Microsatellite Analysis and Stock Identification of Pacific Salmon Using Pacific Rim Baselines

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Keywords: Chum salmon, microsatellites, population structure, sockeye salmon, stock identification

DNA-level variation has been demonstrated to be very effective in determining population structure in Pacific salmon, and when applied to problems of estimation of stock composition in mixed-stock samples, has provided unprecedented levels of accuracy and precision in stock composition estimates based upon biological variation. It is also possible to identify the origins of individual salmon with a high degree of resolution. DNA-level variation (both microsatellite and major histocompatability complex (MHC)) has been extensively applied in determination of stock composition and individual identification in samples from fisheries, providing detailed resolution of origin of fish in the samples not previously available from biological characters.

For sockeye salmon, tissue samples were collected from populations with a Pacific Rim distribution, and DNA was extracted from the samples as described by Withler et al. (2000). For the survey of baseline populations, PCR products at 14 microsatellite loci: *Ots2*, *Ots3* (Banks et al. 1999), *Ots100*, *Ots103, Ots107, and Ots108* (Beacham et al. 1998; Nelson and Beacham 1999), *Oki1* (two loci), Oki6, *Oki10, Oki16,* and *Oki29* (Smith et al. 1998), *One8* (Scribner et al. 1996), and *Omy77* (Morris et al. 1996) were size fractionated on denaturing polyacrylamide gels and allele sizes determined with the ABI 377 automated DNA sequencer. Allele sizes were determined with Genescan 3.1 and Genotyper 2.5 software (PE Biosystems, Foster City, CA). Genetic variation at the MHC class II *DAB*-β1 locus (Miller et al. 2001) was surveyed by denaturing gradient gel electrophoresis (DGGE). β1 alleles were separated by DGGE with the Bio-Rad (Hercules, CA) D Gene™ or D Code™ electrophoresis systems, with conditions determined by the methods of Miller et al. (1999). Fluorescently-multiplexed (FM)-DGGE (Miller et al. 2000) was used in the population survey.

The baseline survey consisted of analysis of over 48,000 sockeye salmon from 298 populations from Japan, Russia, Alaska, British Columbia, and Washington. Allele frequencies and sample sizes for all locations surveyed in this study are available at http://www-sci.pac.dfo-mpo.gc.ca/mgl/default_e.htm. Multipopulation mixtures of known origin were analyzed with Bayesian statistical techniques similar to those outlined by Pella and Masuda (2000). The estimated lake of origin was defined as the lake assigned the highest probability in the analysis.

Analysis of individuals within samples of known origin indicated that individual salmon can be identified to specific lake of origin with an accuracy > 90%, even with a Pacific Rim distribution of possible natal lakes. Accurate estimates of individual identification by lake of origin will be available as long as a particular lake is represented in the baseline used in the estimation.

The baseline survey for chum salmon consisted of analysis of over 24,000 individuals from over 200 populations in a Pacific Rim distribution of the populations. For the survey of baseline populations, PCR products at 13 microsatellite loci: *Ots3* (Banks et al. 1999), *Oke3* (Buchholz et al. 2001), *Oki2* (Smith et al. 1998), *Oki100* (Miller et al. unpublished data), *One101, One102, One103, One104, One111,* and *One114* (Olsen et al. 2000), Ssa419 (Cairney et al. 2000), Omy1011 (Rexroad et al. 2002) and OtsG68 (Williamson et al. 2002) were size fractionated on denaturing polyacrylamide gels with the ABI 377 automated DNA sequencer.

Genotypic frequencies were determined at each locus in each population and the statistical package for the analysis of mixtures software program (SPAM version 3.7) was used to estimate stock composition of each mixture (Debevec et al. 2000). The Rannala and Mountain (1997) correction to baseline allele frequencies was used in the analysis in order to avoid the occurrence of fish in the mixed sample from a specific population having an allele not observed in the baseline samples from that population All loci were considered to be in Hardy-Weinberg equilibrium, and expected genotypic frequencies were determined from the observed allele frequencies. Each baseline population was resampled with replacement in order to simulate random variation involved in the collection of the baseline samples before the estimation of stock composition of each simulated mixture. Simulated fishery samples of 150 fish were generated by randomly resampling with replacement the baseline populations in each drainage. Estimated stock composition of a simulated mixture was then determined, and the whole process was repeated 100 times to estimate the mean and standard deviation of the individual stock composition estimates.

Analysis of population structure indicated a strong regional component, with Japanese, Russian, Yukon River, southeast Alaska, British Columbia, and Washington populations forming distinct regional groups. Analysis of

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simulated mixtures showed a high level of resolution among populations and regions. Regional estimates of stock composition were within 4% of actual values, and within 3% for specific populations in the baseline. Microsatellites have the potential to provide accurate estimates of stock composition to quite local areas, even if a Pacific Rim baseline is applied in the estimation procedure. The baselines outlined for both sockeye salmon and chum salmon would likely resolve the origin of salmon caught in Bering Sea locations.

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