded pattern typical of a dimeric enzyme (Figure 1). On the basis of relative electrophoretic mobility relative to the most common allele, which is designated as 100 (Shaklee et al. 1990), the other two HEX-2 alleles are designated as 75 and 80. The difference in mobility between these two alleles was not recognized in earlier studies in this laboratory; in addition, 75/80 heterozygotes were not used to test inheritance in this study because of the difficulty in distinguishing the resulting progeny phenotypes (e.g.,

80/100 versus 75/100). Thus, in order to present genotypes more clearly in the tables, I have used A and A' to designate alternative alleles at HEX-2; A always refers to the 100 allele, but A' refers to either the 75 or 80 allele. Similarly, B and B' have been used to designate the 100 and 150 alleles at sSOD-1.

Pairwise Segregation

HEX-2 phenotypes of progeny were consistently associated with sex when the male parent was heterozygous (Table 1). The less frequent linkage class was assumed to be the recombinants if the null hypothesis of independent assortment between HEX-2 and sex in the progeny was rejected using a chi-square test (Bailey 1961); for example, in family M1 it is assumed that the A allele was on the paternal Y-chromosome and the A' allele was on the paternal X-chromosome. See Nordheim et al. (1983) for a discussion of detecting linkage when the parental linkage phases are not known. A significant association between HEX-2 and sex was found in one of four families in which only the maternal parent was heterozygous at HEX-2 (M2). However, this deviation is not statistically significant if corrected for the four independent tests of this association (Cooper 1968).

In family L25 both parents were heterozygous at HEX-2. The significant association between HEX-2 and sex in these progeny was assumed to be caused by linkage in the male parent. Progeny heterozygous at HEX-2 were ignored in the classification as parental or recombinant type in the

progeny because of the ambiguity of determining which allele was inherited from which parent.

The sSOD-1 phenotypes of progeny tended to be associated with sex when the male parent was heterozygous in those families that allowed pairwise analysis of sex and sSOD-1 (Table 2). All analyses were performed as described above for associations between HEX-2 and sex. This association between sSOD-1 and sex was confirmed in the three-point testcrosses that are presented later.

A series of crosses was made to estimate the rate of recombination between HEX-2 and sSOD-1 in both males and females. These progeny were sampled before they were large enough to determine their sex. The null hypothesis of independent assortment can be rejected in three out of four families in which the male parent was heterozygous for both HEX-2 and sSOD-1 (Table 3). However, there is no indication of non-independent assortment when the female parent was doubly-heterozygous.

Some joint-segregation results in salmonids have shown an excess of recombinant over parental types. The process responsible for this result, known as pseudolinkage, results from meiotic abnormalities due to the residual tetraploidy that exists among salmonid species (Wright et al. 1983; Allendorf and Thorgaard 1984).

We tested for pseudolinkage by mating two males heterozygous at HEX-2 and sSOD-1 with known linkage phase (A B /A'B'; Figure 2) to doubly-homozygous females. These progeny were sampled before their sex could be determined. A significant excess of parental types at HEX-2 and sSOD-1 occurred in both families (Table 4).

Three-point testcrosses

Males heterozygous for HEX-2 and sSOD-1 were used to construct five three-point testcrosses to determine gene order (Table 5). There are four recombinant classes, each with two gamete types, resulting from these three-point crosses: parental class, two single crossover classes, and the double crossover class. The most frequent recombinant class was assumed to represent the parental type (i.e., the result of no crossovers). The parental gamete types were designated as X A B and Y a b to reduce the number of different genotypes represented in the table. That is, the allele on the paternal X-chromosome is designated as A or B regardless of its electrophoretic mobility; similarly, the allele on the Y-chromosome is designated as a or b.

Gametic disequilibria

A substantial set of data is available to test for non-random associations among genotypes at HEX-2, sSOD-1, and sex in the Arlee strain of rainbow trout. Sexually mature males and females are collected each year for experimental matings and examined at some 15 polymorphic enzyme loci. The males and females that are used in these matings are often of different ages (e.g., 2 year-old males and 3 year-old females). Therefore, males and females of each cohort have not been examined. For example, data from only males arising from 1985 matings are available, while only females are available from 1986.

Gamete frequencies and the coefficient of gametic disequilibrium (D) were estimated using the algorithm of Hill (1974). The significance of

deviations were tested by the Q statistic that is distributed as a chi-square with one degree of freedom.

The strongest evidence for non-random association was found between HEX-2 and sex (Table 6-8). Significant positive values of D were found in 2 of the four samples (1982 and 1984 cohorts). Significant associations between HEX-2 and sSOD-1 as well as sSOD-1 and sex are present in the 1982 cohort.

DISCUSSION

Sex-linkage of HEX-2 and sSOD-1

Significant non-random associations between HEX-2 and sSOD-1 were found in 9 of the 11 families for which the male parent was doubly heterozygous (Table 9); the probability of the distributions observed in the other two families (N7 and Q5) is less than 0.07. If we include these two families in our calculations, the average recombination rate between HEX-2 and sSOD-1 in males is 0.236. May and Johnson (1990) have reported that these loci are also linked in salmonid fish of the genus Salvelinus; they report recombination rates of 0.15 and 0.27 in crosses using interspecific hybrids between brook trout (S. fontinalis), lake trout (S. namaycush), and Arctic char (S. alpinus).

There is no evidence of non-random association between HEX-2 and sSOD-1 from female parents (Table 3). Previous salmonid linkage studies have shown that recombination rates are generally greater in females than males (May et al. 1979; Johnson et al. 1987; May and Johnson 1990). The reduction of autosomal recombination in the heterogametic sex is expected because of selection for X-Y crossover suppression (Haldane 1922; Huxley 1928; reviewed in Bull 1983; Trivers 1988). Thus, sex differences in recombination rate can evolve by the pleiotropic effects of recombination modifiers acting upon the X and Y (Nei 1969).

These data indicate that HEX-2 and sSOD-1 are both on the chromosome carrying the major sex-determining locus (SEX) in rainbow trout. Significant non-random association between HEX-2 and sex was found in every family for which the male parent was heterozygous at HEX-2 (Table

9). The average recombination rate between these loci in 10 families is 0.081.

The three-point testcrosses results alone do not allow us to determine the gene order. Double crossovers are expected to be less frequent than either of the single crossover classes. However, two of the crossover classes are approximately equally frequent in the five three-point families (Table 5). Thus, HEX-2 and the sex determining locus are very near each other, and both of these loci are somewhat distant from sSOD-1. A consideration of other information suggests a gene order of (SEX)-(HEX-2)-(sSOD-1).

May et al. (1989) have reported sex-linkage of three tightly linked enzyme loci (LDH-1, AAT-5, and GPI-3) in second generation hybrids between brook trout and Arctic char backcrossed to brook trout. These loci are not associated with sex in hybrids between brook trout and lake trout; this is in contrast to the general pattern of strong conservation of linkages among salmonids. On this basis, these authors conclude that the sex linkage of these three loci is caused by a chromosomal rearrangement (i.e., centric fusion) that is unique to the Arctic char. That is, a chromosomal arm responsible for sex determination has fused with an autosome containing the three enzyme loci.

May et al. (1990) also report that the loci encoding hexosaminidase and superoxide dismutase are not linked to sex in crosses of hybrids between brook trout and lake trout. This suggests that the association between HEX-2 and sex results from a centric fusion in rainbow trout that

is not present in Salvelinus. If this is true, then SEX would be across the centromere from the HEX-2 sSOD-1 chromosome arm.

Gene-centromere mapping via gynogenesis (Allendorf et al. 1986) has indicated that HEX-2 in rainbow trout is very near the centromere (0.017 recombination in females) and that sSOD-1 is distal (0.497 recombination in females). The most likely gene order based on this analysis is shown in Figure 3. The proximity of SEX to the centromere in rainbow trout is in agreement with cytogenetic results (Thorgaard 1977) and with evidence from other species (reviewed in Gold, 1979).

Gametic disequilibrium

The association between HEX-2 and sex (Table 6) is likely due to the origin of the Arlee strain of rainbow trout. This strain was created in 1955 by mating males from the University of Washington strain with females from a strain maintained by the Missouri Department of Fish and Game (personal communication from George Holton, Montana Department of Fish, Wildlife and Parks). The number of parents involved in these initial crosses is not known. We would expect initial non-random association between sex and any locus for which these two founding parents had different allele frequencies. These associations are expected to decay at a rate of one minus the recombination rate (i.e., 1-r) per generation. Thus, for loci unlinked to SEX, this association should be reduced by half each generation. However, this process will be considerably delayed for HEX-2 which is closely linked to SEX.

This strain has been maintained largely by mating two year-old males with three year-old females. Thus, there were approximately ten generations between 1955 and 1980, the first year for which we have data (Table 6). There is a tendency for males to have a lower frequency of HEX-2*100. These data are therefore compatible with the males from the University of Washington strain having a lower frequency of this allele than the females from the Missouri strain.

Sex chromosome evolution

Centric fusions between sex-chromosomes and autosomes have apparently occurred many times in salmonid evolution. May et al. (1989) describe such a fusion in Arctic char that is not shared by the congeneric related brook and lake trout. As discussed above, the apparent fusion of the chromosome arm containing HEX-2 and sSOD-1 and the sex-chromosome in rainbow trout is also not shared by the brook and lake trout. In addition, Thorgaard (1978) has described a centric fusion between the Y-chromosome and an autosome in sockeye salmon (Oncorhynchus nerka).

Several instances of isozymes exhibiting sex linkage are found in amphibians (Elinson 1983; Ferrier et al. 1983; Wright and Richards 1983, 1984; Graf 1989a). Both male and female heterogamety was found, and in all species both sexes expressed the same number of alleles, indicating presence of functional loci on both the X and Y.

Interestingly, the locus encoding the cytosolic form of SOD is sex-linked in Rana pipiens (Wright and Richards 1983) and resides in a

linkage group of Xenopus laevis known to contain the sex determining region (Graf 1989b).

In reptiles, Goux and Pasteur (1986); Salvidio et al. (1990) found an unusual association of mannose phosphate isomerase (MPI) phenotypes with sex in several populations of the common lizard (Lacerta vivipara). Alleles are expressed on both the Z and W chromosomes - use of the ZW nomenclature denotes female heterogamety - but the absence of one electromorph on the W implies that crossing over is rare or absent in females. These results would seem to place this species at an intermediate stage of X-Y divergence, with the presence of diallelic expression in both sexes, but an absence or reduction of recombination between the sex chromosomes.

Female heterogamety is well known from cytological studies of birds, but scant inheritance data exists concerning Z or W-linked loci. Baverstock et al. (1982) reported a sex-linked locus encoding the cytosolic form of aconitase in several avian species. No heterozygous females were found and aconitase activity was approximately twice as great in males as females. These results indicate Z linkage with no functional allelic counterpart on the W-chromosome. Morizot et al. (1987) report a sex-linked locus for creatine kinase in Harris' hawk (Parabuteo unicinctus). Different electromorphs were expressed on the Z and W-chromosomes. These results are similar to the previously discussed result with a lizard species (i.e., intermediate divergence with diallelic expression in both sexes, but an absence of recombination between the sex chromosomes).

Somewhat divergent paths for sex chromosome evolution have occurred among mammals, with the paternal-X being inactivated in female marsupials and monotremes, rather than random X-inactivation observed in placental species (VandeBerg et al. 1987). Despite these differences in X-activation mechanisms, only recently has a sex-linked locus in placental mammals been found to be autosomal in marsupials and monotremes (Sinclair et al. 1987). A large number of genes exhibit a consistent pattern of X-linkage among mammalian species due to X/Y recombination suppression and concomitant Y degeneration (Ohno 1967, 1973; O'Brien and Nash 1982; Roderick et al. 1984; Womack and Moll 1986).

The genetic mechanisms that determine the sex of rainbow trout may represent an early stage toward complete X/Y divergence. The existence of sex-linked genes in rainbow trout, along with diallelic expression and suppression of recombination in the heterogametic sex, suggests that this species is in the interim stages of a two-factor mechanism for chromosomal sex determination.

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TABLE 1

Joint inheritance of HEX-2 and sex

Family	Parental genotypes female	Parental genotypes male		Progeny genotypes AA	Progeny genotypes AA'	Progeny genotypes A'A'	r[a]
L25	AA'	AA'	male	2	7	9	0.118**
			female	6	8	0	
L26	AA	AA'	male	15	2	-	0.103***
			female	1	11	-	
L29	AA'	A'A'	male	-	3	2	NS
			female	-	7	5	
L30	AA'	A'A'	male	-	8	4	NS
			female	-	6	7	
M1	A'A'	AA'	male	-	23	5	0.136***
			female	-	3	28	
M2	AA'	A'A'	male	-	43	27	0.407*
			female	-	23	30	
M4	AA'	A'A'	male	-	21	31	NS
			female	-	20	19	
M5	A'A'	AA'	male	-	1	25	0.038***
			female	-	26	1	
M6	AA'	AA	male	3	8	-	NS
			female	3	1	-	
M7	A'A'	AA'	male	-	5	32	0.135***
			female	-	32	5	

NS = not significant; * P < .05; ** P < .01; *** P < 0.001.

[a] estimated rate of recombination.

Heterozygous parental genotypes are underlined.

TABLE 2

Joint inheritance of sSOD-1 and sex

Family	Parental genotypes female	Parental genotypes male		Progeny genotypes BB	Progeny genotypes BB'	Progeny genotypes B'B'	r[a]
L25	BB'	BB'	male	4	9	5	NS
			female	4	7	3	
L26	B'B'	BB'	male	-	3	13	0.192**
			female	-	8	2	
L29	BB	BB'	male	1	4	-	NS
			female	2	7	-	
L30	BB	BB'	male	8	4	-	0.231**
			female	2	12	-	
M7	BB'	BB'	male	13	20	4	0.250*
			female	4	20	11	

NS = not significant; * P < .05; ** P < .01.

[a] estimated rate of recombination.

Heterozygous parental genotypes are underlined.

TABLE 3

Joint inheritance of HEX-2 (A) and sSOD-1 (B)

Family	Parental genotypes		Progeny genotypes						
	female	male	AA BB	AA' BB	A'A' BB	AA BB'	AA' BB'	A'A' BB'	r^a
N1	A'A'BB	AA'BB'	-	53	22	-	27	52	0.318***
N2	AA'BB'	AA BB	46	54	-	45	38	-	NS
N3	A'A'BB	AA'BB'	-	28	71	-	71	20	0.253***
N4	AA'BB'	AA BB	41	38	-	47	54	-	NS
N5	A'A'BB	AA'BB'	-	57	13	-	9	65	0.153***
N6	AA'BB'	AA BB	50	45	-	40	44	-	NS
N7	A'A'BB	AA'BB'	-	15	16	-	10	26	NS
N8	AA'BB'	AA BB	49	33	-	46	52	-	NS

NS = not significant; *** $P < 0.001$.

[a] estimated rate of recombination.

Doubly-heterozygous parental genotypes are underlined.

TABLE 4

Joint inheritance of HEX-2 (A) and sSOD-1 (B) with known linkage phase

Family	Parental genotypes		Progeny genotypes				
	female	male	AA BB	AA' BB	AA BB'	AA' BB'	r[a]
Q104	A B / A B	A' B' / A B	16	7	6	11	0.325*
Q106	A B / A B	A' B' / A B	17	6	5	9	0.297*

* $P < .05$

[a] estimated rate of recombination.

TABLE 5

Three-point test crosses for sex (X;Y), HEX-2 (A;a), and sSOD-1 (B;b) using males as the segregating parent

Crossover Region[a]	Paternal Gamete	Family P1	P5	Q2	Q4	Q5	Total
None	X A B	35	30	19	36	23	301
	Y a b	33	29	35	36	25	
I	X a b	2	0	0	0	0	12
	Y A B	1	3	5	1	0	
II	X A b	4	14	10	6	13	94
	Y a B	12	10	10	0	15	
I,II	X a B	1	3	0	0	2	12
	Y A b	0	2	1	1	2	
		88	91	80	80	80	417

[a] See Figure 3.

TABLE 6

Gametic disequilibrium between sex and HEX-2 in Arlee rainbow trout

		HEX-2 genotype						
Cohort	sex	AA	AA'	A'A'	freq(A)	F[a]	D[b]	D'(%)[c]
1980	female	19	26	5	0.640	-0.13		
1981	female	18	29	13	0.542	0.03		
	male	36	38	13	0.632	0.06		
		54	67	26	0.595	0.05	-0.031	25.8
1982	female	11	69	17	0.469	-0.43		
	male	17	69	101	0.275	0.07		
		28	138	118	0.032	-0.08	0.056	49.7***
1984	female	36	35	9	0.669	0.01		
	male	35	61	19	0.569	-0.08		
		71	96	28	0.610	-0.03	0.045	24.8**
1985	male	13	63	67	0.311	-0.03		
1986	female	8	10	2	0.650	-0.10		
1987	female	12	16	2	0.666	-0.20		
	male	9	8	2	0.684	0.03		
		21	24	4	0.673	-0.11	-0.005	8.3
1988	male	4	9	7	0.425	0.08		

** P < .01 ; *** P < .001

[a] Fixation index

[b] Coefficient of gametic equilibirum

[c] D/D_{max}

TABLE 7

Gametic disequilibrium between sex and sSOD-1 in cohorts of Arlee trout

Cohort	sex	sSOD-1 genotype BB	BB	B'B'	freq(A)	F[a]	D[b]	D'(%)[c]
1980	female	24	25	5	0.675	-0.06		
	male	59	34	7	0.760	0.07		
		83	59	12	0.730	0.03	-0.028	32.4
1981	female	39	20	1	0.816	-0.11		
	male	61	26	3	0.822	0.01		
		100	46	4	0.820	-0.04	-0.002	4.2
1982	female	63	35	2	0.805	-0.11		
	male	107	78	19	0.716	0.06		
		170	113	21	0.745	0.02	0.032	19.2**
1984	female	47	28	5	0.762	0.03		
	male	84	32	4	0.833	0.04		
		131	60	9	0.805	0.04	-0.025	42.0
1985	male	81	55	4	0.775	-0.13		
1986	female	8	10	2	0.650	-0.10		
1987	female	16	13	1	0.750	-0.15		
	male	11	8	1	0.750	-0.07		
		27	21	2	0.750	-0.12	0.000	0.0
1988	male	9	9	2	0.675	-0.03		

** $P < .01$

[a] Fixation index

[b] Coefficient of gametic equilibirum

[c] D/D_{max}

TABLE 8

Gametic disequilibrium between HEX-2 and sSOD-1 among cohorts of the Arlee rainbow trout.

Cohort	sSOD-1	HEX-2 genotype AA	AA'	A'A'	D[a]	D'(%)[b]
1980	BB	9	10	1		
	BB'	9	11	4	0.029	12.9
	B'B'	1	4	0		
1981	BB	42	41	20		
	BB'	18	25	6	-0.011	15.4
	B'B'	2	2	0		
1982	BB	15	73	77		
	BB'	7	58	40	-0.045	21.4***
	B'B'	20	76	22		
1984	BB	43	64	21		
	BB'	24	28	7	-0.020	26.3
	B'B'	4	5	0		
1985	BB	6	30	40		
	BB'	5	24	22	-0.006	4.1
	B'B'	0	1	3		
1986	BB	3	4	1		
	BB'	4	5	1	-0.026	20.9
	B'B'	1	1	0		
1987	BB	11	13	2		
	BB'	9	9	3	0.002	1.1
	B'B'	1	1	0		
1988	BB	2	3	4		
	BB'	2	5	2	0.001	1.1
	B'B'	0	1	1		

*** P < .001

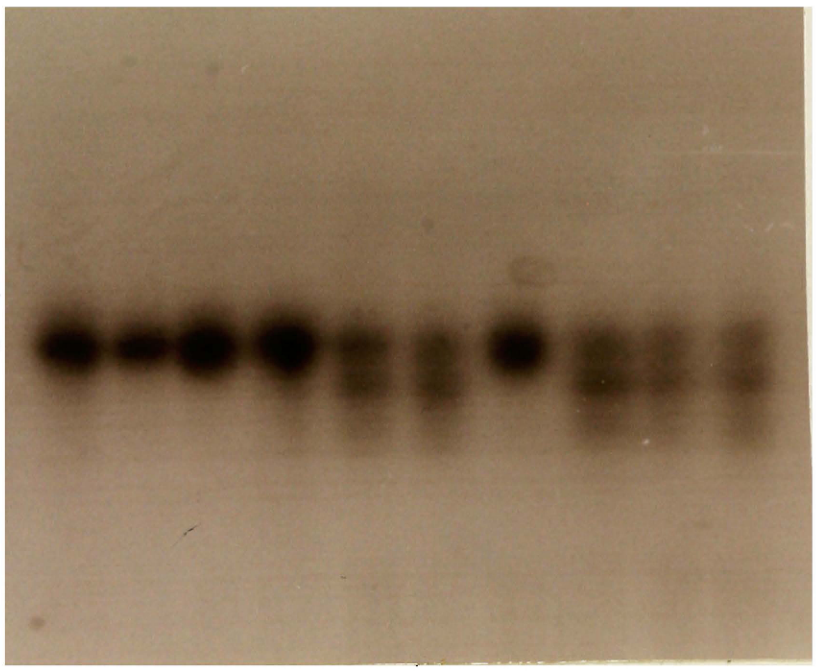
TABLE 9

Summary of recombination rates in males

Family	Total Individuals	HEX-2:sex	sSOD-1:sex	HEX-2:sSOD-1
L25	32	0.118***	NS	---
L26	26	0.115***	0.192**	---
L29	14	---	NS	---
L30	26	---	0.231***	---
M1	59	0.136***	---	---
M5	53	0.038***	---	---
M7	74	0.135***	0.250*	---
N1	154	---	---	0.318***
N3	192	---	---	0.253***
N5	144	---	---	0.153***
N7	67	---	---	NS
P1	88	0.045***	0.216***	0.193***
P5	91	0.088***	0.297***	0.319***
Q2	80	0.075***	0.313***	0.263***
Q4	80	0.025***	0.088***	0.088***
Q5	80	0.050***	0.350**	NS
Q104	40	---	---	0.325*
Q106	37	---	---	0.297*

NS = not significant; * P < .05; ** P < .01; *** P < 0.001.

FIGURE 1. HEX-2 phenotypes in a family segregating 1:1 for *100/100 (1) and *100/75 (2).



1 1 1 1 2 2 1 2 2 2

FIGURE 2. Breeding scheme to test for pseudolinkage.

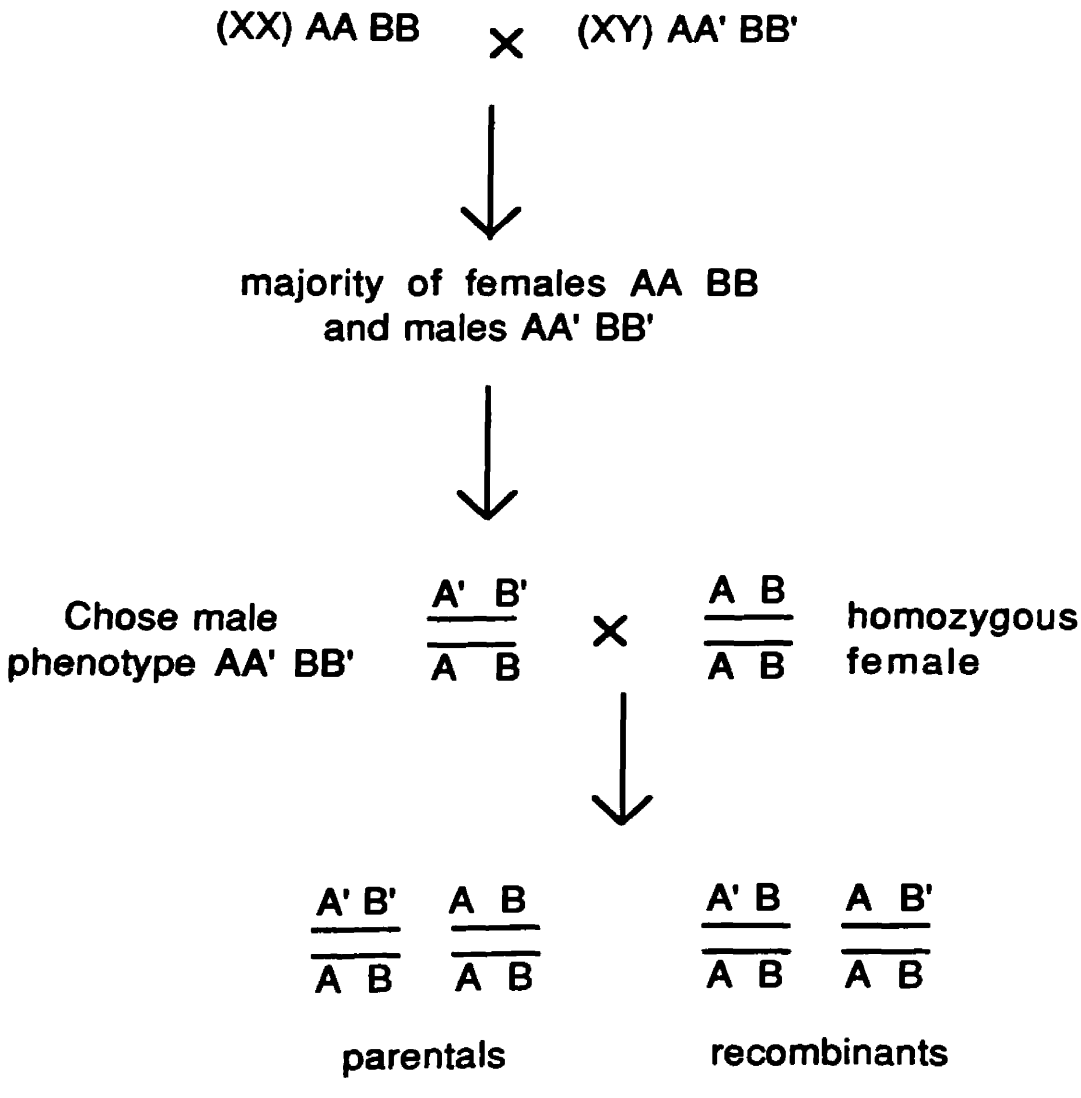
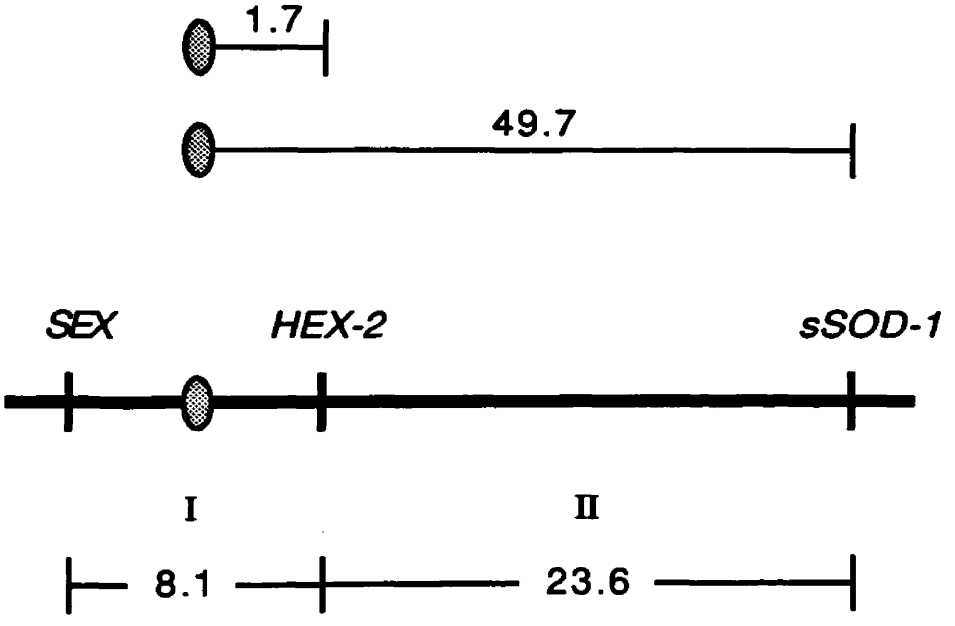

(XX) AA BB × (XY) AA' BB'
majority of females AA BB and males AA' BB'
Chose male phenotype AA' BB'
A' B' / A B × A B / A B
homozygous female
A' B' / A B
A B / A B
parentals
A' B / A B
A B' / A B
recombinants

FIGURE 3. Genetic map of the rainbow trout sex chromosome. Gene-centromere map distances from females are above (Allendorf et. al. 1986), and average recombination rates in males from this study are below.


1.7
49.7
SEX
HEX-2
sSOD-1
I
II
8.1
23.6