Species Identification of Pacific Salmon by Means of a Major Histocompatibility Complex Gene

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Abstract.—A rapid genetic test to identify Pacific salmonid tissue samples to the species level is described. An exon (coding DNA) and its adjacent intron (noncoding DNA) of a major histocompatibility complex (MHC) class II gene were amplified by the polymerase chain reaction from eight *Oncorhynchus* species and the two *Salmo* species that have been transplanted to British Columbia. Among Pacific salmonids, the length of the amplified sequence was between 809 and 826 base pairs (bp) for cutthroat trout *Oncorhynchus clarki*, rainbow trout and steelhead *O. mykiss*, chinook salmon *O. tshawytscha*, coho salmon *O. kisutch*, masu *O. masou*, and some sockeye salmon *O. nerka*; it was between 993 and 1,034 bp for pink salmon *O. gorbuscha*, chum salmon *O. keta*, and other sockeye salmon. Sequence length ranged from 1,000 to 3,000 bp for brown trout *Salmo trutta* and from 1,500 to 3,000 bp for Atlantic salmon *S. salar*. Amplified sequences from all Pacific salmonids except rainbow trout-steelhead and cutthroat trout displayed species-specific restriction fragment length polymorphisms (RFLPs) after independent digestion with three restriction enzymes (*AvrII*, *BglII*, *BsoI*). Restriction patterns of Pacific salmon sequences between 993 and 1,034 bp distinguished them from the 1,000-bp brown trout sequences. Intraspecific RFLP variability revealed regional differentiation in phenotypic frequencies in three species: coho salmon populations in southern British Columbia differed from those in northern British Columbia and the Fraser River; sockeye salmon from Kamchatka and Bristol Bay differed from those of British Columbia; and Japanese and North American chum salmon were well differentiated, enabling an accurate classification to continent of origin.

Correct species identification is of fundamental importance in the management and conservation of Pacific salmon *Oncorhynchus* spp. Identification of species is routine when the fish are approaching sexual maturity, given the distinctive physical changes that occur. However, species identification can be more problematical when juveniles are to be identified, even for experienced observers. In some cases, species identification is required when only a portion of a fish (e.g., a fillet) is available after having been processed and possibly cooked, or when a sample has been preserved in formalin or ethanol.

Species identification of fish independent of morphological characteristics has centered on the use of genetic and immunological variation. When adequate tissue samples of suitable quality are available, electrophoretic analysis of isozymes can be an effective method of species discrimination (Mork et al. 1983; Keenan and Shaklee 1985; Graves et al. 1989). However, when there is an inadequate amount of tissue for analysis or the tissue is of poor quality, amplification of selected DNA fragments via polymerase chain reaction (PCR) may be required (Pendas et al. 1995; Unseld et al. 1995). Species identification of tunas *Thunnus* spp. was accomplished initially by direct sequencing of an amplified mitochondrial DNA fragment (Bartlett and Davidson 1991). Subsequently, analysis of restriction fragment length polymorphisms (RFLPs) of a longer mitochondrial fragment was developed for tuna species identification (Chow and Inoue 1993), enabling relatively simple laboratory analysis. The PCR amplification of a DNA sequence and subsequent RFLP analysis of the fragment has also been applied to a number of other species identification problems (Silberman and Walsh 1992; Chow et al. 1993).

Substantial species differences have been reported in the nucleotide sequence of a major histocompatibility complex (MHC) class II β1 gene in Atlantic salmon *Salmo salar* and Pacific salmon (Hordvik et al. 1993; Grimholt et al. 1994; Miller and Withler 1996). The MHC genes are involved in the function of the immune system; T cells recognize a foreign antigen only in association with class I or II molecules encoded by the MHC. The MHC class II gene sequenced in Atlantic and Pacific salmon displays both intra- and interspecific polymorphism. Species-specific DNA sequences occur in both the DNA sequences that encode proteins (exons) and the interspersed noncoding DNA sequences (introns). In this study, we developed techniques to amplify a contiguous MHC class II
β1 exon and intron by PCR and then to identify known sequence variants (Miller and Withler 1996) from RFLPs. This proved to be a relatively simple method of differentiating *Salmo* from *Oncorhynchus* species and of identifying tissue samples from six of the eight Pacific salmonids to species. We then conducted a broad geographic survey of the five *Oncorhynchus* species that are harvested commercially to confirm the accuracy of species identification over the species' range. Finally, we applied the method to several species identification problems, including a collection of juvenile salmon sampled at sea, a group of 24 fish confiscated by a fishery officer and the liquid residue from a cooler used to transport fish, and a putative Atlantic salmon carcass recovered from a British Columbia stream.

**Methods**

**DNA samples.**—Blood or liver samples were collected from individual Pacific salmon representing, for most species examined, a broad geographic range of populations (Table 1). The *Oncorhynchus* species examined were chinook salmon *O. tshawytscha*, coho salmon *O. kisutch*, sockeye salmon *O. nerka*, chum salmon *O. keta*, pink salmon *O. gorbuscha*, the masu and amago races of *O. masou* (cherry salmon), cutthroat trout *O. clarki*, and rainbow trout and steelhead *O. mykiss*. Liver samples were also obtained from 20 Atlantic salmon being reared on commercial farms in British Columbia and eight hatchery-reared brown trout *Salmo trutta* from a naturalized population in the Cowichan River. The methods of DNA extraction have been outlined by Withler et al. (1994)

Once the methods for species identification had been determined, we applied them to three problems of species identification. Another investigator provided us with fins from 15 salmon caught on the high seas, among which were two fish of known (initially only by the investigator) identity and 13 fish of uncertain species identity. A fishery officer provided us with one scale from each of 24 scales of a fish that was recovered dead from a British Columbia stream and tentatively identified as an Atlantic salmon.

**PCR analysis.**—An MHC class II β1 exon and the succeeding intron sequence were amplified with a sense primer located at the 10th codon of β1 (5'-CCGATACTCCTAAAGGACCTGCA-3'). The antisense primer incorporated the last nucleotide of the intron and the first seven codons of the β2 exon (5'-TCAGTCTGACATGGGGCTCA-ACT-3'). Each PCR reaction contained 1 mg of template DNA, 10 pmol of each of the forward and reverse primers, 200 mmol of each deoxynucleotide, and 2.5 units of *Taq* polymerase. Amplification of the gene was achieved by running 40 cycles of 1 min at 94°C, 2 min at a 55°C annealing temperature, and 4 min at 72°C. The amplified fragment was then digested with 5 units/mg DNA of each of the restriction endonucleases *AvrII*, *BclI*, or *Bsofl*. Five to 10 mL of each reaction were analyzed by gel electrophoresis on a 2% agarose gel, with a 100-base-pair (100-bp) size marker ladder run in three lanes of a 20-lane gel. The gel was stained with ethidium bromide, and sizes of the resultant DNA fragments were recorded.

**Data analysis.**—After digestion of the amplified gene with the appropriate endonuclease, frequencies of occurrence of the observed restriction fragment phenotypes were recorded. Because the allelic products of two (or more) loci were amplified in at least some species (Miller and Withler 1996), the restriction fragment patterns could not be used to provide allele frequencies (i.e., there was no indication of copy number for each allele producing a restriction pattern). Thus, regional differentiation in the frequencies of restriction fragment phenotypes, rather than genotypes, for coho salmon, sockeye salmon, and chum salmon was evaluated by the chi-square test with Monte Carlo simulations of the distribution of the chi-square statistic (Roff and Bentzen 1989). We made 1,000 simulations of the original data using the MONTE program of the REAP software package (McElroy et al. 1992).

**Results**

**PCR of the MHC Gene**

The lengths of the MHC class II sequences amplified in the 443 *Oncorhynchus* samples examined in this study were between 809 and 826 bp ("short" alleles) in cutthroat trout, rainbow trout—steelhead, chinook salmon, coho salmon, masu, and some sockeye salmon; they were between 993 and 1,034 bp ("long" alleles) in pink salmon, chum salmon, and other sockeye salmon (Figure 1). The primers used in this study likely amplified alleles from at least two closely related loci in most *Oncorhynchus* species, as described by Miller and Withler (1996).
The sequences amplified encode 72 amino acids of an MHC β1 exon followed by an intron between 548 and 773 bp long in Pacific salmon. All Pacific salmon long alleles are distinguished from short ones by insertion of a 199-bp HpaI short interspersed repetitive DNA element (SINE) near the 3' end of the intron (Miller and Withler 1996). Sockeye salmon is the only species known to be polymorphic for long and short alleles, and individual sockeye salmon possessing both long and short alleles have been observed (Figure 1). No cutthroat trout MHC class II sequences were examined by Miller and Withler (1996), but three cutthroat trout alleles sequenced in the present...
study (unpublished data) were all short alleles (i.e., they lacked the *HpaI* SINE insert).

The MHC sequences amplified in Atlantic salmon were between 1,500 and 3,000 bp long, resulting in a clear distinction between the Pacific salmon species and Atlantic salmon (Figure 1). However, brown trout sequences of 1,000 bp and 1,500–3,000 bp were obtained. Individual brown trout possessing only 1,000-bp sequences, only sequences longer than 1,500 bp, and both length categories were observed. Thus, neither Pacific nor Atlantic salmon could be distinguished invariably from brown trout on the basis of length of amplified products. The undigested 1,000-bp brown trout sequences were indistinguishable from the long Pacific salmon alleles (unpublished data).

### RFLP Analysis of *Oncorhynchus* and *Salmo trutta*

All of the *AvrII*, *BclI*, and *Bsofl* restriction patterns predicted on the basis of known sequences of the MHC class II gene in seven *Oncorhynchus* species (Miller and Withler 1996) were observed in the geographic survey (Figure 2; Table 2). Digestion with *AvrII* produced monomorphic patterns for all species except sockeye salmon, in which the short allele (undigested) differed from the long allele (one restriction site in the SINE insert). Only *AvrII* consistently distinguished chinook from coho salmon. None of the 120 chinook salmon that were examined produced an MHC sequence that was digested with *AvrII*, but the amplified products from all 117 coho salmon surveyed had a single *AvrII* restriction site, resulting in two fragments of 260 and 561 bp. Similarly, pink salmon and chum salmon were readily discriminated by the presence of an *AvrII* restriction site in pink salmon (Table 2; Figure 2). However, digestion with *AvrII* alone failed to distinguish among cutthroat trout, rainbow trout–steelhead, chinook salmon, sockeye salmon (short allele), and masu. It also provided no differentiation between the amplified products of sockeye salmon (long allele) and pink salmon. Moreover, *AvrII* failed to distinguish the brown trout 1,000-bp sequences from those of chum salmon (Figure 2).

Restriction sites for *BclI* were present in sockeye salmon alleles (both long and short) and masu alleles, and in some coho salmon, rainbow trout–steelhead, and cutthroat trout alleles (Table 2). Digestion with *AvrII* produced monomorphic patterns for all species except sockeye salmon, in which the short allele (undigested) differed from the long allele (one restriction site in the SINE insert). Only *AvrII* consistently distinguished chinook from coho salmon. None of the 120 chinook salmon that were examined produced an MHC sequence that was digested with *AvrII*, but the amplified products from all 117 coho salmon surveyed had a single *AvrII* restriction site, resulting in two fragments of 260 and 561 bp. Similarly, pink salmon and chum salmon were readily discriminated by the presence of an *AvrII* restriction site in pink salmon (Table 2; Figure 2). However, digestion with *AvrII* alone failed to distinguish among cutthroat trout, rainbow trout–steelhead, chinook salmon, sockeye salmon (short allele), and masu. It also provided no differentiation between the amplified products of sockeye salmon (long allele) and pink salmon. Moreover, *AvrII* failed to distinguish the brown trout 1,000-bp sequences from those of chum salmon (Figure 2).

Restriction sites for *BclI* were present in sockeye salmon alleles (both long and short) and masu alleles, and in some coho salmon, rainbow trout–steelhead, and cutthroat trout alleles (Table 2). Digestion with *AvrII* alone failed to distinguish the amplified products of masu, rainbow trout–steelhead, and cutthroat trout, and it did not discriminate coho salmon from chinook salmon. Nor did *BclI* digestion distinguish among pink salmon, chum salmon, and brown trout or between coho salmon and sockeye salmon.

### Table 2.—Fragment sizes of DNA (in base pairs) observed after amplification of a major histocompatibility complex class II gene and restriction with *AvrII*, *BclI*, or *Bsofl* for eight *Oncorhynchus* species and brown trout (the brown trout 1,000-base-pair alleles only).

<table>
<thead>
<tr>
<th>Species</th>
<th><em>AvrII</em></th>
<th><em>BclI</em></th>
<th><em>Bsofl</em></th>
</tr>
</thead>
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<tr>
<td>Chinook salmon</td>
<td>821</td>
<td>821</td>
<td>203, 246, 372</td>
</tr>
<tr>
<td>Coho salmon</td>
<td>260, 561</td>
<td>821</td>
<td>246, 575</td>
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<td></td>
<td></td>
<td>278, 543</td>
<td>203, 246, 372</td>
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<td>135, 858</td>
<td>281, 712</td>
<td>206, 258, 529</td>
</tr>
<tr>
<td>Long allele</td>
<td>809</td>
<td>281, 528</td>
<td>206, 603</td>
</tr>
<tr>
<td>Short allele</td>
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<td>1,034</td>
<td>175, 178, 285, 396</td>
</tr>
<tr>
<td>Chum salmon</td>
<td>1,034</td>
<td>1,034</td>
<td>175, 285, 574</td>
</tr>
<tr>
<td>Pink salmon</td>
<td>147, 852</td>
<td>999</td>
<td>267, 732</td>
</tr>
<tr>
<td>Masu–amago</td>
<td>823</td>
<td>197, 626</td>
<td>823</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>206, 617</td>
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<td>Rainbow trout–steelhead</td>
<td>825</td>
<td>825</td>
<td>102, 247, 476</td>
</tr>
<tr>
<td></td>
<td></td>
<td>201, 625</td>
<td>247, 578</td>
</tr>
<tr>
<td>Cutthroat trout</td>
<td>826</td>
<td>826</td>
<td>100, 247, 479</td>
</tr>
<tr>
<td></td>
<td></td>
<td>201, 625</td>
<td>247, 579</td>
</tr>
<tr>
<td>Brown trout</td>
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<td>1,000</td>
<td>16, 204, 231, 549</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>204, 247, 549</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>451, 549</td>
</tr>
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</table>
Joint examination of the fragment patterns generated by *Avr II* and *Bcl I* digestion enabled the differentiation of chinook salmon, coho salmon, sockeye salmon, pink salmon, and chum salmon. However, digestion with these two enzymes did not differentiate consistently either chinook salmon or masu from cutthroat trout and rainbow trout–steelhead nor the two trout species from each other. Chum salmon and brown trout were also indistinguishable (Table 2).

The MHC sequences of all *Oncorhynchus* species possessed at least one *Bsof I* restriction site, and rainbow trout–steelhead, cutthroat trout, coho salmon, chum salmon, and masu were polymorphic for *Bsof I* sites. Digestion of the MHC gene with *Bsof I* alone did not allow complete differentiation of coho salmon from chinook salmon nor of coho salmon, rainbow trout–steelhead, and cutthroat trout; sockeye salmon and masu were not differentiated consistently (Table 2). However, in combination with the *Avr II* and *Bcl I* restriction patterns, *Bsof I* provided the required discrimination among chinook salmon, rainbow trout–steelhead, and cutthroat trout and between chum salmon and brown trout (Table 2). The *Bsof I* patterns also differentiated masu from rainbow trout–steelhead and cutthroat trout, although a wider survey might reveal overlap among these three polymorphic species. The only two sympatric species not separable on the basis of digestion with all three enzymes were rainbow trout–steelhead and cutthroat trout. Two rainbow trout–steelhead alleles not sequenced in the survey of Miller and Withler (1996) were detected in this study. Two Skeena River steelhead possessed both the known MHC sequence containing two *Bsof I* sites at positions 476

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**Figure 2.**—Amplified *Oncorhynchus* and brown trout genes of major histocompatibility complex class II digested with *Avr II*, *Bcl I*, or *Bsof I*. Lanes are as follows: molecular weight markers (1, 20), coho salmon (2–4), chinook salmon (5), rainbow trout–steelhead (6, 7), masu (8–10), cutthroat trout (11), sockeye salmon (12–14), chum salmon (15–17), pink salmon (18), and brown trout (19). For *Bcl I* and *Bsof I*, sockeye salmon monomorphic for the short allele (lane 12), monomorphic for the long allele (lane 13), and possessing both alleles (lane 14) are shown. Coho salmon (lanes 2, 4) and rainbow trout–steelhead (lane 7) possessing both *Bcl I* alleles are shown. Heterozygotes for *Bsof I* alleles are shown for coho salmon (lane 4), rainbow trout–steelhead (lane 7), masu (lane 10), and chum salmon (lane 17). The molecular weights are in base pairs.
and 578 of the 825-bp sequence and a second sequence lacking the cut site at position 476 (Table 2; Figure 2). Thus, the new allele produced a two-banded (247 and 578 bp) restriction fragment pattern identical to one of the coho salmon alleles. However, because the two steelhead each possessed one copy of the known rainbow trout–steelhead allele, and because the new allele did not restrict with Avr II or Bcl I, these fish would have been identified as rainbow trout–steelhead or cutthroat trout even if their identity had not been known. Two of six domesticated rainbow trout from the Fraser River drainage possessed both the known MHC sequence that does not restrict with Bcl I and a sequence that digested into fragments 201 and 625 bp long (Figure 2). This Bcl I restriction pattern was also observed in cutthroat trout (Table 2).

In sockeye salmon, which were polymorphic for the long and short alleles, and in rainbow trout–steelhead, cutthroat trout, coho salmon, chum salmon and masu, which were polymorphic for Bcl I or Bsof I sites (or both), individuals possessing two restriction patterns occurred and could be identified (Figure 2).

Identification of Unknown Samples

Amplification of MHC sequences from fin clips of 15 juvenile salmonids sampled at sea produced fragments that were either approximately 820 bp (eight fish) or 1,000 bp long (seven fish). No individuals heterozygous for the short and long alleles were observed. Treatment of the eight 820-bp products with Avr II resulted in no digestion, and treatment with Bcl I produced two restriction fragments, approximately 280 and 530 bp long, in every case. Thus, these fish were identified as sockeye salmon homozygous for the short allele. Digestion of these samples with Bsof I produced the expected restriction pattern with fragments approximately 205 and 600 bp long. Among the seven amplified 1,000-bp products, one was digested only with Bsof I, producing fragments approximately 175, 285, and 575 bp long (chum salmon); four samples were not digested by Bcl I but displayed two fragments about 150 and 850 bp long after Avr II digestion (pink salmon); and two samples were digested by both Avr II and Bcl I, producing fragments of the sizes expected from the sockeye long allele. Digestion of the latter six samples with Bsof I was consistent with the species designation. One pink salmon and the chum salmon were the two samples identified independently on the basis of morphology, and the genetic analysis confirmed the morphological classification.

Amplification and digestion of the DNA extracted from the 24 scales supplied by a fisheries officer revealed that all fish were coho salmon. For each scale sample, and for DNA extracted from the water sample collected from a cooler in which fish had been transported, amplification with the MHC primers produced DNA fragments approximately 820 bp long. In each case, digestion with Avr II resulted in fragments approximately 260 and 560 bp long. For the scale samples, independent digestion with Bcl I and Bsof I produced fragment patterns indicating that each fish was either homozygous or heterozygous for the two possible coho salmon patterns for each enzyme (Table 2). Both coho salmon restriction patterns for both enzymes were present in DNA from the cooler sample. Thus, we determined that coho salmon had been transported in the cooler, although none of the fish was recovered for morphological confirmation.

Amplification of MHC sequences from the putative Atlantic salmon found dead in a stream produced fragments 2,000 bp long, confirming that the fish was either a brown trout or Atlantic salmon. We then amplified the 5S ribosomal DNA sequences, which differ in length between Atlantic salmon and brown trout (Pendas et al. 1995), and confirmed that the fish was an Atlantic salmon.

Population Variation

Frequencies of the three sockeye salmon phenotypes (short sequences only, long sequences only, short and long sequences) varied significantly (P < 0.001) among five regions ranging from Kamchatka to Vancouver Island (Table 3).
Greater proportions of sockeye salmon from Kamchatka and Bristol Bay possessed long alleles, either exclusively or together with short alleles, than did sockeye salmon from the Skeena and Fraser river systems of British Columbia. Sockeye salmon from Fulton River, a Skeena River tributary, were monomorphic for the short allele. Although both the short and long phenotypes were relatively common among Vancouver Island sockeye salmon, only one individual possessed both long and short sequences. Further sampling is required to determine whether this shortage of “heterozygotes” resulted from small sample sizes, heterogeneity in phenotype frequencies among Vancouver Island populations, or some novel genetic mechanism.

Frequencies of the two chum salmon MHC alleles detected by Bsof I digestion differed significantly between Japanese and North American populations. Of the 24 Japanese chum salmon examined, 23 fish possessed only sequences with two Bsof I sites and 1 fish possessed sequences with both two and three sites. The 28 North American chum salmon were monomorphic for sequences possessing three Bsof I sites. Thus, all of the Japanese chum salmon possessed at least one copy of the MHC allele possessing two Bsof I sites, and North American chum salmon were distinguished by its absence. Larger sample sizes of fish from both continents will be required to determine the frequency of the “North American” MHC allele and fish homozygous for it in Asian chum salmon populations and to determine if the “Japanese” allele occurs in North American populations.

Coho salmon MHC alleles sequenced by Miller and Withler (1996) and detected in the current study were polymorphic for both a Bcl I restriction site and an independent Bsof I site (Table 2). Thus, four coho salmon MHC alleles potentially exist: one possessing no Bcl I site but one Bsof I site (allele A), one possessing a Bcl I and a Bsof I site (B), one possessing no Bcl I site but two Bsof I sites (C), and one possessing one Bcl I and two Bsof I sites (D). Of the five known sequences (Miller and Withler 1996), two were allele A, two were allele B, and one was allele C. Allele D was not present among the coho genes sequenced in that study and was not observed in the current survey.

Coho salmon heterozygotes BD and CD would have provided distinctive patterns after individual digestion with Bcl I and Bsof I, but the AD and BC genotypes (each heterozygous in separate digestions with Bcl I and Bsof I) could be distinguished only by simultaneous (double) digestion with the two enzymes. No DD homozygotes or BD or CD heterozygotes were observed. Ten coho salmon from southern British Columbia displayed the ambiguous double heterozygote pattern, but all were determined to be BC heterozygotes by double digestion. No fish carrying all three of the A, B, and C alleles were detected in the double digestions, but coho salmon carrying three B1 alleles distinguished by Mbo I digestion have been observed (Miller and Withler 1996).

The distribution of MHC restriction phenotypes varied significantly among coho salmon from three regions of British Columbia (P < 0.001) (Table 4). Two northern populations possessed only the A and B alleles. Fraser River populations possessed the A, B, and (at a low frequency) C alleles, and southern populations (Vancouver Island and mainland Strait of Georgia) were characterized by a high frequency of the C allele.

### Table 4: Regional counts of major histocompatibility complex class II restriction fragment phenotypes in coho salmon of British Columbia (B.C.), as identified by single and double digestions with Bcl I and Bsof I endonucleases. The three alleles detected in the double digestions have been available before this study, and steelhead trout, a species for which no sequence data had been available before this study, and steelhead trout. Two steelhead were heterozygous for an allele with a Bsof I restriction site loss that resulted in a Bsof I pattern identical to one observed for coho salmon. However, the new steelhead allele did not possess
any Avr II or Bcl I sites, as would be expected of a rainbow trout–steelhead allele but not a coho salmon allele.

All three cutthroat trout MHC sequences obtained in this study possessed a single Bsof I site that produced a restriction pattern identical to that of the new rainbow trout–steelhead allele, as well as a single Bcl I site that has not yet been observed in rainbow trout–steelhead. However, the subsequent digestion of MHC sequences of five cutthroat trout from each of three strains that have been supplemented by hatchery production revealed that cutthroat trout also possess sequences without the Bcl I site and sequences with the common steelhead Bsof I restriction pattern. Thus, digestion of the MHC B1 and intron sequences with the three enzymes used in this study does not reliably differentiate rainbow trout–steelhead from cutthroat trout. The two steelhead sequences (Miller and Withler 1996) can be distinguished from the three cutthroat trout sequences obtained in this study with Hinf I, which digests the rainbow trout–steelhead sequences twice and the cutthroat trout sequences at the same two and an additional site. However, a broader survey of purebred populations, as well as those in which introgression between the species has occurred (Carmichael et al. 1993), will be required to confirm the technique. A broader survey of masu populations may also reveal overlap with the trout sequences. However, differentiation of the weakly anadromous Asian masu from the North American trout species will rarely be necessary.

The PCR–RFLP analysis is independent of morphological or scale characteristics, which sometimes are ambiguous, and can be conducted with minute amounts of fresh or preserved tissue. This makes the technique very useful for unanticipated species identification of fresh or frozen tissue, identification of preserved historical samples (including scales and otoliths), and forensic identification of confiscated fresh or processed tissue. The coho salmon examined in this study for a fisheries officer were each identified to species after the two-step amplification of the MHC and used to confirm an Atlantic salmon identity through the two-step amplification of the MHC and SS ribosomal sequences.

Among the Pacific salmon species, most of the variability in intron length results from the presence or absence of the 199-bp Hpa I SINE, which distinguishes the “short” alleles of cutthroat trout, rainbow trout–steelhead, masu, chinook salmon, and coho salmon from the “long” alleles of pink salmon and chum salmon. Only sockeye salmon is polymorphic for the SINE, possessing both long and short alleles. Various classes of SINEs are common in salmonid fish genomes (Kido et al. 1991; Spruell and Thorgaard 1996) as well as in mammalian intones, including those of the MHC (Del Pozza et al. 1991). The 1,000-bp brown trout sequences, although the same length as the Pacific salmon “long” alleles, do not possess the Hpa I SINE.

In British Columbia, where Atlantic salmon are the predominant aquacultural species, discrimination between Pacific and Atlantic salmon is important because it is otherwise difficult to identify Atlantic salmon farm escapees or hybrids that might result from interbreeding of escapees with native salmonids. However, the presence of a small, introduced brown trout population in the Cowichan River necessitates a test that can distinguish Atlantic salmon from brown trout. The MHC exon amplified in this study is also polymorphic and under balancing selection in Atlantic salmon (Hordvik et al. 1993; Grimholt et al. 1994), but no sequence data are available for the adjacent intron. The results of this study indicate that the Atlantic salmon intron is consistently longer than those of the Pacific species (resulting in amplified sequences at least 1,500 bp long), whereas brown trout sequences overlap those of Pacific salmon long alleles and Atlantic salmon alleles (1,500–3,000 bp).

Digestion with the three enzymes used in this study failed to differentiate consistently between brown trout and Atlantic salmon sequences that were in the 1,500- to 3,000-bp size range (unpublished data). The MHC sequences from both species were undigested by Avr II and Bcl I, and the Bsof I patterns were not species specific. Thus, identification of a putative purebred or hybrid Atlantic salmon requires two steps: amplification of the MHC sequences with no digestion to produce sequences of 1,500 bp or longer; and amplification of the 5S ribosomal sequences with the primers of Pendas et al. (1995) to confirm that the long sequences are of Atlantic salmon rather than brown trout origin. The putative Atlantic salmon that was recovered dead from a stream and examined in this study yielded highly degraded DNA from liver tissue that was unsuitable for amplification of the 2,000-bp MHC sequence, whereas high-quality DNA extracted from scales was amplified easily and used to confirm an Atlantic salmon identity through the two-step amplification of the MHC and SS ribosomal sequences.
Although the emphasis of our study was on establishing a rapid, reliable method of species identification, the PCR–RFLP analysis of the MHC class II gene revealed intraspecific regional variation in restriction pattern frequencies that might be exploited for stock identification of sockeye salmon, chum salmon, and coho salmon. If assignment of coho salmon to fairly broad geographic areas (e.g., northern British Columbia, Fraser River, southern British Columbia) is sufficient for management purposes, observed differences in MHC RFLP frequencies may enable the same level of stock composition estimates routinely obtained for other species from isozyme analysis (Beacham et al. 1987; Wood et al. 1989). Similarly, if Fulton River sockeye salmon (monomorphic for the short MHC allele) represent all sockeye populations in the Skeena River watershed, regional estimates of sockeye salmon stock composition based on amplification of the MHC gene may be possible.

Japanese chum salmon are reportedly distinct from Russian and North American populations at both isozyme loci (Winans et al. 1994) and minisatellite DNA loci (Taylor et al. 1994; Beacham 1996). However, application of single-locus minisatellite DNA probes in the classification of individual chum salmon to continent of origin has resulted in only about 80–95% accuracy (Taylor et al. 1994; Beacham 1996). In the present study, digestion of the MHC class II gene with BsoI enabled 100% accuracy in classifying Japanese and North American chum salmon to continent of origin. Although only 52 chum salmon were examined in this study and no Russian populations were surveyed, the results indicate that PCR–RFLP analysis may provide a relatively rapid and effective method of distinguishing North American from Asian chum salmon.

As more classical MHC genes are isolated from Pacific salmonids, and as more of the nucleotide sequence variability among alleles can be detected by sensitive techniques such as denaturing gradient gel electrophoresis (Fischer and Lerman 1983), the usefulness of these highly polymorphic genes for stock identification in Pacific salmonids may increase.

Acknowledgments

We appreciate the assistance of many individuals in obtaining samples, particularly M. Kaeriyama for samples from Japan, N. Varanavskaya for samples from Russia, Ray Billings (Duncan Trout Hatchery) for the cutthroat trout and brown trout samples, and W. Barner, C. Murray, A. Thompson, and J. Candy for samples from British Columbia and the Yukon Territory. D. Tuck assisted with the laboratory analysis. Funding was provided by the Department of Fisheries and Oceans.

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