Genetic diversity determined by Microsatellites and Single Nucleotide Polymorphism Markers: case study of two native cattle breeds

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Abstract

Highly informative genetic markers are essential for efficient management in populations of domestic animals (i.e. individual identification, establishing genetic variability and structure, parentage verification, etc.) and for food traceability. During the last decade, microsatellites were the most used marker system, although replaced graduadly in the last few decades by single nucleotide polymorphisms (SNPs). The aim of the paper was to compare the degree of information provided by microsatellites with those gained by SNPs for native Croatian cattle breeds. Both markers system have been proven as a useful tool for determination of genetic variability in Istrian cattle and Slavonian Syrmian Podolian cattle. The application of both marker systems is advantageous in simultaneously addressing a variety of questions related to breeding and selection. However, decission which method should be used depends on the purpose and objective of the research and available equipment. The chosen method should be, above all, practical and user-friendly.

Introduction

The application of molecular markers has revolutionized management of domestic animals in many segments: from both, individual and population identification, assesing relationship between two or more individuals to parentage verification and food traceability. At the beginig, there were several types of molecular marker systems as a matter of choice (protein polymorphisms, blood group, etc.). In the last two decades, microsatellites or short tandem repeats (STRs) have been used the most. Microsatellite markers are usually di-or three-nucleotide repeat (e.g. CACACA), repeated several times in tandem. They are highly polymorphic, informative and interspersed throughout the entire genome what makes them a good tool for genetic analyses. They do not encode proteins and are thus assumed selectively neutral. However, these markers have disadvatages such as appearance of null-alleles (existing alleles that are not observed using standard assays), they are specific to species and therefore more difficult to compare, time-consuming and expensive to develop (VIGNAL et al., 2002). At the beginning, microsatellite pannel was primarly developed and used for cattle genotyping (with the exception of human and mice genotypng) and now they are available for most livestock species: sheep, goats, horses, donkeys, pigs, and chicken. For easier comparison of research results and to overcome inconsistencies by different laboratories (i.e. allele size calling and errors in size determination) FAO and the ISAG – FAO Advisory

Group on Animal Genetic Diversity proposed panels of 30 microsatellite markers (FAO, 2011).
The most recent tool for studying DNA sequence variation is single nucleotide polymorphism (SNP) which is a single base-pair variation that exists between individuals. SNPs gained high popularity due to greater abundance, small mutation rate (1×10^{-9}), automated analysis and data interpretation (VIGNAL et al., 2002). Genome-wide studies using SNPs have enabled the mapping of quantitative trait loci (QTL) and prediction of animal's genetic merit for the traits of interest in different animal species (SCHMID and BENNEWITZ, 2017). Permanent progress in genomic selection has encouraged the development of high density chips for almost all domestic animal species, i.e. BovineHD 777K with > 777,000 evenly spaced SNPs (Illumina) or Affymetrix Axiom Equine HD array with 670,000 SNPs (Neogen). However, because typically SNP loci are biallelic, heterozygosity cannot exceed 0.5, and such low heterozygosity is disadvantageous for analysis which requires high statistical power (i.e. parentage).

In Croatia, over the last 20 years, attention has been given to protecting autochthonous breeds of domestic animals. Therefore, National Programme with the aim of protection and conservation of livestock was brought in 2010 (NATIONAL PROGRAMME, 2010). This program includes 27 native breeds, of which two, Istrian cattle (IC) and Slavonian Syrmian Podolian cattle (SSP) were the subject of this study. Systematic monitoring and inventarisation for IC started early in 1989 and for SSP latter, in 2008. This comprehensive process included engagement of breeders, breeders associations, State and Public administration bodies as well as scientific and educational institutions. According to the latest ANNUAL REPORT (CAA, 2017) IC counts 866 individuals (823 femaes, 42 males) and SSP counts199 individuals (189 females, 10 males). Although population of IC and SSP increased, estimated effective population size suggests that IC is highly endangered (*Ne* =159.8) and SSP (*Ne* =37.9) critically endangered breed (CAA, 2017).
Important measure of population protection includes the assessment of preserved neutral genetic variability (evident as number of alleles, allelic richness, heterozygosity, etc.) that are mainly accumulated in nonselected native breeds (MEDUGORAC et al., 2009). It was demonstrated that unselected Busha strains show higher alleleic diversity (in terms of total number of alleles, private and rare alleles) than some European breeds. In addition, reduced genetic variation that results from inbreeding and small population size has also been found to correlate with a range of defects, many of which are associated with reproductive traits, fitness and decreased production. This has been demonstrated by GONZÁLEZ-RECIO et al. (2007) using pedigree-based inbreeding of Holstein cattle where pregnancy rate decreased by 1.68% for cows with inbreeding level from 6.25 to 12.5%. PRYCE et al. (2014) found that increase in inbreeding by 1% based either on pedigree or genomic data was associated with a decrease in milk, fat and protein yields of around 0.4 to 0.6% and an increase in calving interval of 0.02 to 0.05% in population of Holstein and Jersey cattle in Australia. Therefore, genetic variations displayed by genetic differences between individuals and populations within a given species are the basis for future livestock management. There is a growing need to maintain animal genetic diversity to be able to facilitate rapid adaptation considering production and environmental demands and challenges in the future. In this respect, it is important to use accessible, feasible and cheap analyses to facilitate comparing results from other surveys with a high percentage of reliability. Therefore, the aim of this paper was to compare genetic diversity results of IC and SSP using two different marker systems from previous researches. In addition, we also want to see which marker system seems more effective and appropriate for some analyses (i.e. determine genetic diversity, parentage verification, etc.).

Material and methods

This survey included data set from previous published research with 105 microsatellites and 51 unrelated animals of Istrian cattle (IC) and Slavonian Syrmian Podolian cattle (SSP) as described in RAMLJAK et al. (2011).
Bovine SNP50 BeadChip (iScan SY101-1001, 189 Illumina) was used for the second research (RAMLJAK et al., in press) and after passing filtering criteria (call rates < 95%, minor allele frequencies < 0.025, and samples with more than 10% of missing genotypes) 45.454 SNPs were used. In order, to reduce ascertainment bias 4-SNP haplotype blocks were defined and considered as multi-allelic markers with their haplotypes as alleles as described in SIMČIČ et al. (2015). These multi-allelic markers and derived allele frequencies were used to infer unbiased allelic diversity and heterozygosity. The number of analysed animals for SNP analyses was 30 for IC and 24 for SSP.
According to NEI (1987) mean number of alleles (*mA*, in second case *mA* per block), observed (H_O) and expected heterozygosity (H_E) were estimated. More information about methodology used in the estimation of genetic diversity is described in SIMČIČ et al. (2015) and RAMLJAK et al. (in press).

Results and discussion

Description of some of genetic diversity parameters based on SNP and microsatellite analysis in two Croatian native cattle breeds, IC and SSP is shown in Table 1. In general, both breeds have similar values for estimated parameters of genetic variability. The average *mA* for SNP showed a higher value for about 1.06 times compared to *mA* for microsatellites. Precisely, for SNP an average *mA* in IC was of 7.19 and in SSP 5.17 while average *mA* for microsatellites was 6.82 and 4.85. The same situation happened conserning observed and expected heterozygosity values. Higher H_O for SNP were 0.713 for IC and 0.675 and SSP compared to H_O for microsatellites of 0.635 and 0.593. Microsatellite-based diversity analysis revealed low values for H_E of 0.677 (IC) and 0.583 (SSP) compared to SNP-based results of 0.705 and 0.636 for IC and SSP, respectively.

Table 1. Average number of alleles (*mA*), observed (H_O) and expected (H_E) heterozygosity for the SNP and microsatellites sets of markers in Istrain cattle (IC) and Slavonian Syrmian Podolian cattle (SSP) cattle. Numbers in bold show the highest value.

Marker type	*mA*		H_O		H_E	
	IC	*SSP*	*IC*	*SSP*	*IC*	*SSP*
$SNP_{(4\text{-}SNP)}$	**7.19**	**5.17**	**0.713**	**0.675**	**0.705**	**0.636**
Microsatellites	6.82	4.85	0.635	0.593	0.677	0.583

Almost identical values of *mA,* H_O and H_E for IC and SSP were obtained in SIMČIČ et al. (2015), i.e. H_O and H_E for IC were 0.719 and 0.711 while for the SSP were 0.681 and 0.642. Therefore, parameters of genetic variability are comparable since similar number of SNPs (44 496) were used in SIMČIČ et al. (2015). If we compare diversity measures using SNPs (without being classified into blocks) for IC (H_O = 0.325, H_E = 0.321) and SSP (H_O = 0.306,

H_E = 0.289; RAMLJAK et al., in press) with other research results, both of these are comparable. In such a way, IC and SSP as typical Podolian breeds show lower level of diversity compared to a group of four Podolic cattle breeds (H_E = 0.386; PARISET et al., 2010). Indigenous Croatian SSP populaton had similar genetic diversity values compared to indigenous and locally-developed breeds from South Africa (0.28-0.30 for H_E; MAKINA et al., 2014) or even lower compared to Spanish beef cattle (0.299-0.319 for H_E; CAÑAS-ÁLVAREZ et al., 2015) but expected heterozigosity for indigenous IC was higher. Although clarification of diversity results of Crotian indigenous breeds is not a topic of this paper, it will contribute to a better understanding and results interpretation of used markers. Both, microsatellite and SNPs show lower variability in SSP population as a result of severe bottleneck and genetic drift that were reflected in lower *Ne* and lower heterozygosity (RAMLJAK et al., 2011; RAMLJAK et al., in press). Moreover, a founder effect is occurred as a consequence of the low number of first reproducers (only four bulls) at the beginning the 1990s when revitalization of this breed started. On the other hand, greater diversity in IC reflects centuries of systematic breeding and care implemented in breeding program at the end of the 19th. At the beginning of the 19th century, crossbreeding of IC with the Italian breeds (Maremmana) is abandoned due to lower resistance and worse characteristics as working animals (at that time famous were Istrian oxen for field work) and breeding of Istrian cattle in pure-blood was continued.

Placing in relationship diversity results between microsatelites and SNPs, EDEA et al. (2013) reported lower values for observed (0.382) and expected (0.385) heterozygosity in five native Ethiopian cattle populations based on SNP study than those obtained using microsatellites (H_O = 0.674; H_E = 0.726) in 10 Ethiopian cattle populations by DADI et al. (2008). Althoug, using only six SNPs and 20 microsatellite markers in analysis, CARRUTHERS et al. (2011) obtained similar findings of higher observed and expected heterozygosity values using microsatellite markers than SNPs in nine Angus cattle populations. The difference in the results using these two approaches could be explained due to multi-allelic nature of microsatellites. It is well known that microsatelites have large numbers of alleles per locus (between 3 up to 17 alleles; see cited papers) compared with two allels for each SNP. According to FAO standards, proposed panels of 30 microsatellite markers provide sufficient and comparable population structure results, parentage analysis and identity verification. Microsatellites with core repeats 3 to 5 nucleotides long, probability of exclusion > 0.999 are preferred in parentage analysis while number of microsatellite can vary (VIEIRA et al., 2016). SCHNABEL et al. (2000) demonstrate high efficiency of 12 microsatellite markers (exclusion probabilities of 0.999) for determining parentage in domestic cattle. In populations that might be expected to have a high level of homozygosity due to traditional method of breeding (i.e. horse breeds), the number of markers of 13 show to be sufficient for parentage testing with high efficiency (KHANSHOUR et al., 2013).

However, as stated above, the lower polymorphism of SNP markers does not necessarily mean a weaker result in genetic sturucture analysis. Numerous studies reveald that the 2-2.5 SNPs are sufficient for replacing one polymorphic microsatellite locus. The comparison of the two types of markers showed that about two SNPs were necessary to provide the same statistical power as one microsatellte marker. WERNER et al. (2004) reported that 37 SNPs provided the same power as a commonly used microsatellite set, while HERRÁEZ et al. (2005) found that 2.6 SNPs matched one microsatellite for prentage assessment in Galloway cattle. FISHER et al. (2009) observed that 40 SNPs were equivalent to the 14 microsatellites (ratio 2.5 SNPs to 1 microsatellite) for parentage testing. More recently, FERNÁNDEZ et al. (2013) reported that 24 SNPs were equivalent to the ISAG minimal recommended set of 12 microsatellite markers (match probability10^{-11}) for parentage verification. Nevertheless, there

are also cases when one marker system is not good enough to detect the genetic structure. TOKARSKA et al. (2009) reported unsuccessful microsatellite-based paternity and identity analysis compared to a panel of 50–60 bovine SNPs characterized by high heterozygosity and even distribution in the genome in European bison.

Decision which method for genotyping to choose depends on many criteria and several aspects have to be taken into account. HERRÁEZ et al. (2005) mention geneticist's aspect (i.e. simple and unexpensive procedure due to large amounts of genotypic data) and statistician's aspect (precision and accuracy of used statistic method). In general, both microsatellite and SNP analysis are similarly suited for cattle genotyping. The choice of the method depends to the purpose and desired objective of the study and the available equipment as well. In the case of parentage verification and individual identification good and reliable result is provided by the microsatellite analysis (e.g. cost ~23€/sample, personal communication GeneControl, Grub). On the other hand, more complex research (selection, QTLs, diseases) requires complex and demanding analysis and data processing, which automatically requires higher financial resources (e.g. cost ~45€/sample for SNP50 BeadChip, personal communication GeneControl, Grub).

The development of both marker systems is advantageous for simultaneously addressing a variety of questions related to breeding and selection. However, practical considerations and commercial purpose should be carefully considered too.

Conclusion

Micrsatellites and SNPs are important and very useful marker system in animal breeding. Approximately twice as many SNP markers were needed to provide the same effectiveness as microsatellites for genetic studies. Beneficiary of the genetic test (private person, association, institution, etc.) has to be aware that each marker system (microsatellites or SNPs) has advantages and disadvantages. No matter whether the genetic markers will be used for practical purpose or for scientific reserch, each of them has to reflect efficiency, applicability, easy data analysis and interpretation. Moreover, the decision concerning which type of marker to use should be carefully considered based on cost and labor time. Only with such an approach they fulfill will their original purpose.

References

CAÑAS-ÁLVAREZ, J.J. – GONZÁLEZ-RODRÍGUEZ, A. – MUNILLA, S. – VARONA, L. – DÍAZ, C – BARO J.A. – ALTARRIBA, J. – MOLINA, A. – PIEDRAFITA, J. (2015): Genetic diversity and divergence among Spanish beef cattle breeds assessed by a bovine high-density SNP chip. Journal of Animal Science 93 (11): 5164-5174.

CARRUTHERS, C. R. – PLANTE, Y. – SCHMUTZ, S. M. (2011): Comparison of Angus cattle populations using gene variants and microsatellites. Canadian Journal of Animal Science 91: 81-85.

CAA – CROATIAN AGRICULTURE AGENCY (2017): Annual report 2016. Cattle Breeding. Križevci.

DADI, H. – TIBBO, M. – TAKAHASHI, Y. – NOMURA, K. – HANADA, H. – AMANO, A. (2008): Microsatellite analysis reveals high genetic diversity but low genetic structure in Ethiopian indigenous cattle populations. Animal Genetics 39: 425-431.

EDEA, Z. – DADI, H. – KIM, S.W. – DESSIE, T. – LEE, T. – KIM, H. – KIM, J-J. –KIM, K. S. (2013): Genetic diversity, population structure and relationships in indigenous cattle

populations of Ethiopia and Korean Hanwoo breeds using SNP markers. Frontiers in Genetics 4: 35. doi: 10.3389/fgene.2013.00035.

FAO (2011): Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Guidelines. No. 9. Rome.

FERNÁNDEZ, M. E. – GOSZCZYNSKI, D. E. – LIRÓN, J. P. – VILLEGAS-CASTAGNASSO, E. E. – CARINO, M. H. – RIPOLI, M. V. – ROGBERG-MUÑOZ, A. – POSIK, D. M. – PERAL-GARCÍA, P. –GIOVAMBATTISTA, G. (2013): Comparison of the effectiveness of microsatellites and SNP panels for genetic identification, traceability and assessment of parentage in an inbred Angus herd. Genetics and molecular biology 36 (2): 185-191.

FISHER, P. J. – MALTHUS, B. – WALKER, M. C. – CORBETT, G. –, SPELMAN, R. J. (2009): The number of single nucleotide polymorphisms and on-farm data required for whole-herd parentage testing in dairy cattle herds. Journal of Dairy Science 92: 369-374.

GONZÁLEZ-RECIO, O. – LÓPEZ DE MATURANA, E. – GUTIÉRREZ, P. (2007): Inbreeding Depression on Female Fertility and Calving Ease in Spanish Dairy Cattle. Journal of Diary Science 90: 5744-5752.

HERRÁEZ, D.L. – SCHÄFERA, H. – MOSNERB, J. – FRIESC, H. R. – WINK, M. (2005): Comparison of Microsatellite and Single Nucleotide Polymorphism Markers for the Genetic Analysis of a Galloway Cattle Population. Zeitschrift fur Naturfordchung 60c: 637-643.

KHANSHOUR, A. M. – CONANT, E. K. – JURAS, R. – COTHRAN, E. G. (2013): Microsatellite analysis for parentage testing of the Arabian horse breed from Syria. Turkish Journal of Veterinary and Animal Sciences 37: 9-14.

MAKINA, S.O. – MUCHADEYI, F.C. – VAN MARLE-KÖSTER, E. – MACNEIL. M.D. – MAIWASHE, A. (2014): Genetic diversity and population structure among six cattle breeds in South Africa using a whole genome SNP panel. Frontiers in Genetics 5:333. doi: 10.3389/fgene.2014.00333.

MEDUGORAC, I. – MEDUGORAC, A. – RUSS, I. – VEIT-KENSCH, C.E. – TABERLET, P. – LUNTZ, B. – MIX, H.M. – FÖRSTER, M. (2009): Genetic diversity of European cattle breeds highlights the conservation value of traditional unselected breeds with high effective population size. Molecular Ecology 18(16): 3394-3410.

MINISTRY OF AGRICULTURE, FISHERIES AND RURAL DEVELOPMENT (2010): National programme for the Protection of autochthonous - native and protected breeds of domestic animals in the Republic of Croatia.

NEI, M. (1987): Molecular EvolutionaryGenetics. Columbia University Press.New York.

PARISET, M. – MARIOTTI, M. – NARDONE, A. – SOYSAL, M.I. – OZKAN, E. – WILLIAMS, J. L. – DUNNER, S. – LEVEZIEL, H. – MARÓTI-AGÓTS, A. – BODÒ, I. – VALENTINI, A.(2010): Relationships between Podolic cattle breeds assessed by single nucleotide polymorphisms (SNPs) genotyping. Journal of Animal Breeding and Genetics 127: 481-488.

PRYCE, J. E. – MEKONNEN, H-M. – GODDARD, M. E. – HAYES, B. (2014): dentification of genomic regions associated with inbreeding depression in Holstein and Jersey dairy cattle. Genetic Selection Evolution 46 (1): 71.

RAMLJAK, J. – BUNEVSKI, G. – BYTYQI, H. – MARKOVIĆ, B. – BRKA, M – IVANKOVIĆ, A. – KUME, K. – STOJANOVIĆ, S. – NIKOLOV, V. – SIMČIČ, M. – SÖLKNER, J. – KUNZ, E. – ROTHAMMER, S. – SEICHTER, D. – GRÜNENFELDER, H.P. – BROXHAM, E. T. – KUGLER, W. – MEDUGORAC, I. (2018): Conservation of a domestic metapopulation structured into related and partly admixed strains. Molecular Ecology. DOI: 10.1111/mec.14555. (in press)

RAMLJAK, J. – IVANKOVIĆ, A. – VEIT-KENSCH, C.E. – FÖRSTER, M. – MEDUGORAC, I. (2011): Analysis of genetic and cultural conservation value of three indigenous Croatian cattle breeds in a local and global context. Journal of Animal Breeding and Genetics 128: 73-84.

SCHMID, M. – BENNEWITZ, J. (2017): Invited review: Genome-wide association analysis for quantitative traits in livestock – a selective review of statistical models and experimental designs. Archives Animal Breeding 60: 335-346.

SCHNABEL, R. D. – WARD, T. J. – DERR, J. N. (2000): Validation of 15 microsatellites for parentage testing in North American bison, Bison bison and domestic cattle. Animal Genetics 31: 360-366.

SIMČIČ, M. – SMETKO, A. – SÖLKNER, J. – SEICHTER, D. – GORJANC, G. – KOMPAN, D. – MEDUGORAC. I. (2015): Recovery of native genetic background in admixed populations using haplotypes, phenotypes, and pedigree information-using Cika cattle as a case breed. PloS One 10(4): e0123253. doi: 10.1371/journal.pone.0123253.

TOKARSKA, M. – MARSHALL, M. – KOWALCZYK, R. – WÓJCIK, J. M. - PERTOLDI, C. - KRISTENSEN, T. N. - LOESCHCKE, V. – GREGERSEN, V. R. – BENDIXEN, C. (2009): Effectiveness of microsatellite and SNP markers for parentage and identity analysis in species with low genetic diversity: the case of European bison. Heredity 103: 326-332.

VIEIRA, M. L. – SANTINI, L. – DINIZ, A. L. – MUNHOZ, F. (2016): Microsatellite markers: What they mean and why they are so useful. Genetics and Molecular Biology 39: 312–328.

VIGNAL, A. – MILAN, D. – SAN CRISTOBAL, M. – EGGEN, A. (2002): A review on SNP and other molecular markers and their use in animal genetics. Genetics Selection Evolution 43: 275-305.

WERNER F.A. – DURSTEWITZ, G. – HABERMANN, F. A. – THALLER, G. – KRÄMER, W. – KOLLERS, S. – BUITKAMP, J. – GEORGES, M. – BREM, G. – MOSNER, J. – FRIES, R. (2004): Detection and characterization of SNPs useful for identity control and parentage testing in major European dairy breeds. Animal Genetics 35: 44-49.