Monitoring genetic variability of Bulgarian cattle biodiversity

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ABSTRACT - The present paper aimed to characterize, using 19 microsatellite markers, three native Bulgarian cattle breeds, Iskar, Rhodope Shorthorn and Bulgarian Rhodope and to clarify their population structure. The three breeds own a genetic variability comparable with other European cattle breeds, nevertheless they showed a significant deviation from Hardy-Weinberg equilibrium in terms of heterozygote deficiency. Allelic frequencies distribution among breeds was highly significant confirming their genetic uniqueness. The population structure of Rhodope Shorthorn was complex and is probably the cause of its rather high FIS estimate (0.111); Iskar breed structure is also rather fragmented and should be studied more deeply while Bulgarian Rhodope population seemed to be the less variable. Presented results helped to clarify the present situation of Bulgarian cattle biodiversity giving interesting suggestions for their management and conservation.

Key words: Bulgarian cattle, Biodiversity, Microsatellite, Population structure.

Introduction – The three Bulgarian native breeds: Iskar (ISK), Rhodope Shorthorn (RHS) and Bulgarian Rhodope (BRH) are located mainly in the southern part of Bulgaria, the Rhodope mountain region. These breeds are well adapted to live in mountain areas in difficult environmental conditions. Bulgarian Rhodope is a synthetic breed created crossing ISK, RHS, Brown Swiss and later Jersey in order to increase the rather poor milk production of these breeds. Rhodope Shorthorn cattle is considered the ancestor of many Balkan breeds; nowadays its population is very small and fragmented and to be maintained, a conservation programme is needed. The actual situation of ISK is rather complex; because of its similarity and of crosses carried out with Bulgarian grey cattle, its genetic uniqueness and features are not sure anymore. Aim of this study is to genetic characterize these breeds for determining their genetic uniqueness and population structure for better management and set up conservation priorities.

Material and methods – The dataset was composed of 195 individual samples: RHS (n=73), ISK (n=82) and BRH (n=40). Samples were collected in several farms and stored
in 5 ml tubes at 4°C until analysis were performed. DNA was extracted from 300μl of blood using a modified commercial kit (Gentra System PUREGENE DNA purification kit). Samples were amplified by PCR in correspondence of the following 19 microsatellite loci: ILSTS008, BM1818, TGLA57, ETH3, RM12, INRA006, MM12, TGLA126, INRA016, TGLA122, CSSM14, TGLA53, INRA64, ETH152, BM203, ETH10, ETH185, BL42, SPS115. Details on the amplification protocol are available upon request. Allele size was determined with a CEQ™8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA). Molecular data were analyzed using different software for population genetics in order to: estimate observed and expected heterozygosity (Genetix version 4.05.2, Belkhir et al., 1996 – 2004), test for deviation from Hardy-Weinberg equilibrium (HWE, GENEPOP 3.4, Raymond and Rousset, 1995), estimate the inbreeding coefficients within population and breed and estimate allelic richness (FSTAT 2.9.3, Goudet, 1995), and analyze population structure (STRUCTURE, Pritchard et al., 2000). In particular, STRUCTURE version 2.2.3 was implemented to detect the most likely number of clusters (K) in the dataset. The estimation was assessed using an admixture model with correlated allele frequencies. One to ten inferred clusters were performed with 25 independent runs each. All analysis used a burn-in period of 100,000 iterations followed by 250,000 iterations for data collection.

**Results and conclusions** – The estimates of expected and observed heterozygosity within each breed are shown in Table 1. Each breed showed values of heterozygosity lower than expected and a highly significant (P<0.001) deviation from HWE. Expected heterozygosity estimates are comparable with what observed in other European cattle breeds (Del Bo et al., 2001; Dalvit et al., 2008). Rhodope Shorthorn seemed to own a slightly greater diversity respect to ISK and BRH, in fact it exhibited higher heterozygosity estimates and higher allelic richness (number of observed alleles per locus corrected by sample size).

| Breed   | Sample size | H. exp. | H. obs. | FIS          | AR  |
|---------|-------------|---------|---------|--------------|-----|
| RHS     | 73          | 0.670 ± 0.119 | 0.596 ± 0.154*** | 0.111 (0.068 – 0.138) | 7.6 |
| ISK     | 82          | 0.666 ± 0.152 | 0.591 ± 0.151*** | 0.113 (0.072 – 0.144) | 7.0 |
| BRH     | 40          | 0.619 ± 0.177 | 0.576 ± 0.228*** | 0.069 (0.001 – 0.105) | 6.3 |

*** P<0.001.

The FIS value, an estimation of the degree of inbreeding in the population, was rather high especially in RHS and ISK. However, high FIS estimates could also be caused by the presence of population substructures; in this case both RHS and ISK showed a rather complex structure as shown in Figure 1. The most probable number of clusters detected in the population was six, as evidenced in Figure 1. Rhodope Shorthorn showed the most complex pattern as it was fragmented in three subpopulations and part of RHS was assigned to the...
same cluster of ISK. Iskar exhibited a particular pattern as a part of the population was very homogenous and did not present any level of admixture with the other clusters, while a second one was completely different and fragmented. This particular structure supports the hypothesis that collected samples derived also from Bulgarian Grey cattle; to clarify this situation and to conserve ISK breed, a deeper study, including more samples is needed. On the other hand, BRH did not show any substructure as all animals were assigned to only one cluster with high proportion of membership. It is known that BRH was obtained crossing, among the others, RHS and ISK, however, the present population is genetically well differentiated from the others and can be surely considered as a separate, distinct breed. Concluding, this study highlighted the complex structure of Bulgarian native cattle breeds and suggested to study in depth the structure of RHS and ISK before setting up an appropriate conservation scheme.

Figure 1. Structure clustering of Bulgarian cattle breeds: Rhodope Shorthorn (RHS), Iskar (ISK) and Bulgarian Rhodope (BRH).

REFERENCES – Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., Bonhomme, F., 1996–2004. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions. CNRS UMR 5000, Université de Montpellier II, Montpellier, France. Available at: http://www.genetix.univ-montp2.fr/genetix/intro.htm. Dalvit, C., De Marchi, M., Dal Zotto, R., Zanetti, E., Meuwissen, T., Cassandro, M., 2008. Genetic characterization of the Burlina cattle breeds using microsatellite markers. J. Anim. Breed. Genet., 125: 137-144. Del Bo, L., Polli, M., Longeri, M., Cerotti, G., Looft, C., Barre-Dirie, A., Dolf, G., Zanotti, M., 2001. Genetic diversity among some cattle breeds in the Alpine area. J. Anim. Breed. Genet., 118: 317-325. Goudet, J., 1995. FSTAT (version 2.9.3): a computer programme to calculate F-statistics. J. Hered. 8: 485–486. Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945–959. Raymond, M., Rousset, F., 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86: 248–249.