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# Genome analysis in five Italian beef cattle breeds

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**Abstract:** Chianina, Marchigiana, Maremmana, Podolica and Romagnola are the main Italian beef cattle breeds, and the quality of their products is largely recognized worldwide. This paper aims to determine the genetic variability and population differentiation by heterozygosity and fixation indices using SNPs data. The dataset was composed of 3,581 animals (Chianina, n = 909; Marchigiana, n = 879; Maremmana, n = 334; Podolica, n = 555; Romagnola, n = 904). The blood samples were collected in ANABIC performance testing station from 1985 to 2019. All the animals were genotyped with the GeneSeek GGP-LDv4 33k SNP chip containing 30,111 SNPs. The genotype quality control for each breed was conducted separately, and SNPs with call rate smaller than 0.95 and minor allele frequency larger than 5% were used for further analysis. Heterozygosity and $F_{IS}$ index were estimated in PLINK v1.9, and $F_{ST}$ index was estimated using the hierstat package of R 4.0.1 software. The genetic analysis highlighted low values of heterozygosity in the improved beef breeds compared to the heritage breeds; moreover, the low values of $F_{IS}$ indicated a positive effect of controlled genetic inbreeding in the studied breeds. The $F_{ST}$ analysis confirmed the historical origin of Marchigiana breed and the values are consistent with their common breeding programmes. In this study, the importance of monitoring genetic variability of Italian beef cattle breeds was emphasized in order to maintain breed identity and genetic diversity in the selection process.

**Keywords:** bovine, SNPs, inbreeding.

## 1 Introduction

Beef cattle are recognized as an important component of world biodiversity because the genes and gene combinations they carry may remain useful to agriculture in the future (Holsinger & Weir, 2009). Italy has a long history and tradition in beef cattle production and local beef cattle breeds (Chianina - CHI, Marchigiana - MAR, Maremmana - MRM, Podolica - POD and Romagnola - ROM) have always been connected with rural and ethnic traditions. Therefore, they represent an historical and cultural heritage which exceeds their economic value. These breeds are the main specialized for beef production and the quality of their products is widely recognized all over the world (Lasagna et al., 2015). CHI beef is internationally recognized as a top-quality product, and the most famous cut is the Fiorentina steak (Bongiorni et al., 2016). The MAR and ROM are excellent breeds for beef production: in MAR a mutation in the *myostatin* gene (*MSTN*) that originates a double muscle phenotype is detected (Vincenti et al., 2007), and the ROM is very efficient grazing cattle (Cosentino et al., 2018). The MRM and POD are typical heritage breeds used both for beef and milk production. Milk of POD is used to produce Caciocavallo cheese, while the MRM has never been milked (Moioli et al., 2004). In all these breeds, performance testing is the method of young bulls' selection since 1985 (Sbarra et al., 2009).

Maintaining genetic diversity and minimizing the inbreeding are important aims of all successful beef cattle breeding programs (Hall & Brandley, 1995). The single nucleotide polymorphism (SNP) is a

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modern tool to estimate genetic diversity (Smaragdov et al., 2018). The *F*-statistics ($F_{IS}$, $F_{IT}$ and $F_{ST}$) are usually estimated for the analysis of population structure (Wright, 1965). Specifically, $F_{IT}$ is the inbreeding coefficient of an individual relative to the total population; $F_{IS}$ is the inbreeding coefficient of an individual relative to the sub-population, using the above for sub-populations and averaging them; and $F_{ST}$ is the effect of sub-populations compared to the total population (Holsinger & Weir, 2009). The $F_{ST}$ analysis allows to quantify the existing genetic differentiation among bovine populations. The analysis of deficit or excess of heterozygotes permits an approximate estimation of inbreeding in each breed, comparing it subsequently with those obtained from pedigree data (Jordana et al., 2003).
This study aims to characterize the genetic variability and population differentiation by SNP molecular markers of 3,581 young bulls of five Italian beef cattle breeds (CHI, MAR, MRM, POD, and ROM) during performance test.

## 2 Material and methods

### *2.1 Blood samples collection and genotypic characterisation*

Individual blood samples (n = 3,581) from five Italian beef cattle breeds (CHI, n = 909; MAR, n = 879; MRM, n = 334; POD, n = 555; ROM, n = 904) were collected from 1985 to 2019 in the Italian Beef Cattle Breeders Association (ANABIC) genetic station during the performance test. The samples were collected using vacutainer tubes containing EDTA as an anticoagulant and stored at −20 °C until analyses were performed. Genomic DNA was extracted from blood using the GenElute Blood Genomic DNA kit (Sigma Aldrich, St. Louis, MO, USA). All the animals were genotyped using the GeneSeek® Genomic Profiler™ Bovine LDv4 33k chip (Illumina Inc., San Diego, CA, USA) and processed at Agrotis lab (LGS, Cremona, Italy) using standard multi-sample protocols and reagents according to manufacturer's instructions.

### *2.2 Statistical analysis*

Data consisted of 30,111 SNPs genotyped for the overall breeds. Only markers located on the 29 autosomes were considered (n = 28,289). The quality control (QC) of genotypes was conducted separately for each breed, and SNPs with call rate smaller than 0.95 and minor allele frequency larger than 5% were used for further analysis. After the QC, a total of 25,007 SNPs were retained and any missing genotypes for these SNPs were replaced by the most frequent genotype at that locus. Data on a total of 198 bulls were discarded, of which 48 were replicates or had inconsistent Mendelian inheritance information, and 38 had a low overall call rate (lower than 95%) (Table 1).

**Table 1** Number of autosomal SNPs and animals before (Pre) and after (Post) quality control (QC) per breed

| Breed | SNPs Pre-QC | SNPs Post-QC | Animals Pre-QC | Animals Post-QC |
|---|---|---|---|---|
| Marchigiana | 28,298 | 25,276 | 879 | 817 |
| Chianina | 28,298 | 24,298 | 909 | 876 |
| Romagnola | 28,298 | 26,332 | 904 | 868 |
| Maremmana | 28,298 | 24,002 | 334 | 312 |
| Podolica | 28,298 | 25,127 | 555 | 510 |
| Total | 28,298 | 25,007 | 3,581 | 3,383 |

The PLINK 1.9 software (Purcell et al., 2007) was used for calculation of gene diversity, tests for deviation from Hardy-Weinberg equilibrium at $p < 10^{-6}$, observed ($H_o$) and expected ($H_e$) heterozygosity, and $F_{IS}$ statistic estimate per locus, per breed, and in the total sample. The $F_{ST}$ values were estimated with "hierstat" package in R 4.0.1 software (Goudet, 2005).

## 3 Results and discussion

Population differentiation was examined by fixation indices $F_{IS}$ and $F_{ST}$ for each locus and across all loci. Results of the *F*-statistics, $H_o$ and $H_e$ for each Italian beef cattle breed are shown in Table 2.

**Table 2** Results of the F-statistical analysis for each breed. ($H_o$ = observed heterozygosity; $H_e$ = expected heterozygosity; SD = standard deviation; $F_{IS}$ = inbreeding coefficient of an individual relative to the sub-population; $F_{ST}$ = measure of population divergence)

| Breed | $H_o$±SD | $H_e$±SD | $F_{IS}$ | $F_{ST}$ |
|---|---|---|---|---|
| Marchigiana | 0.350±0.022 | 0.350±0.019 | 0.005 | 0.085 |
| Chianina | 0.360±0.023 | 0.356±0.020 | -0.011 | 0.076 |
| Romagnola | 0.343±0.021 | 0.350±0.019 | -0.023 | 0.079 |
| Maremmana | 0.383±0.024 | 0.390±0.021 | -0.023 | 0.074 |
| Podolica | 0.399±0.025 | 0.390±0.021 | -0.030 | 0.073 |
| Overall | 0.367±0.023 | 0.368±0.020 | -0.016 | 0.077 |

The overall heterozygosity among breeds ($H_o$ = 0.367, $H_e$ = 0.368) was comparable to that reported for the same breeds in a previous study using few SNPs in candidate genes (Lasagna et al., 2015). The $H_o$ and $H_e$ were lower for the more highly improved CHI (0.360 and 0.356), MAR (0.350 and 0.350) and ROM (0.343 and 0.350) than for heritage POD (0.399 and 0.390) and MRM (0.383 and 0.390) breeds. The average $F_{IS}$ value was relatively low for CHI, ROM, MRM and POD, indicating a positive effect of controlled genetic inbreeding in the breeds (Lasagna et al., 2015), whereas it was slightly positive for MAR (0.005). The low values of heterozygosity and the high inbreeding of MAR compared with other breeds, are attributable to the extensive use of small number of improved bulls (Maretto et al., 2012). The extensive use of artificial insemination in MAR, CHI and ROM breeds could be responsible for lower values of heterozygosity compared to the two heritage breeds. Thus, it is recommended a responsible use of the mating plans in these breeds to avoid the loss of variability and the increase of inbreeding (D'Andrea et al., 2011).

**Table 3** Pairwise $F_{ST}$ estimates between breeds (CHI = Chianina; MAR = Marchigiana; ROM = Romagnola; MRM = Maremmana; POD = Podolica)

| | CHI | MAR | ROM | MRM | POD |
|---|---|---|---|---|---|
| CHI | - | | | | |
| MAR | 0.030 | - | | | |
| ROM | 0.010 | 0.081 | - | | |
| MRM | 0.135 | 0.129 | 0.130 | - | |
| POD | 0.130 | 0.129 | 0.130 | 0.045 | - |

The $F_{ST}$ analysis in the overall sample (Table 2) was higher ($F_{ST}$ = 0.077; $p$=0.001) than the value of 0.049 obtained in a comparable study on the same breeds (Dalvit et al., 2008). The pairwise $F_{ST}$ also detected a higher degree of between-breed variability (Table 3). The highest pairwise $F_{ST}$ was estimated between CHI and MRN (0.135), and the lowest between CHI and ROM (0.010). The higher similarity among the breeds of the Central Italy (CHI, MAR and ROM) is consistent with both their known history and common selection programmes. In particular, the closeness of MAR with CHI and ROM was expected based on historical origin of crossing among local cattle with the two specialized breeds (Bonadonna, 1976). On the other hand, as reported by Maretto et al. (2012) the higher similarity among the MRM and POD breeds is consistent with their common origin.

## 4 Conclusions

Our study is a preliminary analysis and emphasizes the importance of monitoring genetic variability in Italian beef cattle breeds to maintain breed identity and genetic diversity. Trends of genetic diversity indices and population structure over time have been investigated to have a clearer picture of the development of the breeds and help breeding associations to maintain a good reservoir of variability.

 

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