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TECHNICAL NOTE

A genetic marker for the maternal identification of Atlantic salmon × brown trout hybrids

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Abstract Interspecific hybridization between Atlantic salmon and brown trout is well documented, but why it should vary so much among populations is not clear. Determining the maternal origin of hybrids can provide insights into the mechanisms underlying interspecific hybridization, but this information is lacking in many studies. Here we present a species-specific mitochondrial DNA marker for the identification of the maternal origin of hybrids. This marker involves only one PCR step followed by fragment analysis, can be integrated within PCR multiplexing for existing nuclear markers for hybrid identification, and is therefore faster and more cost-effective than previous methods.

Keywords Interspecific hybridization · Atlantic salmon · Brown trout · Mitochondrial DNA

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Interspecific hybridization between Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) can result from multiple reasons and determining the direction of hybridization can shed light on the most plausible explanations (e.g. Garcia de Leaniz and Verspoor 1989; McGowan and Davidson 1992; Youngson et al. 1992, 1993; Jordan and Verspoor 1993; Hindar and Balstad 1994; Hartley 1996; Jansson and Öst 1997; Gephard et al. 2000; Matthews et al. 2000; García-Vázquez et al. 2001; Ayllon et al. 2004). The objective of this study was to develop a new species-specific mitochondrial DNA marker for identifying the maternal origin of hybrids between Atlantic salmon and brown trout. The study was conducted in three steps: (1) Alignment of complete mitochondrial genome sequences of Atlantic salmon and brown trout (2) Design of PCR primer sequences for candidate loci followed by pilot testing on a limited number of fish, and (3) Testing of the most promising candidate locus on a large set of Atlantic salmon and brown trout from a wide geographical range.

The complete mitochondrial sequences of Atlantic salmon (Hurst et al. 1999) and brown trout (Duc et al. unpublished) were obtained from GenBank (accession number U12143 and NC_010007, respectively) and aligned using the nucleotide Blast program (NCBI). One region in the 16S ribosomal RNA had a three base pairs deletion in Atlantic

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Table 1 Primer sequences with expected and observed amplicon sizes of two candidate loci for differentiating between Atlantic salmon and brown trout in the mitochondrial DNA

Primer id—Primer sequences (5′–3′)	Expected Size (bp) Salmon/Trout	Observed size (bp) Salmon/Trout
Salmo_Mito-3378F-AGCGGACATAAACACATAAGAC	228/231	228.23/228.23
Salmo_Mito-3378R-CGGATCATTTTTGGTCAGA		
Salmo_Mito-951F-ACCCCTAAACCAGGAAGTCT	232/239	231.98/239.46
Salmo_Mito-951R-TGCTTTAGTTAAGCTACGCCAACT		

salmon compared to brown trout starting at position 3378 (Hurst et al. 1999), and one region in the D-loop had seven deletions in a broken array in Atlantic salmon compared to brown trout starting at position 951 (Hurst et al. 1999). Primer pairs were designed using Primer3 V0.3.0 (Rozen and Skaletsky 2000) and Netprimer (http://premierbiosoft.com/netprimer). The two primer pairs (Table 1) were tested on four Atlantic salmon and four brown trout from the rivers Lierelva and Glomma (Norway), respectively. The PCR was conducted using the Qiagen® Multiplex PCR kit, according to protocol 2, with the forward primers labeled with FAM (Invitrogen). The PCR products were separated and visualized on an ABI 3130xl DNA analyzer (Applied Biosystems) and sized using Genemapper ver. 3.7 (Applied Biosystems). The primer pairs in the 16S rRNA region (Salmo_Mito-3378) amplified well but there was no difference in fragment length between salmon and trout. The primer pairs in the D-loop region (Salmo_Mito-951) also amplified well and, as expected, the salmon had a fragment, seven base pairs shorter than the brown trout. The Salmo_Mito-951 locus was successfully integrated in a multiplex with three nuclear genetic markers (5S rDNA: Pendas et al. (1995), SsOSL438: Slettan et al. (1996), Ssa197: O'Reilly et al. (1996)), used for differentiating between Atlantic salmon and brown trout, and for identifying hybrids. The PCR multiplex was carried out in a total volume of 10 µl, containing 1 µM of Ssa197 primers, 8 µM of SsOsl438 primers, 0.4 µM of 5Sr primers, 1 µM of Salmo_Mito-951 primers, 0.25 mM of each dNTP, 1X reaction buffer (Thermo Scientific High Performance, VWR), 1 unit of Thermo start DNA polymerase (VWR), and 2 µl of template (Range: 20–400 ng). The following PCR program was run on a Quattro Cycler (VWR): Denaturation for 15 min at 95 °C, 11 cycles of touchdown PCR, including denaturation at 94 °C for 30 s, annealing at 63–52 °C for 30 s with a decrease of 1 °C for each cycle, and extension at 72 °C for 1 min, followed by 25 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C. PCR ended by a final step of extension at 72 °C for 10 min. The Salmo_Mito-951 locus was further tested on a large set of salmon and trout samples with a wide geographical distribution (Fig. 1) using the PCR multiplex protocol described above. The nuclear genetic markers were included to ensure correct species identification. DNA was extracted from ethanol preserved fin-clips or from dried scales, using the E.Z.N.A™ tissue DNA kit (E.Z.N.A.®) or the extraction robot GeneMole®, following the MoleStrip™ DNA tissue protocol.

A total of 134 salmon and 92 brown trout (Fig. 1; Table S1, Supplementary material) were successfully genotyped for the Salmo_Mito-951 locus. All salmon had haplotype *232* and all brown trout had haplotype *239*, except for seven out of nine salmon from the Conne River (Canada) where haplotype *234* was found, instead of haplotype 232.

The mitochondrial marker described here identifies the maternal origin of Atlantic salmon and brown trout hybrids and will be useful for addressing the potential reasons for breaking of reproductive barriers between these two species. In contrast to the previous RFLP method employed, e.g. using the primers of Nielsen et al. (1998), our method involves only one PCR multiplex and simultaneously provides nuclear markers for hybrid identification and a mitochondrial marker for determining the direction of

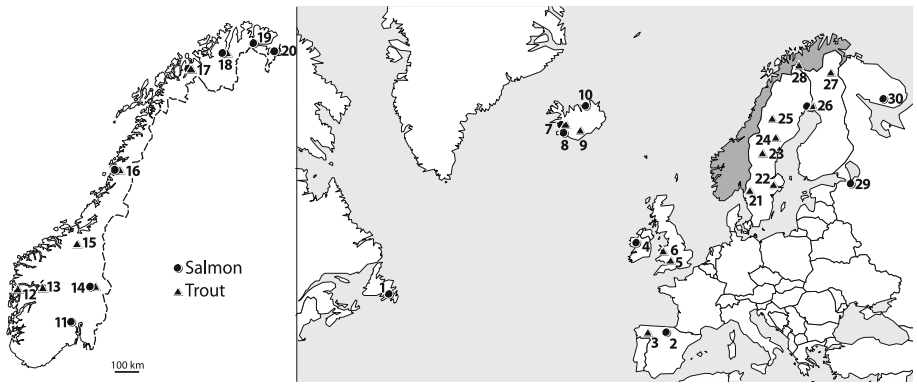

Fig. 1 Map showing the sample sites of Atlantic salmon and brown trout

hybridization. The relatively large geographical coverage of our study suggests that the new species-specific mitochondrial marker is likely to work in most, if not all, populations.

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