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The selection of the 'wild': A combined molecular approach for the identification of pure indigenous fish from hybridised populations ☆

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Abstract

The identification of pure indigenous fish from hybridised populations represents a key issue in fisheries management and conservation biology. In the present study an approach for selection of purebred marble trout (*Salmo trutta marmoratus* C.) individuals out of admixed populations was set up and assessed. In a first step, baseline data sets of pure marble trout and pure brown trout specimens based on twelve microsatellite loci were used to simulate five consecutive generations of admixture. The baseline and the resulting simulation data sets were then combined with data of a 'real' hybridised marble trout population to perform a single individual assignment test as implemented in STRUCTURE. By this procedure the assignment approach was calibrated and it was possible to compare admixture coefficients obtained for individuals from different populations. The ranking of individual admixture coefficients on a plot and comparison with simulated data revealed that the test population was composed of pure marble trout individuals, first generation hybrids between marble trout and brown trout, and hybrid backcross specimens between both groups. However, by defining a critical *q*-value of 0.1 and additionally integrating individual sequence data of the mtDNA control region, it was possible to indicate individuals, which could be selected for the establishment of a pure marble trout strain.

Keywords: Brown trout; Hybridisation; Individual assignment test; Introgression; Marble trout; *Salmo trutta*; *Salmo trutta marmoratus*

1. Introduction

Over 40% of all investigated world's fish species are listed as endangered and many have already become extinct (IUCN, 2004). A restricted natural range, diseases, habitat alterations, over-exploitation and finally the introduction of non-native species and/or populations can be seen as the five major categories of threats to fish (Ono et al., 1983). Introductions and translocations of fish have been shown to heavily compromise the genetic integrity of recipient native fish populations by displacing locally adapted gene pools (Ryman et al., 1995). In the worst case introgression can lead to 'genomic extinction' — the disappearance of a species' population or genetic lineage (Epifanio and Philipp, 2001). Thus, although thought to sustain declining wild fish populations, stocking can present a serious threat when carried out with fish of allochthonous origin.

Many indigenous fish populations are locally restricted, suffer from low genetic variability and show a high degree of interbreeding with stocked gene pools. The development of a reliable and efficient method for identification of 'pure' wild fish specimens out of already introgressed populations is thus one of the major challenges. These specimens could then be used as founders for the establishment of autochthonous breeding strains for restoration of wild populations.

In some cases morphological or meristic characters may be useful for selection, but often they have a polygenic basis and are heavily influenced by environmental factors (Allendorf et al., 1987). In addition, morphological traits are not always unambiguous, especially not beyond the species level. For this reason hypervariable microsatellite markers combined with

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new statistical approaches, such as individual assignment tests, have become the method of choice for identification of indigenous populations or individuals, and studying genetic introgression between wild and stocked populations (Cornuet et al., 1999; Pritchard et al., 2000; Hansen, 2002; Susnik et al., 2004).

The marble trout, *Salmo trutta marmoratus* C., represents one of the five major lineages of *Salmo trutta* and shows a restricted distribution range within the Adriatic basin (Northern Italy and Slovenia). This lineage used to be the predominant salmonid fish of Southern Alpine running waters (Sommani, 1961). However, during the last decades, most indigenous marble trout populations were heavily introgressed by hatchery reared brown trout, all belonging to the Atlantic lineage (Giuffra et al., 1996; Berrebi et al., 2000; Snoj et al., 2000; Meraner et al., 2007). Thus, conservation and fisheries practitioners have to be provided with a reliable tool for selection of purebreds out of hybridised populations. Therefore, the main objective of the present study was to set up and assess an approach in order to address this important issue.

2. Materials and methods

2.1. Populations studied

In the present study, three different categories of data sets were used: baseline data sets, simulation data sets, and a test data set.

2.1.1. Baseline data sets

The specimens for the baseline data sets were collected from trout populations without hybridisation. One population was the pure marble trout from the Predelica River (PRE), a tributary of the Soca drainage system in Slovenia. Recently it was shown that this population represents one of the eight pure marble trout populations known so far (Berrebi et al., 2000; Fumagalli et al., 2002). DNA extracts were kindly provided by Ales Snoj (University of Ljubliana, Snoj et al., 2000). The second population was a pure brown trout population sampled in the Tierserbach (TIE), a small tributary of the Adige/Etsch in South Tyrol (Northern Italy). This brook has been stocked intensively with hatchery reared brown trout for several decades (H. Grund, personal communication) (for details see Table 1).

2.1.2. Simulation data sets

The two baseline data sets where used for the generation of simulation data sets of the generations G0, G1, and G5 (for details see below).

2.1.3. Test data set

The samples for the test data set were taken from the Rienza/Rienz River (RIE), another tributary of the Adige/Etsch drainage in South Tyrol (Table 1). The Rienza/Rienz River was originally populated by marble trout, and in the past has been stocked with hatchery reared brown trout. Nowadays, individuals with characteristic phenotypic traits of both marble and brown trout as well as hybrids with intermediate characters can be found. Our data set included typical phenotypic marble trout specimens as well as fishes displaying additional red spots, since no clear consensus exists whether this trait is unique for brown trout and its hybrids, or it also lies within the spectrum of phenotypic polymorphism of pure marble trout (Delling et al., 2000). To make simulation and test samples comparable, and to always apply the same baseline, the RIE data set was combined with the TIE baseline data set for calculation of possible Hardy–Weinberg deviations, the inbreeding coefficient and the admixture parameter α (data set defined as: RIE+TIE).

2.2. Molecular techniques

Total nucleic acid was extracted from fin tissue following a standard salting-out procedure (Bruford et al., 1998). The complete mtDNA control region of 61 individuals was amplified using primer combinations L19 and Troutdlp2H (Bernatchez et al., 1992; Duftner et al., 2003) as well as Troutdlp2L and HN20 (Bernatchez and Danzmann, 1993; Duftner et al., 2003) in 20 µL reaction volumes containing 1 U HotMaster Taq DNA Polymerase (Eppendorf, Hamburg, Germany), 1× HotMaster Taq Buffer with Mg^{2+} (2.5 mM, Eppendorf), 0.25 µM of each primer, 0.2 mM dNTP Mix (Eppendorf) and approximately 50 to 70 ng of template DNA. Amplification was performed in a Mastercycler Gradient (Eppendorf) using one initial denaturation step at 94 °C for 2 min, followed by 38 cycles of template denaturation at 94 °C for 20 s, primer annealing at 52 °C for 20 s, primer extension at 65 °C for 45 s and a final extension at 65 °C for 4 min. PCR products were purified using Montage PCR Filter Units

Table 1
Samples of *Salmo trutta* investigated in the present study

Sampling locality	Code	River system	Coordinates		Status of population	Sample size	*A*	H_E	H_O
			Latitude	Longitude					
Predelica (Slovenia)	PRE	Soca	[a]	[a]	Pure marble trout population	15	2.17 (±1.27 SD)	0.21	0.25
Tierserbach (South Tyrol, N-Italy)	TIE	Adige/Etsch	46° 28' N	11° 31' E	Brown trout of hatchery-origin	21	8.00 (±5.05 SD)	0.72	0.62
Rienza/Rienz (South Tyrol, N-Italy)	RIE	Adige/Etsch	46° 49' N	11° 43' E	Marble trout population stocked with hatchery-reared brown trout	25	8.17 (±4.61 SD)	0.60	0.54

Mean values (over all 12 microsatellite loci) are indicated for the number of alleles per locus (*A*), as well as the expected (H_E) and observed (H_O) heterozygosity.
[a] Exact position of the sampling site can be obtained from Fig. 1 in Fumagalli et al. (2002) (DNA extracts from the Predelica population were kindly provided by A. Snoj; Snoj et al., 2000).