

Single Nucleotide Polymorphisms (SNPs) Provide Standard DNA Data for Bering-Aleutian Salmon International Survey (BASIS) Studies

James E. Seeb¹, Richard L. Wilmot², Shigehiko Urawa³, Syuiti Abe⁴,
Lisa W. Seeb¹, and Christian T. Smith¹

¹Alaska Department of Fish and Game,

333 Raspberry Road, Anchorage, Alaska 99518, USA

²U.S. Department of Commerce, NOAA, NMFS,
Alaska Fisheries Science Center, Auke Bay Laboratory,
11305 Glacier Highway, Juneau, Alaska 99801, USA

³National Salmon Resources Center,

2-2 Nakanoshima, Toyohira-ku, Sapporo, Hokkaido 062-0922, Japan

⁴Graduate School of Fisheries Sciences, Hokkaido University,
3-1-1 Minato, Hakodate, Hokkaido 041-8611, Japan



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Migratory studies of Chinook *Oncorhynchus tshawytscha*, chum *O. keta*, and sockeye *O. nerka* salmon require markers for which a large number of individuals can be processed in a relatively short time. Genetic markers, especially allozymes, have provided substantial insight into key questions asked by BASIS investigators (see Kondzela et al. 2002; Urawa et al. 2004; Seeb et al. 2004). However, issues of sample collection and preservation as well as a desire for increased resolution have driven efforts to develop DNA markers to describe discrete aggregations of stocks. Given the multi-jurisdictional geographic range of these species, it is desirable that genetic markers and the corresponding data be transportable across laboratories. Allozymes meet these criteria while most DNA markers do not. To solve this DNA standardization dilemma, we are continuing to develop single nucleotide polymorphism (SNP) genotyping assays based upon the 5'-nuclease reaction.

Various approaches to DNA analysis, each with advantages, were used to study Pacific salmon during the last decade.

Initially the most common approach was the collection of SNP data that was obtained by restriction length polymorphism assays (RFLPs; e.g. Cronin et al. 1993; Park et al. 1993; Seeb and Crane 1999), amplification fragment length polymorphism assays (AFLPs; see Flannery et al. 2002), or DNA sequencing (e.g. Sato et al. 2004). SNP data were collected on mitochondrial DNA, nuclear DNA, neutral genes, and selected genes such as MHC (Kim et al. 1999), providing opportunities for extremely high resolution. Despite the great potential for these markers, application to fisheries issues was often hampered by slow throughput.

Minisatellite analysis (Beacham 1996) was explored and discarded; however, microsatellite analysis became popular because of both its relatively high throughput and resolution (e.g., Scribner et al. 1998; Beacham et al. 2001; Habicht et al. 2004). An important limitation of microsatellites in the context of inter-agency BASIS research is the difficulty of data standardization among laboratories. Standardization of data among laboratories is essential for two reasons. First it obviates the need for every agency to spend the resources to create independent and redundant baseline data. Second, standardization is prerequisite for reproducibility of data among laboratories, thus enabling treaty partners to independently evaluate one another's fishery estimates. Despite over a decade of use, no inter-laboratory standardized microsatellite database exists for any species of Pacific salmon. Technical hurdles have proven daunting and expensive to overcome.

Recent developments in DNA chemistry produced high throughput SNP genotyping technologies such as the 5'-nuclease reaction (see Morin et al. 1999, Ranade et al. 2001) and DNA microarray analysis (Moriya et al. 2004). These approaches offer efficient genotyping to capture the high resolution provided by the various RFLP, AFLP, and sequencing assays. No technically difficult or expensive standardization is required because resulting data are the actual DNA sequence and are automatically standard from lab to lab. We conducted a comparison between the SNP scores obtained using the 5'-nuclease reaction at Alaska Department of Fish and Game and DNA microarray at Hokkaido University and observed 99.4% identity (see Fig. 1). The fact that both laboratories are using SNPs allows the two baselines to be merged, saving each laboratory the resources that would otherwise be spent to genotype each other's collections.

The most significant limitation of SNPs presently is that the paucity of DNA sequence data in some salmonid species means that extensive development is required to identify informative loci. Using a targeted gene approach (cf., Elfsrom et al. in press) and the wealth of sequence data available for rainbow trout *O. mykiss* and Atlantic

salmon *Salmo salar*, we have identified in excess of 100 SNPs each in Chinook, chum, and sockeye salmon. From these we have designed 5'-nuclease assays for 19 SNPs in Chinook salmon, 27 in chum salmon, and 26 in sockeye salmon (see Smith et al. 2004a, 2004b). Using these assays a single technician with one thermal cyclener can generate 3840 genotypes in a 7.5hr day. Based on studies in other organisms, we anticipate that 20–40 SNPs will be sufficient to accurately and precisely discriminate salmon populations at a scale useful for Pacific Rim analyses (Werner et al. 2004, i.e., see Fig. 2).

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Fig. 1. Automatic Standardization. SNP data reflects the actual DNA sequence regardless of the hardware and chemistry used to collect those data. We compared SNP scores using DNA microarray at Hokkaido University with those obtained using the 5'-nuclease reaction at Alaska Department of Fish and Game. We examined three SNPs that discriminate Asian and Alaskan stocks of chum salmon; 1142 of 1149 bases were scored identically (99.4% accuracy).

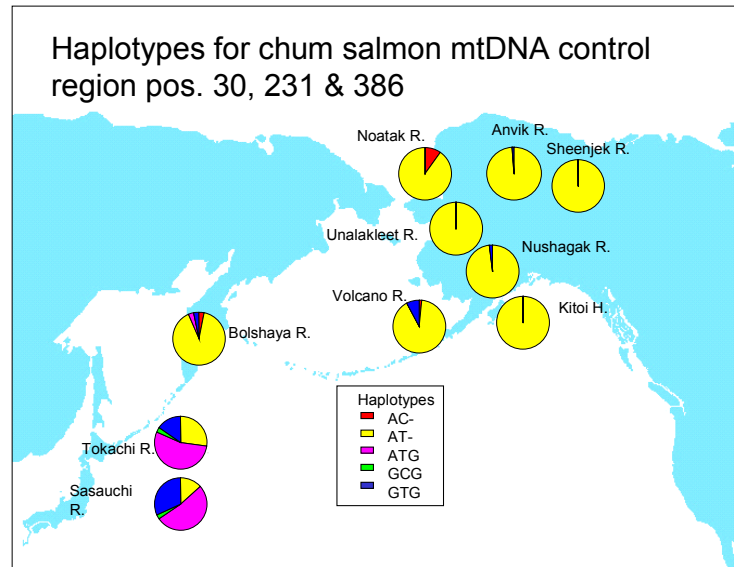
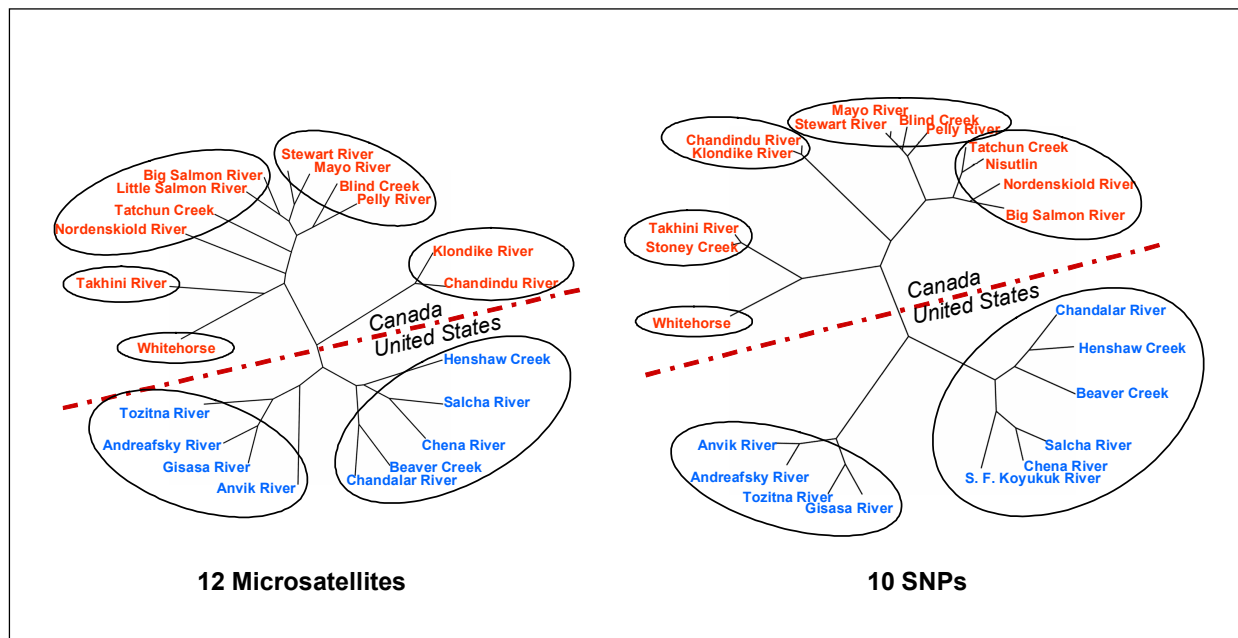


Fig. 2. Resolution. SNPs and microsatellites show similar resolution for Canadian (red) and Alaskan (blue) stocks of Chinook salmon from the Yukon River.



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