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Occurrence of length polymorphism and heteroplasmy in brown trout

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ABSTRACT

Length polymorphism was observed in the mitochondrial DNA (mtDNA) control region (CR) of brown trout (*Salmo trutta*). Length variability was due to the presence of an 81 or 82 bp tandem duplication segment located in the 3′ hypervariable domain of the mtDNA CR in specimens from two European river basins, in the northwestern Iberian Peninsula and in north-eastern Germany. Tandem repeated motifs were present in one to three extra copies. In the Miño river basin of the Iberian Peninsula some specimens revealed heteroplasmy. Such unique genetic structures of brown trout may provide useful information about the adaptive evolution of single populations and further explain the influences causing heteroplasmy.

1. Introduction

The mitochondrial DNA (mtDNA) is a circular double stranded molecule, which has widely been used as a genetic marker for phylogenetic studies, the analysis of population genetic structures and species delineation (e.g. Nielsen et al., 1998; Wang et al., 2007; Vera et al., 2010; Schenekar et al., 2014). The mtDNA is almost exclusively maternally inherited (Lee et al., 1995) and presumed to lack of recombination, which prevents the redistribution of mutations in descendants. Accumulations of such mutations on the same molecule then allow the mtDNA to be used as an efficient marker to identify different evolutionary traces (Guyomard, 1993). Furthermore, the higher mutation rate of the noncoding mtDNA control region (CR) compared to coding regions of the nuclear DNA, due to an inefficient replication repair, leads more frequently to variable number tandem repeats, duplications or rearrangements of genes (Wang et al., 2007; Jamandre et al., 2014).

The CR is a hypervariable, fast evolving, noncoding segment of the mitochondrial genome (Zhaoxia et al., 2010; Shi et al., 2013). In salmonids, the CR is generally located between the tRNA-*Pro* and tRNA-*Phe* genes and seems to regulate the replication and transcription of the mtDNA strands (Hurst et al., 1999, Zhaoxia et al., 2010, Wang et al., 2011a, 2011b). The CR is divided into three domains, a termination associated sequence (TAS) domain, a conserved central domain (CCD), and a conserved sequence block (CSB) domain (Wang et al., 2011a, 2011b; Terencio et al., 2012). The transition to DNA synthesis is supposed to occur in the region adjacent to the CSB-II (Jamandre et al., 2014).

Mitochondrial DNA length polymorphisms have previously been reported in various species of teleost fish including salmonid species (Nielsen et al., 1998, Sell and Spirkovski, 2004, Wang et al., 2011a, 2011b). Even though the mechanisms are not completely understood, tandem duplications differ from regular indels as the inserted segments are much bigger in these duplications, and furthermore, they contain the pattern of the repeated motifs in the respecting sequence just adjacent to the original (Sammeth and Stoye, 2006). However, heteroplasmy (single organisms having multiple mtDNA haplotypes) was described in extremely rare instances. Although often linked to diseases in the past (Grzybowski, 2000), heteroplasmy is currently known to occur also in healthy individuals. Generally, two types of heteroplasmy are reported: site heteroplasmy and length heteroplasmy (Vollmer et al., 2011), whereas length heteroplasmy is more frequently described (Brown et al., 1992; Nesbø et al., 1998).

In this study, we amplified and sequenced the mtDNA CR of brown trout from two river basins in Europe and provide first evidence of perfect tandem duplications (duplication segments that are to 100% identical) and length heteroplasmy in wild *S. trutta* populations. Tandem repeated motifs identified in this study are discussed and compared at both the intra- and interspecific level with similar structures of other salmonids.

Abbreviations: AD, Adriatic; AT, Atlantic; bp, base pair; CCD, conserved central domain; CR, control region; CSB, conserved sequence block; DA, Danubian; dG, Gibbs free energy (kcal/mol); dNTP, deoxyribonucleotide triphosphate; DU, Duero; *h*, haplotype diversity; H_2O, water; H_2O_{dd}, double distilled water; HSP, heavy strand promoter; LSP, light strand promoter; MA, Marmoratus; MgCl, magnesium chloride; mtDNA, mitochondrial DNA; mTF, mitochondrial transcription factor; NaCl, sodium chloride; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; TAS, termination associated sequence.

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2. Methods

2.1. Sampling

Brown trout were collected by electrofishing from two different regions in Europe, the Bubal River (Miño River basin), in the northwestern part of the Iberian Peninsula (42°31′N, 7°49′W Galicia, Spain) in 1988 and the Stepenitz River system (Stepenitz and Dümnitz in the Elbe basin), in north-eastern Germany (53°17′N, 12°5′E and 53°9′N, 12°11′E, Brandenburg, Germany) in 2014. The population of the Bubal River was isolated by several waterfalls, which prevent fish migration from downstream waters. In the Stepenitz system, fish migration between the upper reaches and the sea is possible. The area was moderately stocked with a resident strain of brown trout from the Fläming Heath region and is stocked with migratory sea trout, mainly from the Stör River system, since 1999. All donor populations belong to the Elbe basin. Fish were either immediately stored at −20 °C after being caught, and kept frozen until they were analysed or fin-clips were directly taken and stored in 99% ethanol until analysis.

2.2. DNA extraction, amplification and sequencing

DNA extraction, amplification and sequencing of 24 Iberian specimens were carried out according to Cortey and García-Marín (2002) with the modifications described by Sanz et al. (2006). DNA of 36 German specimens was extracted using a modified protocol after Aljanabi and Martinez (1997). Main modifications were: for homogenisation 450 μL salt extraction buffer, 100 μL SDS and 5 μL Proteinase K were used and 350 μL NaCl (5 M) was added. Centrifugation was done at 13,000 rpm. The last two centrifugation steps were performed at 6 °C and the pellets were resuspended in 50 μL sterile H_2O. PCR (Polymerase Chain Reaction) was performed with the Primers Str-H17 and Str-L19 (Bernatchez et al., 1992). The 20 μL reaction volumes contained 2 μL DNA Template, 2 μL GoFlexi-Buffer, 0.2 μL Go-Flexi-Taq Polymerase (5 U/μL), 14.3 μL H_2O_{dd}, 2 μL $MgCl_2$ (25 mM), 0.5 μL dNTP's (10 mM) and 1 μL of each Primer (10 μM). For the amplification an initial denaturation (94 °C) was followed by 30 cycles at 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1.5 min and a final elongation step at 72 °C for 10 min. Sequencing of the DNA strands of these samples was conducted in forward and reverse direction by Seq IT GmbH & Co.KG in Kaiserslautern, Germany. To exclude methodological artefacts and possible contamination, DNA was re-extracted and PCR amplification and sequencing were carried out twice for each specimen containing tandem repeat structures or showing heteroplasmy. The bands from the agarose gel (Fig. 1) were cut out and then sequenced separately to identify the exact length of the duplicated regions and the different patterns being present in the specimens.

2.3. Sequence analysis

Generated sequences were aligned using the program Geneious 6.0.6 and ranged between 963 bp (without duplications) to 1258 bp in length. Promoter elements (LSP and HSP) initiating the transcription (Wang et al., 2011a, 2011b), TAS elements and CSB's of the CR were localised by aligning the sequences. For comparison, reference sequences of *S. trutta* and other salmonids were used from the genetic sequence database GenBank. Haplotype (*h*) diversity was estimated via DNAsp v 5.10.1 (Librado and Rozas, 2009).

2.4. Secondary structure

Secondary structure prediction and folding analysis were carried out with the program mfold (Zuker, 2003) as secondary structures are supposed to be involved in the formation and maintenance of tandem repeat structures. Three calculations were performed on (1) the entire generated CR sequence including and excluding the perfect repeat motifs, (2) only the perfect repeat segments, and (3) degenerated imperfect repeat motifs.

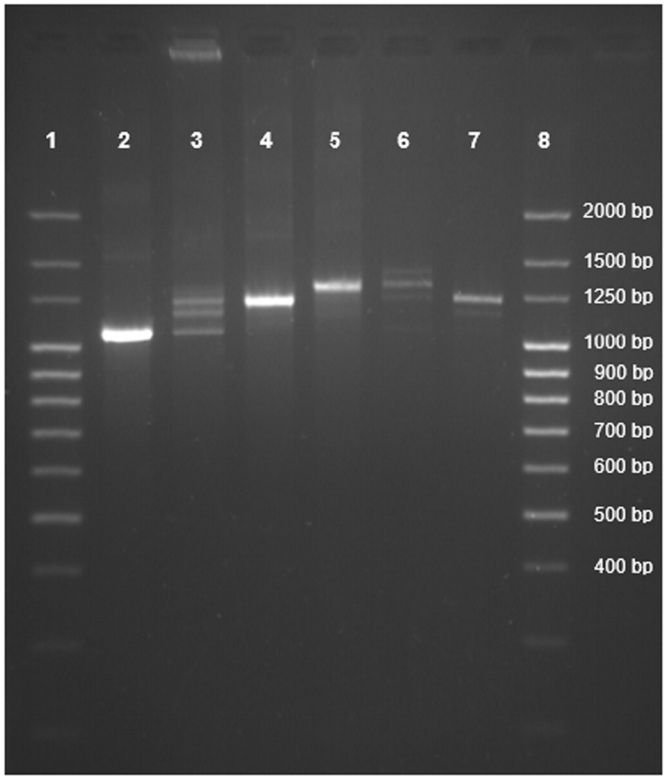

Fig. 1. Length polymorphism in brown trout mtDNA control region. Weight markers are displayed in lane 1 and 8. Standard length control region is placed in the second lane. Lane 3 shows a heteroplasmic individual with standard length plus one and two repeat haplotypes. Lane 4 to 7 show individuals with haplotypes having two and lane 5 and 6 having three tandem repeats.

3. Results

3.1. mtDNA haplotypes

All 60 specimens belonged to the Atlantic (AT) lineage (Bernatchez, 2001). Most of the specimens of the German population exhibited haplotypes generally reported for the central European area (Table 1). In addition, eight specimens revealed newly identified haplotypes. Of

Table 1
MtDNA CR haplotypes and haplotype diversity (*h*) of the Iberian and German brown trout populations.

		Population	
Haplotype	Acc. No.	Iberian	German
H1 (At1a)	AF273086	1	3
H2 (At1b)	AF273087	–	7
H3 (At1d)	AF274574	–	10
H4 (At1c)	AF274575	–	4
At1l	AF253542	–	2
At1r	HQ848361	–	1
At1aa	KT360971	–	1
At1bb	KX987148	–	7
DupA	KT360973	–	1
DupB	KT360974	–	1
DupC	KT360975	–	1
Dup1	EF530536	5	–
Dup2	EF530537	19	–
Dup3	EF530538	8	–
N		33	36
h		0.605	0.752