



A new species-specific nuclear DNA marker for identification of hybrids between Atlantic salmon and brown trout

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(Received 9 October 1995, Accepted 11 January 1996)

The banding pattern of *AluI*-digested prepro-gonadotropin releasing hormone gene is species-specific in Atlantic salmon and brown trout and can be applied for identification of their hybrids.

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Key words: hybrids; Atlantic salmon; brown trout; prepro-gonadotropin releasing hormone gene.

Atlantic salmon *Salmo salar* L. and brown trout *S. trutta* L., can interbreed and produce viable hybrid offspring. Because of their morphological similarity, the identification of hybrids based only on morphometric and meristic characters is highly uncertain (Jansson *et al.*, 1991; Wilkins *et al.*, 1994) and has hindered screening for their presence in natural populations in earlier years. Electrophoretic investigation of allozyme polymorphisms has contributed significantly to the solution of this problem and altogether seven species-specific loci suitable for identification of hybrids have been revealed (Nyman, 1970; Guyomard, 1978; Beland *et al.*, 1981; Vuorinen & Piironen, 1984; Crozier, 1984; Semyonova & Slyn'ko, 1988). However, in most cases the tissue samples must be taken from muscle, liver, or blood after killing the fish and only glucosephosphate isomerase and esterase D exhibit adequate activity in fin biopsies (Vuorinen & Piironen, 1984; Semyonova & Slyn'ko, 1988). Recently developed methods and techniques of DNA analysis (especially, PCR-based techniques) have a potential of detecting new species-specific genetic markers using only minute amounts of any tissue (fresh, frozen or alcohol preserved). Pendas *et al.* (1995) demonstrated that even degraded DNA obtained from scales, bones and commercial smoked salmon was satisfactorily amplified and they found that the size of the amplification products at the 5S rDNA gene was species-specific in *S. salar* and *S. trutta* and suitable for identification of the F_1 hybrids. However, for estimating the extent of introgressive hybridization, F_1 and post- F_1 hybrids must be distinguished and the minimum requirement for identification of the post- F_1 hybrids is two unlinked diagnostic loci (Verspoor & Hammar, 1991). For this reason, we describe here another nuclear DNA marker, the prepro-gonadotropin releasing hormone (*GnRH*) gene, which shows a species-specific banding pattern of *AluI* restriction fragments in *S. salar* and *S. trutta*.

Samples of brown trout were obtained from the Baltic Sea basin [the Swedish rivers Umeälven (60 individuals), Lögdeälven (16 individuals), Oxsjöån (six individuals), and Skellefteälven (six individuals); the Estonian rivers Öngu (five individuals) and Altja (20 individuals)], and from the Mediterranean basin [River Reverotte, a tributary of the

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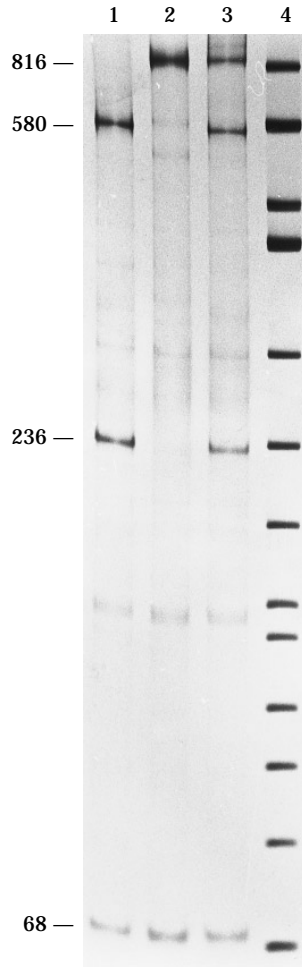


FIG. 1. Banding patterns of the *AluI* digested *GnRH* gene 918 bp segment in 8% non-denaturing polyacrylamide gel: lane 1, Atlantic salmon; lane 2, brown trout; lane 3, hybrid between salmon and trout; lane 4, Φ X174 *HinfI* molecular weight marker (Promega). Fragment sizes are in base pairs. 34 bp fragment not shown.

River Doubs, France (six individuals); River Gesso, Italy, a representative of the *S. trutta marmoratus* (four individuals)]. Samples of Atlantic salmon were obtained from the Baltic Sea basin [the Swedish rivers Umeälven (330 individuals) and Skellefteälven (19 individuals)] and from the Atlantic basin [River Laxa i Adaldal, Iceland (five individuals) and a relict landlocked population of Lake Vänern, Sweden that spawns in the River Gullspångsälven (five individuals)]. Thus, both species were sampled only in R. Skellefteälven and R. Umeälven. Total genomic DNA was isolated from the muscle or fin samples according to the simplified procedure of Laird *et al.* (1991). A pair of 20-mer oligonucleotide primers was designed to match the conserved sequences of the Atlantic salmon *GnRH* gene (locus SSGNRH under accession number X74957 in GenBank):

5'-CGGTTGTGCAGGTGGTGGTG-3' (*GnRH-a*), position 2799 to 2818 according to GenBank;

5'-GGACAGAACTGTAGTTGCTC-3' (*GnRH-b*), position 3696 to 3715 according to GenBank.

In Atlantic salmon, this primer pair is expected to amplify a 918 bp segment which includes partly the exons 2 and 4, and completely the introns 2 and 3, and the exon 3. Amplification reactions were performed in 25 µl containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 2.3 mM MgCl₂, 0.1 mg/ml BSA, 100 µM each of dATP, dCTP, dGTP and dTTP, 0.2 µM of each primer, 5–50 ng of genomic DNA template, and 0.625 unit of Taq DNA polymerase (MBI). After a denaturing step of 5 min at 95° C, samples were processed through 35 cycles consisting of 30 s at 95° C, 30 s at 62° C and 1 min at 72° C. The last elongation step was lengthened to 10 min. Aliquots of the PCR-products were subjected to restriction endonuclease digestion by *AluI*. Amplified and digested gene fragments were separated by polyacrylamide gel electrophoresis (Gross & Nilsson, 1995) and were visualized by the silver staining method according to Klinkicht & Tautz (1992).

AluI digested the amplified segment of the *GnRH* gene in Atlantic salmon into four fragments: 580, 236, 68 and 34 bp (Fig. 1, lane 1) while in brown trout, only three fragments were observed (Fig. 1, lane 2). The two smallest fragments corresponded to the 68 bp and 34 bp fragments of salmon, but the size of the largest fragment corresponded approximately to the sum of the 580 bp and 236 bp fragments of salmon that was obviously due to the absence of the *AluI* restriction site between these two fragments. In hybrids, both salmon and trout fragments (altogether five fragments) were observed (Fig. 1, lane 3). The larger fragments are clearly visible also when separated on 1.2% agarose gel and we found this gel type to be useful in routine screening for hybrids.

No variation of banding patterns was observed among the studied populations within each species. The hybrids (seven individuals) were detected only among the fish sampled in the River Skellefteälven. They were confirmed also by the pattern of heteroduplex bands in *HinfI*-digested *GH2* gene fragment that was identical to that obtained in experimental mixtures of DNA from Atlantic salmon and brown trout (Gross & Nilsson, 1995). Thus, we can conclude that the *AluI* restriction pattern of the *GnRH* gene is species-specific in Atlantic salmon and brown trout, and can be applied for identification of these two species and their hybrids. Also, in connection with other diagnostic markers, F_1 and post- F_1 hybrids can be distinguished.

We thank Dr A. Ferguson, Dr R. Guyomard and F. Árnason for providing us with brown trout and Atlantic salmon samples. Financial support to R. Gross was provided by a scholarship from the Nordic Council of Ministers.

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