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Summary of salmon DNA stock identification work at the Pacific Biological Station

by

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ABSTRACT

Staff at the Pacific Biological Station have investigated three main areas of research in applying DNA technology to problems of salmon stock discrimination and identification. Progress to date in applying variation at minisatellite loci, microsatellite loci, and major histocompatibility complex genes to population structure and stock identification is summarized.

SUMMARY

Research activities on applying DNA variation to problems of stock identification in Pacific salmon have encompassed three main areas of research at the Pacific Biological Station, namely minisatellite DNA variation, microsatellite DNA variation, and major histocompatability complex (MHC) gene variation. Brief summaries of activities to date in these three areas are as follows:

Minisatellite DNA Variation

Minisatellite DNA consists of arrays of tandemly repeated nucleotide sequences, with each array containing a distinctive core sequence of between 10 and 75 nucleotides (Jarman and Wells 1989). Variable numbers of the repeated core sequence occur between restriction enzyme sites, resulting in allelic variation. Individual minisatellite loci can be quite variable in salmonids, with heterozygosities between 35 and 90%. Minisatellite variation is usually surveyed by loading genomic DNA that has been digested with a specific restriction enzyme on to an agarose gel, and the DNA electrophoresed for a period of time. Electrophoresis results in a separation of the DNA fragments by size along in gradient in the agarose gel. The DNA is then transferred and bound to a nylon membrane, and hybridized with a probe that is labelled with either a radioactive or chemical marker. The probe used in the analysis contains a DNA sequence that is complementary to the core repeat sequence at the minisatellite locus. The probe hybridizes to DNA fragments of different sizes in fish with different alleles. Variation is detected by exposure of the membrane to X-ray film, and in our laboratory the autoradiograph is subsequently scanned with a digital camera, translated to digital image files, and analyzed on a workstation using BioImage software.

We have examined minisatellite DNA variation in sockeye, chum, coho, and chinook salmon and steelhead trout. Results for sockeye salmon have been reported by Beacham et al. (1995) and Taylor et al. (1995), and results for chum salmon reported by Taylor et al. (1994) and Beacham (1996). In chinook salmon,

minisatellite DNA variation has been used to identify offspring to specific families as outlined by Stevens et al. (1993), and for stock identification by Withler et al. (1994) and Beacham et al. (1996a). Structure of one of the minisatellite loci surveyed was outlined by Withler and Miller (1996). Minisatellite DNA variation in coho salmon and applications to stock identification have been reported by Miller et al. (1996) and Beacham et al. (1996b). A survey of minisatellite DNA variation in steelhead trout was reported by Taylor (1995).

Our surveys of minisatellite variation at the loci examined indicated that accurate and precise estimates of stock composition were possible for chinook and coho salmon on a stock basis, with virtually no error observed in simulated mixtures of known composition and with the characteristics of the baseline stocks considered fixed. For example, in the Skeena River drainage, with 7 chinook salmon stocks in the baseline and 6 stocks contributing to a 100-fish mixture, the average accuracy of estimation of individual stock compositions was 100% when the distribution of band (allele) frequencies were considered fixed, and 99.6% when the distributions of band frequencies in the baseline populations were resampled (simulated annual and sample variability by bootstrapping) (Beacham et al. 1996a). Chum and sockeye salmon were less variable than chinook and coho salmon at the minisatellite loci we examined, and estimates of stock composition for individual chum and sockeye salmon stocks were less precise than those for chinook and coho salmon. Although variation at minisatellite loci is clearly useful for stock identification for Pacific salmon, the method of laboratory analysis to detect the variation (Southern blotting) is sufficiently complex and time consuming that it is not practical to use in applications where estimates of stock composition are required in less than 72 hours.

Microsatellite DNA Variation

Microsatellite DNA variation is similar to minisatellite variation in that it consists of arrays of a core sequence of nucleotides that is tandemly repeated. However, the core sequence that is repeated is much smaller than at minisatellite loci, and typically ranges from 2 to 4 nucleotides. As the microsatellite alleles are shorter in length than minisatellite alleles, and are usually less than 1,000 base pairs (bp), the laboratory analysis of variation at microsatellite loci can be done via the polymerase chain reaction (PCR) technique. In PCR, if the nucleotide sequence of the DNA flanking or adjacent to the tandemly repeated array is known, primers complementary to this flanking region can be developed and used to selectively amplify specific loci. The microsatellite alleles are amplified by placing genomic DNA and the specific primers in a DNA thermal cycler for an appropriate number of cycles. The amplified products (microsatellite alleles at the specific locus) are then loaded onto an agarose or polyacrylamide gel, electrophoresis conducted, and the alleles visualized with a chemical stain. PCR analysis has the advantage that, as the microsatellite alleles are short relative to minisatellite alleles, poorer quality

(degraded) genomic DNA can be used as the source of the samples. Samples amenable to DNA extraction for subsequent DNA analysis via PCR could include fish scales or otoliths collected previously for other purposes and stored for many years.

Evaluation of microsatellite variation is the current major emphasis of stock identification research in the molecular biology laboratory at the Pacific Biological Station. Drs. R. J. Nelson and M. Small are developing and applying primers to examine variation at microsatellite loci in Pacific salmon. Although the research projects are still at a stage at which variation at microsatellite loci is being screened in a number of salmon stocks in British Columbia to acquire baseline data for chinook, coho, and sockeye salmon, preliminary results suggest that variation at microsatellite loci will be a very effective method to apply to stock identification problems.

Major Histocompatibility Complex (MHC) Genes

The polymorphisms observed at minisatellite and microsatellite loci have no known function, and can simply be exploited as genetic markers to describe population structure. However, we have also investigated variation in MHC genes as a method of elucidating population structure, and applied MHC polymorphism to problems of species and stock identification. MHC genes are involved in the function of the immune system, as T cells recognize a foreign antigen only in association with class I and II molecules encoded in the MHC. Initial work required the development of suitable primers to amplify MHC genes in Pacific salmon, and investigators at the Pacific Biological Station were the among the first to accomplish this task (Miller and Withler 1996). Minisatellite and microsatellite alleles differ in size, but MHC alleles differ in nucleotide sequence. Once specific portions of several MHC genes were amplified, the genes were sequenced in order to identify alleles by their nucleotide composition. Relationships among the various Oncorhynchus species were investigated using variation at a MHC class II gene (Miller and Withler 1996), and differences among the species were exploited to develop a PCR-RFLP (restriction fragment length polymorphism) method of species identification based upon MHC variation (Beacham et al. 1996c). Based upon somewhat limited surveys, the PCR-RFLP analysis indicated that individual Japanese chum salmon were able to be discriminated from individual North American chum salmon (Beacham et al. 1996c). MHC variation within chinook salmon has been used to discriminate all individuals from a lower Fraser River stock from all individuals from an upper river stock (Miller et al. in prep.). Direct DNA sequencing is a labour-intensive method of determining nucleotide sequence variation, and current research in the laboratory is directed towards the development of rapid methods to screen sequence variation in MHC genes. Evaluation of variation at several MHC loci in a larger number of populations is anticipated.

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