Introduction

The tench (*Tinca tinca*) is a freshwater fish native to Europe and Asia, now widespread in all continents except Antarctica, so that it can be considered a sub-cosmopolitan species. Traditionally farmed in co-culture with common carp (*Cyprinus carpio*), in recent years the tench is gaining attention, especially in Central and Eastern European countries, for the increasing interest in diversifying the productions (Kohlmann et al., 2007).

In Italy, most of the tench populations are wild and live in many rivers and lakes. The only exception is a population called *Tinca Gobba Dorata del Pianalto di Poirino* (Golden humped tench of Poirino highland) (PO), which has long been farmed in monoculture in the hundreds of artificial ponds distributed all over the large plain of clayey soils between Turin, Asti and Cuneo provinces, in the Piedmont region. This population has always been very important in the local economy; for example, it is documented that in the 13th century tench were used to pay taxes as well (Julini and Zoccarato, 2000). After a decline during the last century, with many ponds neglected, the *Tinca Gobba Dorata* is gaining a renewed interest within the rediscovery and qualification of traditional products (Gasco et al., 2001).

As for the morphology, this variety differs from the wild type for the presence of the characteristic dorsal sail gibbosity and the goldish coat, probably due to the red colour of the soil. As for the organoleptic traits, its meat is especially appreciated for the delicate flavour, lacking the *mud* aftertaste typical of the species. For its quality it fetches a quite high price, compared to the wild type. Many initiatives have been undertaken to promote this important niche product: it is one of the Slow Food presidia, aimed at protecting the producers and preserving the product quality (Slow Food, 2010); moreover, it has been included in the *Basket of the typical products of the Turin province*, a trademark created to safeguard agricultural products with recognized organoleptic, technological and historic distinctiveness (Province of Turin, 2010); finally, it has obtained the Protected Designation of Origin (PDO) recognition (European Commission, 2008).

If some data exist on morphometric and slaughtering traits, as well as on fillet chemical composition (Gasco et al., 2007; 2010), genetic data on the PO are still lacking. Therefore, the aim of this study was to describe its genetic structure, using microsatellite markers, and to compare it with other tench populations living in Italy.

Abstract

The *Tinca Gobba Dorata del Pianalto di Poirino* (Golden humped tench of Poirino highland, [PO]), the only Italian fish with the Protected Designation of Origin, was characterized by seven microsatellites and compared to three wild populations living in Italian lakes (Valagola [VA]; Trasimeno [TR]; Bolsena [BO]). The PO population showed high variability values (number of effective alleles: 2.70 vs. 1.62 to 2.20; expected heterozygosity: 0.49 vs. 0.29 to 0.40). The analysis of between-population differentiation indicated that PO significantly differed from the others (FST = 0.039 to 0.097, P<0.05), while BO and TR were the most similar, consistently with their geographic proximity. The Neighbour-Joining tree revealed a clear separation between Northern and Central populations, with a bootstrap support of 97%. The population differentiation was reflected by the results of the assignment test, with 64% to 92% of the individuals correctly assigned to the original population, and a probability ranging from 76% to 95%. No individuals belonging to other populations were erroneously assigned to PO. A more detailed analysis of the PO population showed a similar genetic variability within the 15 considered ponds and a low degree of differentiation between ponds, with the exception of one “historical” pond, which significantly differed from most of the others, thus deserving to be preserved. The results indicate that the PO, despite being farmed, has a high level of within-population diversity and is greatly differentiated from the other populations considered. The possibility of applying the assignment test in the framework of the product traceability deserves further investigation.
Results and discussion

In absence of information on the genomic location of the considered loci, the linkage disequilibrium was tested and all the markers were found to be independent; so, the significant linkage disequilibrium between 2MT-2 and 2MT-6 loci, observed by Lajbner et al. (2009) using various methods, including the one adopted in the present study, was not confirmed. Moreover, none of the deviations from the Hardy-Weinberg equilibrium, estimated for each locus -population pair, were significant, thereby suggesting that the 7 microsatellites were neutral markers. Therefore, all loci were considered suitable for the population analysis.

A total of 49 alleles were found, with 2 of them not yet described, 242 at 2MT-2, which is private for PO, and 213 at 2MT-3 locus, which is private for VA. Considering that 66 alleles have been reported by Kohlmann et al. (2009), the total number of alleles found in tench so far comes to 68. The mean number of alleles per locus was 7.00, ranging from 2 (2MT-3) to 17 (2MT-9), which was confirmed as the most polymorphic marker (Table 1).

2MT-3 was polymorphic only in PO, confirming its low variability, already reported by Kohlmann et al. (2009), who found 16 populations out of 21 monomorphic. The number of the effective alleles was in general much lower, for the very low frequency of many alleles.

As for the within-population variability (Table 2), a wide range was observed for the mean number of observed alleles (2.29 to 6.00), mainly due to the high value of PO, where seventeen alleles not found in the other three populations were observed, as a consequence of the larger sample examined. However, as these alleles had frequencies lower than 0.03, the range for the effective number of alleles was limited. A higher genetic variability, estimated by the heterozygosity values, was observed in PO and VA, compared to BO and TR.

The observed heterozygosity was higher than the expected one in all the populations, except for PO. Consistently, the FST statistics showed for PO a significant deficit of heterozygotes; as the PO samples were collected from different ponds, the heterozygote deficiency could depend on the substructuring of the population more than on inbreeding. However, the whole genotypic distributions were in Hardy-Weinberg equilibrium for all the populations.

The variability detected for the Italian populations was of the same magnitude order reported for tench (Kolmann et al., 2009) and other freshwater fish (DeWoody and Avise, 2000). These authors carried out a survey on 524 microsatellite loci in about 40,000 individuals of 78 species and reported for freshwater fish a mean heterozygosity value of 0.46±0.34 at population level and 0.54±0.25 at species level.

The FST statistics indicated that PO was significantly different from the other populations (Table 3).

In general, the situation highlighted by D\textsubscript{A} and FST was similar; the lowest genetic distance was observed for the BO-TR pair, consistently with their geographic proximity; similarly, the FST showed that the only not significant comparison was the one between the same two
The Neighbour-joining tree, constructed on the basis of the $D_A$ genetic distance, which is the most efficient in obtaining a correct branching pattern (Takezaki and Nei, 1996; 2008), revealed a clear separation between Northern and Central populations, with a bootstrap support of 97% (Figure 1).

On the basis of the assignment test, 63.6% to 92.3% of the individuals were correctly assigned to the original population, with a probability ranging from 76.4% to 95.1% (Table 4). The lowest values were observed for BO and TR populations, for which the highest number of reciprocal wrong assignment were obtained, consistently with their low degree of differentiation. Considering the limitations due to the low sample size, the satisfactory results can be ascribed to the high level of differentiation between the populations. In fact, the results of a study on horse breeds (Bjørnstad and Roed, 2002) demonstrated that a precision of assignment around 95% can be obtained with 6 microsatellites, when the breed differentiation has an intermediate value ($0.08 \leq F_{ST} < 0.14$). For other tench populations Kohlmann et al. (2009) found a mean of 69.3% of individuals correctly classified and a precision of assignment lower that the lowest value obtained in the present study (26-60% ex. 64%) for 8 populations out of 21, even if with higher sample size.

A result seems worth underlying for its practical implications: no individuals belonging to other populations were erroneously assigned to PO population; this preliminary result suggests the need for further investigating the possibility to apply the assignment test in the framework of the product traceability, also in the light of the frequent imports of tench from abroad. This would be especially important to protect the PDO PO, which is mainly marketed abroad. This would be especially important to protect the PDO PO, which is mainly marketed abroad. This would be especially important to protect the PDO PO, which is mainly marketed abroad. This would be especially important to protect the PDO PO, which is mainly marketed abroad. This would be especially important to protect the PDO PO, which is mainly marketed abroad. This would be especially important to protect the PDO PO, which is mainly marketed abroad. 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