

SOME ASPECTS OF DNA ANALYSIS IN THE SELECTION OF SMALL RUMINANTS

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Review paper

Abstract: DNA analysis can have great practical application in the management and successful operation of the farm. The application of DNA technology is becoming a tremendous challenge for farm breeding of domestic animals. In recent years, traditional selection methods have been supplemented by the results of molecular analysis of the genome. Determining the genetic distance of sheep and goat breeds had practical and multiple significance. Microsatellites are used widely in the selection, and genomic selection is becoming an increasing challenge for breeders. The development of SNP chips brings an immense advanced for rapid and comprehensive analysis of the genome, which is of great importance for the successful selection and Genomic selection in sheep concentrated on all aspects of genetic and production directions. However, it should be noted that genomic selections in sheep breeding, and especially in goat breeding, take place at a slower pace compared to cattle breeding.

Key words: selection, molecular genetics, microsatellites, small ruminants

Introduction

The traditional selection of domestic animals has based on the application of quantitative and population genetics methods. To estimate the value of the population, it needs mathematical-statistical ways with computer software required (Caro Perovic *et al.*, 2012a, 2012b, 2013a, 2013b, 2014).

In sheep and goat breeding, traditionally selection methods are increasingly supplemented by modern DNA analyzes to detect genes that affected expressions of certain production traits or have located in the genome near the place responsible for a given trait (Carillier *et al.*, 2013, 2014; Petrović *et al.*, 2015).

The application of DNA analysis can have great practical application in the management and successful operation of the farm in the following way: Formation of an appropriate multilocus genotype for the identification of each animal; determining susceptibility to diseases such as Scrapie in sheep; introduction of DNA pedigree for use in long-term genetic improvement programs; resolving genealogical and ownership disputes, as well as confirming parenthood (paternity / maternity). In addition to the above, molecular genome analysis is valuable in forming a multilocus genotype database for use in forensic research and monitoring of animal products.

It is well known that the use of genetic markers has made it possible to detect the responsible genes for exhibiting significant traits or to determine their approximate location in the genome. So, genetic markers are not genes that find production or other traits but show a specific place in the genome where are those genes potentially located. In addition, genes have been identified in recent years.

Microsatellite markers could use to analyze genetic diversity in sheep breeds (Gaouar et al., 2012; Liu et al., 2014; Ebrahimi et al., 2017; Xia et al., 2021), goat (Bindu et al., 2012; Hussain et al., 2013; Seilsuth et al., 2016; Asrroush et al., 2018), and the other domestic animals (Petrovic et al., 2018).

This review paper aims to clarify some of the more important methods used in sheep and goat selection.

Genetic diversity of populations

In consideration of the eighth decade of the 20th century, with the development of molecular genetics methods, sheep breeding was among the first to meet modern selection procedures. Most of the known genetic markers are being used experimentally and in practice.

The use of microsatellites had a special echo in the selection of sheep and goats to characterize certain breeds and determine their genetic distance. These studies are still relevant today (Liu et al., 2014; Zinovieva et al., 2015).

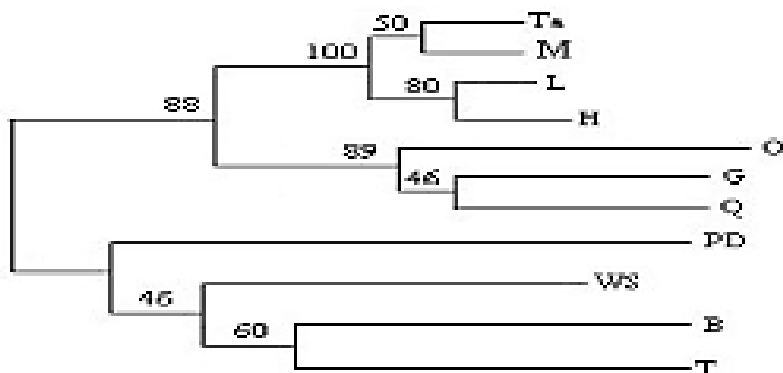


Figure 1. A neighbour-joining dendrogram of 11 mutton sheep populations based on Nei's standard genetic distances (Liu *et al.*, 2014)

Table 1. Observed and expected levels of heterozygosity at microsatellite loci (Zinovieva *et al.*, 2015).

Breed	F_{is}	Degree of heterozygosity		p
		observed	expected	
Grozny (GR)	0.2495	0.619±0.039	0.830±0.017	p<0.001
Stavropol (ST)	0.3520	0.567±0.031	0.881±0.012	p<0.001
Soviet Merino (SM)	0.2702	0.614±0.030	0.850±0.021	p<0.001
Edilbaevskaya (ED)	0.2776	0.595±0.032	0.853±0.023	p<0.001
Karakul (KR)	0.0627	0.724±0.035	0.808±0.036	p>0.05
Romanov (RO)	0.0591	0.707±0.029	0.779±0.031	p>0.05

Since 2010, when the international consortium for genome research of 23 sheep breeds developed the Illumina Ovine SNP50K chip, a new phase of genomic selection of sheep has begun.

In recent years, more than 50,000 SNPs have been tested to see their association with individual production traits. By the way, the Illumina SNP50K chip is a small glasses plate that has 12 panels where 50,000 SNPs have for each animal. It reveals which nucleotide is present at a particular SNP site.

The obtained signals for each of the 50,000 SNP markers have transformed into the "SNP marker genotype" (AA, AB, or BB). In this way, the final result obtained in the form of a genotype for 50,000 SNP markers in each genotyped sheep.

Since 2011, the chips are also being used in goat breeding, and the Illumina SNP50K chip has been developed using the genome of 25 breeds of goats.

In a relatively short period from the discovery of this method until today, thousands of SNPs have been discovered for which the exact position in the genome is known, as well as the consequence of the change in the nucleotide base.

For example, some studies have shown that sheep with heavier fleece in a particular SNP have an adenine (A) base, with animals with a lighter fleece in the same SNP having a guanine (G) base (*Petrovic and Pantelic, 2015*). The use of the SNP chip technique has begun to be applied in the selection of sheep and the determination of the genetic connection of populations (*Deniskova et al., 2019*).

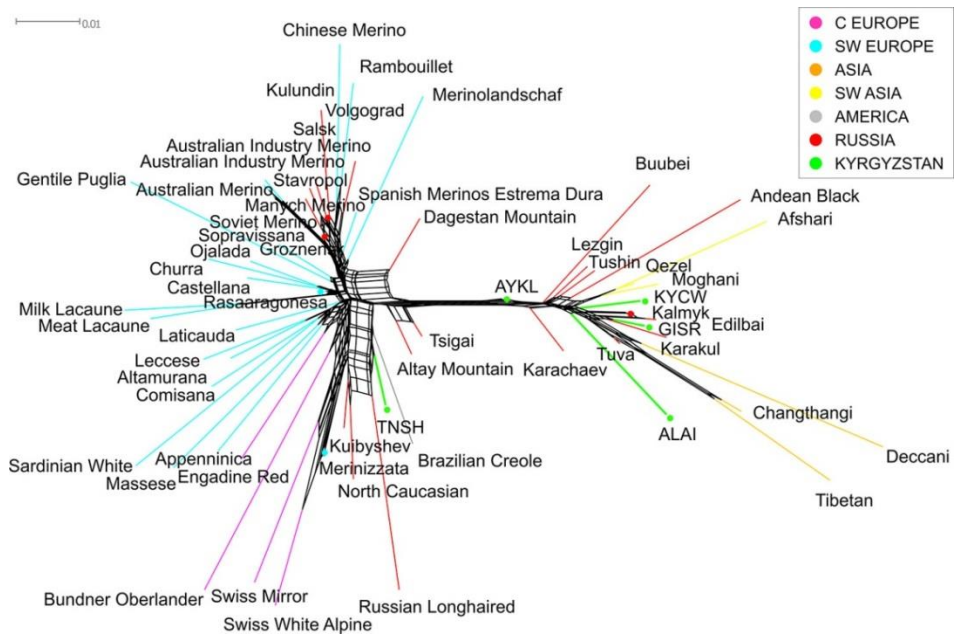


Figure 2. Characterization of the population structure sheep breeds, and to study their genetic connections using the ovinesnp50k beadchip and the Ovine Infinium HD beadchip (Illumina Inc., USA).

The application of the SNP genome detection procedure may have different applications. For example, *Kijas et al. (2009)* developed a set of SNPs distributed throughout the sheep genome. Relied on re-sequencing over 2600 genomic targets that have a known location within the virtual genome of sheep to use such a SNP set, the mentioned researchers performed genotyping. They thus determined the level of polymorphism between the examined sheep populations. The authors state that the results led to the knowledge of how sheep populations are grouped on the basis of geographical origin, whereby a modest number of SNPs can successfully identify the population substructure within individual breeds. Part of the results is shown in Figure 3.

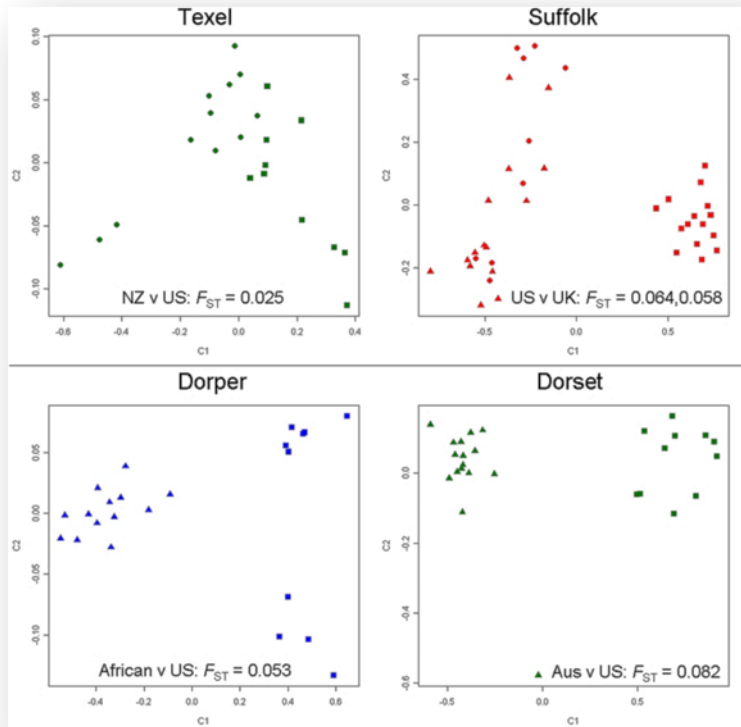


Figure 3. Multidimensional scaling shows genetic differences between geographically separated populations (Kijas *et al.*, 2009)

Application of genomic selection in sheep breeding

Genomic selection application in sheep breeding is gaining momentum, and among the leading countries of such as Australia, New Zealand, Russia, China, more precisely the countries with the most developed sheep breeding. When it comes to European countries, France stands out the most, especially with the dairy breed Lacon.

Chinese researchers have discovered SNPs58995.1 (position 3858663 in contig) located in the regulatory zone of the myocyte enhancer gene; factor-2 (MEF2B) which has a large impact on meat quality (Zhang *et al.*, 2013). The MEF2B gene encodes a protein from the MEF2 family. Proteins interaction from the MEF2 family with the promoter myostatin genome of sheep has a stimulating effect on the expression of myostatin, a protein that restricts muscle growth in mammals (Du *et al.*, 2007). Therefore, a mutation in the MEF2B gene can affect

sheep meat production by altering myostatin production. It's been confirmed in some other studies (*Chen et al., 2015*).

Milk production and traits in the Spanish breed Churra were studied by *Garcia-Gamez et al. (2012)* and found a link to certain genes. Large numbers of regions identified associated to milk traits (*Usai et al., 2019*).

Carillier et al. (2015) state that the availability of the SNP54k chip for goats, enabled the genotyping of 825 goats for the Alpine and San goat breeds in France. In both races, genomic selection can improve annual genetic progress by reducing the length of the father-son time interval. The quality of predicting the value of an individual in this way is sensitive to the size of the reference population, which can affect the accuracy of genomic indicators.

Genomic selection in sheep concentrates on all genetic aspects and production directions. However, it should be noted that genomic selections in sheep breeding, and especially in goat breeding, take place at a slower pace compared to cattle breeding. However, this is only conditionally said because each of these branches of animal husbandry has its own significance, goals and selection requirements depending on different natural and social influences.

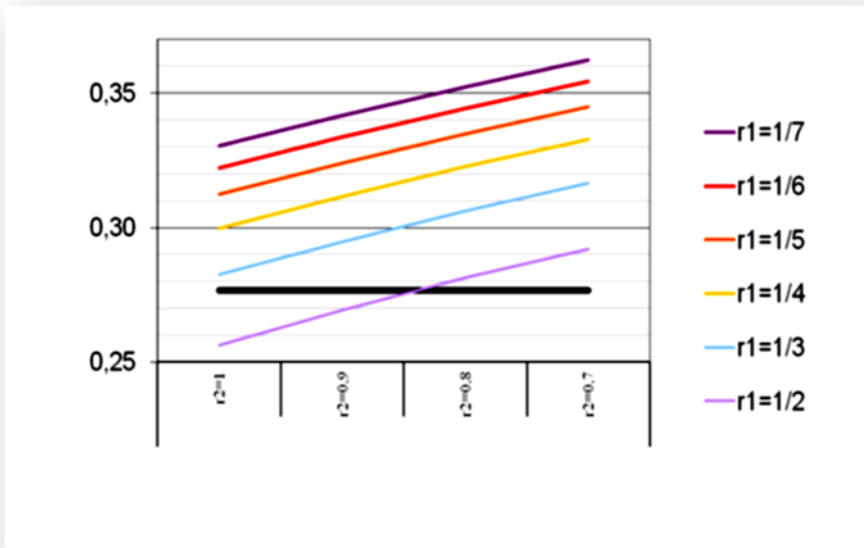


Figure 4. Annual genetic progress (in genetic standard deviation) according to r_1 (genomic selection) and r_2 (selection after progeny test) for Lacon sheep breed (*Buisson et al., 2013*)

Conclusion

The use of molecular DNA analysis techniques in sheep and goats is of great theoretical and practical importance. Knowledge of the genetic distance of populations is necessary in order to avoid homozygosity but also in crossbreeding programs for sheep breeds. Microsatellites, in this sense, played an important role in selection.

The results of the obtained SNP genotypes could apply to determine the history and diversity of sheep and goat populations within our country and the world. It is also possible to determine the origin of individuals, genetic variations that are associated with some diseases, with horns, some characteristics of carcasses, etc.

Finally, we can summarize how the genomic selection of sheep and goats had the following advantages: More accurate prediction of genetic value for the desired breeding goal; in traits that are usually difficult to improve; for properties that are difficult or expensive to measure; for properties that cannot be measured early; in traits with low heritability, for example: yield and meat quality traits, lifelong wool production, reproductive rate, parasite resistance.

Neki aspekti DNK analize u selekciji malih preživara

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Rezime

DNK analiza može imati veliku praktičnu primenu u upravljanju i uspešnom poslovanju farme. Primena DNK tehnologije postaje veliki izazov za selekciju domaćih životinja, pa se poslednjih godina tradicionalne metode selekcije dopunjuju su rezultatima molekularne analize genoma. Određivanje genetske distance rasa ovaca i koza ima višestruki praktični značaj. Mikrosateliti se široko koriste u selekciji, a genomska selekcija postaje sve veći izazov za odgajivače. Razvoj SNP čipova donosi veliki napredak u brznoj i sveobuhvatnoj analizi genoma, što je od velikog značaja za bržu selekciju ovaca i koza. Genomska selekcija je skoncentrisana na sve genetske aspekte i pravce proizvodnje. Posebno na osobine koje se teško mere ili se ne mogu izmeriti u ranom uzrastu jedinke. Međutim, treba napomenuti da se genomska selekcija u ovčarstvu, a posebno u kozarstvu, odvija sporijim tempom u odnosu na govedarstvo.

Ključne reči: selekcija, molekularna genetika, mikrosateliti, mali preživari

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