

Population Structure and Stock Identification of Chum Salmon (*Oncorhynchus keta*) Based upon Microsatellite Analysis

Terry D. Beacham, Khai D. Le, and John R. Candy
Fisheries and Oceans Canada, Pacific Biological Station,
Nanaimo, British Columbia V9T 6N7, Canada



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

Stock identification of chum salmon (*Oncorhynchus keta*) migrating through particular locations on the high seas can be of scientific and management interest. Although allozyme-based methods of stock identification have proven useful in estimation of chum salmon stock composition in mixed-stock fisheries (Shaklee et al. 1999), and differentiation at allozyme loci occurs among chum salmon (Beacham et al. 1987; Seeb and Crane 1999), the level of discrimination available in some applications is not sufficient for fisheries management decisions. Variation in microsatellite loci has been applied in other species requiring discrimination among salmonid populations within watersheds (Small et al. 1998; Beacham and Wood 1999; Beacham et al. 2001), and has been shown to be useful in stock discrimination in chinook salmon (Banks et al. 2000). Variation at microsatellite loci has been particularly useful for population-specific estimates of stock composition of Fraser River chinook salmon (Beacham et al. 2003), and may work well for chum salmon. In the present study, we survey variation at 13 microsatellite loci in chum salmon, and evaluate the utility of using microsatellite variation for stock identification on a regional and local basis. This is accomplished by analysis of simulated mixtures containing chum salmon from different regions, and on a local basis by incorporation of specific chum salmon populations.

Tissue samples were collected from adult chum salmon from populations in Japan, the Yukon River, southeast Alaska, and British Columbia and DNA extracted from the samples as described by Withler et al. (2000). 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. unpub), One101, One102, One103, One104, One106, One108, One109, One111, and One114 (Olsen et al. 2000), Ssa419 (Cairney et al. 2000), and OtsG68 (Williamson et al. 2002) were size fractionated on denaturing polyacrylamide gels 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). Cavalli-Sforza and Edwards (1967) chord distance was used to estimate distances among populations. An unrooted neighbor-joining tree was generated with PHYLIP (Felsenstein 1993).

Genotypic frequencies were determined at each locus in each population and the statistical package for the analysis of mixtures software program (SPAM) (Debevec et al. 2000) was used to estimate stock composition of each mixture. A recent version of SPAM (3.7) that incorporates a 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 (SPAM software available at <http://www.cf.adfg.state.ak.us/geninfo/research/genetics/software/spampage.htm>). 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 mixtures composed of chum salmon from different regional groups were examined in order to evaluate accuracy and precision of the estimated stock compositions. 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. A baseline incorporating 90 populations ranging from Japan, the Yukon River, southeast Alaska, and British Columbia was used in analysis of the simulated mixtures.

The dendrogram analysis indicated that there was regional structure in the chum salmon populations analyzed. Regional structure was observed for chum salmon populations from Japan, the Yukon River, southeast Alaska, the Taku River, the east and west coast of the Queen Charlotte Islands in British Columbia, the central coast of British Columbia, the east and west coasts of Vancouver Island, the southern British Columbia mainland, and the Fraser River.

Analysis of simulated mixtures was conducted to evaluate the utility of microsatellites for estimation of stock composition in mixed-stock samples. Samples containing only Japanese chum salmon from four populations (Abashiri, Chitose, Horonai, Tokachi) were well estimated on both an individual population and region level. With

each of the four populations comprising from 10–40% of the mixture, mean errors of estimation for each of the 4 populations were generally within 3% of the actual values, and mean error of the regional estimate was 1.2% (estimate of 98.8% Japanese component versus 100% actual component). Similar results were observed for four Yukon River populations (Kantishna, Toklat, Fishing Branch, Kluane) comprising 10–40% of the mixtures, with mean population-specific errors generally < 3% of actual values, and with the error in the regional estimates 0.2%. For west coast Queen Charlotte Islands populations (Botany, Clapp Basin, Gold Harbour, Government), mean population-specific errors for populations comprising 10–40% of the mixtures was < 3%, and mean error of the regional estimate was 4%. Similar results were again observed for east coast of Vancouver Island populations (Cowichan, Goldstream, Nanaimo, Campbell River), with population specific errors < 6% of actual values, and the regional estimate in error by 7% (93.2% versus 100%). When multi-regional mixtures were evaluated, mean regional estimates were in error by < 2% for all regions.

Analysis of actual mixed-stock fishery samples from the vicinity of the Saanich Inlet on the south east coast of Vancouver Island provided a practical way to evaluate fishing boundary locations designed to reduce exploitation of Cowichan River chum salmon. Microsatellites appear to be a very effective method to provide a regional estimate of stock composition in samples comprising populations from multiple regions, and may also provide population-specific estimates if the baseline survey of populations in the region has been adequate.

REFERENCES

- Banks, M.A., M.S. Blouin, B.A. Baldwin, V.K. Rashbrook, H.A. Fitzgerald, S.M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in chinook salmon (*Oncorhynchus tshawytscha*). *J. Hered.* 90: 281–288.
- Banks, M.A., V.K. Rashbrook, M.J. Calavetta, C.A. Dean, and D. Hedgecock. 2000. Analysis of microsatellite DNA resolves genetic structure and diversity of chinook salmon (*Oncorhynchus tshawytscha*) in California's Central Valley. *Can. J. Fish. Aquat. Sci.* 57: 915–927.
- Beacham, T.D., A.P. Gould, R.E. Withler, C.B. Murray, and L.W. Barner, L.W. 1987. Biochemical genetic survey and stock identification of chum salmon (*Oncorhynchus keta*) in British Columbia. *Can. J. Fish. Aquat. Sci.* 44: 1702–1713.
- Beacham, T.D., and C.C. Wood. 1999. Application of microsatellite DNA variation to estimation of stock composition and escapement of Nass River sockeye salmon (*Oncorhynchus nerka*). *Can. J. Fish. Aquat. Sci.* 56: 1–14.
- Beacham, T.D., J.R. Candy, K.J. Supernault, T. Ming, B. Deagle, A. Schultz, D. Tuck, K. Kaukinen, J.R. Irvine, K.M. Miller, and R.E. Withler. 2001. Evaluation and application of microsatellite and major histocompatibility complex variation for stock identification of coho salmon in British Columbia. *Trans. Am. Fish. Soc.* 130: 1116–1155.
- Beacham, T.D., J.R. Candy, K.J. Supernault, M. Wetklo, B. Deagle, K. Labaree, J.R. Irvine, K.M. Miller, R.J. Nelson, and R.E. Withler. 2003. Evaluation and application of microsatellites for population identification of Fraser River chinook salmon (*Oncorhynchus tshawytscha*). *Fish. Bull.* 101: 243–259.
- Buchholz, W., S.J. Miller, and W.J. Spearman. 2001. Isolation and characterization of chum salmon microsatellite loci and use across species. *Anim. Genet.* 32: 162–165.
- Cairney, M., J.B. Taggart, and B. Hoyheim. 2000. Characterization of microsatellite and minisatellite loci in Atlantic salmon (*Salmo salar* L.) and cross-species amplification in other salmonids. *Mol. Ecol.* 9: 2175–2178.
- Cavalli-Sforza, L.L., and A.W.F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. *American Journal of Human Genetics.* 19: 233–257.
- Debevec, E.M., R.B. Gates, M. Masuda, J. Pella, J. Reynolds, and L. W. Seeb. 2000. SPAM (Version 3.2): Statistics program for analyzing mixtures. *J. Hered.* 91: 509–510.
- Felsenstein, J. 1993. PHYLIP: Phylogeny Inference Package. University of Washington, Seattle.
- Olsen, J.B., S.L. Wilson, E.J. Kretschmer, K.C. Jones, and J.E. Seeb. 2000. Characterization of 14 tetranucleotide microsatellite loci derived from Atlantic salmon. *Mol. Ecol.* 9: 2155–2234.
- Seeb, L.W., and P.A. Crane. 1999. Allozymes and mitochondrial DNA discriminate Asian and North American populations of chum salmon in mixed-stock fisheries along the south coast of the Alaska Peninsula. *Trans. Am. Fish. Soc.* 128: 88–103.
- Shaklee, J.B., T.D. Beacham, L. Seeb, and B.A. White. 1999. Managing fisheries using genetic data: Case studies from four species of Pacific salmon. *Fisheries Research* 43: 45–78.
- Small, M.P., T.D. Beacham, R.E. Withler, and R.J. Nelson. 1998. Discriminating coho salmon (*Oncorhynchus kisutch*) populations within the Fraser River, British Columbia using microsatellite DNA markers. *Mol. Ecol.* 7: 141–155.

- Smith, C.T., B.F. Koop, and R.J. Nelson. 1998. Isolation and characterization of coho salmon (*Oncorhynchus kisutch*) microsatellites and their use in other salmonids. *Mol. Ecol.* 7: 1613–1621.
- Williamson, K.S., J.F. Cordes, and B.P. May. 2002. Characterization of microsatellite loci in chinook salmon (*Oncorhynchus tshawytscha*) and cross-species amplification in other salmonids. *Mol. Ecol. Notes* 2: 17–19.
- Withler, R.E., K.D. Le, R.J. Nelson, K.M. Miller, and T.D. Beacham. 2000. Intact genetic structure and high levels of genetic diversity in bottlenecked sockeye salmon, *Oncorhynchus nerka*, populations of the Fraser River, British Columbia, Canada. *Can. J. Fish. Aquat. Sci.* 57: 1985–1998.