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Short communication

Sequence analysis of MHC class I α2 from sockeye salmon (*Oncorhynchus nerka*)

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Most studies assessing adaptive MHC diversity in salmon populations have focused on the classical class II DAB or DAA loci, as these have been most amenable to single PCR amplifications due to their relatively low level of sequence divergence. Herein, we report the characterization of the classical class I UBA α2 locus based on collections taken throughout the species range of sockeye salmon (*Oncorhynchus nerka*). Through use of multiple lineage-specific primer sets, denaturing gradient gel electrophoresis and sequencing, we identified thirty-four alleles from three highly divergent lineages. Sequence identity between lineages ranged from 30.0% to 56.8% but was relatively high within lineages. Allelic identity within the antigen recognition site (ARS) was greater than for the longer sequence. Global positive selection on UBA was seen at the sequence level (dN:dS = 1.012) with four codons under positive selection and 12 codons under negative selection.

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Genes of the major histocompatibility complex (MHC) play a vital role in the recognition of parasitic, bacterial and viral pathogens. MHC class I molecules have specific antigen recognition sites (ARS) that bind peptides of endogenous origin and present them to T-cells as part of the cellular immune response. Polymorphisms in the ARS allow MHC variants to bind different infectious agents [1]. Understanding allele diversity of MHC molecules is of increasing interest for management and conservation of wild salmon populations, particularly as pathogen distributions are shifting under a changing climate and increasing the exposure of populations to novel, more abundant and/or virulent disease agents [2]. Salmonids have only one classical class I and one classical class II MHC locus which are unlinked [3,4]. Diversity of the classical MHC class II DAB locus for sockeye salmon (*Oncorhynchus nerka*) has been previously described [5]; here we present the results of a species wide sequencing effort to identify sockeye MHC class I alleles.

Tissue samples were collected from adult sockeye in 137 lakes and streams throughout the North Pacific from Washington to Japan [see 6 for sample collection details]. Tissue samples were taken from between 19 and 336 individuals per population and preserved in 95% ethanol for DNA extraction. Total genomic DNA was extracted using the chelex method outlined in Small et al. [7] or using a Qiagen DNeasy extraction kit (Qiagen, Valencia CA, USA).

MHC class I UBA α2 (hereafter referred to as UBA) was amplified using primers designed from lineages detected in rainbow trout and Atlantic salmon [8; Fig. 1]. PCRs were carried out in a 25 μl volume containing 100–200 ng/μl of DNA, 10 pmol each primer, 0.2 mM each dNTP, 2.5 μl 10× buffer (Qiagen) and 1 U *Taq* polymerase (Qiagen). The PCR profile included an initial 2 min denaturing step at 94 °C, followed by 35 cycles of 94 °C for 1 min, 50 °C for 1 min and 68 °C for 2 min, with a final 10 min extension at 72 °C. The antisense primer was tagged with fluorescent molecules (Hex, 6FAM, or Rox) to enable multiplexing of runs and post-run visualization; sense primers included a 5′ 42 bp GC-clamp segment (5′-CGCCCGCCG CGCCCCGCGCCCGTCCCGCCGCCCCCGCCCG-3′) to maximize separation of alleles using denaturing gradient gel electrophoresis (DGGE) [9]. Alleles were separated by DGGE with the BioRad D-Gene™ (Hercules, CA) electrophoresis system. DGGE gels containing 7% polyacrylamide and 40–65% denaturant gradient were run at 60 V and 56 °C for 16 h. After electrophoresis, gels were scanned with the Hitachi FMBIO™-II fluorescent imaging system (MarioBio, Alameda, CA, USA) and analyzed using Phoretix (Nonlinear Dynamics, Newcastle, UK). In order to determine if primers were amplifying all UBA alleles present in study populations, or in other words, to ensure that null alleles were not affecting results, populations were tested for an excess of homozygosity using the Hardy–Weinberg test in Genepop [10]. Initially, 75 populations were found to have an excess of homozygotes (data not shown); thus, another forward primer was designed (sense primer 5; Fig. 1) in order to amplify previously unidentified alleles and populations were re-screened.

Each band differentiated by DGGE was sequenced in 4–8 individuals from each population in which that allele was found. Alleles were excised from DGGE gels, frozen and re-thawed in 200 μl of

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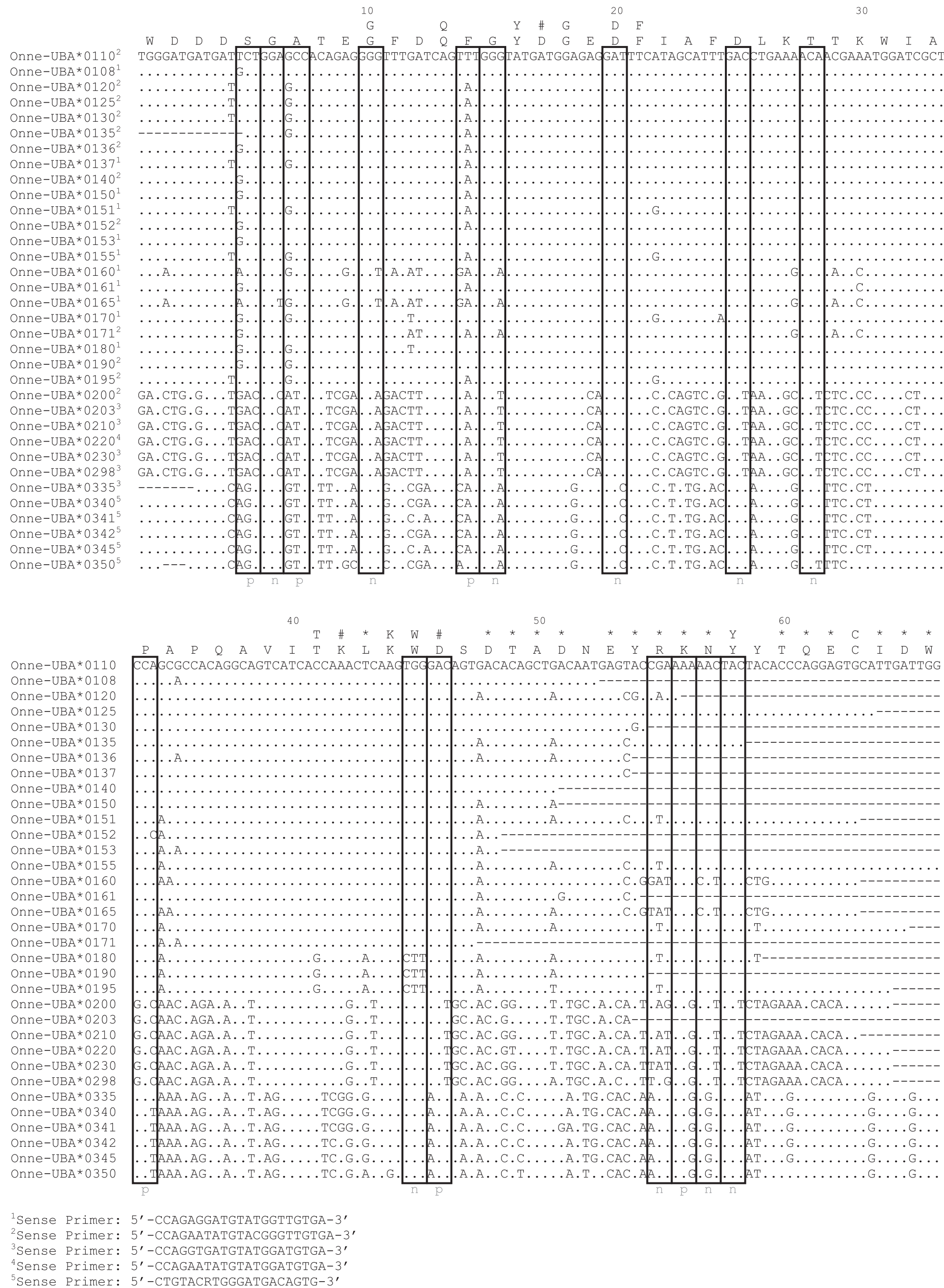

Fig. 1. Nucleotide sequences of *O. nerka* MHC UBA-α2 alleles. Five sense primers (above) and a single antisense primer (5′-GATACTTCTTAAGCCAATCAATGCA-3′) were used to amplify alleles. Sequence names follow the nomenclature used by Shum et al. [19]. Groups of cryptic alleles are indicated by bars to the left of the names. Dots indicate identity with the top sequence and hyphens indicate missing sequence (at the beginning or end of a sequence) or gaps inserted to improve alignment. Shown above the sequence is the amino acid sequence, numbered beginning at the first aa, with residues of the antigen recognition site (*), charged amino acids that form salt bridges (#), and residues generally conserved across species (letter code) [8]. Codon's under selection are boxed with the mode of selection, positive (p) or negative (n), indicated below the box.

dH20, amplified, and cleaned using a Qiagen PCR purification kit (Qiagen, Valencia CA, USA). Purified PCR products were sequenced using BigDye Terminator chemistry with the sense and antisense primers used for initial amplification. Sequences were visualized using ABI377 or 3700 sequencers, and assembled with Sequencher 2.0 (Gene Codes Corp, Ann Arbor, MI). If cryptic alleles that were not separable by the DGGE conditions used here were found during sequencing, additional individuals were sequenced to confirm that differences were not due to PCR errors.

Sequences were aligned using CLUSTAL and relationships between sequences were evaluated using the UPGMA method of clustering in Mega v. 4.1 [11]. Missing data was eliminated in a pairwise manner and clusters were tested with 10,000 bootstrap replicates. Representative samples of UBA alleles from Atlantic salmon and Rainbow trout were included in the UPGMA tree to allow for identification of previously described lineages [8,12]; use of additional representative salmonid sequences did not substantially change the configuration of the tree beyond that shown here. Analyses of molecular evolution were implemented using the Datamonkey webserver [13]. The Felsenstein-81 nucleotide substitution model best fit the data (AIC = 2549.66) and was used throughout the other analyses. The PARRIS method [14]

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