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**Summary of Chum Salmon Stock Identification Research Conducted by the  
Molecular Genetics Laboratory, Pacific Biological Station**

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## Abstract

Variation at 13 microsatellite loci was surveyed in over 34,000 chum salmon (*Oncorhynchus keta*) sampled from 283 localities ranging from the Washington to Japan. The observed regional population structure enabled an evaluation of the utility of using microsatellite variation for estimation of stock composition of chum salmon in mixed-stock fisheries. Stock compositions were estimated for three simulated mixed-stock samples, with the 283-population baseline used to estimate stock compositions. Application of microsatellite variation clearly has the potential to provide reliable estimates of stock composition for chum salmon even when there is a potential of a Pacific Rim distribution of populations contributing to the fishery sample.

## Introduction

Reliable, accurate, effective, and practical methods of stock identification are a key requirement in the assessment and management of Pacific salmon fisheries and populations. Stock identification of chum salmon (*Oncorhynchus keta*) is of particular concern to many management agencies. Genetic methods of stock identification have several advantages, among them the level of differentiation among populations and the stability of the genetic characters surveyed. As annual variation in allele frequencies in salmonid microsatellite loci is substantially less than differentiation among populations (Tessier and Bernatchez 1999; Beacham et al. 2000a,b; Beacham et al. 2004), there is no requirement for annual updating of baseline populations once sufficient surveys have been conducted to characterize adequately the genetic differentiation among populations. Allozyme-based applications generally provide regional estimates of stock composition in a species, provided that there is a regional basis in population structure (Shaklee et al. 1999). Microsatellites can provide regional estimates of stock composition, but may possibly provide population-specific estimates in some applications if the survey of baseline populations has been adequate (Beacham et al. 2001, Beacham et al. 2003). This greater population discrimination ability is a consequence of the higher heterozygosity and the larger number of alleles at microsatellite loci compared with allozyme loci.

## Materials and Methods

### Collection of DNA samples and laboratory analysis

Tissue samples were collected from adult fish from chum salmon populations in the Pacific Rim and DNA extracted from the samples as described by Withler et al. (2000). The MGL laboratory currently surveys variation at 15 microsatellite loci: *Ots3* (Banks et al. 1999), *Oke3* (Buchholz et al. 2001), *Oki2* (Smith et al. 1998), *Oki100* (Miller et al. unpub), *Omm1070* (Rexroad et al. 2001), *Omy1011* (Spies et al. 2005), *One101*, *One102*, *One103*, *One104*, *One111*, and *One114* (Olsen et al. 2000), *Ots103* (Beacham et al. 1998), *Ssa419* (Cairney et al. 2000), and *OtsG68* (Williamson et al. 2002). The loci *Omm1070* and *Ots103* have now been added to the previous 13 loci that had been initially surveyed. These 15 loci constitute a standardized set of loci developed by our laboratory and the Washington Department of Fish and Wildlife under a project supported by the Pacific Salmon Commission. Products amplified via PCR are size fractionated on the ABI 3730 automated DNA analyzer. Allele sizes are determined with GeneMapper 3.7 and Data Collection 2.0 software (Applied Biosystems, Foster City, CA).

### Baseline populations

The baseline survey consisted of analysis of over 34,000 chum salmon from 283 populations from Japan, Russia, Alaska, British Columbia, and Washington (Table 1).

### Estimation of stock composition

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). Thirteen loci were used

to estimate stock compositions. 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.

## Results and Discussion

The regional structure observed in chum salmon populations resulted in good discrimination among regions in the Pacific Rim distribution of the species. We evaluated whether the genetic differentiation observed among the 283 sampling sites or populations included in the baseline was sufficient for mixed-stock analysis aimed at estimating population or regional contributions to fishery samples. Three fishery mixture samples were simulated, and stock compositions were estimated for the individual population and regions. Estimated stock compositions of a simulated mixture containing fish from Russia, Japan, and the Yukon River were usually within 4% of the specific site or population, and within 2% of the specific region of origin (Table 2, mixture 1). Similar but improved results were observed for a mixture comprised of Japanese and Russian populations (Table 3, mixture 2). For example, for the five populations from Japan, estimates of the individual population contributions were within 1% of the actual values, as was the regional estimate (50%) for Japan. Regional compositions of a simulated mixture of fish from the Yukon River and the Alaska Peninsula were generally within 4% of the actual population and regional contribution (Table 3, mixture 3). Accurate estimates of stock composition should be available when the baseline is applied to any sample drawn from the Pacific Rim distribution of Pacific salmon, particularly if the objective is to obtain an estimate of the local regional contribution to the sample.

Ideal technologies for mixed-stock analysis are those based on biological variation in characters which differ substantially among stocks, show little temporal or annual variation within stocks relative to stock differences, and can be screened in a rapid, non-lethal, and cost-effective manner for both baseline and mixed-stock samples. The PCR-based survey of single-locus microsatellite DNA markers meet these criteria, and can be readily used for in-season fishery management decisions requiring stock composition analysis. Application of microsatellite variation clearly has the potential to provide reliable estimates of stock composition for a local group of chum salmon even when there is a potential of a Pacific Rim distribution of populations contributing to the fishery sample. Regardless of the current baseline status, the results outlined in the present study are an example of the power of microsatellite variation that will likely be applied to an increasing number of species and fisheries for which the management concerns of identifying population structure and detecting specific populations or stocks in mixed-stock fisheries arise.

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Table 1. Regions and number of populations sampled within regions included in a survey of microsatellite loci in chum salmon.

Region	Number of populations	Populations
China	2	Amur, Suifen
Hokkaido, Japan Sea	3	Teshio, Chitose, Toshibetsu
Pacific, west	4	Yurappu, Shikiu, Shizunai, Shiriuchi
Pacific, east	2	Tokachi, Kushiro
Nemuro Strait	2	Shibetsu, Nishibetsu,
Ohotsk Sea	5	Shari, Abashiri, Tokushibetsu, Tokoro, Horonai
Honshu, Pacific Coast	5	Orikasa, Sakari, Tsugaruishi, Koizumi, Ohkawa
, Japan Sea Coast	5	Gakko, Uono, Kawabukora, Miomote, Hayatsuki
Sakhalin	4	Kalininka, Naiba, Tym, Udarnitsa
Primorye	3	Avakumovka, Narva, Ryazanovka
Magadan	3	Tauy, Ola, Magadan
Northern Sea of Okhotsk	3	Penzhina, Oklan, Belaya
West Kamchatka	6	Vorovskaya, Pymta, Hairusova, Kol, Utka, Kikchik
East Kamchatka	3	Kamchatka, Ossora, Nerpichi
Northeast Russia	3	Anadyr, Kanchalan
Mackenzie River	1	Peel R.
Kotzebue	2	Kelly Lake, Noatak R.
Norton Sound	4	Pilgrim R., Picniktalik R., Niukluk R., Kwiniuk R.
LowerYukon River (summer)	10	Andreafsky R. (east, mixed), Chulinak R., Tozitna R., Otter Cr., California Cr., Anvik R., Nulato R., Melozitna, Gisasa
Koyukuk River (summer)	4	Jim R., Henshaw Cr., Koyukuk R. (south, late)
Tanana River (summer)	2	Cheena R., Salcha R.
Upper Alaska (summer)	1	Big Salt
Tanana River (fall)	3	Delta River, Toklat River, Kantishna River
Upper Alaska (fall)	3	Sheenjek R., Black R., Chandalar R.
Mainstem Yukon (Canada)	4	Tatchun, Pelly, Big Creek, Minto
White River	3	Kluane R., Kluane L., Donjek R.
Porcupine River	1	Fishing Branch R.
Teslin River	1	Teslin R.
North Alaska Peninsula	2	Moller Bay, Frosty Cr.
South Alaska Peninsula	2	American R., Volcano Bay
Kodiak Island	1	Sturgeon
Southeast Alaska	13	Breezy Bay, Fish Cr., DIPAC hatchery, Gambier, Well's Bridge, Herman Cr. Green's Cr., Sawmill, Kennell, Disappearance, Carroll, Neets Bay (ear, lat)
Taku River	4	Taku, Tuskwa, Yellow Bluff, Shustnini
Queen Charlotte Islands	28	Gold Harbour, Mace Cr., Botany Cr., Clapp Basin, Security, Dawson Inlet, Mountain Cr., Fairfax In., Thorsen Cr., Pallant Cr., Sedgwick Cr., Lagoon In., Bag Harbour, Salmon R., Little Goose Cr., Pacofi, Surprise, Ain, Awun, Naden, Stanley, Slatechuck, Government, North Arm, Tarundl, Deena, Lagins, Buck Channel
Area 3	11	Kshwan, Khutzeymateen, Lachmach, Stagoo, Toon, Dak, Kateen, Kitsault, Ensheshese, Wilauks, Illiance
Skeena River	9	Nangeese, Date, Kitwanga, Ecstall, Whitebottom, Kalum, Dog-tag, Andesite, Kispiox
Area 5	4	Wilson, Markle Inlet, False Stewart, Pa-aat R.
Area 6	16	Kemano, Gill, Turn, Quaal, Kitimat, Green, Khutze, Foch, Blackrock, Nias, West Arm, Kitasoo, Barnard, Tyler, Gilttoyes, Kultuish
Area 7	12	Bella Bella, Clatse, Quarcha, McLoughin, Roscoe, Mussel, Kwakusdis, Neekas, Duthie, Kainet, Lard, Cooper Inlet
Area 8	11	Martin R., Snootli, Cascade, Bella Coola, Dean, Jenny Bay, Kimsquit, Elcho, Skowquiltz, Frenchman, Kimsquit
Area 9	8	McNair, Chuckwalla, Clyak, Lockhart-Gordon, Quap, Ashlum, Draney, Milton
Area 10	2	Walkum, Nekite
Area 11	1	Waump
Johnstone Strait	10	Heydon, Klinaklini, Wortley, Ahta, Viner Sound, Glendale, Nimpkish, Bird Cove, Algard, Ahnuhati
Southern BC Mainland	11	Tzoonie, Cheakamus, Sliammon, Mamquam, Squamish, Indian, Theodosia, Southgate, Orford, Shovelnose, Masiter
Vancouver Island (east)	9	Goldstream, Cowichan, Nanaimo, Chemainus, Puntledge, Big Qualicum, Little Qualicum, Campbell, Cold
Vancouver Island (west)	11	Smith, Kirby, Demamiel, Nitinat, Hathaway, Pegattum, Goodspeed, Cayeghle, Colonial, Sugsaw, Nahmint
Fraser River	12	Wahleach, Chilliwack, Chehalis, Stave, Alouette, Vedder, Harrison, Inch, Lower Lillooet, Norrish/Worth, Alouette, Widgeon
North Puget Sound	6	Nooksack, Skagit, County Line, Tulalip, Grant, Siberia,
South Puget Sound	2	Kennedy Cr., Minter Cr.
Hood Canal	2	Salmon Cr., Elwha R.
Coastal Washington	4	Ellsworth, Bitter Cr., Quinault, Satsop

Table 2. Estimated percentage stock compositions of simulated mixtures of chum salmon as may be encountered in marine samples. Each mixture of 150 fish was generated 100 times with replacement, and stock compositions of the mixtures were estimated by resampling each of the 283 baseline populations with replacement to obtain a new distribution of allele frequencies. Standard deviation is in parentheses. Expected regional composition is obtained by adding true population components, with estimated regional composition listed in the Region column for each mixture.

Population/Region	True	Mixture 1	
		Population	Region
Gisasa (L. Yukon summer)	10	6.9 (2.1)	10.3 (2.4)
Kantishna (Tanana fall)	20	17.0 (3.5)	17.9 (3.3)
Shibetsu (Japan)	20	15.9 (3.0)	
Ohkawa (Japan)	10	6.4 (2.0)	28.7 (4.0)
Magadan (Magadan)	20	15.6 (3.4)	17.4 (3.5)
Pymta (west Kamchatka)	10	6.3 (2.2)	10.8 (3.0)
Quaal (BC, Area 6)	10	6.6 (2.4)	7.8 (2.5)
		Mixture 2	
Hairusova (west Kamchata)	10	8.6 (2.5)	
Vorovskaya (west Kamchatka)	20	19.3 (3.3)	29.2 (3.6)
Anadyr (northeast Russia)	20	16.9 (3.1)	17.6 (3.2)
Tokachi (Japan)	10	9.8 (2.4)	
Tokushibetsu (Japan)	10	8.1 (2.4)	
Yurappu (Japan)	10	9.9 (2.5)	
Abashiri (Japan)	10	9.1 (2.2)	
Chitose (Japan)	10	10.7 (2.9)	49.7 (3.8)
		Mixture 3	
Fishing Branch	20	17.3 (3.5)	17.3 (3.5)
Kluane	20	19.6 (3.2)	19.9 (3.2)
Cheena	10	7.8 (2.2)	9.0 (2.2)
Moller Bay	50	46.2 (3.0)	46.2 (3.0)