Development of a standardized microsatellite baseline for Yukon River Chinook Salmon

Report to Yukon River Panel : Project CRE 79-05

January 9, 2006

Terry D. Beacham

Pacific Biological Station

Department of Fisheries and Oceans

Nanaimo, B. C.

V9T 6N7

T. Beacham: Phone: 250 756-7149 Fax: 756-7053 Email: beachamt@dfo-mpo.gc.ca

Abstract

In 2004, the Genetic Analysis of Pacific Salmon (GAPS) laboratories acting through the Chinook Technical Committee of the Pacific Salmon Treaty agreed upon a set of 13 microsatellite loci to include in a standardized database for application to mixed-stock analysis of chinook salmon. The Yukon River Panel subsequently funded a project to survey variation for Yukon River chinook salmon populations at the 13 microsatellite loci included in the survey conducted by the GAPS collaborators. These 13 microsatellite loci were screened for over 2500 fish surveyed from populations in the Canadian portion of the Yukon River drainage, and the data combined with a survey of populations in the Alaskan portion of the drainage conducted by the Alaska Department of Fish and Game. This microsatellite baseline is shared among all agencies conducting mixed-stock analysis of chinook salmon on the Yukon River drainage.

Introduction

Chinook salmon (*Oncorhynchus tshawytscha)* are widely distributed within the Yukon River drainage, spawning in tributaries ranging from the headwaters (for example, near Whitehorse, Yukon Territory) to near the mouth of the river (for example, Andreafsky River, Alaska). Management for conservation of biodiversity within the drainage requires knowledge of genetic variation among populations as well as population-specific information from fisheries. Effective management of fisheries within major drainages like the Yukon River generally requires that information on the harvesting and timing of specific populations be known, should managers wish to change exploitation rates on specific stocks for conservation purposes. For example, the Canada/U.S. Yukon River Salmon Agreement established specific escapement targets and harvest sharing provisions for Canadian-origin chinook salmon stocks. It is therefore important to develop a management system that allows managers to assess accurately the status of specific stocks in fisheries throughout the drainage during the season so that management decisions can ensure that Treaty obligations are met. Accurate post-season run reconstructions are essential in evaluating whether management actions were consistent with meeting overall objectives and Treaty obligations. Run reconstructions are also important in monitoring the productivity of stocks and assessing the adequacy of current escapement targets and both pre-season and in-season run forecasting techniques. Suitable techniques for identifying specific stocks of chinook salmon in fisheries had not been found either for post-season analysis or for in-season use in fisheries management. Without this knowledge, managing to achieve Treaty obligations is difficult and it

severely limits the assessment of factors influencing stock productivity, which appears to have fluctuated widely in recent years.

Stock identification of chinook salmon migrating through the lower river is a continuing issue of management concern. Although allozyme-based methods of stock identification have proven useful in estimation of chinook salmon stock composition in mixed-stock fisheries (Shaklee et al. 1999), and differentiation at allozyme loci occurs among Yukon River chum salmon (Beacham et al. 1989; Wilmot et al. 1992), the level of population discrimination available in the Yukon River was not yet sufficient for population-specific applications. Variation in microsatellite loci has been applied in other species requiring discrimination among salmonid populations within watersheds (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).

Application of microsatellites in the Yukon River drainage requires that a common standardized baseline be available. A multi-agency work group, the Genetic Analysis of Pacific Salmonids collaborators (GAPS), developed a 13-locus suite of microsatellite loci to survey in chinook populations in areas of interest under the Pacific Salmon Treaty. The Yukon River Panel funded a project to survey variation for Yukon River chinook salmon populations at the 13 microsatellite loci included in the survey conducted by the GAPS collaborators. This report outlines the results of the survey for Canadian populations.

Methods

Collection of DNA samples and laboratory analysis

Genomic DNA was extracted from either liver, scales, operculum punches or fin clips from chinook salmon sampled between 1982 and 2004. Extractions were conducted with a chelex resin protocol outlined by Small et al. (1998). Samples were derived from adults in all areas. Samples of adults were obtained from hatcheries during egg collections, from wild spawning fish or carcasses on the spawning grounds, or from a mixed sample of fish from the specific tributary to the Yukon River. Total genomic DNA was isolated from approximately 10-20mg of tissue using the chelex procedure.

For the survey of baseline populations, 13 microsatellite loci were surveyed for genetic variation (Table 1). These 13 loci were: Ots3 (Banks et al. 1999), Ots208b (Grieg et al. 2003), Otsg474 (Williamson et al. 2002), Ots212 (Grieg et al. 2003), Oki100 (Miller et al., unpub), Ots9 (Banks et al. 1999), Ogo2 (Olsen et al. 1998), Ogo4 (Olsen et al. 1998), Omm1080 (Rexroad et al. 2001), Ots201b (OSU, unpub), Ots211 (Grieg et al. 2003), Ots213 (Grieg et al. 2003), and Ssa408 (Cairney et al. 2000). Primer sequences for the loci are outlined in Table 1.

In general, PCR reactions were conducted in 10 µl volumes consisting of 0.06 units of Taq polymerase, 1µl of 30ng DNA, 1.5-2.5mM $MgCl_2$, 1mM 10x buffer, .8mM dNTP's, 0.006-0.065µM of labeled forward primer (depending on the locus), 0.4µM unlabeled forward primer, 0.4µM unlabeled reverse primer, deionized H_2O, and 1M Betaine (majority of loci). PCR was completed on an MJResearch™ DNA Engine™ PCT-200 or a DNA Engine Tetrad™ PCT-225. The amplification profile involved one cycle of 2 min @ 92°C, 30 cycles of 15 sec @ 92°C, 15 sec @ 52-60°C (depending on

the locus) and 30 sec @ 72°C, and a final extension for 10 min @ 72°C. Specific PCR conditions for a particular locus could could vary from this general outline.

PCR fragments were separated by size on denaturing polyacrylamide gels and allele sizes were determined with the ABI 377 automated DNA sequencer in conjunction with Genescan 3.1 and Genotyper 2.5 software (PE Biosystems, Foster City, CA).

Results

Laboratory activities

The analysis proceeded by way of extracting DNA from all available samples, amplifying specific fragments of genomic DNA as defined by the primers outlined in Table 1, and using an automated DNA sequencer to estimate sizes in base pairs of the amplified products. Surveys of microsatellite variation were conducted for the 13 loci listed in Table 1 across all available samples of Canadian Yukon River chinook salmon. Approximately 2500 fish were surveyed from 35 sampling sites or populations, with sample sizes ranging from 1 to 575 fish per population (Table 2). Observed genotypes at each locus were recorded from the automated sequencer, and reported in the format approved by the GAPS consortium of agency genetics laboratories.

The genotypes recorded at each locus for each individual surveyed were assembled for the Canadian populations, and provided to genetics laboratories of the Alaska Department of Fish and Game and the United States Fish and Wildlife Service. The genetic data from the Canadian populations was merged with the comparable data from Alaska populations which had been generated by the Gene Conservation Laboratory of the Alaska Department of Fish and Game. A drainage-wide baseline of genetic

variation observed in chinook salmon at the 13 microsatellite loci included in the GAPS survey was thus established, and the baseline shared among genetics laboratories in agencies with an interest in management of Yukon River chinook salmon (Alaska Department of Fish and Game, United States Fish and Wildlife Service, Canadian Department of Fisheries and Oceans). It is anticipated that this Yukon River baseline will become part of the larger GAPS baseline that is being assembled for chinook salmon populations ranging from southeast Alaska to California.

Discussion

A project sponsored by the Pacific Salmon Commission (PSC) Chinook Technical Committee (CTC) was initiated prior to the start of the current project that had as an objective development of a standardized microsatellite database for chinook salmon that could be applied to estimate stock composition in mixed-stock fisheries. This intent of the PSC project was to develop a microsatellite database that could be applied to estimate stock composition in fisheries of interest to the PSC, which would include fisheries from southeast Alaska to Oregon. Agency laboratories from ADF&G, CDFO, Washington Department of Fish and Wildlife, National Marine Fisheries Service, and the Columbia River Inter-Tribal Fish Commission formed a consortium known as the Genetic Analysis of Pacific Salmon (GAPS) to evaluate a number of microsatellite loci. This consortium was to agree on a set of loci to include in a standardized database, and develop this standardized database for application to mixed-stock fishery analysis of PSC interest. In 2004, the GAPS laboratories agreed upon a set of 13 microsatellite loci to include in a standardized database for application to mixed-stock analysis of chinook salmon. The

laboratories also commenced surveying microsatellite variation at these 13 loci in populations ranging from California to southeast Alaska.

The Yukon River Panel in the fall of 2004 decided that a standardized baseline developed for application to estimation of stock composition in chinook salmon mixed-stock fisheries in the Yukon River drainage should be compatible with the baseline being developed by the GAPS consortium. The Yukon River Panel supported a project to survey microsatellite variation in the same microsatellite loci as was being conducted by the GAPS laboratories. With these loci surveyed, the baseline data for the Yukon River populations should be compatible with the baseline being assembled under the GAPS initiative. Estimation of stock composition from chinook salmon over a wide geographic range should be possible with the baseline being assembled under the GAPS initiative.

Acknowledgments

Baseline samples were either collected or coordinated by staff in a variety of organizations, the Canadian Department Department of Fisheries and Oceans, and other organizations supported by the Yukon River Restoration and Enhancement Fund Financial support for the project was provided by the Yukon River Restoration and Enhancement Fund as well as the Department of Fisheries and Oceans.

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*). Journal of Heredity 90: 281-288.

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. Transactions of the American Fisheries Society 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*). Fishery Bulletin 101: 243-259.

Beacham, T.D., C.B. Murray, and R.E. Withler. 1989. Age, morphology, and biochemical genetic variation of Yukon River chinook salmon. Transactions of the American Fisheries Society 118: 46-63.

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*). Canadian Journal of Fisheries and Aquatic Sciences 56: 1-14.

Cairney M., J. B. Taggart, B. Hoyheim. 2000. Atlantic salmon (*Salmo salar* L.) and cross-species amplification in other salmonids. Molecular Ecology 9: 2175- 2178.

Grieg, C., D. P. Jacobson, and M. A. Banks. 2003. New tetranucleotide microsatellites for fine-scale discrimination among endangered Chinook salmon (*Oncorhynchus tshawytscha*). Molecular Ecology Notes 3: 376-379.

Olsen, J. B., P. Bentzen, and J. E. Seeb. 1998. Characterization of seven microsatellite loci derived from pink salmon. Molecular Ecology 7: 1083-1090.

O'Reilly, P. T., L. C. Hamilton, S. K. McConnell, and J. M. Wright. 1996. Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. Canadian Journal of Fisheries and Aquatic Sciences 53: 2292-2298.

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. Molecular Ecology 7:141-155.

Williamson K., S. Cordes, F. Jan, B. May. 2002 Characterization of microsatellite loci in Chinook salmon (*Oncorhynchus tshawytscha*) and cross-species amplification in other salmonids. Molecular Ecology Notes, 2, 17-19.

Wilmot, R. L., Everett, R. E., Spearman, W. J., and Baccus, R. 1992. Genetic stock identification of Yukon River chum and chinook salmon 1987 to 1990. Prog. Rep. U.S. Fish and Wild. Serv., Anchorage. 132p.

Table 1. Microsatellite loci surveyed for chinook salmon

Locus	Primer Sequence (5'→ 3') **F** > Forward, **R** > Reverse	Citation
Ots201b	F- CAGGGCGTGACAATTATGC R- TGGACATCTGTGCGTTGC	OSU unpublished
Ots208b	F- GGATGAACTGCAGCTTGTTATG R- GGCAATCACATACTTCAACTTCC	Greig et al. 2003
Ots211	F - TAGGTTACTGCTTCCGTCAATG R - GAGAGGTGGTAGGATTTGCAG	Grieg et al. 2003
Ots212	F- TCTTTCCCTGTTCTCGCTTC R- CCGATGAAGAGCAGAAGAGAC	Grieg et al. 2003
Ogo4	F- GTCGTCACTGGCATCAGCTA R- GAGTGGAGATGCAGCCAAAG	Olsen et al. 1998
Ogo2	F- ACATCGCACACCATAAGCAT R- GTTTCTTCGACTGTTTCCTCTGTGTTGAG	Olsen et al. 1998
Ots3M	F- TGTCACTCACACTCTTTCAGGAG R- GAGAGTGCTGTCCAAAGGTGA	Banks et al. 1999
Ots213	F- CCCTACTCATGTCTCTATTTGGTG	Grieg et al. 2003
Omm1080	F- GAGACTGACACGGGTATTGA	Rexroad et al. 2001
Ssa408UO	F- AATGGATTACGGGTACGTTAGACA	Cairney et al. 2000
Ots9	F- ATCAGGGAAAGCTTTGGAGA	Banks et al. 1999
OtsG474	F- TTAGCTTTGGACATTTTATCACAC	Williamson et al.
Oki100	F- CCAGCACTCTCACTATTT	DFO unpublished

Table 2. Populations surveyed for microsatellite variation at the 13 microsatellite loci included in the GAPS survey of chinook salmon microsatellite variation

Population	Number of Fish
Yukon mainstem	27
Nisutlin	56
Big Salmon	119
Morley	20
Tatchun	369
Little Salmon	100
Takhini	168
Blind Creek	162
Whitehorse	242
Mayo	205
Stewart	112
Pelly	150
Michie	47
Klondike	114
Fishing Branch	1
Wolf	59
Chandindu River	575
Chena River	81
Porcupine River	12

Crow River	1
Miner River	1
Ollie Lakes	5
Tincup	32
Earn River	44
Big Campbell	7
Nordenskiold	106
Little Kalzas	40
Big Kalzas	24
Glenlyon	24
Hoole	2
Janet	7
Fifty-Mile	4
Gladys	4
Primrose	3
North Big Salmon	12