PAPER

Tinca Gobba Dorata del Pianalto di Poirino: genetic characterization by microsatellite markers

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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.

Introduction

The tench (*Tinca tinca L.*) 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 L.*), 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 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.

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Key words: *Tinca tinca*, *Tinca Gobba Dorata del Pianalto di Poirino*, Genetic characterization, Microsatellites.

Acknowledgments: the authors are very grateful for the assistance in sample collection to Pianalto's tench producers, Aquaprogram s.r.l., Fishery Cooperative of Bolsena Lake, Massimo Lorenzoni and Deborah Celauro.
The study was supported by the Turin University grant ex-60%.

Received for publication: 2 September 2010.
Revision received: 9 October 2010.
Accepted for publication: 5 November 2010.

Italian Journal of Animal Science 2010; 9:e85
doi:10.4081/ijas.2010.e85

Materials and methods

The investigation was carried out on 217 PO, belonging to 15 ponds; three wild populations living in different areas of Italy were also included in the study, for a total of 45 individuals (Valagola lake, VA: 13; Trasimeno lake, TR: 10; Bolsena lake, BO: 22). The whole PO sample was used for a detailed analysis of the population, while a random sub-sample of 55 individuals, representative of the entire farming area, was used for the comparison with the other populations.

From fish caught by electrofishing a small piece of dorsal fin was collected and preserved in ethanol at -20°C. Total genomic DNA was extracted using the NucleoSpin Tissue kit (Macheray-Nagel, Düren, Germany).

The nine microsatellite *loci* (*MTT-1* to *MTT-9*) described by Kohlmann and Kersten (2006) were considered. As a previous study on 21 tench populations indicated that the *MTT-4* and *MTT-7 loci* were monomorphic (Kohlmann *et al*., 2009), about 50 samples were preliminary tested for all the microsatellites and still no polymorphism was observed for these two *loci*; therefore the subsequent analyses were performed only for the 7 polymorphic microsatellites (*MTT-1, MTT-2, MTT-3, MTT-5,*

MTT-6, MTT-8, MTT-9), as described in Kohlmann and Kersten (2006).

The Hardy-Weinberg equilibrium for each *locus*-population pair and for each population across *loci* was tested by the exact test (Guo and Thompson, 1992) implemented in Genepop 4.0 (Rousset, 2008); the same software was also used to estimate the pairwise genotypic associations among *loci* pairs across populations, computing a G test by the Markov chain algorithm of Raymond and Rousset (1995).

Allele frequencies, observed and effective number (Kimura and Crow, 1964) of alleles per *locus*, as well as observed and expected heterozygosity were computed using the Popgene software version 1.32 (Yeh *et al*., 1999).

F_{IS} statistics for each breed across *loci* were computed with the FSTAT software (Goudet, 2002), testing the statistical significance with permutation tests. The FSTAT software was also used to evaluate the between-breeds differentiation by the pairwise F_{ST}. The sequential Bonferroni correction (Rice, 1989) was applied to correct for the effects of multiple tests. In addition, the D_A distances proposed by Nei *et al*. (1983) were calculated and used to construct the Neighbour-Joining tree, as implemented in the DISPAN software (Ota, 1993); the robustness of the tree topology was tested by bootstrapping analysis (1000 replicates). The GeneClass2 software (Piry *et al*., 2004) was employed for population assignment, using the Bayesian option (Cornuet *et al*., 1999).

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 *MTT-2* and *MTT-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 *MTT-2*, which is private for PO, and 213 at *MTT-5 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 (*MTT-3*) to 17 (*MTT-9*), which was confirmed as the most polymorphic marker (Table 1).

MTT-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 F_{IS} 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 to 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 F_{ST} statistics indicated that PO was significantly different from the other populations (Table 3).

In general, the situation highlighted by D_A and F_{ST} was similar: the lowest genetic distance was observed for the BO-TR pair, consistently with their geographic proximity; similarly, the F_{ST} showed that the only not significant comparison was the one between the same two

Table 1. Descriptive statistics of the markers used.

Locus	Size range, bp	Na	Ne	Ho	He
MTT-1	167-177	5	4.32	0.71	0.77
MTT-2	236-242	4	1.10	0.09	0.09
MTT-3	148-160	2	1.03	0.03	0.03
MTT-5	207-215	5	1.57	0.38	0.36
MTT-6	160-176	7	2.05	0.47	0.51
MTT-8	178-236	9	1.88	0.44	0.47
MTT-9	130-182	17	6.56	0.83	0.85
Mean (SD)		7.00 (4.93)	2.64(2.05)	0.42 (0.29)	0.44(0.31)

Na, number of observed alleles; Ne, number of effective alleles; Ho, observed heterozygosity; He, expected heterozygosity.

Table 2. Within-population variability.

Population	Na	Ne	Ho	He	FIS	P_{HW}
BO	3.29	1.73	0.31	0.30	-0.047	0.57
PO	6.00	2.70	0.44	0.49	0.108*	0.36
TR	2.29	1.62	0.31	0.29	-0.103	1.00
VA	3.14	2.20	0.44	0.40	-0.101	1.00
Mean	3.68	2.06	0.38	0.37		
SD	1.61	0.49	0.08	0.09		

Na, number of observed alleles; Ne, number of effective alleles; Ho, observed heterozygosity, He, expected heterozygosity; BO, Bolsena; PO, *Tinca Gobba Dorata;* TR, Trasimeno; VA, Valagola; SD, standard deviation. *P<0.05.

Table 3. Genetic relationships between populations: F_{ST}, (above the diagonal), D_A (below the diagonal).

	BO	PO	TR	VA
BO	-	0.089*	0.000	0.104*
PO	0.103	-	0.097*	0.039*
TR	0.043	0.170	-	0.088*
VA	0.140	0.101	0.154	-

BO, Bolsena; PO, *Tinca Gobba Dorata;* TR, Trasimeno; VA, Valagola. *P<0.05.

populations. On the other hand, the high degree of genetic similarity between BO and TR had already been detected by the analysis of the mtDNA polymorphism (Lo Presti *et al*., 2010). The Neigbour-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 Røed, 2002) demonstrated that a precision of assignment around 95% can be obtained with 6 microsatellites, when the breed differentiation has an intermediate value ($0.08>F_{ST}>0.14$). 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% *vs.* 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 as processed food, in order to avoid fraudulent misdescription of food contents on product labels, which is a widespread problem, particularly with high added-value products (Woolfe and Primrose, 2004).

A more detailed analysis of the PO population, based on the 217 individuals sampled, showed a similar genetic variability within the 15 ponds they were coming from, with mean values of 3.94 (SD 0.44) and 2.39 (SD 0.23) for the number of observed and effective alleles respectively; the same mean values were obtained for the observed and the expected heterozygosity, 0.44 (SD 0.04). Interestingly, in the few cases of data diverging from the general situation, the genetic results were consistent with information obtained during the sample collection. For example, the highest values for most of the diversity indices (Na=4.83; Ne=2.96; He=0.51) were observed for a pond whose owner is used to get fries from all over the area, thus increasing the existing variability; on the other hand, the lowest observed heterozygosity (0.35) was obtained for a pond neglected for more than 20 years, where very few individuals were found during the sampling, which had been interpreted as a sign of a critical situation. In general, the values obtained for PO are comparable with those obtained for many wild populations, indicating that the farming in the Poirino area did not induce the reduction of variability often observed in other species, including carp (*Cyprinus carpio*), which has a longer history of domestication (Kohlmann *et al*., 2005). On the other hand, the PO tench is farmed under an extensive breeding system, with the almost complete absence of selection and artificial reproduction, so that it can be considered at an intermediate level between wild and cultured populations.

A low degree of genetic differentiation between the 15 ponds was also observed, with 90% of pairwise comparisons being not significant. The only exception was one "historical" pond, which significantly differed from most of the others (10 significant comparisons out of 14), confirming its genetic originality, already highlighted by the analysis of mitochondrial DNA polymorphism and possibly corresponding to a different maternal evolutionary lineage (Lo Presti *et al*., 2010); therefore, this is the pond which mainly contributes to the genetic variability of the *Tinca Gobba Dorata*. This situation was reflected on the assignment of the individuals to the original pond: only the 'historical' pond had a moderate percentage of corrected assignments (65%), with a mean probability of 91%; a lower accuracy was obtained for the other ponds, with a mean percentage of correct assignments of 20%, as expected from the low genetic differentiation.

Table 4. Assignment test.

Original population	n.	Individuals assigned to BO	PO	TR	VA	Individuals correctly assigned, %	Mean P of assignment, %
BO	22	14	-	6	2	63.6	85.0
PO	55	3	50	1	1	90.9	90.7
TR	10	1	-	9	-	90.0	76.4
VA	13	-	-	1	12	92.3	95.1

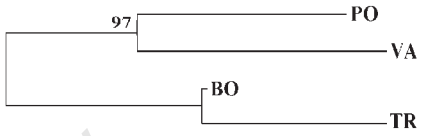

Figure 1. Neighbour-joining tree. BO, Bolsena; PO, *Tinca Gobba Dorata*; TR, Trasimeno; VA, Valagola.

Conclusions

The present investigation is the first contribution to the detailed genetic description of the PO. The data revealed a high level of within-population diversity, in some cases even higher than that of wild Italian and European populations. Therefore, the limited area it lives in and the farming practices have not reduced its variability, which is maintained also in the single ponds. Moreover, the genetic information allowed identifying one of the ponds as mainly contributing to the population variability, thus deserving to be preserved.

The PO was also significantly differentiated from the other Italian populations considered, which was reflected on the high individual assignment accuracy. This could possibly be exploited for the product traceability, in order to protect the PDO label.

References

Bjørnstad, G., Røed, K.H., 2002. Evaluation of factors affecting individual assignment precision using microsatellite data from horse breeds and simulated breed crosses. Anim. Genet. 33:264-270.

Cornuet, J.M., Piry, S., Luikart, G., Estoup, A., Solignac, M., 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153:1989-2000.

De Woody, J.A., Avise, J.C., 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other

animals. J. Fish Biol. 56:461-473.
European Commission, 2008. Commission Regulation No 160/2008 of 21 February 2008 registering certain names in the Register of protected designations of origin and protected geographical indications (Pane di Matera (PGI), Tinca Gobba Dorata del Pianalto di Poirino (PDO)). In: Official Journal, L 48, 22/2/2008, pp 27-28.
Gasco, L., Zoccarato, I., Lussiana, C., Azzi, L., Julini, M., 2001. Valorizzazione del territorio attraverso l'allevamento della tinca: il caso del Pianalto di Poirino (Piemonte). In: G.F. Greppi and G. Enne (eds.) "Prodotti di origine animale: qualità e valorizzazione del territorio". MG Publ., Milano, Italy, pp 109-114.
Gasco, L., Sabia, C., Lussiana, C., Guo, K., 2007. Morphometric characteristics of "Tinca Gobba Dorata del Pianalto". Ital. J. Anim. Sci. 6(suppl.1):800-802.
Gasco, L., Gai, F., Lussiana, C., Lo Presti, R., Malfatto, V., Daprà, F., Zoccarato, I., 2010. Morphometric, slaughtering performances, chemical and fatty acid composition of the protected designation of origin "Golden hump tench of Poirino highland" product. Rev. Fish Biol. Fisheries 20:357-365.
Goudet, J., 2002. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3.2). Home page address: http://www2.unil.ch/popgen/ softwares/fstat.htm
Guo, S.W., Thompson, E.A., 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361-372.
Julini, M., Zoccarato, I., 2000. Le tinche di Ceresole d'Alba. In: L. Bertello (ed.) "Le terre rosse". Associazione Artistico-culturale del Roero Astisio, Bra (CN), Italy, pp 31-55.
Kimura, M., Crow, J.F., 1964. The number of alleles that can be maintained in a finite population. Genetics 49:725-738.
Kohlmann, K., Kersten, P., 2006. Microsatellite loci in tench: isolation and variability in a test population. Aquacult. Int. 14:3-7.
Kohlmann, K., Kersten, P., Flajšhans, M., 2005. Microsatellite-based genetic variability and differentiation of domesticated, wild and feral common carp (Cyprinus carpio L.) populations. Aquaculture 247:253-266.
Kohlmann, K., Kersten, P., Flajšhans M., 2007. Comparison of microsatellite variability in wild and cultured tench (Tinca tinca). Aquaculture 272:S147-S151.
Kohlmann, K., Kersten, P., Panicz, R., Memis, D., Flajšhans, M., 2009. Genetic variability and differentiation of wild and cultured tench populations inferred from microsatellite loci. Rev. Fish Biol. Fisher. 20:279-288.
Lajbner, Z., Kohlmann, K., Linhart, O., Kotlìk, P., 2009. Lack of reproductive isolation between the Western and Eastern phylogroups of the tench. Rev. Fish Biol. Fisher. 20:289-300 .
Lo Presti, R., Gasco, L., Lisa, C., Zoccarato, I., Di Stasio, L., 2010. PCR-RFLP analysis of mitochondrial DNA in tench (Tinca tinca L.). J. Fish Biol. 76:401-407.
Nei, M., Tajima, F., Tateno Y., 1983. Accuracy of estimated phylogenetic trees from molecular data. J. Mol. Evol. 19:153-170.
Ota, T., 1993. DISPAN: Genetic distance and phylogenetic analysis. Pennsylvania St. Univ., University Park, PA, USA. Home page address:. ftp://ftp.bio.indiana.edu/molbio/ibmpc
Piry, S., Alapetite, A., Cornuet, J.-M., Paetkau, D., Baudouin, L., Estoup, A., 2004. GeneClass2: a software for genetic assignment and first-generation migrant detection. J. Hered. 95:536-539.
Province of Turin, 2010. Home page address: http://www.provincia.torino.it/agrimont/sapori/tipici
Raymond, M., Rousse, F., 1995. An exact test for population differentiation. Evolution 49:1283-1286.
Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43:223-225.
Rousset, F., 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol. Ecol. Resources 8:103-106.
Slow Food, 2010. Home page address: http://www.presidislowfood.it
Takezaki, N., Nei, M., 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144:389-399.
Takezaki, N., Nei, M., 2008. Empirical tests of the reliability of phylogenetic trees constructed with microsatellite DNA. Genetics 178:385-392.
Woolfe, M., Primrose, S., 2004. Food forensics: Using DNA technology to combat misdescription and fraud. Trends Biotechnol. 22:222-226.
Yeh, F.C., Yang, R., Boyle, T., 1999. Population genetic analysis. Home page address: http://www.ualberta.ca/~fyeh