GENETIC CHARACTERIZATION OF ISTRIAN CATTLE USING MICROSATELLITE MARKERS

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Scientific paper

Summary

Istrian cattle breed is one of the three Croatian autochthonous cattle breeds, which was on the edge of survival by the end of the twentieth century. The genetic structure of 159 animals of Istrian cattle was estimated using nine microsatellites. The number of alleles per microsatellite locus was in the range from five to fourteen, with a relatively high average number of alleles of 9.1. The mean observed heterozygosity was 0.648, while the mean expected heterozygosity was 0.729. These parameters indicate that Istrian cattle preserved a considerable amount of genetic variability, although a heterozygote deficit of 11.5% was established. This deficit is mainly the result of inbreeding and/or population subdivision. In this breed, no serious demographic bottleneck has occurred. Factorial correspondence showed genetic homogeneity in the population of Istrian cattle. Our results suggest usefulness of molecular information for preservation of the genetic variability of Istrian cattle.

Key words: *Istrian cattle, microsatellite markers, genetic variability*

INTRODUCTION

Autochthonous breeds of domestic animals are valuable genetic and cultural heritage. Over a long period of time, they have had a major role in agriculture and the social life of rural populations, influencing the agricultural production and the environment, inspiring artistic works and folklore. Thanks to centuries of coexistence with humans, Istrian ox (boškarin) has become a recognizable symbol of Istria. Due to the loss of their economic function, autochthonous breeds are in danger of extinction, which may result in a permanent loss of the accumulated genetic value. In the future, a possible change of environmental factors might motivate utilization of the rare genes possessed by autochthonous breeds, whose values still cannot be fully evaluated today. The breeding area of Istrian cattle is the peninsula of Istria and Kvarner islands. The actual population size is around 1000 breeding individuals, which represents a meaningful breeding step in comparison to the one hundred heads registered twenty years ago, when the preservation program for Istrian cattle was launched. Effective population size, population dispersion, age of farmers and public interest, identify the breed as highly endangered. In 2008, a breeding program for Istrian cattle was accepted and conducted by the Istrian Cattle Breeders Association (SUIG) and the Croatian Agricultural Agency. The breeding program is supported by activities within the Agency for Rural Development of Istria (AZRRI) project titled "Permanent preservation of Istrian cattle by commercial exploitation through the system of rural development of Istria," whose main aim is economic reaffirmation of Istrian cattle through meat and milk production, tourism and other activities. The present studies of phenotype and genetic structure indicate that Istrian cattle breed belongs to the Podolian cattle group, and point out some specific characteristics of the breed (Ivanković et al., 2007; Dalvit et al., 2009; Ivanković et al., 2009). A targeted introduction of Italian Podolian breeds from Romagna, Puglia, Marche and Polesine in the late eighteenth century to improve production characteristics left a mark on the genetic profile of the breed. Therefore, the aim of this study is characterization of the genetic structure of Istrian cattle in order to optimize breeding guidelines.

MATERIAL AND METHODS

The survey covered 159 individuals from the population of Istrian cattle. Blood samples were collected from several locations in the area of Istria, and stored at -20°C until analysis implementation. Nine microsatellite markers from ISAG/FAO list were included in the analysis (*ETH3, ETH10, ETH152, ETH185, INRA023, MM12, TGLA122, TGLA126, TGLA227*). For DNA extraction, the QIAamp Blood-Kits was used. PCR amplifications were performed in the reaction mixture of 15µl (1 µl DNA, 4.2 µl 10 pM primers, 0.2 µl Taq polymerase 100 IU, 1.5 µl 100 mM PCR buffer, 1.5 µl 2 mM dNTP, 0.9 µl 25 mM $MgCl_2$). Electrophoresis was conducted on an ABI Prism 310/3100 DNA Sequencer, and the size of DNA fragments was preformed with the ABI GeneScan and ABI Genotyper software. The number of alleles, observed and expected heterozygosity, deviation from Hardy-Weinberg equilibrium and fixation index (F_{IS}) were calculated with Fstat v2.9.3 (Goudet, 2001) program. The graphical display of factorial analysis was done with Genetix4.05 program (Belkhir et al., 2004).

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RESULTS AND DISCUSSION

The number of alleles per locus was in the range from 5 (*ETH10*) to 14 (*TGLA122*), with an average of 9.1. The largest number of alleles (39%) had a frequency from 1% to 10%. Seven loci displayed 17 rare alleles with frequencies below 1%. *TGLA122* locus has four rare alleles (139, 157, 166, 169), whereas *INRA023* (201, 211, 221), *ETH3* (109, 115, 131), *MM12* (171, 185, 195) have 3 rare alleles; *ETH185* locus has two rare alleles (228, 242) and *TGLA126* (114) and *TGLA227* (126) locus have one. PIC values per locus were in the range from 0.297 to 0.701. Three loci had a moderate informativeness level (*ETH10, ETH3, TGLA126*), while the other six loci were highly informative. The main parameters of the genetic variability of Istrian cattle are shown in Table 1.

The average allele number of 9.1 in this study was higher than in the previous reported study (Dalvit et al., 2009; Ivanković et al., 2009) of Istrian cattle. Istrian cattle breed has a higher average number of alleles in comparison to some Italian Podolian cattle breeds (Chianina, 6.53; Marchigiana, 7.76; Romagnola, 6.65 and Piedmontese, 8.06; Ciampolini et al., 1995; Moioli et al., 2004). A lower level of polymorphism was recorded in Belgian cattle breeds (Peelman et al., 1998), Spanish cattle breeds (Martín-Burriel et al., 1999; Martín-Burriel et al., 2007; Rendo et al., 2004), France (Maudet et al., 2002) and Portuguese cattle breeds (Beja-Pereira et al., 2003).

Tab. 1: Size and number of alleles, informativeness of markers (*PIC*), observed and expected heterozygosity (H_O, H_E), deviation from Hardy-Weinberg equilibrium (*HWE*), fixation index (F_{IS}) with significance level

Locus	Allele size	Number of alleles	*PIC*	H_O	H_E	HWE	F_{IS}
INRA023	199-221	10	0.624	0.636	0.804	***	0.211***
ETH10	210-222	5	0.297	0.500	0.532	ns	0.064
ETH152	287-301	6	0.577	0.758	0.782	ns	0.034
ETH185	222-242	10	0.618	0.756	0.803	***	0.061***
ETH3	109-131	9	0.341	0.497	0.558	**	0.113
MM12	171-195	11	0.552	0.705	0.768	ns	0.085
TGLA122	139-169	14	0.563	0.632	0.741	***	0.150***
TGLA126	114-124	6	0.495	0.569	0.727	***	0.221**
TGLA227	102-126	11	0.701	0.778	0.850	*	0.087
Average		9.1	0.530	0.648	0.729	***	0.115***

*P<0.05; **P<0.01; ***P<0.001; ns – not significant

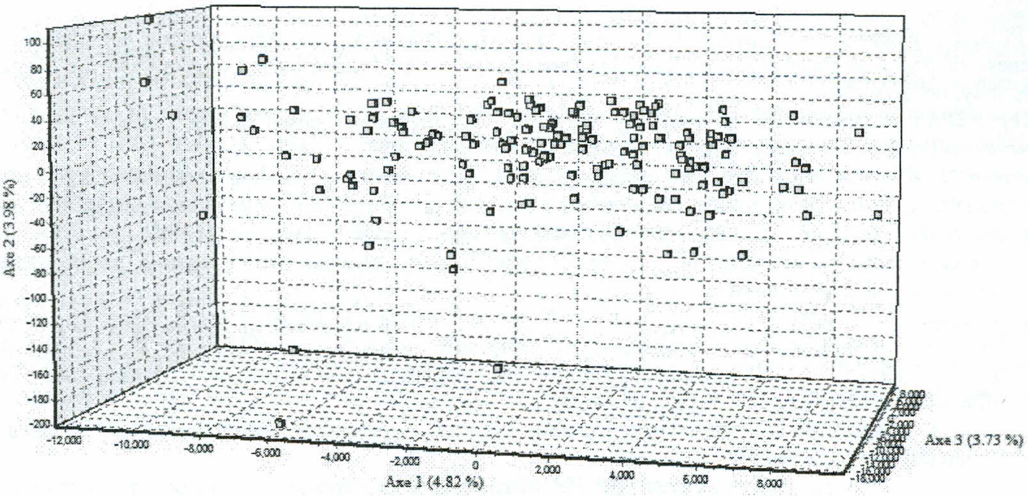

Fig. 1: Factorial correspondence analysis of Istrian cattle

The values of observed heterozygosity (HO) per locus were in the range from 0.497 to 0.778. Four loci (INRA023, ETH185, TGLA122, TGLA126) significantly deviated from HWE (P<0.001); ETH3 locus deviated at the level of P<0.01, and TGLA227 locus deviated at the level of P<0.05. The significant deviation from Hardy-Weinberg equilibrium on the six loci, probably as a result of deficit of heterozygous individuals, may be due to the presence of null alleles, Wahlund effect, inbreeding or selection towards homozygotes (Maudet et al., 2002). The most likely reason for the heterozygote deficit in the population of Istrian cattle was inbreeding, which was confirmed by a high and statistically significant value of FIS coefficient. The average observed and expected heterozygosity (HO=0.648, HE=0.729) was higher compared to the earlier studies of Istrian cattle (Dalvit et al., 2009; Ivanković et al., 2009). Istrian cattle have higher values of the observed and expected heterozygosity in comparison to the Italian cattle breeds of Podolica and Maremmana (Moioli et al., 2004) and some Spanish breeds (Martín-Burriel et al., 1999; Martín-Burriel et al., 2007).

An average FIS value was 11.5%, indicating a statistically significant deficit of heterozygous individuals in the population of Istrian cattle ($P<0.001$). All loci displayed a deficit of heterozygotes, ranging from 3.4% to 22.1%. Factorial correspondence analysis indicates genetic homogeneity in the population of Istrian cattle (Figure 1). The first axis accounts for 4.82% of genetic variability, the second axis accounts for 3.98%, while the third axis accounts for 3.73% of the genetic variability within the population.

The values of FIS coefficient in Italian cattle breeds were slightly higher: Podolica 0.106 and Maremmana 0.138 (Moioli et al., 2004). D`Andrea et al. (2009) indicate lower values of inbreeding coefficient in some Italian breeds: Podolica 0.047, Piedmontese 0.031, Chianina 0.022 and Romagnola 0.016, compared to Istrian cattle. The lower values of inbreeding coefficient were found by Martín-Buriell et al. (2007) in six Spanish autochthonous cattle breeds and by D`Andrea (2009) in two German cattle breeds (German Original Black Pied-West and German Brown Original). In the population of Istrian cattle, four basic lines (Ras, Bodul, Bakin and Boškarin) are represented, whose crossing contributes to a higher level of inbreeding.

CONCLUSION

In the population of Istrian cattle, a significant proportion of genetic variability is maintained. The highest number of alleles had a frequency from 0.01 to 0.10, and only 2 alleles had a frequency greater than 0.50. Seventeen rare alleles with a frequency less than 0.001 were observed. Deviation of the Istrian cattle population from Hardy-Weinberg equilibrium was significant due to a deficit of heterozygotes. The value of F_{IS} fixation index indicates the presence of inbreeding in the Istrian cattle population of 11.5%.

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