Preliminary genetic variability analysis of the native Garfagnina goats based on microsatellite polymorphism

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SUMMARY
For the development of an appropriate programme for conservation of animal genetic resources, genetic typifying is considered an important preliminary step. In this paper, we have done a preliminary genetic variability analysis of 48 adult Garfagnina goats belonging to a single flock of Tuscany using 12 STR markers (MAF065, SRCRSP05, INRA023, McM527, CSRDR247, SRCRSP23, OarFCB20, TGLA53, INRA005, INRA063, ETH10, ILSTS87) some of which belonged to a markers panel validated by the International Society of Animal Genetics (ISAG) and others routinely used by the facilities of the Laboratorio di Genetics e Servizi (Associazione Italiana Allevatori, Migliaro, Italy). Garfagnina is an Italian native goat breed registered on the Tuscan regional repertory of genetic resources at risk of extinction and have a total of about 745 animals belonging to 17 flocks. Garfagnina breed is important for livestock biodiversity preservation, being a key animal for specialized cheese market in the Tuscan region.

For each marker the following parameters were computed: number of alleles, effective allele size, observed heterozygosity and polymorphism information content (PIC). Allelic frequencies were estimated by direct counting. To analyze the genetic variability of the population, the following parameters were computed at population level: molecular co-ancestry coefficients (fij), kinship distance (Dk), and inbreeding coefficient (Fi). Moreover, genetic similarities (GS) among all animals were investigated using the Individual Multilocus Genotype. The number of alleles ranged from 3 to 9 (mean 5.92) whereas the expected heterozygosity ranged from 0.48 to 0.83 (mean 0.69). There was a high genetic similarity within the whole population (0.43) showing the great homogeneity of the sampled animals, as confirmed also by the small kinship distance (0.34). However inbreeding coefficient was low (0.32). The results of this research indicate that, despite the fact that animals are considered to belong to the same breeding, the genetic variability of this Garfagnina goat population is acceptable for a population with a reduced numerical value.

KEY WORDS
Genetic variability, STR markers, Garfagnina goats.

INTRODUCTION
Italy has a long history of goats breeding and, despite a dramatic number contraction occurred in the last century, goat farming is still an important reality on the Italian livestock panorama. Many different goat breeds are diffused throughout the entire Italian territory from the green Alpine regions to the dry southern and island ones and they may represent a unique source of genetic diversity. The knowledge of the genetic variability is essential to preserve and exploit biodiversity; the genetic variability of a population can be estimated from genealogical data or using the short tandem repeat (STR) molecular markers both in livestock and in pet animals. At the molecular level more recently SNPs have also been introduced. In this context, the purpose of this work was that to make a preliminary genetic variability analysis of the native Garfagnina goats based on microsatellite polymorphism. Garfagnina breed is important for livestock biodiversity preservation, being a key animal for specialized cheese market in the Tuscan region. Garfagnina is an Italian native goat population registered on the Tuscan regional repertory of genetic resources at risk of extinction, with about 745 animals belonging to 17 flocks. The origin of this population is still uncertain, even if it seems to derive from crossings between native goats from Alpine Arc and from the Tuscan-Emilian Apennines; local breeders refer that the population was reared for generations for its milk and meat production.

MATERIALS AND METHODS
The study was performed in a Garfagnina goat breed flock consisting of 269 females and 20 males. Age ranged from 2 to 9 years. All animals were registered in the herdbook, but genealogical information was not available. The flock was located in the Garfagnana district (Media Valle del Serchio, Lucca, Italy) and a semi-extensive farming was practiced. The goats grazed during the morning (feed supplements are given mainly over the winter), and were housed overnight, when they received an integration of forage and feed. Flock management was of a family farm type. Milking was practiced twice a day using a trolley milking and the milk was conveyed in refrigerated tanks. Blood samples from the 48 Garfagnina goats, were collected according to the recommendations of the European Council (1986) concerning animal care. Whole blood was collected in Vacutainer tubes with K-EDTA as anticoagulant and stored at −20 °C until genomic DNA was extracted using Qiagen QIAamp DNA blood mini/midi kit (Qiagen, San Diego, CA, USA). Twelve microsatellites (MAF065, SRCRSP05, INRA023, McM527, CSRDR247, SRCRSP23, OarFCB20, TGLA53, INRA005, INRA063, ETH10, ILSTS87) were used for the analysis.
Population are summarized in Table 1. In total, 71 alleles were observed for the 12 microsatellite loci analyzed. All 12 microsatellite markers resulted to be polymorphic. Table 2 reports the percentage of each of the most frequent alleles (>10%) for each marker.

The most polymorphic loci were: MAF065 and SRCRSP23 (9 alleles) (Table 1) but the alleles with a frequency higher than 10% were respectively 3 and 5 (Table 2); on the contrary the less polymorphic loci were: ETH10 (3 alleles), INRA063 and OarFCB20 (4 alleles). All the alleles of ETH10 marker and all the alleles of OarFCB20 had a frequency higher than 10% (Table 2).

The PIC per locus showed only one marker with values under the 50% (INRA063) and an average value of 64.5% (±12.30).

Genetic similarity within the population (GS), the mean molecular co-ancestry (fij), the kinship distance (Dk) and the inbreeding coefficient (Fi) were 0.430, 0.308, 0.304 and 0.318 respectively (Table 3).

RESULTS

The results of the microsatellite analysis in term of number of alleles observed, alleles size, PIC and observed heterozygosity of the analyzed Garfagnina goat population are summarized in Table 1. In total, 71 alleles were observed for the 12 microsatellite loci analyzed. All 12 microsatellite markers resulted to be polymorphic. Table 2 reports the percentage of each of the most frequent alleles (>10%) for each marker.

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INRA063, ETH10, ILSTS87), located in 12 chromosomes and amplified in one multiplex PCR reactions, were investigated. Detailed information of these markers is reported in Table 1. Some STR belonged to a markers panel validated by the International Society of Animal Genetics (ISAG) and others were routinely used by the facilities of the Laboratorio di Genetica e Servizi (Associazione Italiana Allevatori, Migliaro, Italy).

For each marker the following parameters were computed using the Molkin v2.0 program: number of alleles, effective allele size, observed heterozygosity and polymorphism information content (PIC). Allelic frequencies were estimated by direct counting. To analyze the genetic variability of the population, the following parameters were computed at population level by using the MolKin program: molecular co-ancestry coefficients (fij), kinship distance (Dk), and inbreeding coefficient (Fi). The molecular co-ancestry between 2 individuals, i and j, is the probability that two randomly sampled alleles from the same locus in 2 individuals are identical by state (Caballero and Toro, 2002). The molecular co-ancestry of an individual i with itself is self-co-ancestry (si), which is related to the coefficient of inbreeding of an individual i (Fi) by the formula Fi = 2si - 1. In turn, the kinship distance (Dk) between 2 individuals i and j is Dk = (si +sj)/2 - fij. MolKin computes within-breed molecular co-ancestry and Dk by simply averaging the corresponding values for all the within-population pairs of individuals. Moreover, genetic similarities among all animals were investigated by comparing the individual multilocus genotype of each individual. Genetic similarity is defined as P=2A/2L, where P is the proportion of common alleles (A) in relation to the 2L possibilities (L=number of considered loci). The similarities between each pair of individuals were then averaged over the whole population.

Table 1 - Locus, Dye, range, number of alleles, effective allele size (EfAlSize), observed heterozygosity (Ho) and polymorphism information content (PIC), for the 12 microsatellite loci.

Table 2 - Percentage of each of the most frequent alleles (> 10%) for each marker.
DISCUSSION AND CONCLUSION

There is a dearth of published reports on the genetic variability and on the number of alleles, their size and frequencies for microsatellite loci in goats. Although a comparison with other breeds can be biased due to the different marker sets used by different authors, it may be noted how the mean number of alleles per locus was slightly lower than that reported by Ramamoorthi et al. on the Barbari goats, and by Sechi et al. on three Sardinian goat populations, but similar to what observed in Orobita and Girgentana goats by Negrini et al. Obstetric and Girgentana exhibited small genetic variability and therefore evidence of a recent bottleneck. However, our animals derived from a single flock.

The PIC estimated in the present study is comparable with that obtained in other goat breeds, such as Saanen. The PIC was originally introduced by Botstein et al. It refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency and has been proved to be a general measure of how informative a marker is; the higher the PIC value, the more informative a marker is. In the present study, MAF065 and SCRRSP23 and ILSTS87 microsatellites appeared the most informative, whereas INRA063 the less informative.

Ten of the 12 markers considered in this research were used also by Sechi et al. for the study of the genetic variability in Maltese, Sardo autochthonous goats and their mixed blood population, and by Negrini et al. who analyzed the genetic structure of eight Italian goat breeds (Camosciata delle Alpi, Valdostana, and by Negrini et al.) Obstetric and Girgentana exhibited small genetic variability and therefore evidence of a recent bottleneck. However, our animals derived from a single flock.

In conclusion, the analysis performed with the use of 12 microsatellite markers showed that despite all goats originated from the same flock and are presumably subdivided into 3 groups with different ascenders, the inbreeding was lower than that reported in all the paper previously mentioned and the genetic variability was acceptable for a population with a reduced effective numerical value. Future work may include replication of this study with a larger number of animals belonging to different flocks.

Acknowledgements

This work was supported by grants of the University of Pisa (PRA 2016 and Fondi Ateneo).

Table 3 - Within-population diversity.

<table>
<thead>
<tr>
<th>N</th>
<th>Average n° of alleles</th>
<th>Ho*</th>
<th>f*</th>
<th>Fst</th>
<th>Dij</th>
<th>GS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>5.92±1.881</td>
<td>0.693</td>
<td>0.308</td>
<td>0.318</td>
<td>0.340</td>
<td>0.430±0.103</td>
</tr>
</tbody>
</table>


References


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References