STUDY OF GENETIC DIVERSITY OF THREE PORTUGUESE CATTLE BREEDS BY 93 MICRO SATELLITE MARKERS

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Abstract

The objectives of this work were to assess the genetic diversity within and between three Portuguese cattle breeds using 93 microsatellites markers. Blood samples were collected from 50 individuals of each breed, and ninety-three microsatellites were analysed to get thorough information about genetic diversity and interrelationships among three Portuguese cattle breeds: Mirandesa (MIR), Maronesa (MAR), and Barrosã (BAR). Estimates of genetic variability, observed (Ho) and expected heterozygosity (He), allelic richness for each locus were determined. The alleles were classified in three classes according to their frequency: common alleles (observed in the three sub-populations), private alleles (alleles observed in one sub-population) and rare alleles (non-private alleles with a frequency < 0.01 over the whole population). The number of rare alleles found was 52 in MAR, 33 in MIR, and 30 in BAR. The number of private alleles found was 5 in MIR and BAR, and 2 in MAR. The MIR showed the lowest genetic diversity, and the highest genetic distance to the other two breeds. The three breeds could be considered as genetically distinct populations. This study shows that measures should be taken in order to preserve the genetic diversity of MIR, MAR, and BAR cattle breeds.

Key words: Cattle Conservation Genetic diversity Microsatellites.

INTRODUCTION

Considering the animal and plant species used, extensive livestock production systems are highly heterogeneous and contributes to maintain the ecological balance. The European Union (EU), in general, and Portugal, in particular, has interest in the preservation of these production systems since they contributes to reduce environmental pollution, to maintain or increase the biodiversity and to preserve the typical landscape across EU regions. In Portugal, several autochthonous cattle breeds are classified as endangered by the MADRP (2008). In general, they present a good adaptation to adverse environmental conditions, making these breeds particularly suited for the extensive productions systems.

EU consumers are, also, sensitive to the management practices that improves the welfare of livestock animals, and are willing to pay more for these certified animal products. Thus, these high quality products may contribute to the preservation of the rural world and its diversity as well as to increase the profitability of the extensive production systems, which can contribute to the conservation of autochthonous breeds endangered.

Traits, genotypes and alleles with possible economic interest are at risk of being lost (Mateus et al., 2004b). But, genetic diversity is the basis for the sustained ability of a breed to respond to selection programs, for adaptation to environmental changes, like: climate, diseases, management and husbandry practices (Boettcher et al., 2010). Livestock breeds with small population size are prone to a rapid increase of the inbreeding coefficient, and to losses of genetic diversity, which at long term is the primary key to the survival of animal

populations. The reduction of fitness of the populations due to the inbreeding depression effects is well known, and a severe reduction of the populations size (genetic bottleneck) increases the risk genes loses. Thus, the conservation of endangered livestock breeds relies on the conservation of their genetic diversity, and at the initial stage of conservation plan the rate of inbreeding should be minimised (Baumung and Sölkner, 2003) to avoid the losses of genetic variability gained during the breeds differentiation process (Cañón et al., 2011). According to FAO (2007) those livestock breeds classified as endangered should be included in conservation programs, in order to preserve their adaptation characteristics, value for food and agriculture, and because of their cultural and historical value (Ramljak et al., 2011).

Several studies (Jordana et al., 2003; Mateus et al., 2004c; 2004a) have been conducted to study the genetic diversity of Portuguese cattle breeds, however those studies were based in 16 to 30 microsatellite markers.

Thus, the objectives of this work were to assess the genetic diversity of within and between three autochthonous Portuguese cattle breeds using 93 microsatellites markers.

MATERIALS AND METHODS

Samples and microsatellite markers
Blood samples from 131 adult animals were collected, and the animals were selected using the pedigree information in order to ensure that animals were not closely related.

This study was conducted with three Portuguese cattle breeds: Mirandesa (MIR, http://www.mirandesa.pt/caracteristicas.htm), Barrosã (BAR, http://www.carnebarrosa.com /index.asp?p=r) and Maronesa (MAR, http://www.marones.pt/conteudo.php? idm=9); bred at north of Portugal. These breeds where selected because of their geographical proximity, and because of their importance for high quality meat production which is protected by Protection Designation of Origin (PDO).

A total of 93 microsatellite markers previously described by Ramljak et al. (2011) (http://onlinelibrary.wiley.com/doi/10.1111/j.1 439-0388.2010.00905.x/suppinfo) were analyzed to estimate several parameters of genetic diversity. These loci were recommended by the International Society of Animal Genetics (ISAG) /FAO for the analysis of genetic diversity in cattle breeds (FAO/ ISAG 2004).

Samples and microsatellite markers
The genomic DNA was extracted using the QIAamp Blood-Kits (Qiagen) protocols. The summary information concerning the 93 microsatellites markers can be checked at http://onlinelibrary.wiley.com/doi/10.1111/j.14 39-0388.2010.00905.x/suppinfo. The PCR products were analysed on ABI377 and ABI310 DNA Sequencers (Applied Biosystems) at the Animal Genetics and Husbandry laboratory of the Ludwig-Maximilians-University Munich. Genotypes were assigned using GENESCAN ANALYSIS 3.7 NT (Applied Biosystems) and GENOTYPER 3.7 (Applied Biosystems). To ensure the accuracy of genotyping, all animals, including international control samples (as declared by the European Cattle Genetic Diversity Consortium), were genotyped twice in two independent courses.

Statistical analysis
The adegenet package (Jombart and Ahmed, 2012) from the R software (R Development Core Team, 2011) was used to calculate the allele frequencies, the mean number of alleles per locus and breed, the Nei′s genetic distance (DA, Nei, 1987), the observed (Ho) and expected (He) heterozygosities. The Fisher′s exact test, with standard Bonferroni corrections, was used check for the deviation from Hardy-Weinberg equilibrium (HWE).

The Wright F-statistics (FS_{IT}, FS_{ST}, and FS_{IS}; Weir and Cockerham, 1984), were calculated for each locus and across breeds using hierfstat package (Goudet, 2005) and the population pairwise FS_{ST} was computed using adegenet package (Jombart and Ahmed, 2012) from the the R software (R Development Core Team, 2011).

The alleles were classified in three categories according to their frequency: common alleles (cA), observed in all 3 sub-populations; private alleles (pA) alleles observed in one sub-population; and rare alleles (rA) non-private alleles with a frequency < 0.01 over the whole population.

RESULTS AND DISCUSSIONS

Overall genetic variability

Across the 93 microsatellite loci, a total of 554 alleles where detect for BAR, 465 for MIR, and 578 for MAR breed. The number of alleles at the 93 microsatellite loci are shown in Figure 1., and ranged from 3 (L01) to 18 (L75). The mean number of alleles per locus was 6.22 for MAR, 5.96 for BAR, 5.00 for MIR, and the number of private alleles occurred at very low frequencies (< 0.011) for the three breeds. These results are in accordance with those presented by Medurogac et al. (2009) and Ramljak et al. (2011) in studies with Central European cattle breeds and by Costa et al. (2012) in a study with Cuban cattle breeds.
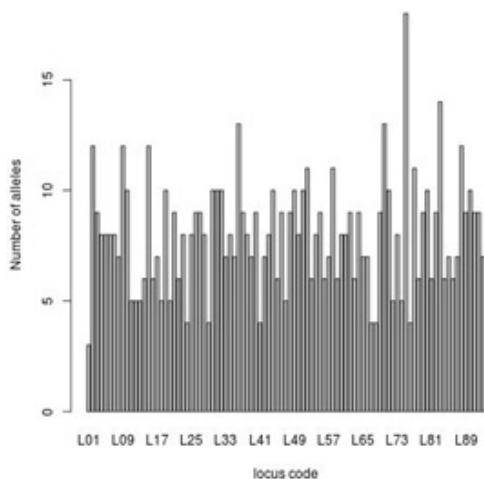

Figure 1. Alleles number per locus at the 93 microsatellite loci

Across breeds, the expected heterozygosity varied from 0.122 (L01) to 0.882 (L75), and the observed heterozygosity ranged from 0.113 (L01) to 0.781 (L57). The mean observed heterozigosity was lower (P< 0.001) than the mean expected heterozigosity as can be observed from the Figure 2.

The overall loci estimates of inbreeding, evaluated by the F_{IS} statistic, showed that the three cattle breeds presents a reduced heterozygosity due to within population inbreeding (FF$_{IS}$ = 0.0724). The breed differentiation, evaluated by the F_{ST} statistic (0.0988), indicates that only 9.88% of the total genetic variation can be attributed to differences among the cattle populations. Thus,

90,12% of the genetic variability can be attributed to the individuals within the populations.

Genetic diversity within breeds

The within-breed genetic variability measures are presented in Table 1. The mean number of alleles per locus was 6.22 for MIR, 5.96 for BAR, and 5.00 for MIR, which is lower than the mean of the three breeds. The MAR breed presented the higher number of total (578) and rare (52) alleles, and the number of private alleles were low for all three breeds (5 for MAR and MIR, and 2 for MAR). These allele richness indicators are lower than those reported by Ginja et al. (2010) in a study with 13 Portuguese cattle breeds with 39 microsatellite markers.
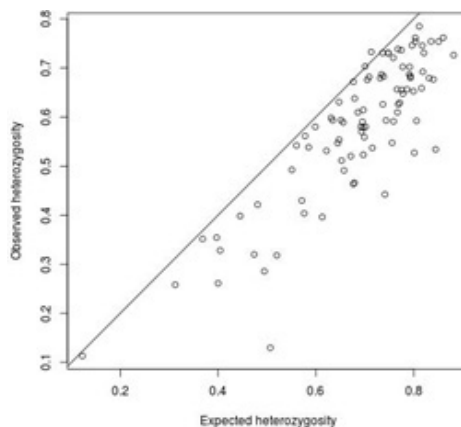

Figure 2. Observed versus expected heterozigosity for the 93 microsatellite loci

However, our results are in line with those presented by Medurogac et al. (2009) and Ramljak et al. (2011) for Central Europe cattle breeds and by Costa et al. (2012) for Cuban cattle breeds.

Table 1. Genetic variability at the 93 microsatellites loci for the three breeds studied

Breed	mA	tA	rA	pA	He	Ho
BAR	5.96	554	30	5	0.64	0.60
MAR	6.22	578	52	2	0.64	0.54
MIR	5.00	465	33	5	0.56	0.49
Mean	5.72	532	38.3	4	0.62	0.54

mA = mean number of alleles; tA = total number of alleles; rA = number of rare alleles; pA = number of private alleles; He = mean

expected heterozygosity (unbiased estimate Nei, 1987); Ho = mean observed heterozygosity.

The MAR presented the highest (Ho = 0.60 and He = 0.64) genetic diversity, and the MIR breed presented the lowest (Ho = 0.49 and He = 0.56) genetic diversity. These results corroborates those attained by Mateus et al. (2004) and Ginja et al. (2010), where the MIR also presented the lowest heterozigosity among all Portuguese cattle breeds. The three breeds presented Ho lower ($P < 0.001$) than the He (Figure 3), and the exact test for HWE within breed showed a deviation ($P < 0.001$) from the equilibrium.
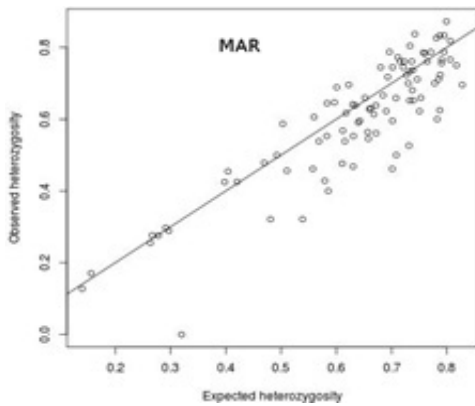

Figure 3. Expected versus observed heterozigosity for the three breeds studied

This observation is common in domestic animal populations (Costa et al., 2012), and the reduction in the heterozigosity can have several causes: selection against heterozygous animals (Wahlund effect) and inbreeding effects (Maudet et al., 2002).

Table 2 shows the frequency distributions of the inbreeding coefficient for three cattle breeds. The mean and median individual inbreeding coefficient for the analysed samples was 12.2 and 10.4% for MIR, 10.1 and 8.1% for MAR, and 10.7 and 6.96 for BAR. These results are in line with the results for heterozigosity. The inbreeding, produced by mating between relatives, is one of the causes for the losses of heterozigosity (Nei, 1987).

Populations under random mating, the genes are equally related within and between individuals, and the $F_{ST} = F_{IT} = 0$. Estimates of F_{ST} and F_{IT} that differ significantly indicate departures from random mating. In our study, both, F_{ST} and F_{IT} are positive ($F_{ST} = 0.131$ and $F_{IT} = 0.219$), thus we can assume that differences in the allele frequencies may be attributed to the effects of random genetic drift. Thus, the genetic differentiation (9.88%) can be attributed to an increase in the mean inbreeding coefficient.

Breeds interrelationships

Pairwise estimates of genetic differentiation (F_{ST}) and Nei's genetic distance (DA) among the three cattle breeds are shown in Table 2. The estimates of pairwise F_{ST} were all significant ($p < 0.01$), thus indicates that the three breeds can be considered genetically independent (Figure 5).
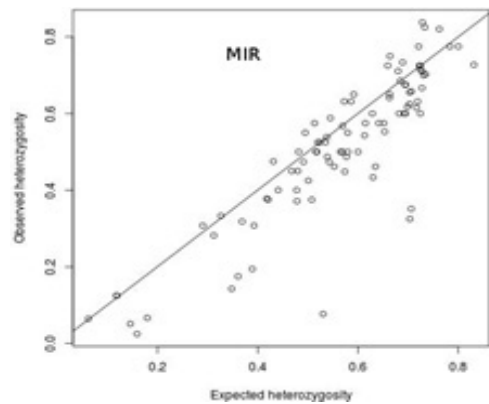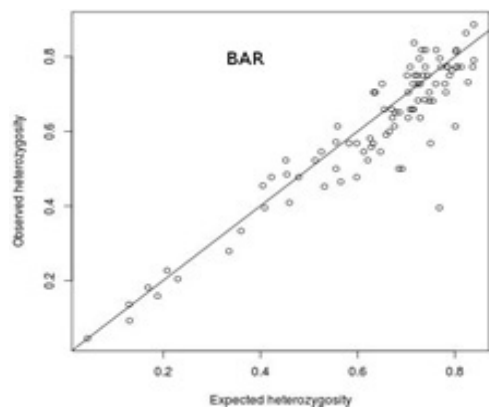

Figure 4. MIR

Figure 5. BAR

The Nei's genetic distance presented the highest values among MIR and BAR (0.477) and MIR and MAR (0.466).

Table 2. Pairwise estimates of FST
below the diagonal, and Nei

Breed	BAR	MIR	MAR
BAR	-	0.477	0.345
MAR	0.174	-	0.466
MIR	0.09	0.157	-

A principal components analysis, based on Nei's genetic distances, corroborates these results, showing that all three breeds are genetically independent (Figure 5). Thus, both MAR and BAR are genetically well differentiated from MIR (F_{ST} = 0.157 and 0.175, respectively), and this clear genetic differentiation of MIR can be attributed to the occurrence of a strong genetic bottleneck. This evidence of a strong genetic subdivision (see F_{ST} values) between MIR and both MAR and BAR corroborates the results attained by Ginja et al. (2010), that showed that MIR presented the higher genetic differentiation among all Portuguese cattle breeds.
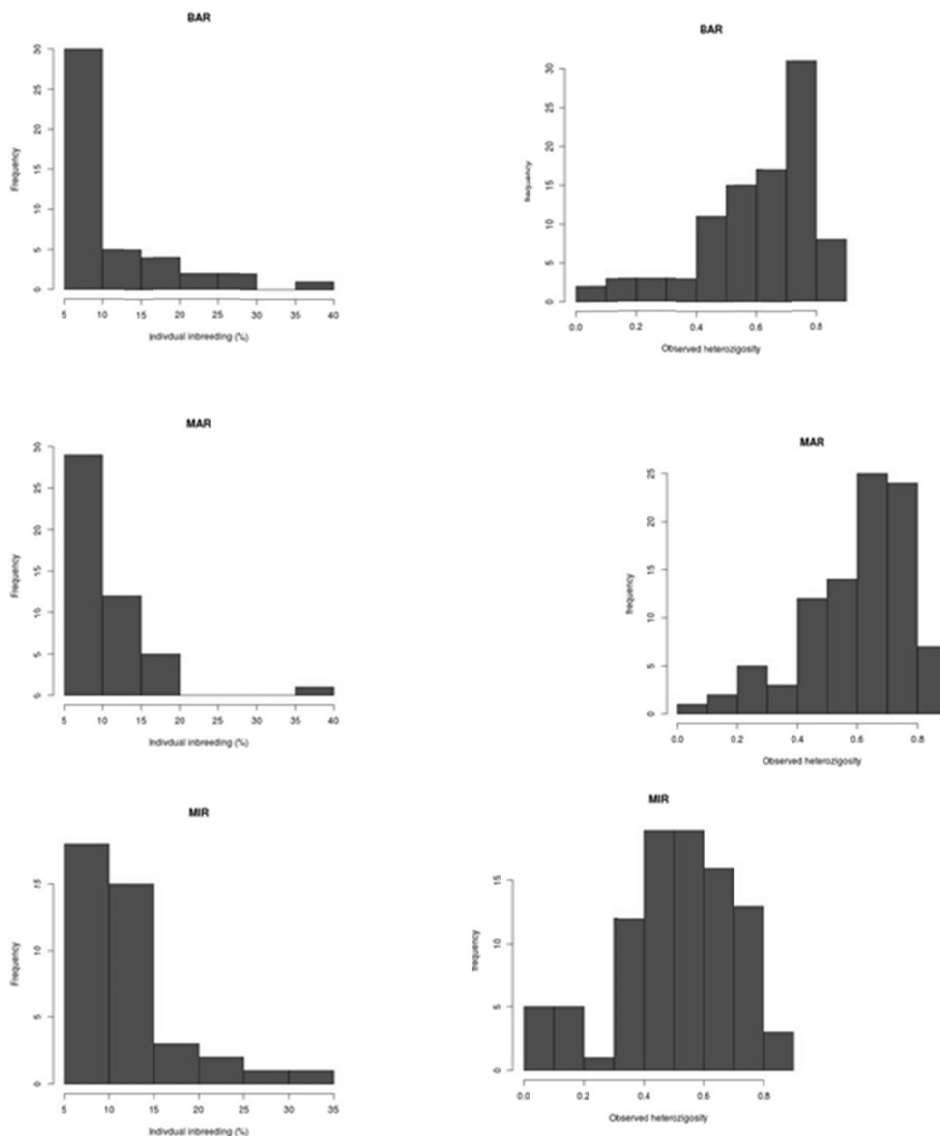

Figure 6. Frequency distributions of the individual inbreeding coefficient and heterozigosity for three cattle breeds

This results for MIR can be attributed to the increase of the inbreeding coefficient, in a short period of time, as stated by Laval et al. (2000). It is well known that populations subjected to genetic bottleneck lead to an increase of the genetic distance, distorting the topology of the evolution trees (Nei et al., 1983; Nei, 1987).
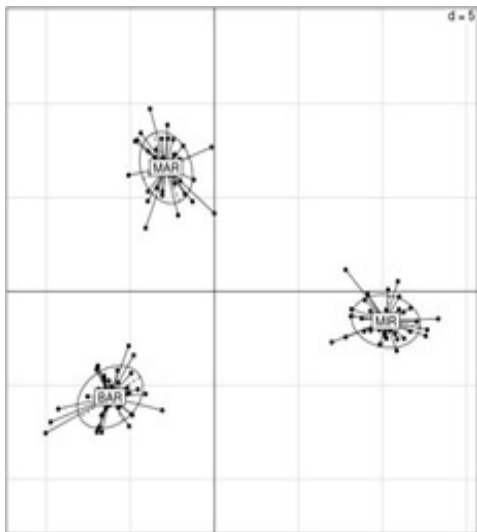

Figure 7. Principal components analysis of the Nei

CONCLUSIONS

The present study showed that a significant amount of genetic variation is maintained in the three cattle populations.

The three breeds could be considered as distinct genetic populations, however the MIR is the more genetically distance from both MAR and BAR. The MIR maintains an important genetic isolation from MAR and BAR. Populations with small effective size, needs breeding programs properly managed to avoid the losses of genetic diversity.

Thus, accurate pedigree records are essential to define matings among individuals in order to minimize the increase of the inbreeding coefficient.

Finally, it is clear that conservation measures should be developed to minimize the inbreeding in these three cattle breeds.

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