Genetic Diversity of Autochthonous Cattle Breeds: Implications on Conservation Strategy

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Introduction

Trends of globalization and industrialization of farm production during the twentieth century have led to significant erosion of farm genetic resources. Croatian autochthonous cattle breeds are part of national, regional and global genetic resources. During the last three decades the number of animals in the Istrian and Slavonian-syrmian podolian cattle population decreased drastically. Measure of public support stopped negative trends in these cattle populations so their representation numbers of adult animals are ≈ 500 for the Istrian cattle and ≈ 120 for the Slavonian-syrmian podolian cattle. Genetic diversity is essential for preservation of adaptive potential of species and improvement of production potentially high selected breeds. Small populations raise several problems when faced with conservation plans: they have lost most of their economic value, and usually show a high inbreeding level which threatens their long term maintenance (Dunner *et al*., 1998).

Monitoring and preservation of genetic diversity is the basis for effective selection and/or conservation programmes (Gutiérrez *et al*., 2003). Managing genetic diversity is one of the primary goals in conservation programmes (Toro *et al*., 2003). Pedigree analysis is an important tool to describe genetic variability and its evolution across generations (Gutiérrez *et al*., 2003). Molecular characterization is an essential prerequisite for the development of an effective and meaningful conservation programme. Microsatellite analysis is now a widespread used technique for the designated genetic variability. The control region of the mitochondrial DNA (mtDNA) is, due to its high mutation rate, lack of recombination and maternal inheritance, a very useful marker system for population and evolutionary biology.

In this study, we analyzed two endangered autochthonous cattle breeds with the aim to ascertain the levels of genetic variability, and their integration in improving of conservation strategy.

Material and methods

Material. Two Croatian endangered autochthonous cattle breeds were include in analysis: Istrian cattle (IG) and Slavonian-syrmian podolian cattle (SSP). Pedigree information data collected between 1994 and 2009 were obtained from central Herd Book register of the Croatian Agricultural Agency. In analysis were included 3017 records for IG, 629 records for SSP. Blood samples for molecular analysis were collected from unrelated animals (50 individuals IG and 50 individual SSP). Animals were chosen from wide, boundless

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geographical areas. DNA was extracted according to manufacter's protocol (QIAamp Blood-Kits, Qiagen).

Pedigree analyses. The analyses of pedigree data were performed using the software ENDOG v4.6 (Gutiérrez and Goyache, 2005).

Microsatellite variability. Thirty microsatellite loci (MoDAD marker sets recommended by FAO) have been used for the analysis of microsatellite variability. Microsatellite genotyping was carried out using ABI PRISM 310/3100 sequencer. The number of alleles (*nA*), observed and expected heterozygosity (H_O, H_E), and within population inbreeding estimate (F_{IS}) value were calculated using Fstat v2.9.3 program (Goudet, 2001). To present the genetic distance matrix in the two-dimensional (2*DD*) space we applied a heuristic approach described in Veit-Kensch *et al*. (2007).

MtDNA variability. Sequences of D-loop region of mtDNA were used to clarify the mtDNA variability. The proximal part of the D-loop region was PCR amplified using two primers (5'-GTAAAACGACGGCCAGTCTCACCATCAACCCCCAAAGC-3') and (5'-GCCCCATGCATATAAGCAAG-3'). PCR fragments were sequenced using ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit and ABI PRISMTM 310 Genetic Analyzer (PE Applied Biosystems, MA). Genetic diversity of mtDNA sequences was performed using MEGA3 (Kumar *et al*., 2004) and Phylip programme package (Felsenstein, 1993).

Results and discussion

In Table 1 are presented basic parameters of pedigree analysis of IG and SSP. In Herd Book data of IG, 80 animals were recognized as founders and 479 as half founders. In Herd Book data of SSP 18 animals were recognized as founders and 94 as half founders. Shorter generation interval was found in SSP population (6.12 year) in comparison with the IG population (6.93 year). Generation interval in IG population through maternal pathway was shorter (6.48 years) in relation to paternal pathway (7.29 years), but in populations of SSP was reverse situation (paternal pathway was shorter that maternal pathway; 5.66 : 6.05 years). The lower average coefficient of inbreeding was found in SSP population (2.12) in relation to IG population (3.53).

Table 1: Basic analysis of pedigree information of two autochthonous cattle breeds

	IG	SSP
Number of herd book data	3017	629
Number of founders	80	18
Number of half - founders	479	94
Expected inbreeding of founders	1.92%	2.59%
Generation intervals (year)	6.93±0.139	6.12±0.101
Comp. equiv. generat. (years)	2.14	1.72
Average *F* in whole population	3.53	2.12
Average relatedness coefficient	4.96	9.11
Inbreed animals	16.71	16.12
Average *F* of inbreed animals	13.01	22.84

The inbreeding coefficient is a relative value that greatly depends on pedigree completeness level. The high average relatedness coefficient was found in SSP population (9.11) suggesting on problem in maintenance of genetic variability. Further conservation strategy of SSP population must accept more careful mating scheme. The relative high average relatedness coefficient in population of IG (4.96) is results of specific breeding management, *i.e.* interchange of the mating sires among herds. Inbreeding increased by equivalent generation in population IG (1.88), suggesting the need for careful mating planning, such as factorial and compensatory mating (Caballero *et al*., 1996) or minimum co-ancestry mating (Sonesson *et al*., 2002).

In Table 2 analysis of population genetic variability based on thirty microsatellite marker are presented. Microsatellite analysis indicates relatively high average numbers of alleles per marker (6.52) in IG population, in regard to SSP population (4.90). Level of observed heterozygosity wasn't significantly different, but expected heterozygosity was significantly higher in IG in regard to SSP population (0.663 : 0.572). Estimated level of inbreeding was significantly higher in IG (0.112), in regard to SSP population (-0.016).

Table 2: Average number of alleles (*nA*), observed and expected heterozygosity (*Ho*, *He*), inbreeding estimates (F_{IS}) in two autochthonous cattle breeds

	IG	SSP
Average number of alleles (***nA***)	6.52	4.90
Average observed heterozygosity (***Ho***)	0.589	0.581
Average expected heterozygosity (***He***)	0.663	0.572
Average inbreeding estimates (F_{IS})	0.112	-0.016

Limited and small part of diversity space which is covered by SPP population (Fig. 1) indicates a strong genetic drift and presence of bottleneck within this population. Covering larger part of diversity space, higher allelic richness and heterozygosity put IG in better position for election of conservation plans than SPP population.

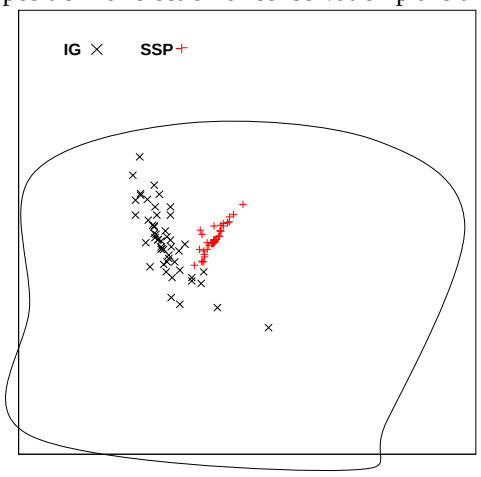

Figure 1: Individual genetic distance matrix in two-dimensional space.

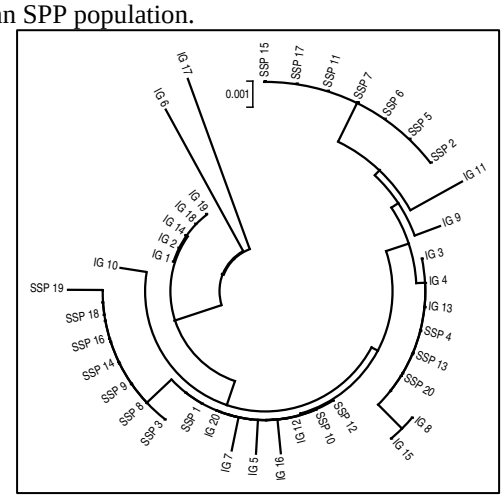

Figure 2: Unrooted tree mtDNA D-loop sequences IG and SSP.

The 783-bp fragment of the mtDNA D-loop region was sequenced and 22 polymorphic sites were observed. Mean number of nucleotide differences and Kimura-2 parameter distance within sequence IG population was significant higher (3.111±0.80; 0.004) than sequence in SSP population (1.805±0.84; 0.0023). Differentiation coefficient of population was 0.074±0.022.

Conclusion

Managing genetic diversity is one of the basic conservation goals for autochthonous, especially endangered breeds. All available information regarding population genetic structure should be well known and used for multiple pusposes. Conservation strategy example of two Croatian cattle breeds shows possibility to use pedigree information in creating mating schemes in order to preserve genetic diversity, assessing genetic interval and other genetic parameters, with necessary attention due to limited depth of pedigree information. Parameters of genetic diversity are more reliably estimated using molecular genetic markers. They reliably point to abundance of genetic variants (alleles, sequences), as well as critical events happened during breed history (bottleneck, etc.). Genotyping of microsatellite markers and mtDNA sequences is efficient toll in evaluation of pedigree information.

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