

Analysis of genetic diversity in newly created sheep populations and their maternal breeds

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The aim of the study was to provide a genetic characterization of the synthetic lines BCP and SCP in relation to the breeds used to create them. We analysed a total of 37 sheep of the breeds Romanowska, Charollais, Olkuska, Friesian and Suffolk and the lines BCP and SCP. Genetic structure was analysed on the basis of 5 microsatellite markers. The following genetic parameters were calculated: I – Shannon's Information index, H_o – observed heterozygosity, H_e – expected heterozygosity, PIC – Polymorphic Index Content, F_{is} – Wright's coefficient, N_m – gene flow coefficient, I_N – similarity, and genetic distance (D_s) between populations. A dendrogram was also created for the breeds and lines. The results of the research provided comprehensive information on the genetic structure and variation in the groups of sheep (breeds and lines). Genetic differences found in and between the groups indicate a reduction in genetic variation. Evidence of this is the decrease in the total number of alleles and in heterozygosity. The results indicate that the usefulness of the markers used to identify and track changes in the genetic structure of the sheep population is varied.

KEY WORDS: synthetic lines of sheep / genetic diversity

Native populations reared under livestock genetic resources conservation programmes are a source of raw materials used to produce food with substantial health-promoting properties, but often their level of production does not meet the current expectations. On the other hand, native breeds can be used to create synthetic lines, including sheep, which are characterized by a high level of adaptation to their environment and high

productivity, while the raw material obtained from them has specific health-promoting properties.

An example of such populations is the two synthetic lines BCP and SCP, produced in south-eastern Poland [6]. The starting material for these populations was native Uhruska ewes improved with a prolific breed. The next step was to cross the animals with cross-bred sheep whose genotype was 50% meat breed (Berrichon du Cher or Suffolk) and 50% Charollais. The final effect of the breeding work is populations with the following genotype: Uhruska – 37.5%, prolific sheep – 12.5%, meat breed (Suffolk in the SCP line and Berrichon du Cher in the BCP line) – 25% and Charollais – 25%. The animals of both lines are characterized by a high level of reproductive performance, together with high meat performance parameters. A significant limitation on efficient use of such populations for commercial cross-breeding is the lack or scarcity of information on the genetic variation of new lines in relation to the parent breeds.

Therefore a study was undertaken to provide a genetic characterization of the synthetic BCP and SCP lines in relation to the breeds used to create them, which will enable universal generalizations that will be useful in breeding practice.

Material and methods

The study was carried out at the Small Ruminants Teaching and Research Station in Bezek, belonging to the University of Life Sciences in Lublin, and at the University of Warmia and Mazury in Olsztyn. The experiment was conducted on 37 sheep. Samples were collected from 7 randomly selected, unrelated individuals of the Romanowska breed and 5 individuals each of the Charollais, Olkuska, Friesian and Suffolk breeds and the BCP and SCP lines. Peripheral blood was taken from the jugular vein and DNA was isolated according to the manufacturer's recommendations (GeneJet Genomic DNA Purification Kit Thermo Scientific). The genetic structure was analysed on the basis of 5 microsatellite markers selected among those recommended by FAO and ISAG [5, 7]. Characteristics of the loci analysed are presented in Table 1. PCR conditions were the same for all loci: initial denaturation at 95°C for 5 minutes, 40 cycles of 30 s at 95°C, 30 s at 55°C and 1 min at 72°C. Final elongation at 72°C was extended to 7 minutes. Electrophoresis was performed in 3% agarose gel and product profiles were analysed in POLYDOC software. Polymorphism of sequences was evaluated on the basis of differences in their length. Genetic indices (F-statistics) were calculated in PopGEN 1.31 software [16]: I – Shannon's Information index, H_o – observed heterozygosity, H_e – expected heterozygosity, PIC – Polymorphic Index Content, and F_{is} – Wright's coefficient. Gene flow for the whole population (N_m) was estimated according to the formula $N_m = 0.25(1 - F_{st})/F_{st}$ (F_{st} – inbreeding coefficient). A dendrogram was created by UPGMA, and the similarity (I_N) and distance (D_s) between populations were calculated in PopGEN 1.31 software [16]. The N_m index is calculated for two or more populations to show the gene flow between them. The H_o , H_e ,

Table 1
Characteristics of analysed loci

<i>Locus</i>	Location on chromosome	Primer sequence	GenBank accession number	Allele range
Maf65	15	AAAGGCCAGAGTATGCAATTAGGAG CCACTCCTCCTGAGAATATAACATG	M67437	114-138
Maf33	9	GATCTITGT'ITCAATCTATTCCAAT'ITC GATCATCTGAGTGTGAGTATATACAG	M77200	119-141
Maf209	17	GATCACAAAAAGTTGGATAACAACCGTGG TCATGCACTTAAGTATGTAGGATGCTG	...	107-135
Maf214	16	GGGTGATCTAGGGAGGTTTGGAGG AATGCAGGAGATCTGAGGCGGACG	M88160	173-235
OarAE54	25	TACTAAAGAAACATGCTCCAC AGAAACATITATTCTTATCCTCAGTG	L11048	113-141

PIC, I, and Fis indices, which describe the variation within one population, were calculated for each of the breeds and lines analysed.

Results and discussion

Genetic variation is one of the most important traits characterizing every population. Sufficiently high genetic diversity is the basis for preserving the adaptive abilities of a population. Low genetic diversity can lead to a reduction in the population's adaptive capacity [4]. Estimation of the level of genetic variation is crucial in studying populations and extremely important for biodiversity. Factors such as drift and selection, particularly in small populations, may result in a considerable reduction in the number of alleles, leading to a lower level of genetic variation. Therefore monitoring of changes in the genetic structure of a population is recommended.

The heterozygosity index is a parameter characterizing genetic variation in a population. The observed heterozygosity (H_o) for the entire population of animals was 0.3923 and differed considerably from the expected heterozygosity (H_e), which was 0.5168 (Tab. 2). Similar results (H_o 0.4580 and H_e 0.5801) were obtained by Kusza et al. [8] for a population of Bulgarian sheep. Analysis of the differences between H_o and H_e in the lines reveals the greatest divergence between the observed (0.2800) and expected (0.4400) heterozygosity in the SCP line, and the least in the BCP line and in the Suffolk breed. In the Romanowska, Charollais, Olkuska and Friesian breeds observed heterozygosity was higher than expected heterozygosity, which is indicative of additional mechanisms increasing genetic variation, such as a suitable choice of mating pairs. Relatively low values for observed and expected heterozygosity (H_o 0.3334 and H_e 0.3099) were obtained

by Sun et al. [13] in their analysis of native breeds of Asian sheep. In the Suffolk, BCP and SCP sheep the observed heterozygosity is lower than the expected heterozygosity. This result, interpreted as a loss of diversity, may be a consequence of a decrease in the size of the population. Another reason for large discrepancies between the expected and observed value may be the division of the entire genetic pool into subgroups—distinct subpopulations within the species. This can be explained by the ‘founder effect’, where the genetic pool narrows and expands for some time, but only on the basis of the founder pool.

Another indicator defining genetic diversity in a given locus is the Polymorphic Index Content (PIC). The PIC calculated for the entire population of sheep was 0.4491 (Tab. 2), which suggests a relatively low level of genetic variation of these loci, as the value was below 0.5. The lowest PIC was noted in the BCP line (0.2286) and the highest in the Romanowska breed (0.3573). Both the observed heterozygosity and the PIC were highest in the Romanowska sheep, which indicates that variation was highest in this breed. Comparison of the level of variation of the locus with literature data shows that it was lower than the variation reported in the available literature [1, 2, 10].

Table 2
Parameters characterizing genetic variability

Specification	I	Ho	He	PIC	Fis	Nm
Romanowska	0.606	0.4833	0.4370	0.3573	0.0797	–
Charollais	0.352	0.3333	0.2781	0.2410	0.3886	–
Olkuska	0.627	0.5200	0.4756	0.3376	0.0044	–
Friesian	0.412	0.4000	0.3289	0.2463	0.2711	–
Suffolk	0.597	0.4200	0.4489	0.2996	0.1311	–
BCP	0.428	0.2800	0.3067	0.2286	0.4133	–
SCP	0.625	0.2800	0.4400	0.3326	0.2800	–
Population	0.865	0.3923	0.5168	0.4491	0.0909	0.54

I – Shannon’s Information index
 Ho – observed heterozygosity
 He – expected heterozygosity
 PIC – Polymorphic Index Content
 Fis – Wright’s coefficient
 Nm – gene flow coefficient

The heterozygosity deficit in a population is measured by Wright’s coefficient (Fis). In the population as a whole the heterozygosity deficit was fairly low (0.0909), as it was in

the Olkuska and Romanowska sheep (0.0044 and 0.0797, respectively). Similar values for the inbreeding coefficient have also been obtained in three sheep breeds from Egypt and Central Asia [5, 13]. In the BCP, Charollais, SCP and Friesian populations a relatively high F_{is} value was noted. Similar values were obtained in Bulgarian sheep [7, 8]. The most frequent cause of a high F_{is} is that the breed is derived from the genetic pool of one herd, from which the animals were distributed as founders to other herds. A lack of or reduction in the exchange of males between flocks also contributes to the risk of inbreeding [3, 15]. Similar results were obtained in a study on small herds of goats living in groups that were separated from one another [11]. In the present study, the high F_{is} can be explained by the small numbers of animals analysed.

Increasing relatedness of individuals and the resulting inbreeding are a problem for both small, closed populations and for larger ones subjected to intensive selection. An increase in inbreeding is generally an unfavourable phenomenon; it can cause a decline in the vitality, health, and performance of animals. In sheep breeding it usually causes a reduction in body weight and growth rate in lambs, and decreased fertility and fecundity in ewes.

The main factors determining genetic structure and genetic variation between populations of the same species are usually geographical, behavioural and ecological. The existence of such variation between populations is a very common phenomenon, even in the case of species considered to be highly panmictic.

The next parameter used to assess the genetic variation in the sheep population was the *Shannon index* (Shannon-Wiener index). The value for the Shannon-Wiener index ranged from 0.352 in the Charollais breed to 0.627 in the Olkuska sheep (Tab. 2). Similar values for the Shannon biodiversity index indicate relative maintenance of stability, which may be confirmed by unchanging, optimal environmental conditions. On the other hand, if the results differ, it can be concluded that there is an environmental factor which has disturbed the balance in the environment—in this case the factor was breeding selection.

Evaluation of migration of individuals between populations shows that they reduce genetic divergence. The N_m value, at 0.5405, can be interpreted as one migrant for every two generations.

Due to the high degree of polymorphism, microsatellite sequences of DNA are particularly useful in estimating the genetic distance between subpopulations within a species. The most commonly used measure of genetic distance is D_s , developed by Nei [12], which assumes that genetic differences are the result of mutations and genetic drift. The study made it possible to determine the genetic distance between breeds (Tab. 3). The results reflect the origin of the synthetic lines, created using animals from 5 breeds. The greatest distance (0.7360) was noted between the Friesian and BCP populations. The distance between BCP and SCP was 0.0453. Similar results were obtained by Liu et al.

Table 3
Similarity I_N (above diagonal) and genetic distance D_s (below diagonal) between populations

	Romanowska	Charollais	Olkuska	Friesian	Suffolk	BCP	SCP
Romanowska	0	0.8543	0.7860	0.7601	0.7737	0.7050	0.7649
Charollais	0.1575	0	0.6517	0.5237	0.6752	0.5613	0.7006
Olkuska	0.2409	0.4282	0	0.8762	0.9551	0.6868	0.7180
Friesian	0.2743	0.6469	0.1322	0	0.8763	0.4790	0.5306
Suffolk	0.2565	0.3927	0.0459	0.1321	0	0.5852	0.6672
BCP	0.3496	0.5775	0.3757	0.7360	0.5359	0	0.9557
SCP	0,2681	0,3559	0,3314	0.6337	0.4046	0.0453	0

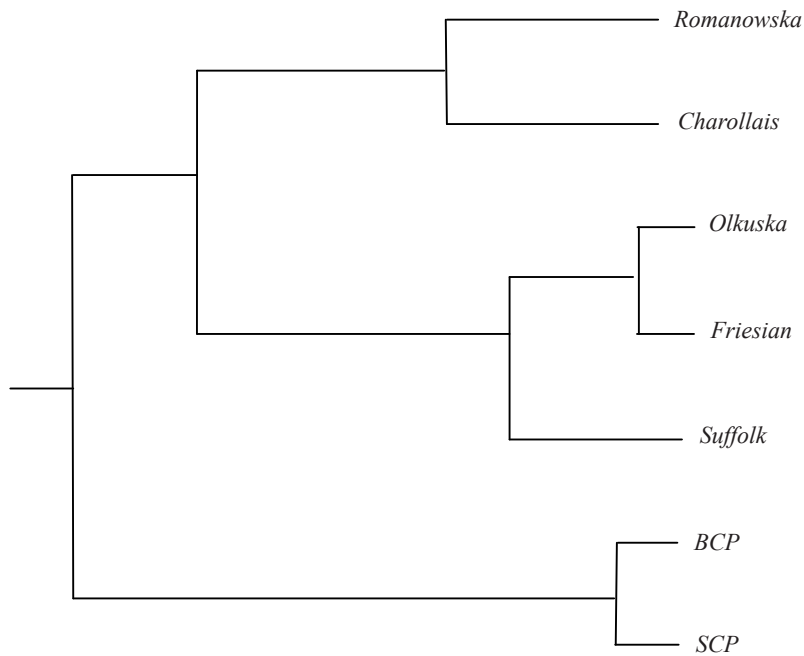


Fig. Dendrogram (cladogram) illustrating 'clades' corresponding to the structure of the sheep populations

[9] (smallest distance 0.132, greatest distance 0.735) in their analysis of the phylogenesis of 11 Asian sheep populations from breeding and native lines, where there was no direct correlation between phylogenesis and phylogeography.

The phylogenetic tree constructed using the 'neighbour-joining' (NJ) method [14] identified two groups among the populations analysed. The first group consisted of the parental lines Romanowska, Charollais, Olkuska, Friesian and Suffolk. The synthetic lines SCP and BCP form a separate clade from the breeds involved in their creation, which indicates differences in the genetic structure of these lines from that of the parent breeds.

Genetic variation between populations is directly linked to the flow of alleles between them; the more intensive the flow of individuals between populations, the lower the genetic diversity. The results obtained showed relatively low genetic variation in the sheep populations analysed. The values for H_e and H_o and for F_{is} indicate a small degree of variation in the genetic markers in the loci analysed, which suggests the need for further monitoring of genetic variation in this population.

To sum up, the results of the study have provided comprehensive information on the genetic structure and variation of the analysed groups (breeds and lines) of sheep. The genetic differences noted within and between groups indicate that a reduction in genetic variation has occurred in the population. Evidence of this is the decrease in the total number of alleles as well as the decrease in heterozygosity. The results indicate that the usefulness of the markers used to identify and track changes in the genetic structure of the sheep populations is varied.

Due to the threat posed by increased inbreeding, particularly in small populations of native sheep breeds, these changes should be monitored, e.g. with the use of microsatellite markers. Another useful tool in breeding practice may be mitochondrial DNA, which will be used in future studies on sheep populations.

The results obtained may be useful in developing breeding programmes aimed at maintaining or increasing the genetic variation of sheep populations.

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