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Genetic identification of wild and domesticated strains of chinook salmon (*Oncorhynchus tshawytscha*) in southern British Columbia, Canada

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Abstract

Chinook salmon (*Oncorhynchus tshawytscha*) are the predominant indigenous salmonid reared in marine aquaculture in British Columbia (BC), Canada. As such, domesticated chinook strains may represent the greatest potential source of genetic interaction between wild and domesticated salmon in BC rivers. This survey of eight domesticated strains (combined $N=1100$) at 13 microsatellite loci confirmed the close relationship of the domesticated strains to east coast Vancouver Island wild populations from which they were derived five to seven generations earlier, but revealed that domesticated strains possess significantly less allelic diversity than their wild progenitors and other wild chinook salmon populations throughout the species range. Maximum-likelihood analysis of simulated mixtures of wild and domesticated chinook salmon and Bayesian classification of individual domesticated chinook salmon indicated that sufficient genetic differentiation exists to enable highly accurate identification of both wild and domesticated chinook salmon to type over the species range from Russia to California. Moreover, the domesticated strains tended to be distinct from each other (average pairwise $F_{ST}=0.05$, range 0.01–0.07), enabling accurate classification (>90%) of domesticated fish to strain of origin. These results indicate that genetic methods can be used to identify escaped domesticated chinook salmon in BC and perhaps to monitor the success of their reproductive activity.

Keywords: Microsatellite; Chinook salmon; *Oncorhynchus tshawytscha*; Identification; Domesticated; Wild

1. Introduction

Chinook salmon (*Oncorhynchus tshawytscha*) are indigenous to British Columbia and support valuable aboriginal, commercial and recreational fisheries. Domesticated chinook salmon are the primary species of Pacific salmon reared in aquaculture facilities in British Columbia and their cultivation in marine netpens raises concerns about genetic interactions between the domesticated strains and sympatric wild and hatchery-supplemented populations (Hindar et al., 1991; Fleming et al., 2000; McGinnity et al., 2003). Chinook salmon are currently cultured in relatively low numbers, especially in comparison with abundances of wild populations. However, wild chinook salmon populations in southern British Columbia, where the both the human population and aquaculture industry are concentrated, face a range of potential pressures including urbanization, fishing pressure and climate change (Slaney et al., 1996; Bradford and Irvine, 2000; Beamish and Mahnken, 2001). Along the west coast of Vancouver Island, chinook salmon populations suffered low marine survival during the el Niño years of the early 1990s, resulting in the imposition of commercial fishery restrictions (Riddell et al., 2002;

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Winther and Beacham, 2006). Thus, while the overall impact of escaped fish may be low, local wild populations near culture facilities could be affected by introgression if ongoing escapes of cultured chinook salmon occurred.

Population structure in chinook salmon throughout their range is strongly geographically based (Gharrett et al., 1987; Utter et al., 1989; Teel et al., 2000; Beacham et al., 2003; Guthrie and Wilmot, 2004; Beacham et al., 2006b), providing information that enables the accurate classification of fish to region and often to population within region (Beacham et al., 2006a). Domesticated chinook salmon, reared predominantly in southern British Columbia, were developed primarily from the Big Qualicum River hatchery-supplemented population on the east coast of Vancouver Island, although fish from other populations were occasionally reared during early industry development (Kim et al., 2004). Early strain development was undertaken by a number of companies that subsequently dissolved or were amalgamated into larger companies. By 2000, chinook salmon were cultured primarily by two companies that had acquired a number of strains during industry reorganization. The companies had initiated a pedigreed breeding program in 1996, into which they attempted to incorporate genetic diversity from the available strains.

Domesticated Atlantic salmon have been shown to be reproductively inferior (producing both fewer and less fit progeny) to local wild fish in the natural environment, both in matings with each other and with wild conspecifics (Fleming et al., 2000; McGinnity et al., 2003). Thus, the introgression of escaped cultured fish into a wild population can reduce the productivity of the wild population both in the first and succeeding generations. Reported escapes of chinook salmon in British Columbia waters (DFO, website) indicate that accidental releases of large numbers of fish are rare, but there is little information on the numbers of domesticated chinook salmon that enter the natural environment due to 'leakage' or low levels of loss during handling or transport. Detection of domesticated fish in river systems or fishery harvests requires an accurate method of identifying them even if they are sampled some years after escape. The genetic differentiation of domesticated chinook salmon at microsatellite loci from their progenitor and other wild populations may provide the required means of identification.

In this study, we have expanded an original study of genetic diversity in two domesticated chinook salmon strains (Kim et al., 2004) to the six available at the initiation of a selective breeding program. In addition, we examined two samples from the selective breeding program after one and two generations of pedigreed breeding to determine the genetic relatedness of the selected strain to the six original strains. We then compared the microsatellite allele frequencies of domesticated chinook salmon strains with corresponding data from a range-wide survey of wild and hatchery-supplemented chinook salmon populations (Beacham et al., 2006a,b) to determine the level of genetic differentiation between the domesticated strains and wild chinook salmon in all geographic regions.

We subjected simulated mixtures of domesticated and wild chinook salmon to mixed-stock analysis to determine the accuracy and precision of estimated contributions of domesticated fish to the mixtures. We also tested our ability to accurately identify individual domesticated fish using fish from each of the eight samples, with the entire sample excluded from the genetic baseline.

2. Materials and methods

2.1. Samples and DNA analysis

Domesticated chinook salmon are generally spawned as three year old fish, often with little gene flow occurring among the three brood cycles of fish maintained by a company. Six broodstock strain samples (adult fish) were obtained from industry sources between 2000 and 2002 (Strains 1–6). These strains, reared by various companies throughout the period of industry development without formal breeding programs, were not pedigreed. In addition, two samples of adult broodstock were obtained in 2002 (SBP 1) and 2005 (SBP 2) from a breeding program implemented in 1996. The SBP1 sample arose from matings conducted in 1999 among three non-pedigreed strains related to those sampled in this study. The SBP2 sample arose from matings among the SBP1 fish and additional crosses among fish from strains 1 and 2, which were incorporated into the breeding program. Samples consisted of operculum punches or fin clips placed in 95% undenatured ethanol. Sample sizes ranged from 42 to 268 fish.

Genomic DNA isolated from tissue samples was amplified for the following 13 polymorphic microsatellite loci: *Ots*100, *Ots*102, *Ots*104, *Ots*107 (Nelson and Beacham, 1999), *Ots*101 (Small et al., 1998), *Ssa*197 (O'Reilly et al., 1996), *Ogo*2, *Ogo*4 (Olsen et al., 1998), *Oke*4 (Buchholz et al., 2001), *Omy*325 (O'Connell et al., 1997), *Oki*100 (K.M. Miller, unpublished data), and *Ots*2, *Ots*9 (Banks et al., 1999). Amplification of each locus was carried out independently except for the following multiplexes: *Omy*325 and *Ogo*4, *Ogo*2 and *Oke*4, and *Ots*2 and *Ots*9. Primer concentrations in the multiplex polymerase chain reactions were adjusted to ensure equal amplification of both loci. Amplification products were size fractionated, 96 samples on a gel, on 4.5% denaturing polyacrylamide gels. The gels were run at 3000 V for 2.25 h at a gel temperature of 51°C on an ABI 377 automated DNA sequencer. Allele sizes were determined with Genescan 3.1 and Genotyper 2.5 software (PE Biosystems, Foster City, CA, U.S.A.).

Expected (H_E) and observed (H_O) heterozygosity were estimated for samples using GDA version 1.0 (Lewis and Zaykin, 2001). FSTAT version 2.9.3.2 (Goudet, 2001) was used to determine the significance of departures of genotype distributions at each locus from Hardy–Weinberg equilibrium and the