The Journal of Heredity 66:227–232. 1975.

Biochemical Genetic Variation in Pink and Chum Salmon

Inheritance of intraspecies variation and apparent absence of interspecies introgression following massive hybridization of hatchery stocks

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THIS PAPER describes an electrophoretic examination of pink salmon (*Oncorhynchus gorbuscha*) and chum salmon (*O. keta*) indigenous to streams of northwest Washington state (U.S.A.). The purposes of this investigation were 1) to seek evidence of introgression of populations of each species from a hatchery where experimental hybrids were released in the early 1960's, and 2) to find additional genetic variation for population studies, particularly in chum salmon from Washington state where no variation had previously been observed.

We have obtained data from field collections of both species and from individual crosses of chum salmon. We describe electrophoretic variation both within and between species including one previously undescribed variant in pink salmon and three in chum salmon. Evidence of disomic inheritance and absence of linkage is presented for the chum salmon variations. A single locus coding for an enzyme in chum salmon is contrasted with the duplication of this locus in rainbow trout (*Salmo gairdneri*). The evolutionary implications of these findings are discussed.

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Materials and Methods

Collection data are summarized in Table I. The adult chum salmon from Brinnon were used in the inheritance studies. Gametes were removed and stored up to 12 hours at 5° C while tissue extracts of the fish were tested electrophoretically. Specific matings were made on the basis of electrophoretic phenotypes. Tissues were extracted with approximately 2:1 volumes of tissue and distilled water by blending with glass rods in 12 × 75 mm culture tubes and subsequently centrifuging the tubes at 1,000 × g for 10 minutes.

Electrophoresis followed the procedures outlined by Utter *et al.*[20]. Staining procedures were modified from Shaw and Prasad[14]. The protein systems examined, tissue specificity, buffer system giving best resolution, and abbreviations used in this paper are listed in Table II. A maximum of 200 volts was required for optimal AAT resolution contrasted with higher voltages and amperages that were routinely used for other proteins during electrophoresis. Stability varied among proteins during frozen storage. AAT had to be tested within the first few weeks of storage—preferably using fresh tissue—in order to obtain reliable results. IDH in the liver had only slightly more stability. Other proteins tested were stable for up to a year or more.

Table I. Collection data

Species	Location	Collection date	Cooperating agency	Maturity
Pink	Hood Canal Hatchery Hoodsport, Washington	9/14/73 4/05/74	Washington State Department of Fisheries	Adult Fingerling
	Dungeness River, Washington	9/21/73	Washington State Department of Fisheries	Adult
Chum	Hood Canal Hatchery Hoodsport, Washington	11/20/73	Washington State Department of Fisheries	Adult
	Brinnon, Washington	12/11/73	U.S. Bureau of Sport Fish and Wildlife	

Genetic Systems

IDH is the only genetic system described in this report that has not been previously described for these species. Our present findings amplify earlier reports through description of newly found variations and place special emphasis on the differences between species in order to more clearly identify possible introgression. Disomic inheritance of these polymorphic systems based on breeding data has been previously reported for these or closely related salmonid species [1,2,4,5,19].

MDH

Pink salmon were polymorphic for MDH-B (the predominant soluble MDH of salmonid skeletal muscle described by Bailey *et al.* [7]) while chum salmon were monomorphic for the common MDH-B form observed in pink salmon (Figure 1). The variant phenotypes appear to be the same as those described in pink salmon by Utter *et al.* [18] and Aspinwall [5] and are typical of MDH-B variants described in other salmonid species. The asymmetrical banding of heterozygous individuals (9:6:1 ratio of AA:AA′:A′A′ bands, Figure 1) results from two loci with common alleles coding for enzymes of identical electrophoretic mobility. Thus the common single-banded electrophoretic phenotype is a reflection of four gene doses and heterozygous individuals at a single locus still retain three doses of the common allele, while expressing only a single dose of the variant allele; this gene dosage is directly reflected by the asymmetrical banding of heterozygotes [7,11,17].

AGP

AGP has been reported to be polymorphic in both pink salmon [4,18] and chum salmon [3]. Only pink salmon had AGP variants in the populations we examined; all chum salmon had single-banded phenotypes of the same mobility as the common pink salmon band (Figure 1).

PGM

PGM was invariant in chum salmon and polymorphic in pink salmon. The variant allelic form of the pink salmon migrated faster than the common form and had the same mobility as the band of the chum salmon (Figure 2). Pink salmon previously collected from the North Pacific Ocean and Southeastern Alaska were monomorphic for the common band [18].

IDH

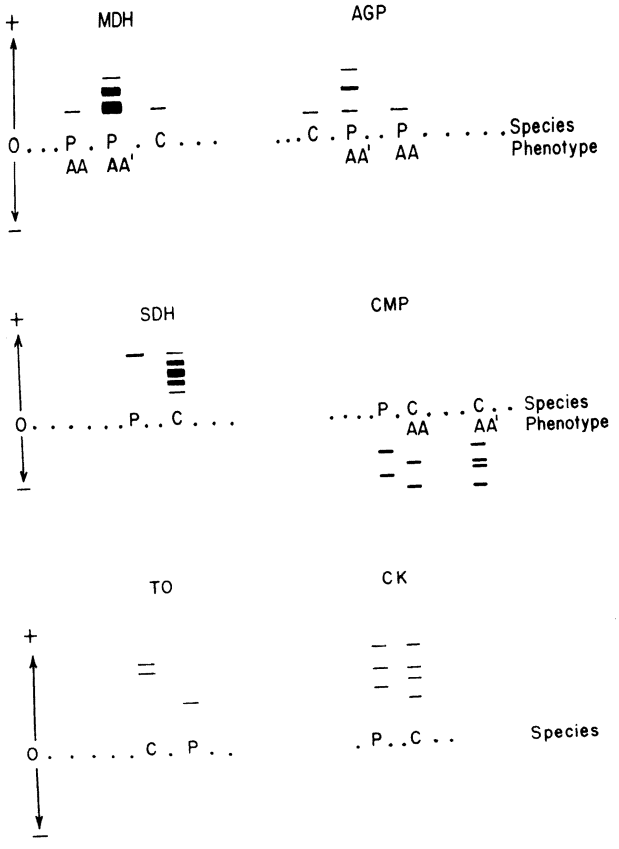
Liver IDH patterns were monomorphic in pink salmon and polymorphic in chum salmon (Figure 3). The three phenotypes observed in chum salmon suggested a single

Table II. Tissue distribution and buffer systems for proteins examined

Protein	Tissue: muscle	Tissue: liver	Buffer system*
B-type malate dehydrogenase (MDH-B)	+		1
Alphaglycerophosphate dehydrogenase (AGP)	+		2,3
Phosphoglucomutase (PGM)	+		1
NADP-dependent isocitrate dehydrogenase (IDH)		+	3
Aspartate aminotransferase (AAT)	+		1
Sorbitol dehydrogenase (SDH)		+	1
Tetrazolium oxidase (TO)	+	+	1,2
Creatine kinase (CK)	+		1
Cathodal muscle protein (CMP)	+		1,2

* 1. Gel buffer: .03 M tris, .005 M citric acid monohydrate, .0006 M LiOH, .003 M boric acid
Tray buffer: .06 M LiOH, .3 M boric acid (Ridgway *et al.*[13])
2. Gel buffer: .045 tris, .025 M boric acid, .001 M Na EDTA
Tray buffer: .18 M tris, .1 M boric acid, .004 M Na EDTA (Markert and Faulhaber[10])
3. Gel buffer: .004 M Na_2HPO_4, .006 M NaH_2PO_4.
Tray buffer: .04 M Na_2HPO_4, .06 M NaH_2PO_4 (Wolf

FIGURE 1—Diagrammatic electropherograms of six protein systems in pink (P) and chum (C) salmon.



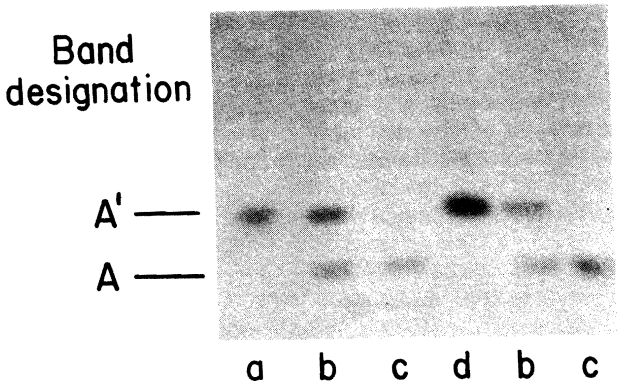


FIGURE 2—PGM phenotypes of pink salmon. *a*—A'A'; *b*—AA'; *c*—AA; and *d*—invariant band in chum salmon (indistinguishable from A'A' phenotype in pink salmon).

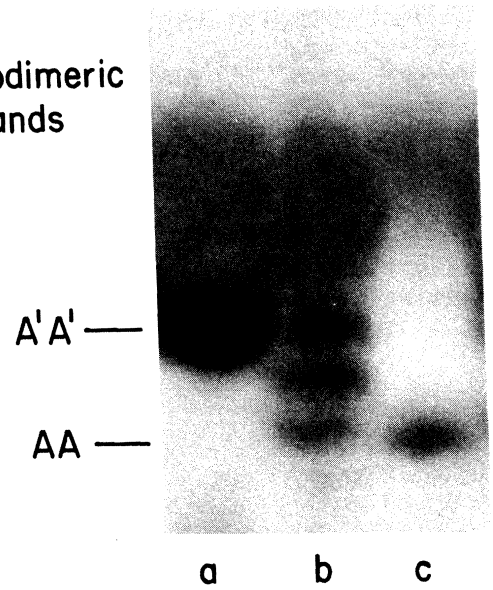


FIGURE 3—IDH phenotypes of chum salmon. *a*—A'A'; *b*—AA'; and *c*—AA phenotype in chum (identical to invariant band in pink salmon).

disomic locus. This hypothesis was confirmed through breeding data (Table III) and is in contrast with rainbow trout IDH where two disomic loci occur[1].

AAT

AAT was originally described as monomorphic in both pink and chum salmon[18]. In the present study, the pink salmon remained monomorphic but the chum salmon displayed variation similar to MDH-B and IDH variation in rainbow trout where two loci code for common alleles of identical electrophoretic mobility (Figure 4). Six crosses (Table IV) clearly demonstrate that both loci segregate disomically. There was no evidence of linkage between the two AAT loci or between one AAT locus and the IDH locus in the two crosses that permitted examination of joint segregation ratios for these respective pairs of loci (Table V). This result is expected since chum salmon have a chromosome number of $2n = 74$ making the chance of detecting linkage between randomly chosen loci quite small.

Other proteins

Four additional protein systems reported by Utter *et al.*[18] were monomorphic in both pink and chum salmon but differed between them. These systems, SDH, TO, CK, and CMP, are illustrated in Figure 1. The only intraspecies variation observed in this study for these systems was a CMP variant observed in one chum salmon. The variant bands did not coincide with the electrophoretic mobility of pink salmon CMP nor did this individual display both pink and chum bands for SDH, TO, or CK. It was therefore concluded that this individual reflected a mutant form of CMP in chum salmon rather than being an indication of pink salmon introgression.

Table III. Observed and expected segregation of IDH alleles from individual matings of chum salmon

	Parental phenotypes		Progeny phenotypes observed (expected)					
Lot no.	Male	Female	*N*	AA	AA'	A'A'	χ^2	*P*
1	A'A'	AA'	29	—	14 (14.5)	15 (14.5)	.03	>.80
2	AA'	AA'	67	19 (16.8)	33 (33.5)	15 (16.8)	.49	>.70
3	AA'	AA'	75	22 (18.8)	36 (37.5)	17 (18.8)	.78	>.50
7	A'A'	A'A'	52	—	—	52 (52)	—	—
8	AA	AA'	240	124 (120)	116 (120)	—	.27	>.50

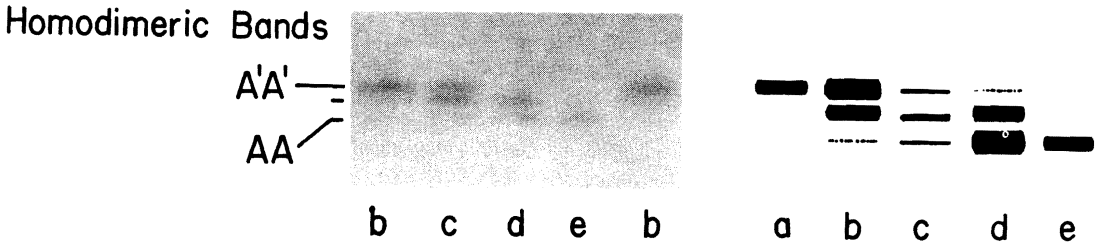


FIGURE 4—AAT phenotypes of chum salmon. *a*—A'A'A'A' (not seen due to low frequency of *A'* allele); *b*—AA'A'A'; *c*—AAA'A'; d—AAAA'; and *e*—AAAA phenotype in chum and invariant band in pink salmon.

Comparison of Populations

The gene frequency data for the two pink salmon populations (Table VI) indicate a stability among three consecutive two-year cycles in the Dungeness River for MDH-B and AGP (using data from Aspinwall[6] and the present study); no significant differences in gene frequency between the populations were observed. A significant difference does exist for the PGM locus between the two populations from our 1973 collections. This is of interest because the Hoodsport population was originally derived from fish taken from the Dungeness River. It is not possible from the present data to determine whether these differences are the result of different selective pressures in the two populations or resulted from stochastic processes. Regardless of cause, however, the PGM variants appear to be useful as population markers in pink salmon.

There were no significant differences in gene frequencies for either polymorphic system in comparisons of the two chum salmon populations sampled (Table VII). The fairly large differences observed for the IDH alleles are insignificant because of the small sample size. The IDH and AAT variants of chum salmon now permit comparisons among populations in the southeastern extremes of this species where such comparisons were previously not possible because of an absence of known genetic variation in these populations.

Introgression

A total of 500,000 F_1 hybrids of pink and chum salmon were released from the Hoodsport hatchery in 1961 and 1963. All identifiable F_1 hybrids were killed upon return to the hatchery and excluded from normal spawning of pink and chum salmon. However, there was a possibility for misidentification because the hybrid progeny tended to resemble one parent or the other[16]. The return of hybrids in 1963 was more than a third of the combined total pink and chum salmon runs[21,22]. A

Table IV. Observed and expected segregation of AAT alleles from individual matings of chum salmon

	Parental phenotypes			Progeny phenotypes observed (Expected with disomic inheritance) (Expected with tetrasomic inheritance)						
Lot no.	Male	Female	*N*	AAAA	AAAA'	AAA'A'	AA'A'A'	A'A'A'A'	χ^2	*P*
2	AAA'A'	AAAA	172	44	85	43	—	—		
				(43)	(86)	(43)			.03	>.98
				(28.7)	(114.7)	(28.7)			22.97	<.0001
3	AAAA'	AAAA	63	32	31	—	—	—		
				(31.5)	(31.5)				.02	>.80
				(31.5)	(31.5)				.02	>.80
4	AAAA	AAAA	20	20	—	—	—	—		
				(20)					—	—
				(20)						
5	AAA'A'	AAAA'	119	16	40	44	19	—		
				(14.9)	(44.6)	(44.6)	(14.9)		1.69	>.50
				(9.9)	(49.6)	(49.6)	(9.9)		14.61	<.003
6	AAA'A'	AAAA'	110		52	58	—	—		
					(55)	(55)			.33	>.50
				(9.2)	(45.8)	(45.8)	(9.2)		22.49	<.0001
8	AAAA	AAAA'	258	138	120	—	—	—		
				(129)	(129)	—	—	—	1.26	>.20
				(129)	(129)				1.26	>.20

purpose of the present study was to evaluate possible introgression of pink and chum salmon in fish returning to the hatchery 10 years (five generations for pink salmon; approximately three generations for chum salmon) after the last release of hybrids.

The present data indicate there is no introgression of pink and chum salmon at the Hoodsport hatchery based on 80 fish of each species that were examined for each of the biochemical systems. There was no introgression detected among the four loci that differed qualitatively between the two species. It could be argued from the Hoodsport data that the higher frequency of the PGM variant in pink salmon (having the same mobility as chum salmon PGM) reflected introgression if there had been some positive evidence from other loci. It seems most likely that this difference is due to causes other than introgression. This conclusion may, nevertheless, not be the final answer to the question of introgression because of the relatively small number of loci and individuals examined. It is safe to assume, however, from the negative data presented here that any introgression occuring in either species is very low.

Some Evolutionary Considerations

The finding of a single IDH locus in chum salmon, contrasted with the duplicated IDH loci of rainbow trout, suggests either loss of activity or deletion of genetic material in chum salmon. This is not an isolated instance but appears to be a relatively common phenomenon in salmonids based on our observations of these and other loci in a number of salmonid species. We have previously mentioned the apparent single locus for MDH-A in rainbow trout contrasted with duplicate MDH-A loci in brown trout, *Salmo trutta*[2,7].

Other apparent instances of limited gene loss or duplication in salmonids (based on our unpublished observations) include different numbers of loci expressed for PGM, transferrin, AGP, and possibly SDH and phosphohexose isomerase among species of

Table VII. Gene frequencies of *AAT* and *IDH* in chum salmon

		*AAT**			*IDH*		
Location	*N*	A	A′	2SE†	A	A′	2SE‡
Brinnon	30	.817	.183	± .071	.450	.550	± .128
Hoodsport	40	.750	.250	± .069	.295	.705	± .102

* *AAT* gene frequencies are calculated on basis of two loci for *AAT* per individual. The gene frequency of each allele is assumed to be the same at each AAT locus

† SE = $\sqrt{p(1-p)/4N}$ for *AAT*

‡ SE = $\sqrt{p(1-p)/2N}$ for *IDH*

Table V. Joint segregation ratios in two chum salmon matings (1) for one AAT locus and the IDH locus (2) for the two AAT loci

Lot no.	Enzyme systems	Parental phenotypes: Male	Female	*N*	Progeny phenotypes				χ^2: IDH	AAT	Joint	*P** ± 2SE†
8	IDH	AA	AA′	209	AA	AA	AA′	AA′	.234	.809	.005	.498 ± .035
	AAT	AAAA	AAAA′		AAAA 57	AAAA′ 51	AAAA 54	AAAA′ 47				
2	AAT	AAA′A′	AAAA	172	AAAA 44	AAAA′ 85	AAA′A′ 43		—	—	.023	.494 ± .038

* *P* = Fraction of recombinants (assuming the largest linkage class is the parental type)

† SE = $\sqrt{P(1-P)/N}$

Table VI. Gene frequencies of *MDH*, *AGP*, and *PGM* in pink salmon

		*MDH**			*AGP*			*PGM*		
Location	*N*	B	B′	2SE†	S	F	2SE	A	A′	2SE
Hoodsport	109	.954	.046	± .028	.959	.041	± .027	.853	.147	± .048

Oncorhynchus and *Salmo* that we have studied. We mention this variability to stress the apparent plasticity of the salmonid genome and the heterogeneity that appears to exist in the number of loci expressed within a group of closely related species.

This plasticity is also found within species. Cueller and Uyeno[8] reported a triploid rainbow trout among 18 individuals whose karyotypes were examined. The external phenotype of this individual was typical of the other 17 individuals examined in both size and appearance. They postulated that this individual resulted from suppression of the second meiotic division with subsequent fertilization of the unreduced oocyte. Davisson *et al.*[9] reported biochemical and cytological evidence of trisomy for an LDH locus in physiologically normal brook trout (*Salvelinus fontinalis*). These workers proposed a mechanism based on centric fusion and recurrent trisomy to explain the extensive gene duplication in salmonids.

The disomic inheritance reported here for the duplicated AAT locus in chum salmon is consistent with the disomic inheritance reported to date for all other duplicated loci of salmonids where actual breeding data have been available[2]. If the extensive gene duplication of salmonids is the result of a recent ancestral tetraploid event[12], sufficient time appears to have elapsed for diploidization to have taken place.

Much of our current research is directed towards a better understanding of the genetic and evolutionary significance of the extensive gene duplication of salmonids. It is hoped that a clearer picture of salmonid evolution will emerge from these and other investigations.

Summary

An electrophoretic examination of inter- and intraspecific genetic variation was carried out in pink salmon (*Oncorhynchus gorbuscha*) and chum salmon (*Oncorhynchus keta*) from Washington state (U.S.A.). Examination of single populations of these species at a hatchery where previous hybridization studies had been carried out failed to detect any residual introgression. Previously unreported intraspecies variants that we observed included phosphoglucomutase (PGM) in pink salmon; and NADP-dependent isocitrate dehydrogenase (IDH), aspartate aminotransferase (AAT, two loci), and cathodal muscle protein (CMP) in chum salmon.

Chum salmon variants were further examined through selected matings. IDH segregated as a single disomic locus contrasted with the duplication of this locus in rainbow trout (*Salmo gairdneri*). AAT segregated at two disomic loci. No linkage was detected between the two AAT loci or between one of the AAT loci and the IDH locus.

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