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Note

Analysis of Genetic Variation in Agerolese Cattle Breed

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INTRODUCTION

The Agerolese is a local Italian cattle breed reared in the province of Naples, in the proximity of the town of Agerola. It is a dual-purpose breed, which originated during the nineteenth century from an autochthonous nucleus of Podolian cows crossed with Swiss Brown, Dutch Friesian, and Jersey bulls. After 1940, the breed was severely threatened by the substitution with purebred Italian Brown and Holstein Friesian cattle (Felius, 1995). In 2002 it numbered only 13 males in natural service and 100 breeding females (EAAP, http://www.tiho-hannover.de/einricht/zucht/eaap/). Therefore, according to the FAO criteria for breed categorization, based on the number of breeding subjects as well as on the trend in population size (http://dad.fao.org), the status of the Agerolese should be classified as critical. Moreover, its effective genetic size, N_e, is approximately 46, slightly below the critical value of 50 that corresponds to a rate of inbreeding of 1% per generation, which is commonly accepted as the maximum tolerable level.

The reasons for conservation of the breed rely on its integration into agricultural production systems of low to medium input. Agerolese is well adapted to mountainous country and can be fed on products of pruning and undergrowth. The milk is used to produce the Provolone del Monaco, a cheese of remote origins protected by the Slow Food Foundation for Biodiversity. Recently, a specification has been developed to apply for the Protected Designation of Origin (Peretti, personal

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[5] He passed away before he could see the completion of this project. The paper is dedicated to him.

communication). As the proposed specification of production states that milk from Agerolese must represent at least 20% of the total milk used, it is likely that the breed–product link will improve the economic profitability of the Agerolese and hence contribute to its conservation.

The aim of this study was to describe the genetic structure of the Agerolese breed and to evaluate its genetic diversity using a set of 16 microsatellite markers, in order to provide data for better genetic management of the population.

MATERIALS AND METHODS

Blood samples from 60 Agerolese individuals were collected. Genomic DNA was extracted by a rapid method using IsoCode Stix (Schleicher and Schuell, Germany). Sixteen microsatellite loci were amplified using two multiplex PCR reactions (BM1824, BM2113, ETH10, INRA023, TGLA122, TGLA126, TGLA227, SPS113, and SPS115 for the first multiplex; AGLA293, CYP21, ETH225, INRA005, MGTG4B, TGLA53, and TGLA57 for the second one). Amplicons were analyzed using an ABI Prism 310 automated sequencing system (Applied Biosystems, California).

The allele frequencies were computed with the GENEPOP software (Raymond and Rousset, 1995). The observed and expected heterozygosities were obtained using GDA software (Lewis and Zaykin, 2001), and departure from the Hardy–Weinberg proportions (HWP) was estimated by F_{IS} statistics according to Weir and Cockerham (1984) and tested with the permutation test implemented by FSTAT software (Goudet, 1995). The Bonferroni procedure was applied over loci. In order to evaluate variability between breeds, Agerolese was compared with Holstein, Swiss Brown, and Simmental breeds using the genotypic data sets obtained from the Cattle Diversity Database (http://www.projects.roslin.ac.uk/cdiv/) with the consent of the donating partners. Differences between breeds for average observed heterozygosity were estimated using the Student t-procedure, as implemented by GraphPad InStat 3.00 software (GraphPad Software). The genetic relationships among breeds were estimated by principal component analysis (PCA), performed using the PCA-GEN software package (http://www.unil.ch/izea/softwares/pcagen.html); the p-values for the percentage of inertia of each axis were computed by 1000 randomizations of genotypes. In addition, analysis of molecular variance (AMOVA) based on F-statistics implemented by the Arlequin software was performed (Schneider *et al.*, 2000). Significance of the differentiation index (F_{ST}) was tested using 1000 permutations of individuals among populations.

RESULTS AND DISCUSSION

All the loci were found to be polymorphic in the Agerolese population (allele frequencies are available upon request). The 16 microsatellites generated a total

Table I. Heterozygosity in the Agerolese Cattle Breed

Locus	H_{obs}	H_{exp}	F_{IS}
ETH10	0.667	0.685	+0.027
BM2113	0.850	0.834	−0.019
BM1824	0.733	0.750	+0.023
INRA005	0.729	0.626	−0.167
ETH225	0.763	0.783	+0.026
SPS115	0.533	0.615	+0.133
TGLA227	0.817	0.857	+0.048
TGLA126	0.633	0.704	+0.101
TGLA122	0.667	0.661	−0.009
TGLA53	0.643	0.791	+0.188**
INRA023	0.733	0.765	+0.042
SPS113	0.797	0.772	−0.032
TGLA57	0.383	0.574	+0.333***
MGTG4B	0.817	0.805	−0.014
CYP21	0.833	0.853	+0.023
AGLA293	0.317	0.343	+0.076
Over loci	0.682 ± 0.039	0.714 ± 0.033	+0.044

Note. H_{obs}: observed heterozygosity. H_{exp}: expected heterozygosity. F_{IS}: estimate of departure from the HWP.
$^{**}p < 0.01$. $^{***}p < 0.001$.

of 131 alleles, with an average number of 8.2 alleles per locus, which is similar to values found in other local and selected cattle breeds reared in Italy (Del Bo *et al*., 2001). Observed heterozygosities ranged from 0.317 to 0.850, and expected heterozygosities ranged from 0.343 to 0.857 (Table I). For most loci, as well as for the total of the loci, the F_{IS} values revealed deficiencies of heterozygosity as regards the expected HWP. However, F_{IS} was significant only for TGLA53 and TGLA57. For these loci, a significant inbreeding effect seems to be excluded, since inbreeding affects all or most loci in a similar way (Jordana *et al*., 2003), whereas in the present investigation the heterozygote deficiency was not significant for all the other markers. A possible factor causing departures from the expected values is the existence of a mutation in a primer sequence, which would lead to the typing of heterozygote individuals as homozygote. Actually, in Agerolese, TGLA53 showed the highest frequency of individuals with no PCR products (0.07). An alternative interpretation could be the close linkage of these two microsatellites with genes under natural or artificial selection. In any case, since the analysis of a population structure is based on the assumption of selective neutrality and absence of genotyping errors at the markers used, TGLA53 and TGLA57 were discarded from the subsequent analysis.

Agerolese was compared with Holstein and Swiss Brown breeds that mainly contributed to its constitution, and with Simmental, which was expected to show the highest distance from the other breeds on the basis of historical information

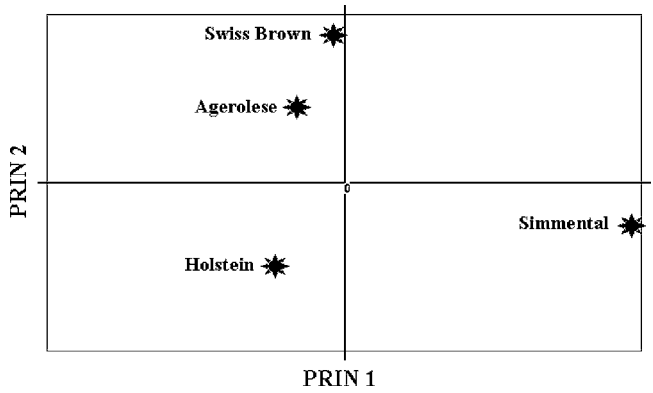

Fig. 1. Principal component analysis (PCA) of relationships among four breeds of cattle. PRIN 1: The first axis accounts for 60.1% ($p = 0.004$) of the diversity among the populations and clearly distinguishes Simmental from the other breeds. PRIN 2: The second axis accounts for 28.3% of the diversity and separates Holstein from Agerolese and Swiss Brown, but not at a significant level.

and genetic data (Del Bo *et al.*, 2001). The statistical analysis was performed considering only the 10 microsatellite loci for which all the four breeds had been typed (BM1824, BM2113, ETH10, ETH225, INRA005, INRA023, SPS115, TGLA122, TGLA126, and TGLA227). For all the loci no significant deviations from HWP were detected in the considered populations.

The average observed heterozygosity in Agerolese was rather high (0.713 ± 0.029) in comparison with the other three breeds (0.738 ± 0.034 in Holstein, 0.686 ± 0.034 in Swiss Brown, 0.582 ± 0.055 in Simmental). Only the difference between Agerolese and Simmental was significant ($p < 0.05$).

The PCA illustrated the relationships among the four breeds (Fig. 1). The first two axes together explained 88.4% of the total genetic variation. The first axis (PRIN 1) explained 60.1% ($p = 0.004$) of the diversity among the populations and clearly distinguished Simmental from the other breeds. The second axis (PRIN 2) accounted for 28.3% of the diversity and separated Holstein from Agerolese and Swiss Brown, but not at a significant level.

Further information about genetic relationships among the breeds was provided by the F_{ST} index. The proportion of genetic variation due to differences among populations was 6.9%, with the significant contribution of all loci ($p < 0.001$). Similar values were reported in the literature for cattle. For example, a value of 7% was obtained in the comparison of 18 European cattle breeds with 16 microsatellites (Cañon *et al.*, 2001) and of 8% in the comparison of 7 French breeds with 23 loci (Maudet *et al.*, 2002). Even if data from other studies should be considered with caution, for differences in the number of included breeds, sample

size, and markers used, the results obtained show an overall variability among breeds comparable to that generally observed in cattle. All the pairwise F_{ST} values (Agerolese vs. Holstein: 0.029; Agerolese vs. Swiss Brown: 0.030; Agerolese vs. Simmental: 0.118) were highly significant ($p < 0.001$), indicating that Agerolese has maintained some genetic diversity in spite of the past introgressions. Of course, random genetic drift could have contributed to such differences, due to the small size of this population.

In order to manage and preserve populations at risk, the basic step is to characterize their genetic structure and to evaluate their variability. As far as we know, the data of the present investigation are the first contribution to the genetic description of Agerolese breed and make it possible to gain an insight into its relationships with the breeds mainly involved in its establishment. The microsatellites used proved to be highly informative in Agerolese and showed that this breed maintains an unexpectedly high variability within and between breeds. Even though the rather high level of heterozygosity is a favorable premise for conservation purposes, the critical N_e of the breed underlines the existence of a considerable risk of excessive inbreeding, with all the negative consequences, especially on reproductive performance. In this respect, the genotypic information, allowing the recognition of the most heterozygous animals, will be useful in planning mating schemes aimed at maximal preservation of the existing variability.

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