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The use of molecular markers for the characterisation and rehabilitation of indigenous trout populations in the Central Alpine region

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Abstract

The brown trout (*Salmo trutta* L.) represents one of the most differentiated vertebrate species. However, human activities such as habitat alteration, overexploitation and introgression of non-native hatchery fish led to an alarming reduction of population variability. This is particularly evident for vast parts of the highly fragmented alpine area. Because of these facts the Interrreg IIIA-project "Trout-Examinvest" was initiated in order to achieve the following goals:

- Genetic characterisation of local trout populations
- Identification of potential autochthonous populations
- Establishment of indigenous hatchery strains for conservation management

In the framework of our project two molecular techniques were applied: (i) sequence analysis of the complete mitochondrial DNA control region and (ii) analyses of a number of variable microsatellite DNA loci. As was shown in previous studies, mitochondrial DNA revealed to be a useful tool in the screening of frequencies and distribution patterns of the major trout lineages. On the other hand, microsatellite DNA data delivered more detailed information about within-population genetic diversity and population structure as well as about hybridisation between native and introduced trout lineages. Based on these findings we point to the necessity of using a combined approach of molecular analyses to select and establish indigenous trout breeding strains for future stocking and repopulation measures.

Keywords

Brown trout, Marble trout, *Salmo trutta* L., *Salmo (trutta) marmoratus* C., microsatellite DNA, mitochondrial DNA

Introduction

The brown trout, *Salmo trutta* L., represents a typical element of the Eurasian fluviatile fauna. It is characterised by high degrees of morphological and ecological plasticity including landlocked as well as migratory life-history forms. In the last decades many local populations have been affected by human influences, such as habitat degradation, overexploitation and the introduction of domesticated hatchery reared trout strains. The severe threat status of a number of brown trout populations throughout Europe was underlined in several studies based on ecological and genetic data (e.g. Garcia-Marin et al. 1991; Ruzzante et al. 2001). Despite the fact that fish species in the alpine region are often restricted to island-like habitats and are thus particularly vulnerable to anthropogenic disturbances, there is only limited information about the genetic composition and the actual endangerment of local trout populations in this area (Weiss et al. 2001; Duftner et al. 2003). For this reason the Interreg IIIA-project "Trout-Examinvest" was started as a common initiative of the Research Centre for Agriculture and Forestry Laimburg (South Tyrol, ITA), the Department of Zoology and Limnology at the University of Innsbruck (AUT), the Alpenzoo Innsbruck (AUT) and the Nationalpark Hohe Tauern (AUT). The main goals of the sub-project being carried out at the Research Centre Laimburg are to (i) genetically characterise the local trout populations in the Italian province of South Tyrol (marble and brown trout) and the Austrian provinces of Tyrol, Salzburg and Carinthia (brown trout only), (ii) to identify potential autochthonous trout populations and (iii) to give scientific support for the establishment of indigenous hatchery strains for future rehabilitation programs.

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The use of molecular markers for conservation management of local trout populations

In the present study two different molecular approaches have been applied: sequence analysis of the entire mitochondrial DNA control region and analysis of variable microsatellite DNA loci.

Analysis of mitochondrial DNA is widely used in phylogenetic, phylogeographic and population genetic studies because of its relatively high degree of variability, the absence of recombination and its rather simple applicability (reviewed in MORITZ 1994). Previous studies analysing highly variable regions of the mitochondrial genome of the brown trout identified five major mitochondrial DNA lineages distributed in different areas of Europe (reviewed in BERNATCHEZ 2001). These studies also demonstrated the impact of fish translocation on local trout populations, which was either caused by unintentional escapes from fish farms or by stocking activities with domesticated trout strains (e.g.: GIUFFRA et al. 1994; HANSEN et al. 2000). Similar findings were also made in our study area in the Central Alpine region, where most of the analysed populations displayed a variable number of individuals with mitochondrial DNA haplotypes belonging to the non-indigenous Atlantic clade, of which some were characteristic for hatchery reared trout strains. In contrast, we identified few unaffected populations in remote creeks of the Northern Alps (see also DUFTNER et al. 2003). Nevertheless, mitochondrial DNA sequence data were an insufficient tool to assess possible hybridisation events between native and introduced trout lineages, since mitochondrial DNA is strictly maternally inherited and thus gives no information about the paternal ancestor (DEGNAN et al. 1993; AVISE 1998).

Therefore, it is necessary to analyse nuclear markers in parallel. Microsatellites are simple tandemly repeated DNA sequence elements exhibiting a high degree of variability based on differences in allele size (i.e. number of repeats per allele). Currently, these markers are widely applied for the identification of individuals, for parentage and pedigree analyses as well as population genetic studies. In addition, microsatellite data were shown to be an efficient tool for addressing past and recent hybridisation events (HANSEN et al. 2001). In our study the analysis of allele frequencies investigated at twelve microsatellite loci by using a Bayesian individual assignment approach (PRITCHARD et al. 2000) revealed varying degrees of genetic introgression of non-indigenous trout lineages into native populations. However, by combining microsatellite and mitochondrial DNA data with information about the colour patterns of single fishes, it was possible to identify pure autochthonous individuals even within hybridised marble trout populations.

Without doubt, the use of a combined approach including different molecular markers and sophisticated data analysis methods will serve as an important tool for the elaboration of conservation management strategies. However, in order to ensure long-term survival of indigenous trout populations, solely scientific efforts will not be sufficient. Therefore, genetic assessments should be part of a broader management framework, involving fisheries practitioners as well as policy-making institutions and local conservation initiatives.

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