

**GENE-CENTROMERE RECOMBINATION RATES OF ALLOZYME LOCI
IN EVEN AND ODD YEAR PINK SALMON**

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Introduction

Gene-centromere recombination rates can be estimated in gynogenetic diploid progeny in which the second polar body of the oocyte is retained by heat-shock or hydro-static pressure treatment. In analyzing these data, it is assumed that at first meiosis (1) separation occurs always between homologous chromatids, (2) crossovers occur only between homologous chromatids, and (3) only one crossover occurs on one chromosomal arm (Volpe, 1970). As a result, the genotype of a particular locus of a gynogenetic diploid progeny from a heterozygous maternal parent is a homozygous if there is no recombination between the locus and the centromere, but heterozygous if there is recombination. The rate of appearance of heterozygous progeny reflects the rate of recombination. In addition, if recombination occurs at any site on a chromosome with equal probability, the rate of recombination reflects the relative distance between the locus and the centromere.

Earlier work for gene-centromere mapping using gynogenesis in vertebrates were conducted in the leopard frog, *Rana pipiens*, with mutant alleles (Nace et al., 1970; Volpe 1970). As far as we know, 28 studies in more than 15 fish species have been reported using not only allozyme loci but also mutant alleles and microsatellite loci. In previous studies, recombination rates at some loci were estimated as close as 1. This result suggests that exactly one crossover takes place between the gene and the centromere on the chromosome arms carrying these loci. The phenomenon is common in fish but unusual in other animals and plants (Thorgaard et al., 1983). The result has been explained by a high level of chiasma interference, in which the first chiasma interferes with the formation of the next chiasma, because the size of fish chromosome is relatively small (Thorgaard et al., 1983).

In this study, we estimated gene-centromere recombination rates of allozyme loci in even and odd year pink salmon, *Oncorhynchus gorbuscha*, using gynogenetic progeny. We compared the estimates between families within a locus and between years within a locus.

Materials and Methods

Gametes and tissue sample collections: All materials for this project were obtained from the Gastineau Hatchery, Douglas Island Pink and Chum, Inc. (DIPAC), Juneau, Alaska. Production of families was conducted for two consecutive years, 1992 and 1993. In each year, eggs from 80 females and sperm of chinook salmon, *O. tshawytscha*, were used for gynogenesis. Tissues of muscle, heart, liver, and eye were taken for electrophoresis then stored at -80°C.

Production of gynogenetic progeny families: The procedure for production of gynogenetic diploid families was according to Smoker et al. (1995). In total, eighty gynogenetic diploid families were produced each year. Ten eggs of each female were not treated for the retention of the second polar body and were incubated as haploid control groups.

Sampling: Samples of alevins and fry for electrophoresis were stored at -80°C until analysis.

Electrophoresis: Horizontal starch gel electrophoresis and histochemical stain techniques (Utter et al. 1986) were employed. Frozen samples from parental fish for 61 loci of 32 enzymes were analyzed first (Table 1). From these data, the most useful families for analysis were chosen. For gene-centromere analysis, at least three families (when possible) were examined for each locus.

Table 1. Protein-coding loci for enzymes resolved in this study and the tissues and buffers in which they were resolved.

Enzyme name	E.C. Number	Locus	Tissues (1)	Buffer (2)	Variability (3)
Acid phosphatase	3.1.3.2	<i>ACP*</i>	L	TG	n
Aconitate hydratase	4.2.1.3	<i>mAH-1*</i>	H	CAME7.2	E
		<i>mAH-2*</i>	H	CAME7.2	n
		<i>mAH-3*</i>	M,H	CAME7.2	O
		<i>mAH-4*</i>	M,H	CAME7.2	B
		<i>sAH*</i>	L	CA6.8	E
Adenosine deaminase	2.7.4.3	<i>ADA-1*</i>	M,H	CA6.1	n
		<i>ADA-2*</i>	M,H	CA6.1	B
Alanine aminotransferase	2.6.1.2	<i>ALAT*</i>	M	MF	O
Aspartate aminotransferase	2.6.1.1	<i>mAAT-1*</i>	M,H	CA6.1	E
		<i>mAAT-2*</i>	M,H	CA6.1	E
		<i>sAAT-1,2*</i>	M,H	CA6.1	n
		<i>sAAT-3*</i>	E	TC	B
		<i>sAAT-4*</i>	H,L	CAME7.4	B
Creatine kinase	2.7.3.2	<i>CK-A1*</i>	M	R	n
		<i>CK-A2*</i>	M	R	n
		<i>CK-B*</i>	E	R	n
		<i>CK-C1*</i>	E	R	E
		<i>CK-C2*</i>	E	R	n

Table.1 (continued)

Enzyme name	E.C. Number	Locus	Tissues (1)	Buffer (2)	Variability (3)
Diaphorase	1.8.1.4	<i>DIA-1*</i>	E	CAME7.2	B
		<i>DIA-2*</i>	E	CAME7.2	E
Fumarate hydratase	4.2.1.2	<i>FH*</i>	M	TC	n
Formaldehyde dehydrogenase	1.2.1.1	<i>FDHG*</i>	M,H	R	O
β -Galactosidase	3.2.1.23	<i>βGALA*</i>	E	TG	n
Glutathione reductase	1.6.4.2	<i>GR*</i>	E	TC-4	n
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	<i>GAPDH-1*</i>	H,E	CAME7.2	n
		<i>GAPDH-2*</i>	H,E	CAME7.2	n
Glucose-6-phosphate isomerase	5.3.1.9	<i>GPI-A*</i>	M,H,E	R	E
		<i>GPI-B1,2*</i>	M,H,E	R	B
Glycerol-3-phosphate dehydrogenase	1.1.1.8	<i>G3PDH-1*</i>	M	CA6.1	B
Guanine deaminase	3.5.4.3	<i>GDA*</i>	L	CAME7.4	B
L-Iditol dehydrogenase	1.1.1.14	<i>IDDH*</i>	L	R	B
Isocitrate dehydrogenase	1.1.1.42	<i>mIDHP-1*</i>	M	CA6.1	n
		<i>mIDHP-2*</i>	M	CA6.1	n
		<i>sIDHP-1,2*</i>	E,L	TC	B
L-Lactate dehydrogenase	1.1.1.27	<i>LDH-A1*</i>	M	R	E
		<i>LDH-A2*</i>	M	R	n
		<i>LDH-B1*</i>	H	R	n
		<i>LDH-B2*</i>	L	R	E
		<i>LDH-C*</i>	E	R	n
Malate dehydrogenase	1.1.1.37	<i>mMDH-1*</i>	M	CA6.1	n
		<i>sMDH-A1,2*</i>	M,H,E	CAME7.2	E
		<i>sMDH-B1,2*</i>	M,H	CAME7.2	B
Malic enzyme	1.1.1.40	<i>sMEP-1*</i>	M,H	CAME7.2	B
Mannose-6-phosphate isomerase	5.3.1.8	<i>MPI*</i>	H,E	MF	B
α -Mannosidase	3.2.1.24	<i>α-MAN*</i>	H	TC-4	n
Peptidase	3.4.*.*				
Glycyl-leucine		<i>PEP-A*</i>	M	MF	O
Leucyl-glycyl-glycine		<i>PEP-B*</i>	M	MF	B
Phenylalanyl-proline		<i>PEP-D1*</i>	M	MF	n
		<i>PEP-D2*</i>	M	MF	B
Leucyl-tyrosine		<i>PEP-LT*</i>	M	MF	B
Phosphoglucomutase	5.4.2.2	<i>PGM-2*</i>	M	CA6.1	B
Phosphoglycerate kinase	2.7.2.3	<i>PGK*</i>	E,L	TG	n
Phosphogluconate dehydrogenase	1.1.1.44	<i>PGDH*</i>	M,L	CA5.7	B
Pyruvate kinase	2.7.1.40	<i>PK-1*</i>	E	TC	n
		<i>PK-2*</i>	E	TC	O
Superoxide dismutase	1.15.1.1	<i>sSOD*</i>	M,H	MF	n
Triose-phosphate isomerase	5.3.1.1	<i>TPI-1*</i>	E	TG	n
		<i>TPI-2*</i>	E	TG	n
		<i>TPI-3*</i>	E	TG	n
		<i>TPI-4*</i>	E	TG	B

- (1) M = muscle, H = heart, L = liver, E = eye.
(2) R (Ridgway et al. 1970),
MF (Markert and Faulhaber 1965),
TG (0.04M Tris, 0.12M Glycine),
CA5.7, 6.1 and 6.8 (Clayton and Tretiak 1972),
CAME7.2 and 7.4 (Aebersold et al. 1987),
TC (Shaw and Prasad 1970),
TC-4 (Schaal and Anderson 1974).
(3) E = even year only, O = odd year only, B = both even and odd year, n = no variation.

Statistical analysis: First, whether the ratio of the number of two types of homozygotes is 1:1 among progeny was tested using the χ^2 -test. Any family with a different ratio was removed from the further analysis. Second, heterogeneity between families within a locus was analyzed using χ^2 -test for $n \times 3$ (n is the number of families) contingency table. Finally, heterogeneity between years within a locus was compared using χ^2 -test for 2×3 contingency table.

Results and Discussions

The recombination rates were estimated for 29 loci in the even year and 24 loci in the odd year and 34 loci (53 loci groups) in combined years (Table 1). One family out of 111 families examined in the even year and 11 families out of 120 families in the odd year were removed from the further analyses because of departure from 1:1 ratio of the two types of homozygous progeny.

The recombination rates at each locus of the pooled data ranged from 0.5% at *MPI** in the odd year to 99.6% at *ADA-2** in the even year (Table 2). Average recombination rate was 60.5% in the even year, 67.8% in the odd year and 63.8% in combined years. Fourteen loci groups out of 53 groups had a recombination rate above 90%. The appearance of loci with a high recombination rate is a common phenomenon in previous studies of fish.

Test for heterogeneity between families was conducted in 36 loci groups, and in 20 loci groups heterogeneity was observed (Table 3). Out of 20 loci groups which showed heterogeneity, 16 groups were homogeneous after one outlier family was removed and one group was homogeneous after two families were removed.

At *sMDH-1,2** and *sMDH-3,4** in the even year, the families were divided into roughly two groups by the recombination rates (Table 4 and 5). However, at *sMDH-3,4** in the odd year, the recombination rates ranged between 91.1 and 100.0% (no statistical test was conducted because of the small expected values; Table 5). Because these loci are isoloci (duplicated loci), one possible explanation for the differences is that the variation occurred on different loci of the duplicated loci. At *mAH-3** in the odd year, the recombination rates were from 11.0% to 72.8% (Table 6). The differences between families were similar to those reported in rainbow trout and could result from differences in the rate of recombination, differences in the amount of interference, chromosomal rearrangements, differential survival of genotypes, or statistical chance (Allendorf et al 1986). In pink salmon, variation between even and odd year in chromosome number and rearrangements have been reported (Phillips, R.B. and A.R. Kapuscinski 1987 and 1988). The variation at this locus might result from chromosomal rearrangement.

Heterogeneity between strains has been tested in rainbow trout, but the results were not consistent (Guyomard 1984; Thompson and Scott 1984; Allendorf et al., 1986). To clarify the nature of the differences between year groups (or strains), continuous studies over generations with multiple strains would be necessary.

Table 2. Summary of gene-centromere recombination rates and heterogeneity between families within a locus in even and odd year pink salmon.

Locus	Even year			Odd year		
	# of family examined	n	G-C rate	# of family examined	n	G-C rate
<i>AAT-3*</i>	5	589	67.9	4	588	68.9
<i>AAT-4*</i>	2	99	87.9	4	299	91.6
<i>mAAT-1*</i>	2	374	63.6	----	----	----
<i>mAAT-2*</i>	1	230	80.9	----	----	----
<i>ADA-2*</i>	5	686	99.6	5	285	97.5
<i>mAH-1*</i>	1	62	81.9	----	----	----
<i>mAH-3*</i>	----	----	----	4	456	46.7
<i>mAH-4*</i>	5	492	95.9	3	180	87.2
<i>sAH-1*</i>	1	127	81.9	----	----	----
<i>ALAT*</i>	----	----	----	2	186	89.8
<i>CK-4*</i>	1	231	39.4	----	----	----
<i>DIA-1*</i>	1	43	27.9	7	584	77.4
<i>DIA-2*</i>	2	182	98.4	----	----	----
<i>FDHG*</i>	----	----	----	5	428	78.3
<i>GDA*</i>	7	513	37.4	3	284	40.5
<i>G3PDH-1*</i>	10	1279	95.1	4	432	92.8
<i>GPI-1,2*</i>	1	223	93.7	----	----	----
<i>GPI-3*</i>	1	225	91.6	2	270	94.4
<i>IDDH*</i>	1	15	53.3	1	90	67.8
<i>sIDHP-1,2*</i>	9	892	67.3	11	957	71.5
<i>LDH-A1*</i>	2	268	1.9	----	----	----
<i>LDH-B2*</i>	1	127	5.5	----	----	----
<i>sMDH-1,2*</i>	5	552	14.9	----	----	----
<i>sMDH-3,4*</i>	6	528	85.4	11	1064	96.9
<i>sMEP-1*</i>	9	739	96.5	3	315	94.3
<i>MPI*</i>	3	408	11.0	1	183	0.5
<i>PEP-A*</i>	----	----	----	1	168	75.0
<i>PEP-B1*</i>	3	254	6.3	3	270	21.5
<i>PEP-D2*</i>	13	879	91.9	12	829	88.7
<i>PEP-LT*</i>	7	557	42.9	6	530	45.7
<i>PGDH*</i>	4	350	68.3	9	959	72.0
<i>PGM-2*</i>	1	229	40.6	6	544	19.1
<i>PK-2*</i>	----	----	----	1	273	46.5
<i>TPI-4*</i>	1	228	26.8	1	179	62.6

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Table 3. Summary of the statistical tests for heterogeneity between families within locus and between years.

Locus	Between families		Between years
	Even year	Odd year	
<i>AAT-3*</i>		+	
<i>AAT-4*</i>		+	
<i>mAAT-1*</i>	+	-----	-----
<i>mAAT-2*</i>	#	-----	-----
<i>ADA-2*</i>	+++		
<i>mAH-1*</i>	#	-----	-----
<i>mAH-3*</i>	-----	+++	-----
<i>mAH-4*</i>	+	+++	+++
<i>sAH-1*</i>	#	-----	-----
<i>ALAT*</i>	-----		-----
<i>CK-4*</i>	#	-----	-----
<i>DIA-1*</i>	#	+	+++
<i>DIA-2*</i>		-----	-----
<i>FDHG*</i>	-----		-----
<i>GDA*</i>	+++		
<i>G3PDH-1*</i>	++	+++	
<i>GPI-1,2*</i>	#	-----	-----
<i>GPI-3*</i>	#		
<i>IDDH*</i>	#	#	
<i>sIDHP-1,2*</i>			
<i>LDH-A1*</i>		-----	-----
<i>LDH-B2*</i>	#	-----	-----
<i>sMDH-1,2*</i>	+++	-----	-----
<i>sMDH-3,4*</i>	+++	\$	+
<i>sMEP-1*</i>		++	
<i>MPI*</i>	+	#	+++
<i>PEP-A*</i>	-----	#	-----
<i>PEP-B1*</i>			+++
<i>PEP-D2*</i>		+	+
<i>PEP-LT*</i>		+++	
<i>PGDH*</i>	+	++	
<i>PGM-2*</i>	#		+++
<i>PK-2*</i>	-----	#	-----
<i>TPI-4*</i>	#	#	+++

Blank: homogeneous; +: heterogeneous at $0.005 < P < 0.05$; ++: heterogeneous at $0.0005 < P < 0.005$; +++: heterogeneous at $P < 0.0005$; #: data is from only one family; \$: no test was conducted because of small expected values; -----: no information for the tests.

Table 4. Recombination rates at *sMDH-1,2** in even year pink salmon.

Family number	Number of progeny examined	Recombination rate (%)
33	86	0.0
34	76	2.6
70	88	13.6
19	88	21.6
50	214	22.9

Table 5. Recombination rates at *sMDH-3,4** in even and odd year pink salmon.

Family number	Even year		Odd year		
	Number of progeny examined	Recombination rate (%)	Family number	Number of progeny examined	Recombination rate (%)
E-72	88	61.4	O-21	90	91.1
E-62	88	67.0	O-10	168	91.7
E-40	88	85.2	O-16	90	94.4
E-47	88	98.9	O-20	131	97.7
E-21	88	100.0	O-31	90	98.9
E-66	88	100.0	O-49	90	98.9
			O-71	90	98.9
			O-9	90	100.0
			O-26	45	100.0
			O-50	135	100.0
			O-80	45	100.0

Table 6. Recombination rates at *mAHD-3** in odd year pink salmon.

Family number	Number of progeny examined	Recombination rate (%)
73	118	11.0
8**	179	14.5
6	90	44.4
9	90	50.0
44	158	72.8

** In this family, a ratio of the number of two homozygotes was not 1:1.

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