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by

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Present status of mitochondrial DNA baseline for stock identification of chum salmon

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ABSTRACT

A baseline of mitochondrial DNA frequencies covering the entire range of chum salmon was developed and evaluated for its resolution to estimate stock of origin of Asian, Russian, and North American chum salmon in complex mixtures. The baseline samples of chum salmon were collected from 6,447 individuals of 116 populations around the Pacific Rim. The mtDNA haplotypes were determined for about 3,200 individuals from 76 populations by direct sequence analysis or DNA microarray methods. In simulation studies using 48 baseline populations, estimates for the Japanese and North American regions showed more than 95% accurate (95.9% Japan and 95.7% North America), whereas an estimate for Russian region was 88.5% accurate.

Keywords: chum salmon, mitochondrial DNA, genetic stock identification, baseline

INTRODUCTION

Chum salmon (*Oncorhynchus keta*) is the most widely distributed species of salmon in the Pacific Rim (Salo 1991). Historically, chum salmon were probably the most abundant Pacific salmonid, contributing almost 50% of annual biomass of all salmonids in the North Pacific Ocean and today are second only to pink salmon (*O. gorbuscha*) in abundance (Heard 1991). Chum salmon also represent an important component in commercial fisheries in Asia and North America. Furthermore, convention for the conservation of anadromous stocks in the North Pacific Ocean is recognized that states of origin of anadromous stock make expenditures and forego economic development opportunities to establish favourable conditions to conserve and manage those stocks. Thus, the salmon stock assessment, including chum salmon, is very important to conservation of salmon stocks and commercial fisheries in the North Pacific Ocean.

Chum salmon from Asia and North America migrate into the North Pacific Ocean and the Bering Sea. The eastward extension of Asian chum salmon shows a more distant migration than the chum salmon of North America, which are not commonly found west of 175°E (Salo 1991). This result indicates that mixed aggregations, often composed of both Asian and North American stocks, form during these ocean migrations. Identification of composite stocks in mixtures has become an important part of chum salmon management and conservation programs.

In the Pacific salmon, stock identification has been attempted by tagging, scale characteristics, parasite tagging, thermal otolith marking, and/or protein genetic (allozyme) characters (Ishida et al. 1989, Winans et al. 1994, Wilmot et al. 1998, Urawa et al. 1998). Recently, the powerful molecular genetic markers, e.g. mitochondrial (mt) DNA, microsatellite DNA, and single nucleotide polymorphisms (SNPs),

are developed and are used for several genetic stock identification (GSI) studies of Pacific salmon (Seeb et al. 1998, Seeb and Crane 1999, Sato et al. 2004). Particularly, analysis of mtDNA has received considerable attention in GSI of Pacific salmon species because of the higher sequence variability than single copy nuclear genes (Brown et al. 1979) and clonal haploid inheritance. In this document, we report the present status of mtDNA baseline and resolution for GSI of chum salmon.

METHODS OF GENETIC STOCK IDENTIFICATION OF CHUM SALMON

Sample collection

Fresh tissues (liver, muscle, or blood) of baseline samples of chum salmon were collected. The fish were captured when they returned to their natal river. Liver and blood samples were stored at -80°C and muscle samples were stored in 100% ethanol at room temperature until DNA extraction.

DNA extraction

DNA was extracted from the collected tissues stored at -80°C or preserved in ethanol, using a conventional phenol-chloroform method (Sambrook et al. 1989) or Puregene™ DNA Purification kit (Gentra Systems, Minneapolis, MN) following the manufacturer's instructions. In brief, DNA was isolated from the stored specimens following the phenol-chloroform methods (Sambrook et al. 1989). Prior to extraction of DNA, the muscle samples were washed twice in 500 μl sodium tris EDTA buffer (STE; 0.1 M NaCl, 10mM Tris-HCl, and 1 mM EDTA, pH 8.0). The frozen liver samples were immediately homogenized in the same solution. About 50 μl of whole blood and homogenates of liver or muscle were added to 500 μl STE buffer containing 500 μg/ml proteinase K and 0.5% SDS, and incubated at 37°C overnight. DNA was extracted three times with a mixture of phenol (250 μl) and 24:1 chloroform:isoamylalcohol (250 μl), and then twice with 500 μl of the chloroform-isoamylalcohol alone. DNA in aqueous phase was recovered by ethanol precipitation, dried in air, and dissolved in tris EDTA buffer (TE; 10 mM Tris-HCl, 1 mM EDTA, pH 7.5).

PCR amplification and nucleotide sequence analysis

The PCR amplification and nucleotide sequence analysis of the mtDNA control region was followed as in the previous studies of Sato et al. (2001) and Sato et al. (2004). In brief, amplifications were performed in 100 μl volumes containing 25-100 ng of template DNA, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM $MgCl_2$, 250 nM each dNTPs, 250nM of forward and reverse primers, 0.001% gelatin, and 1.25 U of *Taq* polymerase (Takara, Tokyo). The PCR products were purified by the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) after confirmation of their sizes by gel-electrophoresis.

Nucleotide sequences were determined directly from PCR products. Approximately 500-bp in the variable position of 5' end of the mtDNA control region was sequenced with a Hitachi SQ-5500L DNA Sequencer (Hitachi, Tokyo). Sequence reaction was preformed using a Thermo Sequenase™ pre-mixed cycle sequencing kit (Vistra Systems, Sunnyvale, CA) according to the manufacturer's instruction.

MtDNA haplotype detection by DNA microarray system

The mtDNA haplotypes of several chum salmon samples were detected by DNA microarray system. DNA microarray analysis of approximately 500 bp in the variable position of the 5' end of the mtDNA control region followed the protocol developed by Moriya et al. (2004) with an OligoArray ® (Chum salmon) Kit (Nisshinbo Industries, Tokyo), according to the manufacturer's instruction. Amplification was carried out in a 25 μl of reaction mixture containing 25-100 ng of template DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.25 mM each dNTP, 1 U Taq DNA polymerase (Shigma-Aldrich Corporation, St.Louis, MO), 1 μM of forward and reverse primers. The condition of PCR amplification using a GeneAmp PCR System 2400 (Applied Biosystems, Foster City, CA) was as follows; preheating at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 1 min, annealing at 45°C for 30 sec,

and elongation at 72°C for 30 sec, and post-cycling extensions at 72°C at 3 min. The PCR products were examined of the fragment size with 1.5% agarose-gel electrophoresis and ethidium bromide staining.

Hybridization of PCR products and signal detection followed the protocol of Moriya et al. (2004), using reagents and buffers supplied in the above kit. 2 µl each of reaction mixture was denatured at 95°C for 2 min, followed by quenching on ice until hybridization. The denatured PCR product was mixed with 16 µl of hybridization buffer, mounted on a DNA microarray with cover film, and hybridized at 37°C for two hours in a moisture chamber. After hybridization, the DNA microarray was washed in a washing buffer at 37°C for 5 min. Then, 1.4 ml of conjugate solution, prepared according to the manufacturer's instruction, was mounted on the DNA microarray and incubated at room temperature for 30 min. The DNA microarray was washed twice a coloring buffer at room temperature for 5 min each, followed by incubation with 1.4 ml of coloring solution at room temperature for 30 min. Coloring reaction was stopped by rinsing of the DNA microarray in distilled water. Air-dried DNA microarray was scanned by a GT-8700F and GT-9300UFS scanner (Seiko Epson Corp., Tokyo) for visual analysis of the signal intensity on a computer. Haplotypes were determined according to the combination of signal positive-oligomer sites, which correspond to the previously identified SNPs in the target region (Sato et al., 2004).

Data analysis

Stock contributions of the mixed samples were estimated via a conditional maximum likelihood (Pella and Milner 1987, Masuda et al. 1991). A conjugate-gradient searching algorithm using a square root transformation was employed, because it provides good performance with large baselines and small stock differences (Pella et al. 1996). Standard deviations were estimated by 1,000 bootstrap resampling of the baseline and 500 mixture samples. Estimates were made to individual stocks and then pooled to regional stock groups: Japan, Russia, and North America. These regional stock groups were categorized based on previous genetic analysis for data set of 48 populations of chum salmon in the Pacific Rim (Sato et al. 2004). Computations were performed with the Statistics Programs for Analyzing Mixtures (SPAM version 3.7b, Alaska Department of Fish and Game, Anchorage, AK).

CONSTRUCTION OF BASELINE FOR CHUM SALMON AROUND THE PACIFIC RIM

The baseline samples of chum salmon were collected from 2,154 individuals of 48 populations from Japan (16 populations), Korea (1 population), Russia (10 populations) and North America including Northwest Alaska (4 populations), Southcentral Alaska (2 populations), Alaska Peninsula (2 populations), Southeast Alaska (6 populations), British Columbia (3 populations), and Washington (5 populations) from 1988 to 2002 (Sato et al. 2004). Additional 1,048 individuals of 27 populations from Russia (19 populations) and North America (8 populations of Northwest and Southcentral Alaska and Alaska Peninsula) were sampled and analyzed (Moongeun et al., 2004). Furthermore, 75 individuals from 2 populations in Russia and 1,475 individuals from 20 populations in North America (4 Northwest Alaska, 5 Southcentral Alaska, 3 Alaska Peninsulas, 3 Southeast Alaska, and 5 British Columbia) were collected. These samples are stored in Hokkaido University (Table 1). The 1,596 samples of 19 Japanese populations and 99 samples of 1 Korean population of chum salmon were archived in the National Salmon Resources Center. The archived samples had been previously used for allozyme analyses. River names, sampling locations, sampling date, kinds of tissue, the number of individuals, archives, and data sources are given in table 1.

BASELINE EVALUATION

The performance of the maximum likelihood model for chum salmon was investigated through simulation studies of three regions, Japan, Russia, and North America. We used the mtDNA data baseline collected from 48 chum salmon populations around the Pacific Rim from Sato et al. (2004). In simulation studies where the true regional contributions were 100%, the average of maximum likelihood estimates was more than 88% accurate. Estimations for the Japanese and North American regions reached more than 95% (95.9% Japan and 95.7% North America), whereas estimate for Russian region was 88.5% accurate (table 2). The previous allozyme studies observed that most maximum likelihood estimates were more than 90% accurate for more than 12 reporting regions (Seeb and Crane 1999, Kondzela et al. 2002). Thus, further baseline of mtDNA should be accumulated for more fine-scale stock identification. Furthermore, other variable regions of mtDNA and new molecular markers, e.g. microsatellite DNA, should be developed for increase baseline resolution.

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REFERENCES

Brown, W.M., M. George, and A.C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA. 76: 967-1971.

Heard, W.R. 1991. Life history of pink salmon (*Oncorhynchus gorbuscha*), p. 231-309. *In*: C. Groot and L. Margolis [eds] Pacific Salmon Life Histories. UBC Press, Vancouver, BC.

Ishida, Y., S. Ito, and K. Takagi. 1989. Stock identification of chum salmon *Oncorhynchus keta* from their maturity and scale characters. Nippon Suisan Gakkaishi. 55: 651-656.

Kondzela, C.M., P.A. Crane, S. Urawa, N.V. Varnavskaya, V. Efremov, X. Luan, W.B. Templin, K. Hayashizaki, R.L. Wilmot, and L.W. Seeb. 2002. Development of a comprehensive allozyme baseline for Pacific Rim chum salmon. NPAFC Doc. 629: 23 p.

Masuda, M., S. Nelseon, and J. Pella. 1991. The computer programs for computing conditional maximum likelihood estimates of stock composition from discrete characters. U. S. Department of Commerce, NOAA/NMFS Auke Bay Laboratories, Juneau, Alaska.

Moongeun, Y., V. Brykov, N. Varnavskaya, L.W. Seeb, S. Urawa, and S. Abe. 2004. Mitochondrial DNA analysis of genetic variation in the Pacific Rim populations of chum salmon. NPAFC Doc. 792. 25 p.

Moriya, S., S. Urawa, O. Suzuki, A. Urano, and S. Abe. 2004. DNA microarray for rapid detection of mitochondrial DNA haplotypes of chum salmon. Mar. Biotechnol. 6: 430-434.

Pella, J.J., and G.B. Milner. 1987. Use of genetic marks in stock composition analysis, p. 247-276. *In*: N. Ryman and. F. Utter [eds] Population genetics and fishery management. University of Washington Press, Seattle..

Pella, J.J., M. Masuda, and S. Nelson. 1996. Search algorithms for computing stock composition of a mixture from traits of individuals by maximum likelihood. USA-DOC-NOAA-NMFS Tech. Memo. NMFS-AFSC-61.

Salo, E.O. 1991. Life history of chum salmon (*Oncorhynchus keta*), p. 231-309. *In*: C. Groot and L. Margolis [eds] Pacific Salmon Life Histories. UBC Press, Vancouver, BC.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A laboratory Manual 2nd edition Cold. Spring Harbor Laboratory Press, New York.

Sato, S., J. Ando, H. Ando, S. Urawa, A. Urano, and S. Abe. 2001. Genetic variation among Japanese populations of chum salmon inferred from the nucleotide sequences of the mitochondrial DNA control region. Zool. Sci. 18: 99-106.

Sato, S., H. Kojima, J. Ando, H. Ando, R.L. Wilmot, L.W. Seeb, V. Efremov, L. LeClair, W. Buchholz, D.-H. Jin, S. Urawa, M. Kaeriyama, A. Urano, and S. Abe. 2004. Genetic population structure of chum salmon in the Pacific Rim inferred from mitochondrial DNA sequence variation. Env. Biol. Fish. 69: 37-50.

Seeb, J.E., C. Habicht, J.B. Olsen, P. Bentzen, J.B. Shaklee, and L.W. Seeb. 1998. Allozyme, mtDNA, and microsatellite variants describe structure of populations of pink and sockeye salmon in Alaska. N. Pac. Anadr. Fish. Comm. Bull. 1: 300-318.

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.

Urawa, S., K. Nagasawa, L. Margolis, and A. Moles. 1998. Stock identification of chinook salmon (*Oncorhynchus tshawytscha*) in the North Pacific Ocean and Bering Sea by parasite tags. N. Pac. Anadr. Fish. Comm. Bull. 1: 199-204.

Wilmot, R.L., C.M. Kondzela, C.M. Guthrie, and M.M. Masuda. 1998. Genetic stock identification of chum salmon harvested incidentally in the 1994 and 1995 Bering Sea trawl fishery. N. Pac. Anadr. Fish. Comm. Bull. 1: 285-299.

Winans, G.A., P.B. Aebersold, S. Urawa, and N.V. Varnavskaya. 1994. Determining continent of origin of chum salmon (*Oncorhynchus keta*) using genetic stock identification techniques: status of allozyme baseline in Asia. Can. J. Fish. Aquat. Sci. 51: 95-113.

Table 1. River names, sampling locations, sampling date, collect tissues, preservation, numbers of samples (N), and archives of chum salmon in the Pacific Rim.

	River name	Location	Sampling date	Tissue	Preservation	N	Archives	Source
1	Chitose	Japan Sea Coast, Hokkaido, Japan	Oct. 14, 1996	Liver	Frozen	51	Hokkaido Univ.	1
2	Chitose	Japan Sea Coast, Hokkaido, Japan	Sep. 4, 2003	Liver	Frozen	80	NASREC	
3	Chitose	Japan Sea Coast, Hokkaido, Japan	Oct. 7, 2003	Liver	Frozen	80	NASREC	
4	Chitose	Japan Sea Coast, Hokkaido, Japan	Nov. 6, 2003	Liver	Frozen	80	NASREC	
5	Chitose	Japan Sea Coast, Hokkaido, Japan	Dec. 5, 2003	Liver	Frozen	80	NASREC	
6	Teshio	Japan Sea Coast, Hokkaido, Japan	Oct. 23, 2001	Liver	Frozen	80	NASREC	
7	Tokushibetsu	Okhotsk Sea, Hokkaido, Japan	Sep. 23, 1997	Liver	Frozen	51	Hokkaido Univ.	1
8	Tokushibetsu	Okhotsk Sea, Hokkaido, Japan	Sep. 2004	Liver	Frozen	80	NASREC	
9	Tokushibetsu	Okhotsk Sea, Hokkaido, Japan	Oct. 2004	Liver	Frozen	80	NASREC	
10	Tokushibetsu	Okhotsk Sea, Hokkaido, Japan	Nov. 2004	Liver	Frozen	80	NASREC	
11	Tokoro (late-run)	Okhotsk Sea, Hokkaido, Japan	Nov. 20, 1998	Liver	Frozen	44	Hokkaido Univ.	1
12	Tokoro (early-run)	Okhotsk Sea, Hokkaido, Japan	Oct. 13, 1999	Blood	Frozen	49	Hokkaido Univ.	1
13	Shari	Okhotsk Sea, Hokkaido, Japan	Oct. 11, 2001	Blood	Frozen	80	NASREC	
14	Abashiri	Okhotsk Sea, Hokkaido, Japan	Oct. 19, 1998	Blood	Frozen	80	NASREC	
15	Nishibetsu	Nemuro Stait, Hokkaido, Japan	Sep. 25, 1997	Liver	Frozen	41	Hokkaido Univ.	1
16	Nishibetsu	Nemuro Stait, Hokkaido, Japan	Oct. 22, 1997	Blood	Frozen	80	NASREC	
17	Shibetsu	Nemuro Stait, Hokkaido, Japan	Oct. 10, 2003	Liver	Frozen	76	NASREC	
18	Kushiro	Pacific Coast East, Hokkaido, Japan	Oct. 22, 1998	Liver	Frozen	49	Hokkaido Univ.	1
19	Tokachi	Pacific Coast East, Hokkaido, Japan	Oct. 17, 1996	Liver	Frozen	46	Hokkaido Univ.	1
20	Tokachi	Pacific Coast East, Hokkaido, Japan	Oct. 22, 2002	Liver	Frozen	80	NASREC	
21	Yurappu	Pacific Coast East, Hokkaido, Japan	Sep. 24, 1997	Liver	Frozen	80	NASREC	
22	Yurappu	Pacific Coast East, Hokkaido, Japan	Nov. 17, 1997	Liver	Frozen	80	NASREC	
23	Yurappu	Pacific Coast East, Hokkaido, Japan	Nov. 17, 1998	Liver	Frozen	40	Hokkaido Univ.	1
24	Shikyu	Pacific Coast East, Hokkaido, Japan	Nov. 25, 1998	Liver	Frozen	80	NASREC	
25	Shizunai	Pacific Coast East, Hokkaido, Japan	Oct. 17, 2002	Liver	Frozen	80	NASREC	
26	Tsugaruishi (late-run)	Pacific Coast, Honshu (Iwate Pref.), Japan	Dec. 10, 1997	Liver	Frozen	44	Hokkaido Univ.	1
27	Tsugaruishi (early-run)	Pacific Coast, Honshu (Iwate Pref.), Japan	Oct. 1999	Blood	Frozen	47	Hokkaido Univ.	1
28	Otsuchi	Pacific Coast, Honshu (Iwate Pref.), Japan	Apr. 8, 1999	Muscle	Frozen	49	Hokkaido Univ.	1
29	Orikasa	Pacific Coast, Honshu (Iwate Pref.), Japan	Oct. 24, 1996	Liver	Frozen	80	NASREC	
30	Sakari	Pacific Coast, Honshu (Iwate Pref.), Japan	Nov. 19, 1997	Liver	Frozen	80	NASREC	
31	Koizumi	Pacific Coast, Honshu (Miyagi Pref.), Japan	Nov. 21, 1996	Liver	Frozen	47	Hokkaido Univ.	1
32	Kawabukuro	Japan Sea Coast, Honshu (Akita Pref.), Japan	Nov. 18, 1997	Liver	Frozen	30	Hokkaido Univ.	1
33	Gakko	Japan Sea Coast, Honshu (Yamagata Pref.), Japan	Dec. 10, 1996	Liver	Frozen	45	Hokkaido Univ.	1
34	Gakko	Japan Sea Coast, Honshu (Yamagata Pref.), Japan	Oct. 25, 2003	Liver	Frozen	80	NASREC	
35	Uono	Japan Sea Coast, Honshu (Niigata Pref.), Japan	Oct. 23-24, 1996	Liver	Frozen	49	Hokkaido Univ.	1
36	Jintsu	Japan Sea Coast, Honshu (Toyama Pref.), Japan	Nov. 7, 1995	Liver	Frozen	49	Hokkaido Univ.	1
37	Namde	Japan Sea Coast, Korea	Nov. 13, 2000	Liver	Frozen	46	Hokkaido Univ.	1

Table 1-continued.

	River name	Location	Sampling date	Tissue	Preservation	N	Archives	Source
38	Namde	Japan Sea Coast, Korea	Oct. 2004	Liver	Frozen	99	NASREC	
39	Anadyr	Anadyr, Russia	1990	Liver	Frozen	43	Hokkaido Univ.	1
40	Anadyr (early-run)	Anadyr, Russia	1993		DNA	33	Hokkaido Univ.	2
41	Hairusova	Kamchatka Peninsula, Russia	1993	Liver	Frozen	41	Hokkaido Univ.	1
42	Kamchatka	Kamchatka Peninsula, Russia	1991	Liver	Frozen	32	Hokkaido Univ.	1
43	Kamchatka (early-run)	Kamchatka Peninsula, Russia	1990	Liver	Ethanol	50	Hokkaido Univ.	2
44	Vorovskaya	Kamchatka Peninsula, Russia	1990	Liver	Frozen	46	Hokkaido Univ.	1
45	Kol	Kamchatka Peninsula, Russia	1991	Liver	Frozen	44	Hokkaido Univ.	1
46	Pymta	Kamchatka Peninsula, Russia	2003	Fin	Ethanol	49	Hokkaido Univ.	2
47	Utka	Kamchatka Peninsula, Russia	2002		DNA	20	Hokkaido Univ.	2
48	Apuka	Kamchatka Peninsula, Russia	2002	Liver	Ethanol	50	Hokkaido Univ.	2
49	Olyutorskiy Bay	Kamchatka Peninsula, Russia	2002	Liver	Ethanol	50	Hokkaido Univ.	2
50	Tigil	Kamchatka Peninsula, Russia	2002	Liver	Ethanol	44	Hokkaido Univ.	2
51	Bolshaya Hatchery	Kamchatka Peninsula, Russia	1999	Liver	Ethanol	50	Hokkaido Univ.	2
52	Bolshaya Malki	Kamchatka Peninsula, Russia	2001	Liver	Ethanol	50	Hokkaido Univ.	2
53	Kalininka	Sakhalin Island, Russia	1994	Liver	Frozen	42	Hokkaido Univ.	1
54	Kalininka (early-run)	Sakhalin Island, Russia	2003	Liver	Ethanol	25	Hokkaido Univ.	2
55	Belaya	Sakhalin Island, Russia	2003	Liver	Ethanol	25	Hokkaido Univ.	2
56	Tymovo	Sakhalin Island, Russia	2003	Liver	Ethanol	25	Hokkaido Univ.	2
57	Taranay	Sakhalin Island, Russia	2003	Liver	Ethanol	24	Hokkaido Univ.	2
58	Naiba	Sakhalin Island	1995		DNA	16	Hokkaido Univ.	2
59	Okhotsk	Magadan, Russia	2003	Liver	Ethanol	25	Hokkaido Univ.	2
60	Ola	Magadan, Russia	1990	Liver	Frozen	33	Hokkaido Univ.	1
61	Ola	Magadan, Russia	1990		DNA	33	Hokkaido Univ.	
62	Ola (early-run)	Magadan, Russia	1991	Muscle	Ethanol	42	Hokkaido Univ.	
63	Arman	Magadan, Russia	1991	Liver	Frozen	37	Hokkaido Univ.	1
64	Arman (early-run)	Magadan, Russia	1991			42	Hokkaido Univ.	2
65	Taui	Magadan, Russia	1991		DNA	39	Hokkaido Univ.	2
66	Amur	Nikolaevsk-na-Amure, Russia	Sep. 9, 2000	Muscle	Ethanol	50	Hokkaido Univ.	1
67	Avakumovka	Primorye, Russia	1994	Liver	Frozen	30	Hokkaido Univ.	1
68	Salmon	Northwest Alaska, United States	1991	Liver	Frozen	48	Hokkaido Univ.	1
69	Sheenjek (fall-run)	Northwest Alaska, United States	1992	Liver	Frozen	45	Hokkaido Univ.	1
70	Andreafsky (summer-run)	Northwest Alaska, United States	1993	Liver	Frozen	45	Hokkaido Univ.	1
71	Togiak	Northwest Alaska, United States	1993	Liver	Frozen	49	Hokkaido Univ.	1
72	Noatak	Northwest Alaska, United States	1991		DNA	50	Hokkaido Univ.	2
73	Tanana	Northwest Alaska, United States	1993	Liver	Ethanol	50	Hokkaido Univ.	2

Table 1-continued.

	River name	Location	Sampling date	Tissue	Preservation	N	Archives	Source
74	Unalakleet	Northwest Alaska, United States	1992		DNA	50	Hokkaido Univ.	2
75	Kwethluk	Northwest Alaska, United States	1994	Liver	Ethanol	50	Hokkaido Univ.	2
76	Upper Nushagak	Northwest Alaska, United States	1993	Liver	Ethanol	49	Hokkaido Univ.	2
77	Toklat	Northwest Alaska, United Stats	1992	Liver	Ethanol	100	Hokkaido Univ.	
78	South Fork Kuskokwim	Northwest Alaska, United Stats	1995	Liver	Ethanol	100	Hokkaido Univ.	
79	Pelly	Northwest Alaska, Canada	1993	Liver	Ethanol	84	Hokkaido Univ.	
80	Donjek	Northwest Alaska, Canada	1994	Liver	Ethanol	72	Hokkaido Univ.	
81	WHN Hatchery	Southcentral Alaska, United States	1992	Liver	Ethanol	92	Hokkaido Univ.	
82	Blossom	Southcentral Alaska, United States	1986	Liver	Ethanol	50	Hokkaido Univ.	
83	Marten	Southcentral Alaska, United States	1986	Liver	Ethanol	50	Hokkaido Univ.	
84	Welly H. Noerenberg Hatchery	Southcentral Alaska, United States	2002	Liver	Ethanol	100	Hokkaido Univ.	
85	Olsen 2004	Southcentral Alaska, United States	Jun. 8, 2004	Fin	Ethanol	70	Hokkaido Univ.	
86	Kizhuyak	Southcentral Alaska, United States	1992	Liver	Frozen	44	Hokkaido Univ.	1
87	Olsen Creek	Southcentral Alaska, United States	1992	Liver	Frozen	45	Hokkaido Univ.	1
88	Kitoi Hatchery	Southcentral Alaska, United States	1993	Liver	Ethanol	49	Hokkaido Univ.	2
89	McNeil River	Southcentral Alaska, United States	1994	Liver	Ethanol	50	Hokkaido Univ.	2
90	Belkofski	Alaska Peninsula, United States	1992	Liver	Frozen	46	Hokkaido Univ.	1
91	Chiginigak	Alaska Peninsula, United States	1991	Liver	Ethanol	50	Hokkaido Univ.	2
92	Joshua Green	Alaska Peninsula, United States	1992	Liver	Ethanol	80	Hokkaido Univ.	
93	Frosty Creek	Alaska Peninsula, United States	1992	Liver	Ethanol	100	Hokkaido Univ.	
94	St. Catherine's Cove	Alaska Peninsula, United States	1992	Liver	Ethanol	80	Hokkaido Univ.	
95	Chunlina (Sustia River)	Southeast Alaska, United States	1993	Liver	Ethanol	87	Hokkaido Univ.	
96	9 Stream	Southeast Alaska, United States	Jul. 8, 2004	Fin	DNA	80	Hokkaido Univ.	
97	Little port	Southeast Alaska, United States	Aug. 8, 2004	Fin	DNA	80	Hokkaido Univ.	
98	Sawmill Creek	Berner's Bay, Southeast Alaska, United States	Jul. 28, 1993	Liver	Frozen	50	Hokkaido Univ.	1
99	Long Bay	Chichigof Island, Southeast Alaska, United States	Aug. 25-26, 1991	Liver	Frozen	49	Hokkaido Univ.	1
100	Whale Bay	Baranof Island, Southeast Alaska, United States	Aug. 12, 1993	Liver	Frozen	48	Hokkaido Univ.	1
101	Port Beauclerc	Kuiu Island, Southeast Alaska, United States	Aug. 20, 1995	Liver	Frozen	45	Hokkaido Univ.	1
102	Fish Creek	Portland Canal, Southeast Alaska, United States	Sep. 25, 1988	Liver	Frozen	49	Hokkaido Univ.	1
103	Disappearance Creek	POW Island, Southeast Alaska, United States	Sep. 25, 1998	Liver	Frozen	50	Hokkaido Univ.	1
104	Ecstall	Skeena River area, British Columbia, Canada	Sep. 12, 1988	Liver	Frozen	45	Hokkaido Univ.	1
105	Bag Harbor	QCI, British Columbia, Canada	Mid-Oct. 1989	Liver	Frozen	50	Hokkaido Univ.	1
106	Nekite Channel	British Columbia, Canada	Sep. 15, 1989	Liver	Frozen	33	Hokkaido Univ.	1
107	Nitinat	British Columbia, Canada	1992	Blood	Ethanol	50	Hokkaido Univ.	
108	Vedder	British Columbia, Canada	2002	Operculum	DNA	50	Hokkaido Univ.	
109	Cowichan	British Columbia, Canada	1997	Operculum	DNA	50	Hokkaido Univ.	

Table 1-continued.

	River name	Location	Sampling date	Tissue	Preservation	N	Archives	Source
110	Nanaimo	British Columbia, Canada	2002	Operculum	DNA	50	Hokkaido Univ.	
111	Harrison	British Columbia, Canada	2002	Operculum	DNA	50	Hokkaido Univ.	
112	Nooksack	Washington, United States	1998	Liver	Frozen	47	Hokkaido Univ.	1
113	Quilcene Bay	Washington, United States	1998	Liver	Frozen	49	Hokkaido Univ.	1
114	Blackjack Creek	Washington, United States	1998	Liver	Frozen	50	Hokkaido Univ.	1
115	Satsop	Washington, United States	1998	Liver	Frozen	49	Hokkaido Univ.	1
116	Hamilton Creek	Washington, United States	1998	Liver	Frozen	43	Hokkaido Univ.	1

Data sources are designated as follows: (1) Sato et al. (2004), and (2) Moongeun et al. (2004).

Table 2. Mean estimated contribution for 1,000 bootstrap resampling where each region composes 100% of the mixtures (N=500 each). Bold italic cells denote correct regional allocation; Standard deviations (SD) are given in parentheses.

	Region		
Regional allocation	Japan	Russia	North America
Japan	***0.959 (0.029)***	0.025 (0.026)	0.014 (0.015)
Russia	0.017 (0.021)	***0.885 (0.099)***	0.0954 (0.096)
North America	0.000 (0.002)	0.041 (0.031)	***0.957 (0.031)***