

Polymorphism of Mitochondrial DNA (mtDNA) of the Cytb/D-loop Region in Pink Salmon Populations

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Pink salmon is the most abundant Pacific salmon species in the North Pacific Ocean, and its commercial role in salmon fisheries of the Russian Far East is paramount. The principal stocks supporting commercial salmon fisheries originate from local populations in Kamchatka, Sakhalin, Kuril Islands and the Amur River basin. The purpose of our research was to determine the level of pink salmon mtDNA polymorphism as a marker to identify the regional origin of pink salmon in mixed-stock marine aggregations. We analyzed the population and regional level genetic variation on the basis of polymorphism of mtDNA Cytb/D-loop region using the following restriction enzymes: *Dde* I, *Hin6* I, *Hinf* I, *Msp* I, *Rsa* I, *Sfr*13 I.

We analyzed 32 samples of pink salmon returning to spawn in even-numbered years (25 populations, 1612 fishes) and 17 samples of pink salmon returning in odd-numbered years (16 populations, 805 fishes) from the rivers of West and East Kamchatka, Sakhalin, Amur River, continental shore of the Okhotsk Sea and Primorye. As a result of restriction fragment length polymorphism (RFLP) analysis, 59 unique Cytb/D-loop haplotypes were found in the 2417 individuals. Analysis of even-year pink salmon revealed 39 haplotype variants, and 34 haplotypes were found in the odd-year samples. Fourteen composite haplotypes were found in common between the odd- and even-year brood lines. Only three haplotypes were detected in all 49 samples analyzed. Most of the haplotypes (39) were rare and detected once in one of the samples.

Based on the variation of composite haplotype frequencies, we confirmed the difference between even- and odd-year brood lines in Asian pink salmon, and we obtained data indicating the heterogeneity of even- and odd-year generations of pink salmon. Pair-wise comparison of all samples indicated significant differences in 55.1% of the comparisons. Analysis of samples from even-year brood lines revealed significant differences in 33.7% of the pair-wise comparisons, and analysis of samples from the odd-year brood line indicated significant differences in 33.8% of the comparisons.

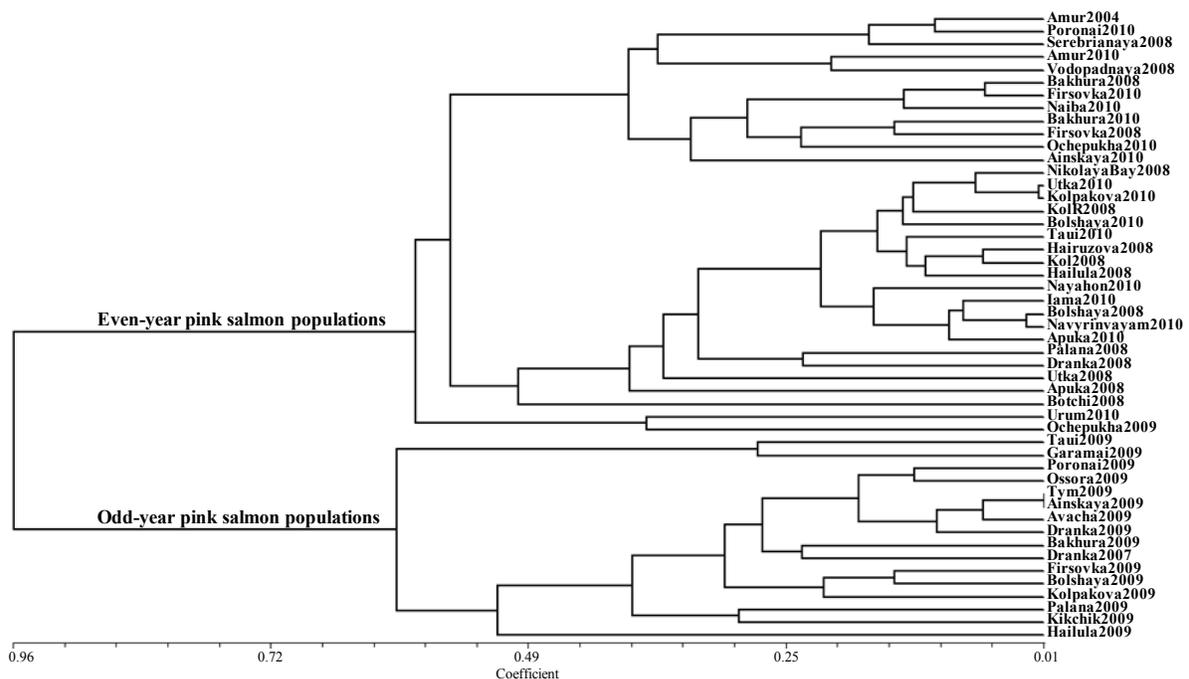


Fig. 1. The unweighted pair-group method with arithmetic mean (UPGMA) dendrogram created using genetic chord distances and based on the frequencies of composite haplotypes of even- and odd-year Asian pink salmon.

The genetic chord distances, calculated from the frequencies of the composite haplotypes, were analyzed using cluster analysis and multidimensional scaling (Figs. 1 and 2). The samples formed two significant divergent clusters, one each for the even- and odd-year brood line. Samples from even-year brood lines clearly expressed a regional division into northern and southern groups of populations. Samples from odd-year brood lines did not demonstrate any regional groups or other clusters.

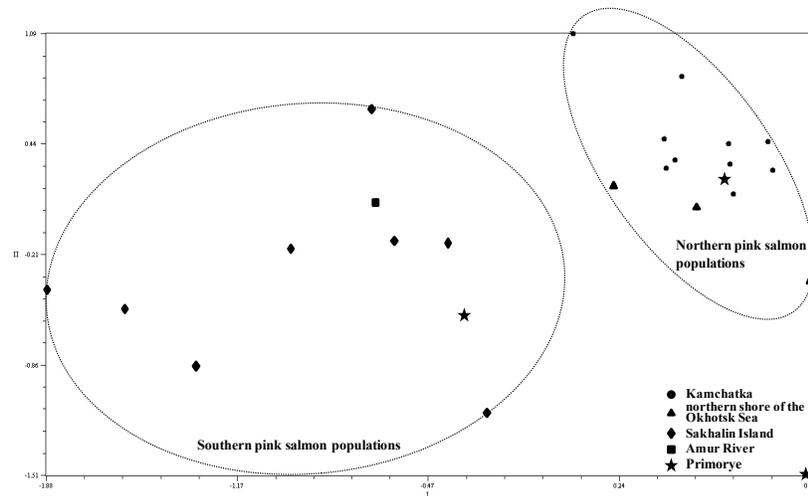


Fig. 2. Multidimensional scaling analysis of genetic distances calculated from composite haplotype frequencies of even-year Asian pink salmon populations.

Table 1. The relative (%) genetic variation among and within populations and regional groups that was observed in even-year Asian pink salmon populations. Variation estimated by hierarchical analysis of molecular variation (AMOVA).

Analyzed pool	Source of variation	Percentage of variation
All samples	Among populations	1.88
	Within populations	98.12
5 regional groups of populations: Kamchatka, Sakhalin, Amur R., Primorye, northern shore of the Okhotsk Sea	Among groups	2.10
	Among populations within groups	0.30
	Within populations	97.60

Within regional groups of populations, hierarchical analysis of molecular variation (AMOVA) of samples collected from even-year brood lines indicated a visibly higher inter-regional variation than inter-population variation (Table 1). Analysis of samples from odd-year brood lines demonstrated higher differentiation at the population level than the even-year brood line. Variation between populations was substantially higher than between regions in samples collected from odd-year brood lines (Table 2).

Table 2. The relative (%) genetic variation among and within populations and regional groups that was observed in odd-year Asian pink salmon populations. Variation estimated by hierarchical analysis of molecular variation (AMOVA).

Analyzed pool	Source of variation	Percentage of variation
All samples	Among populations	3.10
	Within populations	96.90
3 regional groups of populations: Kamchatka, Sakhalin, northern shore of the Okhotsk Sea	Among groups	1.00
	Among populations within groups	2.69
	Within populations	96.31

Judging from these results, we have no doubt there is regional divergence in the even-year brood line of Asian pink salmon. Determination of intra-regional divergence will require additional analysis using a larger number of samples from major spawning rivers. However, the use of mtDNA polymorphism of the Cytb/D-loop region as a regional marker looks promising for analysis of mixed-stock catches of even-year pink salmon.