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RESEARCH PAPER

Microsatellite-Based Genetic Diversity and Admixture History of Rainbow Trout (*Oncorhynchus mykiss* – Walbaum, 1792) Stocks in Trentino (Italy)

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Abstract

Italy is the first producer of rainbow trout in Europe (EU member) and Trentino farmers represent roughly 8% of this production, but genetic information of the stocks employed is still lacking. This study aimed to estimate the genetic variability of the rainbow trout stocks farmed in Trentino and to reconstruct the related admixture history. Sixty hundred and thirty-two animals belonging to 23 different stocks were analysed using microsatellite loci. Seventeen stocks showed negative values of inbreeding coefficient within population (F_{is} ranging from -0.06 to -0.107). NJ tree of genetic distances among stocks showed distinct clusters reflecting, at least partially, the information coming from known history. Discriminant analysis of principal components evidenced only 2 main groups of trout whereas model-based cluster analyses could detect 7 genetically distinct groups, underlining an influence from an old commercial Danish stock. Rainbow trout in Trentino showed a recent history of admixture with a lower level of genetic differentiation among stocks when compared to wild populations (F_{ST} = 7.7%). The results confirmed the rather similar genetic origin of the analysed populations, highlighting the necessity to carefully manage the stocks to prevent phenomena of inbreeding and, more often, outbreeding depression.

Keywords: Rainbow trout; freshwater; fish population; conservation genetics.

Introduction

The pristine distribution of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) after the last glacial period was restricted to the Pacific Ocean coastal drainages of North America, extending from Alaska southward to Mexico (MacCrimmon, 1971). Today, many strains of rainbow trout have been developed worldwide by selective breeding and crossbreeding with the goal of improving economically important traits (Gjedrem, 2000). It is believed that most of the rainbow trout strains cultured around the world originated from McCloud River hatchery in California (Gall & Crandell, 1992). Such strains probably maintained the original genetic variation conserved mainly within populations but also between them reflecting thus substantial sub-division (Silverstein, Rexroad, & King, 2004). With the aim of studying intra and inter-populations genetic differences some applications of genetic markers will place greater emphasis on genetic differences among groups (stock structure) and some will focus on differences among individuals within populations, but the detection of polymorphism remains the key (Ferguson & Danzmann, 1998). As well known, among salmonids, the rainbow trout has one of the greatest measures of average heterozygosity (Allendorf & Utter, 1979), indicating considerable potential for artificial selection.

To our knowledge, only two major studies on European rainbow trout strains have been published (Gross, Lulla, & Paaver, 2007; Glover, 2008) and no research on Italian rainbow trout is available in scientific literature; nonetheless, Italy is the sixth largest trout producer in the world and the first in the European Union, with a total production estimated in 37000 tons (2015 yearly production; FEAP, 2016). Trentino region has a climatic feature suitable for farming of rainbow trout which are reared there since 1885. Currently, about 60 small-medium sized fishplants operate in the Trentino regional district mainly within a regional trade association of trout farmers (ASTRO) and the yearly rainbow trout production amount to 8% (2700 tons, 2015 yearly production; ASTRO data) of national portion-size production.

Mass mating is the breeding programme most commonly applied by the local farmers, usually based

on phenotypic selection of parental fish. Breeders exchange small shares of different fish stocks in order to prevent inbreeding and to maintain high genetic variation. However, the information about the geographical and/or genetic origin as well as the parentage of the shared stocks are often inaccurately tracked by farmers, and therefore relevant stock information is lacking or unavailable.

Such exchange of material could increase the genetic variation of the new stocks and the resulting genetic admixture can create positive genetic combinations both for adaptation and production. Nevertheless, admixture between introduced and wild (or local) population is a rising concern for the management of the species and it could also lead to a loss of local adaptation and a fitness reduction in local populations.

The main aims of the present study are: i) to estimate genetic variability of the rainbow trout stocks farmed in Trentino; ii) to evaluate the genetic relationship of these commercial stocks with each other and with other experimental ones, included in the study as control samples; iii) to validate and complete the local strains traceability, as made available by local farmers, by reconstructing the admixture history of strains, as backtracked by genetic markers information.

Materials and Methods

Sampled Stocks

Twenty-three stocks of rainbow trout were sampled from 13 different farms being part of ASTRO association and geographically included in the Trentino region (Italy). Each sampled stock was identified by a number indicating the farm (plants 1-13) followed by a letter indicating the different stock (A-X) and from here onwards is reported with the term 'stock' both in text and tables. Stocks from the Fondazione Edmund Mach (FEM) experimental fish plant (plant 6) are lineages with known breeding history, bought from commercial foreign companies (6U and 6V), from an Italian hatchery (6T) or from local farmers (6S and 6X), with the exception of the 6R stock, originating from a local self-sustaining population of rainbow trout naturalized in the Travignolo stream, within the Paneveggio wildlife park (Pontalti & Vittori, 2004), through a captive breeding programme (2001-2005). FEM experimental fish stocks (6R-X) were included in the study as tracking-history control samples.

The origin of each trout farm/stock combination is based on the farmer's declarations and on the recorded history of the stock. According to these records, the stocks included in the dataset were expected to be representative of twelve different lineages/strains (Figure 1). Four strains, commonly imported in Trentino through years, were recently bought from commercial hatcheries from the USA (Americano1 and Americano2), the Isle of Man - UK (Man) and Spain (Spagnolo). A single lineage was bred from a locally naturalized population (Paneveggio). Four lineages were locally reared since at least 15 generations from unknown origin (Azzurro, Early, Late) or originating from an old commercial Danish stock (Danese). Finally, two strains were recently bought from Italian hatcheries and locally reared (Salmontrutta and Frola). In Figure 1, each colour represents a different declared origin, and mixed origin was reported with different colours and in different proportions, according to the contribution of different strains.

Trout specimens were sampled from the farms at commercial size, ranging from 500 to 1200 g and corresponding to 16-32 months as age group. For each stock, common name locally used, expected origin of strain selection, according to traceability by local farmers, and available information on breeding history were collected and registered (Table 1). During the three-year sampling campaign, a total of 632 fin clips were collected and subsequently stored in 95% ethanol, until DNA extraction. The number of animals analysed ranged from a minimum of 21 and a maximum of 32 specimens per strain (Table 1).

Total genomic DNA was extracted from fin clips using the DNeasy 96 Blood & Tissue Kit (Qiagen, Valencia, CA, USA), according to manufacturer's instructions. In each extraction plate, a 'no-DNA' negative control was included and used in all the following steps of the analysis to check for possible cross-contaminations. Quantity and quality of extracted DNA was evaluated by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide (0.01%), by comparison with a MassRuler DNA Ladder mix (Fermentas International Inc., Burlington, ON, Canada) reference marker.

Microsatellite Markers

Eight microsatellite loci (OMM1008, OMM5047, OMM1097, OMM5177, OMM1051, OMM1088, OMM1325, OMM5233) were selected from the literature (Johnson, Rexroad, Hallerman, Vallejo, & Palti, 2007) and organized in two multiplex panels. Forward primers were labelled with Fam (Sigma-Aldrich, Taufkirchen, Germany), Hex and Ned (Applied Biosystems, Foster City, CA, USA) fluorescent dyes. The microsatellite loci were amplified in the two separate multiplexes using Multiplex PCR Kit (Qiagen), according to manufacturer's instructions, with 0.2 µM of each primer and 3 mM $MgCl_2$ in a total reaction volume of 25 µl. Reactions were performed by a Geneamp PCR System 9700 thermal-cycler (Applied Biosystems) with the following thermal profile: initial denaturation of 15 min at 95 °C, 29 cycles of 30 s at 94 °C, 90 s at 58 °C and 1 min at 72 °C, and a final extension of 30 min at 60 °C. Amplicons were run on an ABI PRISM 3130xl DNA sequencer (Applied Biosystems) with a

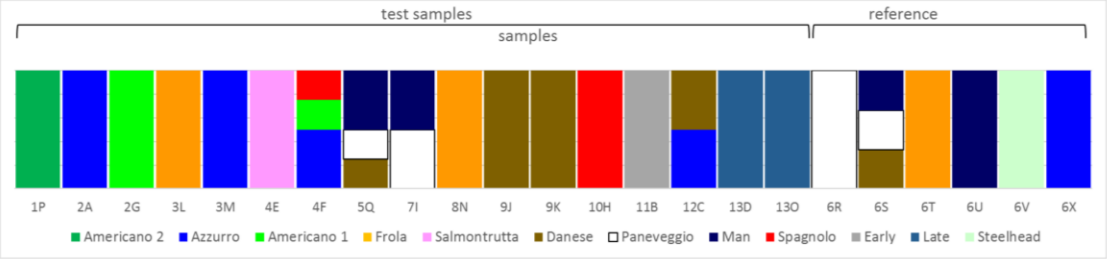

Figure 1. Declared origin of the stocks.

Table 1. Origin, number and characteristics of rainbow trout strains

Farm	Stock	N	Common name[a]	Origin[b]
Test samples				
1	P	24	Americano2	Kamloops strain, USA
2	A	26	Azzurro	unknown, local strain
	G	26	Americano1	unknown, USA
3	L	30	Frola	unknown, ITA
	M	27	Azzurro	unknown, local strain
4	E	23	Salmontrutta	unknown, ITA
	F	32	Mixed1	mixed Americano1/Spagnolo/Azzurro, local strain
5	Q	31	Mixed2	mixed Man/Paneveggio/Danese, local strain
7	I	29	Mixed3	mixed Man/Paneveggio, Fondazione Edmund Mach, local strain
8	N	27	Frola	unknown, ITA
9	J	25	Danese	unknown, local strain
	K	24	Danese	unknown, local strain
10	H	26	Spagnolo	Ovapiscis, ESP
11	B	21	Early spawning	unknown, local strain
12	C	25	Mixed4	mixed Azzurro/Danese, local strain
13	D	31	Late spawning	unknown, local strain
	O	30	Late spawning	unknown, local strain
Reference samples				
6	R	28	Paneveggio	unknown, Fondazione Edmund Mach, local strain from a self-sustaining population in Trentino
	S	32	Mixed2	mixed Man/Paneveggio/Danese, local strain
	T	28	Frola	unknown, ITA
	U	30	Man	Glen Wyllin trout Hatchery, UK
	V	29	Steelhead strain	Troutlodge, USA
	X	28	Azzurro	unknown, local strain

[a] Common name locally used by farmers.
[b] Expected origin of strain selection, according to traceability by local farmers.

GeneScan-500 ROX Size Standard (Applied Biosystems). Alleles were scored using Genescan and Genotyper (Applied Biosystems) software.

Statistical Analyses

The program Micro-Checker 2.2 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004), was preliminary used in order to check for the presence of null alleles in the microsatellite dataset.

Observed number of alleles, allelic richness, heterozygosity, F_{is} and F_{ST} parameters per locus were calculated using the packages *hierfstat* v.0.04-22 (Goudet, 2005) and *poppr* v.2.3.0 (Kamvar, Tabima, & Grünwald, 2014; Kamvar, Brooks, & Grünwald, 2015) in R Version 3.1.2 (R Core Team, 2014). Compliance with the Hardy Weinberg equilibrium for each locus within each stock was performed with the R package *pegas* v.0.9 (Paradis, 2010) applying the classical 2-test based on the expected genotype frequencies calculated from the allelic frequencies. Stock differentiation was evaluated by the Wright's F-statistics as proposed by Weir and Cockerham (1984) using the R package dive*Rsity* v.1.9.89 (Keenan, McGinnity, Cross, Crozier, & Prodöhl, 2013) with 1000 bootstrap replicates. The molecular coancestry within stock (f_{ii}), the heterozygote deficiency within stock (F_{IS}), the uncorrected number of alleles per locus (K) and the rarefacted number of alleles per locus (K(g)) (Hurlbert, 1971) were computed from microsatellite data using the program MolKin v. 3.0 (Gutiérrez, Royo, Álvarez, & Goyache, 2005). To

estimate K(g), g was fitted to 36, which is twice the minimum number of individuals within a stock with genotype known for all the microsatellites (without any missing data). Furthermore, the Reynolds distance (Reynolds, Weir, & Cockerham, 1983) matrix was also computed using the R package *poppr* v.2.3.0 (Kamvar *et al*., 2014; Kamvar *et al*., 2015) and a neighbour-joining consensus tree of the 1000 bootstrapped distances was constructed using the R package *ape* v.3.4 (Paradis, Claude, & Strimmer, 2004).

Population genetic structure was analysed through Discriminant Analysis of Principal Component (DAPC) implemented in the R package *Adegenet* v.2.0.2 (Jombart, 2008). Microsatellite data were scaled in order to account for bias induced by heterogeneous variances (Jombart, Pontier, & Dufour, 2009) and the optimal number of clusters was identified through the Bayesian Information Criterion (BIC). Furthermore, the genetic diversity was also analysed using the Bayesian approach implemented in the software Structure 2.3.3 (Pritchard, Stephens, & Donnelly, 2000). In the analysis, 20 independent Structure runs were performed for each number of clusters (K) considered, from 1 to 20, using 50,000 burn-ins and 250,000 drawings under a model assuming admixture between individuals and correlated allele frequencies. The choice of the most likely number of clusters (K) was made according to recommendations presented in Pritchard *et al*. (2000). CLUMPP (Jakobsson & Rosenberg, 2007) was used to find the optimal alignment of the outcomes of the 20 runs and to check for convergence.

Results

Following evaluation with the program *Micro-Checker* 2.2 (Van Oosterhout *et al*. 2004), one locus (*OMM5233*) was removed from the analysis due to the suggested presence of null alleles. The observed number of alleles per microsatellite ranged from 7 at *OMM5177* to 26 at *OMM1097* (Table 2) with a total number of 91 alleles detected. Among the microsatellites analysed, *OMM1051* showed the highest allelic richness (10.60) whereas *OMM1325* showed the lowest value (3.98) The observed heterozygosity was always higher than 0.7 except for *OMM1325* and *OMM5177* (0.559 and 0.635 respectively).

Looking at the F_{IS} at markers level (Table 2) no locus presented a high level of heterozygote deficiency and the average genetic differentiation (F_{ST}) per locus was 7.5% (Table 2).

At stock level, the observed heterozygosity ranged from 68.3% (stock 6R) to 84.3% (stock 2G) with two out of 23 stocks showing an expected heterozygosity lower than the observed heterozygosity (4E and 13O) even if only for the 4E stock the difference exceeded the 5% (Table 3). In overall, it does not seem that the analysed stocks presented high heterozygote deficiency as also suggested by the F_{IS} indexes (Table 3). Molecular coancestry within stock gives an overview of the self-coancestry of the individuals, which is related to inbreeding coefficient (Table 3). The stocks that showed molecular coancestry higher than 0.35 are 1P and 6R (0.361 and 0.360 respectively). The first belonged to foreign commercial strains imported in Italy from 2005 through 2009, whereas 6R is derived from a naturalized population conserved by FEM from 2001 and managed without gene flow from other broodstocks (Pontalti & Vittori, 2004). The highest difference between the average allele per population (k) and the average rarefacted alleles per populations (g=36) was equal to 0.99, confirming the good representation of the stocks analysed (Table 3).

The genetic diversity among stocks, calculated through Reynolds' genetic distance, is represented with the unrooted NJ dendrogram (Figure 2). The supposed North American commercial stocks, called Americano1 and Americano2 (respectively 2G and 1P) are located in 2 different branches, moreover 2G and the reference stock 6V (called Steelhead) cluster together. The other branches with a bootstrap value higher than 50% are those of the local stocks 13D – 13O (called Late) belonging to a specific farmer forming also a higher order cluster with the stock 11B, and the cluster formed by the wild strain of FEM (6R).

The genetic diversity among individuals via DAPC analysis (and consequently among stocks) is represented in Figure 3. The lowest BIC value corresponds to 14 clusters with a well separated cluster (1) formed by the individuals of 6V plus 8

Table 2. Number of observed alleles (Ao), allelic richness (Arich), heterozygosis (Het), inbreeding (F_{is}) and fixation index (F_{ST}) for each locus

Locus	Ao	Arich	Het	F_{is}	F_{ST}
OMM1008	8	5.64	0.756	-0.025	0.075
OMM5047	9	6.62	0.782	-0.028	0.075
OMM1097	26	10.39	0.854	-0.014	0.064
OMM5177	7	4.03	0.635	-0.076	0.122
OMM1051	22	10.60	0.856	0.006	0.067
OMM1088	12	7.12	0.804	-0.020	0.065
OMM1325	7	3.98	0.559	0.019	0.063
Mean	13			-0.019	0.075

individuals of 2G whereas the new cluster number 3 (the closest to cluster 6) is mainly formed by individuals of 2G stock. The other new clusters represented almost exclusively by individuals of a single populations are the 6 (6R stock) and the 5 (1P stock).

Stock structure and degree of admixture were estimated using the program Structure and the results of the analyses are reported in Figure 4. The across-run average of estimated ln probability of data (ln Pr(X|K)) reached a plateau at K=8 (Figure 4a) where the mean variance of the ln Pr(X|K) estimates was the lowest. Different admixture levels were observed in different stocks when looking at both the mean population (Figure 4b) and the individual membership (Figure 4c) to the eight clusters. The 2G and 6V

Table 3. Number of observed (Ao) and expected (Ae) alleles, observed (Hobs) and expected (Hexp) heterozygosity, molecular coancestry (f_{ii}), inbreeding coefficient (F_{is}), average number of observed allele per locus (k) and average number of alleles per locus corrected using the rarefaction method ($k_{(36)}$) for each stock analysed

Stock	n.	Ao	Ae	Hobs	Hexp	f_{ii}	F_{is}	k	$k_{(36)}$
2A	26	7.71	4.49	0.734	0.704	0.310	-0.046	7.71	7.10
11B	21	8.00	5.28	0.796	0.767	0.248	-0.050	8.00	7.81
12C	25	8.00	5.32	0.833	0.790	0.224	-0.052	8.00	7.55
13D	31	7.29	4.09	0.715	0.714	0.297	-0.048	7.29	6.70
4E	23	7.29	4.96	0.691	0.751	0.266	0.076	7.29	7.05
4F	32	8.14	5.12	0.764	0.739	0.274	-0.025	8.14	7.20
2G	26	7.57	5.05	0.843	0.786	0.229	-0.077	7.57	7.22
10H	26	5.71	3.84	0.743	0.683	0.336	-0.099	5.71	5.43
7I	29	7.86	4.77	0.793	0.766	0.245	-0.033	7.86	7.23
9J	25	7.71	4.84	0.809	0.754	0.248	-0.077	7.71	7.35
9K	24	7.71	5.13	0.808	0.760	0.251	-0.137	7.71	7.37
3L	30	7.71	4.52	0.729	0.703	0.310	-0.038	7.71	6.91
3M	27	8.00	4.62	0.757	0.727	0.287	-0.047	8.00	7.26
8N	27	7.14	4.87	0.784	0.729	0.283	-0.076	7.14	6.74
13O	30	8.43	4.91	0.723	0.751	0.262	0.038	8.43	7.44
1P	24	6.29	3.25	0.684	0.651	0.361	-0.055	6.29	5.87
5Q	31	8.71	5.61	0.783	0.781	0.231	0.001	8.71	7.66
6R	28	5.29	3.30	0.683	0.653	0.360	-0.047	5.29	5.03
6S	32	8.00	5.19	0.791	0.766	0.246	-0.021	8.00	7.14
6T	28	7.86	5.06	0.744	0.741	0.268	0.002	7.86	7.28
6U	30	7.00	4.08	0.740	0.733	0.279	-0.024	7.00	6.25
6V	29	5.00	3.42	0.721	0.699	0.312	-0.034	5.00	4.79
6X	28	9.29	5.91	0.824	0.776	0.230	-0.064	9.29	8.58

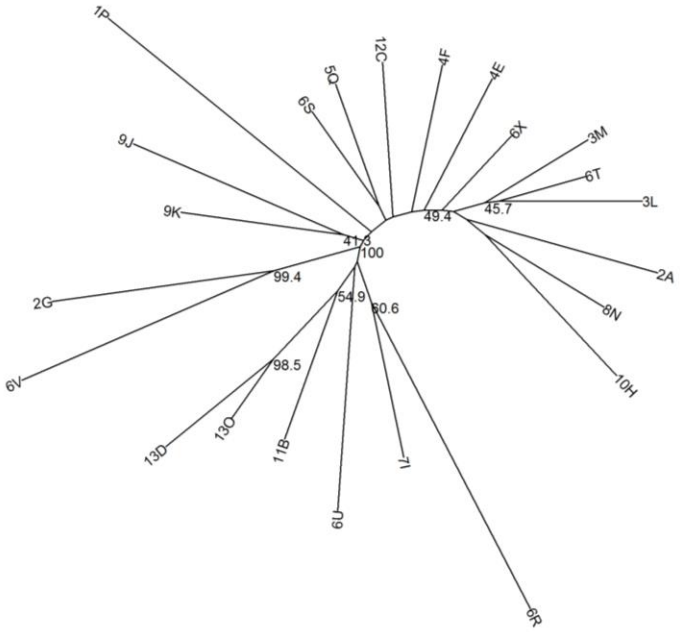

Figure 2. Unrooted neighbour joining tree for the twenty-three stocks studied based on Reynolds' genetic distances.

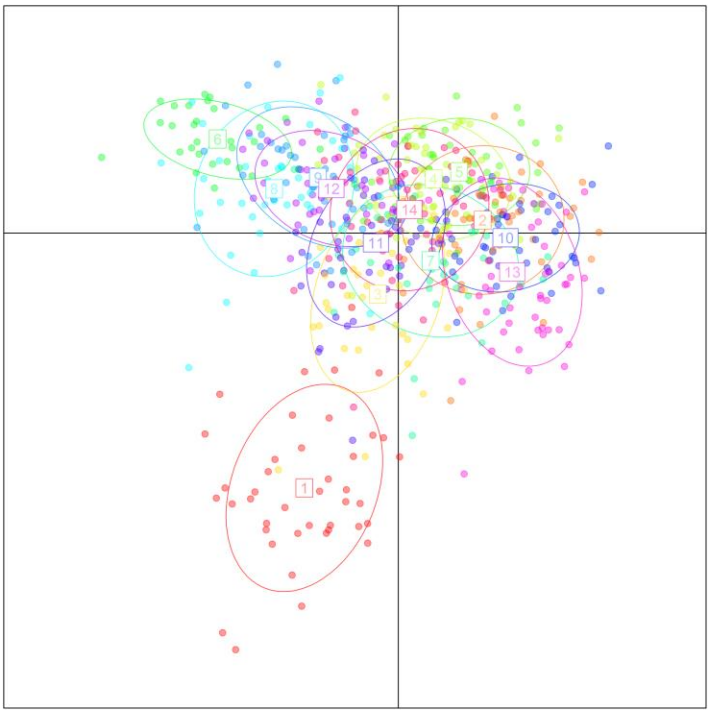

Figure 3. Scatterplot of resulting genetic clusters after Discriminant Analysis of Principal Components. Plot reports the first two components.

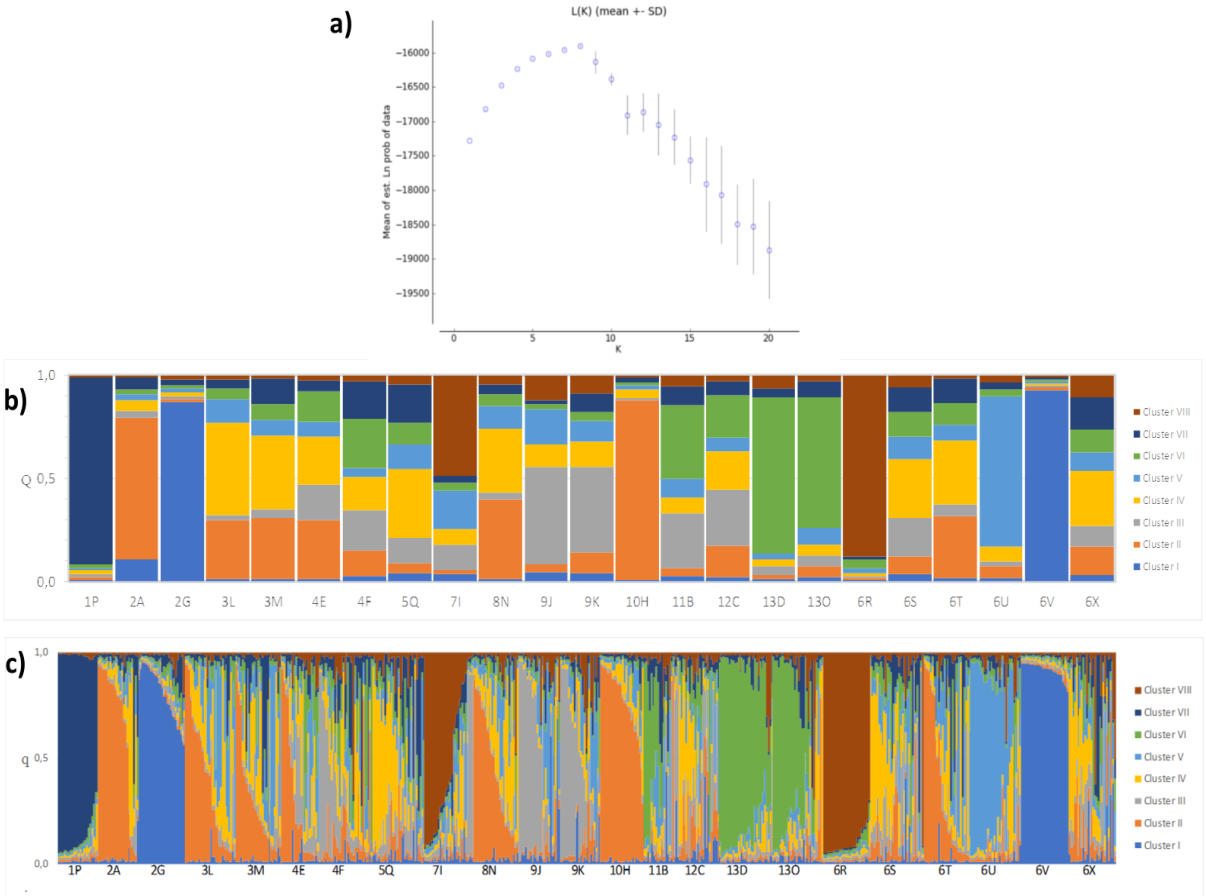

Figure 4. Genetic diversity structure of the 23 stocks. Population memberships for each genotype is shown based on K=8. a) Across-run average of estimated ln probability of data b) Admixture level partitioned into coloured segments in proportion to the estimated mean population to the eight clusters. c) Admixture level partitioned into coloured segments in proportion to the estimated individual membership to the eight clusters.

(reference as *Puget Sound Steelhead* strain) stocks, with North American origin, were almost completely referable to a first cluster (cluster I in Figure 4b-c). A different North American derivation, declared as *Kamloops* strain (1P), and the Isle of Man (UK) derived commercial stock (6U) had maximum ancestry levels in a second (cluster VII) and a third (cluster V) cluster, respectively. The 6R stock, from the self-sustaining local population, was entirely ascribable to a fourth cluster (cluster VIII). Two Spanish derived stocks, namely 2A and 10H (Ovapiscis commercial strain), clustered together with maximum and largely dominant ancestry in a fifth cluster (cluster II). The 13D and 13O stocks, sampled from a single fish farm and locally selected by the farmer, were almost completely referable to a sixth cluster (cluster VI). Again, the majority of individuals from 9J and 9K stocks, sampled from the same local farm and primarily originating, decades ago, from a commercial Danish lineage, clustered primarily with higher q values in cluster III. No stock was found to have a clearly predominant ancestry in the last cluster (Cluster IV).

Discussion

According to our sampling campaign, few farmers maintained in the last decades their own stocks, avoiding mixing different populations and provenances, whereas most farmers refreshed rather frequently their stocks using material from both foreign and local hatcheries. In some cases, the farmers' work of selection is easily recognizable both in the NJ tree and in the populations' structure. For instance, the two strains of the farm 13 tightly clustered and they jointly clustered with farm 11 whose material comes from a genetic line of the farm 13.

The main outcome of the present work is the confirmation, by means of genetic analysis, of the recent history of admixture of the farmed rainbow trout in Trentino with many exchanges of genetic material among farms, which in turns provides a maintenance of high genetic diversity. Nonetheless, some stocks resembling the Spanish commercial strain and others are more similar to the commercial Troutlodge population, even if the distinction is not fully clear. This result was not unexpected considering that all the European rainbow trout are of North American origin (Gall & Crandell, 1992).

For a clearer reading of the outcomes it has to be pointed out that stock 6R was reared in an experimental fish plant (FEM) with well documented past and recent history of breeding (Pontalti & Vittori, 2004). Furthermore, stocks from 6S to 6X were bought from commercial companies and local farmers who assured the genetic origin of the material and thus they could serve as a reference point for the other groups. Overall, the genetic diversity (heterozygosity and allelic diversity) of the studied stocks is rather high (Ho 0.784) and, even if the markers employed were different, it is of the same magnitude than that reported from Silverstein *et al.* (2004) on rainbow trout. It is thus possible to state that our captive trout stocks retained a high genetic variation. At population level, no clear indication of the inbreeding is suggested by the F_{IS} index with all the stocks not statistically different from 0 for this parameter (data not showed). The stocks with the highest f_{ii} values are those clustering rather separately with the model-based clustering method.

Observing the NJ tree, it is possible to recognize some clusters showed good reliability (i.e. 13D, 13O and 11B; 6V and 2G; 6R and 7I) and reflecting the information derived from the known history of the stocks, as well as some others (3L, 3M and 6T; 9J and 9K) with lower confidence levels. However, some sub-clusters gather apparently different stocks (based on the combination farm/sampling site) but the common origin is rather clear analysing the information coming from the survey conducted on each sampled farm. For instance, 2G and 6V clustered together, they shared North American origin in their formation and similar pattern is valid for 7I and 6R that are managed by the same owner. The not perfect alignment between the results of the genetic analysis and the declared origin of the stocks could be probably due to the fact that some stocks are declared as self-produced by the farmers. In some instances, this type of self-production, to maintain diversity, may have provided the inclusion of external germplasm not promptly recorded in the history of the stock.

Spanish (10H) and USA (2G, 1P and 6V) are the only stocks clearly identifiable through membership assignment of Structure software whereas the situation is not so straightforward for all the other stocks analysed. As pointed out before, this is not so unexpected bearing in mind that the rainbow trout is of North American origin (Gall & Crandell, 1992) and the current differences between populations could be ascribed to recent management (during the XX century).

DAPC analysis evidenced an original shared signal among the stocks 2A, 3L, 3M, 4E, 8N, and 10H with most of the individuals assigned to the clusters in the right part of the plot (Figure 3).

According to the farmers' declarations (Table 1 and Figure 1), the 23 stocks included in the dataset were expected to be representative of twelve different lineages/strains, but the admixing of genetic material in most of the farms does not allow a clear differentiation of the 23 stocks analysed and an indication of number of probable putative populations comes from the results of Structure analysis (k=8) making plausible the theory of the common recent history of the Trentino trout which included frequent exchange of animals.

The origin of the two North American clusters is attributable to the *Puget Sound Steelhead* and to

Kamloops strains for 2G-6V and 1P stocks respectively (MacCrimmon, 1971; Pontalti & Vittori, 2004). The 6R stock forms a separate cluster including, at least partially, also the 7I stock which is reported as a cross between 6R and 6U (FEM database). Stocks 10H, 2A, 3L, 3M, 4E, 8N and partially 6T formed a distinct cluster attributable to an original Spanish strain even if only for 10H the declaration of the farmer fully matched with the genetic analysis. Three out of the five remaining stocks (3L, 8N and 6T) matched partially with the known information whereas 2A stock was declared as a local strain known as Azzurro. The 6U stock clustered alone confirming its unique origin led back to the Isle of Man (UK) and a similar pattern is shown by two stocks with a known history of local selection (13D and 13O).

The remaining stocks showed high level of admixture and, at least for three of them (9J, 9K and 12C), it seems recognizable an influence of a not well-defined population (represented by the light grey colour in Figure 4). Stock 9J and 9K, as tracked from the farmer, originating from an old commercial Danish stock, so the grey colour could represent an old Danish lineage. Actually, the Danish commercial strains, where the rainbow trout farming industry began in the 1890s (Gall & Crandell, 1992), had a high impact on the diffusion of the rainbow trout in Trentino after the Second World War (Lappi, 2008). For the remaining stocks (4E, 4F, 5Q, 6S, 6T, 6X and 11B) the situation is complex and it could be a sign of a management program of long-term crossings aimed to achieve specific selection objectives and/or to a simple conservation of genetic variability. In overall, the analysed populations are rather similar from the genetic point of view and the results confirmed the hypothesis of Gross *et al*. (2007) of Californian origin for most of the European stocks.

Conclusions

This research represents the first genetic characterisation of farmed rainbow trout in Trentino and in Italy. Our results indicate that there is some degree of genetic diversity in local rainbow trout, but the admixing among stocks is rather high. Genetic analysis has made possible to clarify the relationships among groups and this could be a useful tool in addressing both the producers' choices in their selection plans and possible buyers in order to maintain sufficient genetic variability of their stocks. At local level, it exists the tendency to a continuous exchange of genetic material among farmers and these uncontrolled crosses did not help both from the practical and genetic point of view to characterize the rainbow trout stocks of the Trentino region.

Most of the farmers know neither the exact history of their animals nor the real origin of the acquired genetic lines. This could be particularly dangerous for the management of the stocks that may be subjected to either inbreeding or outbreeding depression depending on the different management strategies elected by the farmers. As already mentioned in the literature the inbreeding in fish is less accentuated than in other livestock populations whereas uncontrolled admixing and subsequent selection could lead to potentially dangerous levels of outbreeding depression. Furthermore, it is often erroneously believed that stocks coming from distant sites or maintained isolated for long time possess a high commercial and genetic value whereas they are widely diffused on the region by different common names. Such belief led some farmers to maintain some stocks genetically isolated because considered different from the genetic point of view whereas they are very similar among them. The present results are useful to shed some light on the local context, and the presented database could give guidance to the local farmer on management of pure bred broodstocks as to a more aware admixing.

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References

Allendorf, F.W., & Utter, F.M. (1979). Population genetics. In W.S. Hoar, D.J. Randall, & J.R. Brett (Eds.) *Fish Physiology*, (pp. 407–454). New York, USA. Academic Press,

FEAP (2016). European Aquaculture Production Report 2007-2015. Liege, Belgium, FEAP Secretariat, 46 pp.

Ferguson, M.M., & Danzmann, R.G. (1998). Role of genetic markers in fisheries and aquaculture: useful tools or stamp collecting? *Canadian Journal Fisheries Aquatic Science*, *55*(7), 1553–1563. http://doi.org/10.1139/f98-096

Gall, G.A.E., & Crandell, P.A. (1992). The rainbow trout. Gall GAE Edition. Stirling, UK, Elsevier Science, 346 pp.

Gjedrem, T. (2000). Genetic improvement of cold-water fish species. *Aquaculture Research*, *31*(1), 25–33. https://doi.org/10.1046/j.1365-2109.2000.00389.x

Glover, K.A. (2008). Genetic characterisation of farmed rainbow trout in Norway: intra- and inter-strain variation reveals potential for identification of escapees. *BMC Genetics*, *9*(1), 87-97. http://doi.org/10.1186/1471-2156-9-87

Goudet, J. (2005). Hierfstat, a package for r to compute and test hierarchical F-statistics. *Molecular Ecology Notes*, *5*(1), 184–186. https://doi.org/10.1111/j.1471-8286.2004.00828.x

Gross, R., Lulla, P., & Paaver, T. (2007). Genetic variability and differentiation of rainbow trout (*Oncorhynchus mykiss*) strains in Northern and Eastern Europe. *Aquaculture*, *272*(S1), S139-S146. http://doi.org/10.1016/j.aquaculture.2007.08.004

Gutiérrez, J.P., Royo, L.J., Álvarez, I., & Goyache, F. (2005). MolKin v2.0: a computer program for genetic analysis of populations using molecular coancestry information. *Journal of Heredity*, *96*(6), 718-721. http://doi.org/10.1093/jhered/esi118

Hurlbert, S.H. (1971). The non concept of species diversity: a critique and alternative parameters. *Ecology*, *52*(4), 577-586. https://doi.org/10.2307/1934145

Jakobsson, M., & Rosenberg, N.A. (2007). CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, *23*(14), 1801-1806. https://doi.org/10.1093/bioinformatics/btm233

Johnson, N.A., Rexroad, C.E., Hallerman, E.M., Vallejo, R.L., & Palti, Y. (2007). Development and evaluation of a new microsatellite multiplex system for parental allocation and management of rainbow trout (*Oncorhynchus mykiss*) broodstocks. *Aquaculture*, *266*(1-4), 53–62. http://doi.org/10.1016/j.aquaculture.2007.02.054

Jombart, T. (2008). Adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*(11), 1403-1405. http://10.1093/bioinformatics/btn129

Jombart, T., Pontier, D., & Dufour, A.B. (2009). Genetic markers in the playground of multivariate analysis. *Heredity*, *102*(4), 330-341. http://doi.org/10.1038/hdy.2008.130

Kamvar, Z.N., Tabima, J.F., & Grünwald, N.J. (2014). Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *Peer Journal*, *2*, e281. http://doi.org/10.7717/peerj.281

Kamvar, Z.N., Brooks, J.C., & Grünwald, N.J. (2015). Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. *Frontiers in Genetics*, *6*, 1-10. http://doi.org/10.3389%2Ffgene.2015.00208

Keenan, K., McGinnity, P., Cross, T.F., Crozier, W.W., & Prodöhl, P.A. (2013). diveRsity: An R package for the estimation of population genetics parameters and their associated errors. *Methods in Ecology and Evolution*. *4*(8), 782-788. https://doi.org/10.1111/2041-210X.12067

Lappi, E. (2008). La piscicoltura nella Judicaria dalle origini alla metà del '900. [Fish farming in Judicaria from its origins to the middle of '900.]. Tione, Italy, Centro Studi Judicaria press. Italian.

MacCrimmon, H.R. (1971). World distribution of rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board of Canada*, *28*(5), 663–704. http://doi.org/10.1139/f71-098

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, *20*(2), 289-290. http://doi.org/10.1093/bioinformatics/btg412

Paradis, E. (2010). pegas: an R package for population genetics with an integrated-modular approach. *Bioinformatics*, *26*(3), 419-420. http://doi.org/10.1093/bioinformatics/btp696

Pontalti, L., & Vittori, A. (2004). Sperimentazione di miglioramento genetico su ceppi di trota iridea *Oncorhynchus mykiss* (Walb.). In M. Maldini, G.L. Gandolfi, & F. Nonnis-Marzano (Eds.), *Proceedings of the 9° National Congress of Associazione Italiana Ittiologi Acque Dolci* (pp. 181-185). Acquapartita, Italy, C.I.S.B.A. Press., 274 pp.

Pritchard, J.K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from http://www.R-project.org/

Reynolds, J., Weir, B.S., & Cockerham, C. (1983). Estimation of the coancestry coefficient: basis for a short-term genetic distance. *Genetics*, *105*(3), 767–779.

Silverstein, J.T., Rexroad, C.E., & King, T.L. (2004). Genetic variation measured by microsatellites among three strains of domesticated rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Aquaculture Research*, *35*(1), 40–48. https://doi.org/10.1111/j.1365-2109.2004.00979.x

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., & Shipley, P. (2004). Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, *4*(3), 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Weir, B.S., & Cockerham, C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, *38*(6), 1358-1370. https://doi.org/10.2307/2408641