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BIODIVERSITY ASSESSMENT IN INTERSPECIES HYBRIDS OF THE GENUS *Ovis* USING STR AND SNP MARKERS

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Abstract

Introgression of wild and domestic species is regarded as a promising way to improve genetic diversity in populations of farm animals. The aim of our study was to investigate the influence of introgression of the wild species (argali) on genetic diversity of interspecific hybrids with domestic sheep using STR and SNP markers. Samples included original parental forms: Romanov sheep (ROM, $n = 35$), representing the «domestic» form (*Ovis aries*), and argali (OAM, $n = 10$), characterizing the «wild» form (*O. ammon polii*), male hybrid F1, obtained by surgical insemination of Romanov ewe by argali sperm (F_1, $n = 1$), and back crosses obtained by crossing Romanov ewes with hybrid F_1 ram (BC_1, $n = 38$) and hybrid BC_1 rams (BC_2, $n = 14$). The analysis of 11 STR loci (BLT001B, CSRD247, FCB20, CSAP36, MAF65, McM147, OarCP49, D5S2, HSC, BMS2213 and INRA23) was carried out on the ABI PRISM 3130xl genetic analyzer. For SNP genotyping we used Ovine SNP50K BeadChip. After quality control, 9 STR loci and 8591 SNPs were left for the analysis. Statistical calculations were performed in GenAIEx 6.5, PLINK v1.07, HP-Rare 1.1, GENETIX 4.05 and STRUCTURE 2.3.4. Regardless the type of DNA marker, ROM, compared with OAM, was characterized by a higher level of genetic diversity, assessed by observed heterozygosity (H_o) and allelic richness (Ar). Hybridization resulted in an increase in this parameter in the F_1 hybrid. In groups BC_1 and BC_2, the H_o values, calculated per STR and SNP, were higher than similar in the parental forms. In BC_1 and BC_2 groups the Ar values, estimated by SNP-markers, were reducing in comparison with F_1 and were intermediate in comparison with the same in the parental forms. The changes in the Ar values, based on STR data, had the character of a trend in groups BC_1 and BC_2. Principal component analysis (PCA), performed by using SNP-markers, showed a more objective distribution pattern of the studied animals according to their origin in the coordinates space. In case of SNP data, PC1 was sufficient for clearly differentiation of groups OAM, ROM, F_1 and $BC_1 + BC_2$. In summary, the first two components (PC1 and PC2) were responsible for 25.87 % of the SNP variability and for only 12.46 % of the STR variability. Principal component 3 (PC3), which was responsible for 6.16 % of SNP variability, made it possible to differentiate BC_1 and BC_2 groups, whereas at the application of STR these groups were localized as a common cluster. The results of STRUCTURE analysis showed that association of the investigated individuals into clusters, based on STR-profiles, did not match their origin, while the formation of clusters by SNP-markers was corresponded to the actual origin of animals. We found that both types of tested DNA markers were suitable for detecting changes in genetic diversity through hybrids generation. Nevertheless, a significant advantage of using multiple SNP-markers for the differentiation of hybrids from the parental forms was shown.

Keywords: interspecific hybrids, introgression, genetic diversity, SNP, STR, genus *Ovis*

Major evolutionary levers, such as recombinations and natural selection, act slowly and with a few exceptions lead to a definite effect after a long time, but through introgressive hybridization these events can be significantly accelerated [1-5]. Introgressive hybridization is the process of including alleles of one species in the gene pool of another on the basis of a repeated backcrosses of an interspecific hybrid with one of the parental forms [6-9]. In nature, introgression, as a rule, occurs between closely related species [10, 11], and is also characteristic of hybrid zones, or zones of contact of two species [1]. Nevertheless, the greatest interest is remote, or interspecific, hybridization.

Intensification of livestock raising and long-term unidirectional selection, including the use of related lines or crosses, lead to a decrease in genetic diversity and an increase in the negative consequences of inbreeding, in particular, to deterioration in the adaptive abilities of animals. In this case, wild forms are considered as unique reservoirs of genetic variability for their home relatives [12].

The interspecific hybrids are mostly sterile and unable to transfer their unique properties to the offspring [13]. However, there are exceptions. Thus, the descendants of the Pyrenean ibex (*Capra pyrenaica*) and the domestic goat (*Capra hircus*) retain the ability to reproduce [5]. Hybrids of bovine cattle (*Bos taurus*) with Indian cattle (*Bos indicus*) [14], American bison (*Bison bison*), bison (*Bison bonasus*)[13, 15], banteng (*Bos javanicus*) and gaur (*Bos gaurus*) are fertile [16, 17]. The offspring of crossing the domestic sheep with wild species, argali (*Ovis ammon*), mouflon (*Ovis orientalis musimon*), snow sheep (*Ovis nivicola*) is breedy and can be the basis for the creation of a new selection type of sheep, characterized by excellent adaptation to complex relief and natural climatic conditions and serving as a source of dietary meat [18, 19].

The fertility of offspring from the crossing of sheep of different breeds with mouflons has been shown in numerous experiments [20, 21] and in natural conditions, which casts doubt on the existence of genetically pure populations of this ungulate [22]. In 1934-1950 in Kazakhstan, a Kazakh arkharomeris was bred on the basis of interspecies hybridization of argali and domestic sheep of the Kazakh merino breed, which proved to be well adapted for breeding in mountainous areas [19, 23].

Obtaining objective data on the degree of introgression and changes in the breed allele fund under the influence of hybridization is primarily determined by an adequate choice of the type of DNA markers. Until recently, one of the most popular markers for detecting interspecific hybrids was mitochondrial DNA polymorphism (mtDNA) [1, 24-26].

However, this approach has significant drawbacks. First, the genetic contribution of the male to the introgression is not taken into account. Secondly, mtDNA analysis does not allow to estimate the degree and nature of changes affecting nuclear DNA (nDNA) in hybrids. Contrast genetic regularities for nuclear and mitochondrial markers are usually explained by the high genetic drift of mtDNA due to a reduction in the effective size of mtDNA compared to that of nDNA [24, 25].

Microsatellites, or STR (short tandem repeats) markers, are a widespread type of nuclear DNA markers [27]. Microsatellites which are co-dominant, having Mendelian inheritance and analyzed automatically have proved themselves for identification and characteristics of allele fond and genetic diversity in closely related species [28-32]. However, as per M.G. Sovic et al. [33], the use of microsatellites to evaluate the hybridization of more remote species is questionable.

With the development of modern high-performance genotyping technologies, SNP markers (single nucleotide polymorphisms) are becoming promising

tools for investigating introgressive changes in the genome [33-35]. The advantage of SNP is the ability to identify loci with fixed alleles specific for each of the parental species [36, 37]. The detection of such loci, as a rule, is a simple and fast process. The use of whole genome DNA platforms allows finding several hundred loci [38]. The results obtained by comparing two taxa and their hybrids using diagnostic loci are unambiguous and highly reliable [39].

At the L.K. Ernst All-Russian Research Institute of Animal Husbandry was created a unique model population of interspecific hybrids of domestic Romanov sheep (*O. aries*) and argali of the Pamir population (*O. ammon polii*), which to date includes two generations of descendants from the recurrent crossing of Romanov sheep with hybrid males. In the present work, the changes in genetic parameters in interspecific hybrids were studied in detail for the first time as compared to both parental forms in a population obtained under artificial rather than natural conditions.

Our goal was to assess the impact of introgression of the wild species [argali] on the genetic diversity of interspecies hybrids with domestic sheep, performed using SNP and STR markers.

Technique. The object of the research was the original parental forms - Romanov sheep (*O. aries*) (ROM, $n = 35$, maternal "domestic" form) and argali (*O. ammon polii*) (OAM, $n = 10$, paternal "wild" form]; hybrid male F_1 from crossing the Romanov sheep and argali, 50 % argali blood); backcrosses obtained by crossing Romanov gimmers with hybrid F_1 males (BC_1, 25 % argali blood, $n = 38$) and BC_1 (BC_2, 12.5 % argali blood, $n = 140$. The biomaterial was ear tissue samples.

DNA was isolated using Nexttec columns (Nexttec Biotechnology GmbH, Germany) and a DNA-Extran kit (ZAO Sintol, Russia) according to the manufacturer's protocols.

Genetic studies of STR markers were carried out by PCR amplification of 11 microsatellite loci of sheep as per ISAG (International Society for Animal Genetics) combined into two multiplex panels — panel 1 (BLT001B, CSRD247, FCB20, CSAP36, MAF65, McM147) and panel 2 (OarCP49, D5S2, HSC, BMS2213 and INRA23). Reaction mixture (final volume 20 μl) contained 1× PCR buffer, which included 16.6 mM $(NH_4)_2SO_4$, 67.7 mM Tris-HCl (pH 8.8), 0.1 (v/v) Tween 20, and 1.5 mM $MgCl_2$, 200 μM dNTP, 20 pmol of each of the primers, 1 U Taq DNA polymerase (Dialaz Ltd, Russia) and 1 μl of DNA (50-100 ng). After initial denaturation (95 ° C, 5 min), 35 amplification cycles were performed (95 °C, 20 s, 55 °C, 30 s, 72 °C, 1 min) for the panel 1 and 41 amplification cycle (95 °C, 20 s, 63 °C, 30 s, 72 °C, 1 min) — for the panel 2. The PCR products were separated on a PRISM 3131xl genetic analyzer (Applied Biosystems, USA). The fragment sizes were determined using the GeneMapper® Software v4 (Thermo Fisher Scientific, Inc., USA).

For a whole genome SNP scan, a medium-density DNA chip Ovine SNP50K BeadChip (Illumina Inc., USA] was used. With the PLINK v1.07 software [40], quality control was performed which included four steps. In the first stage, a GC score of 0.5 and GT score of 0.3 cutoff were applied. SNP localized on sex chromosomes and with unknown localization were excluded from the analysis. In the second stage, the SNPs polymorphic in the parent forms were determined at the frequency of minor alleles less than 10 % (-maf 0.01), the genotyping efficiency not less than 90 % of animals tested (--geno 0.1), and Hardy-Weinberg equilibrium test $p < 10^{-6}$ (-hwe 1e-6). After filtering, only SNPs common in both parent groups were selected. In the third stage, monomorphic SNPs unique for each group were identified, since in hybrid forms they could become polymorphic. The monomorphic SNPs were added to the poly-

morphs selected at the first stage. In the fourth stage, hybrids were added to the parent forms. Filtering was carried out according to GENO criteria (--geno 0.1) and LD linkage disequilibrium test (--indep-pairwise 50 5 0.5; for 50 SNP, one of the pair of markers for which LD was above 0.5 was deleted, and then the frame was shifted by 5 SNP). The MAF and HWE filters were not applied at this stage. Afterward the LD test, a set of SNPs for statistical calculations was formed.

The main population indicators, i.e. the observed (H_o) and expected (H_e) heterozygosity, inbreeding coefficient (F_{IS}), F_{ST} fixation index [41] and M. Nei distances (D_N) [42], were determined in GenAIEx software 6.5.1 [43] for STR markers, and in GENETIX 4.05 software [44] for SNP markers. The rarified allelic richness (Ar) in each animal group for both markers was calculated in the HP-Rare 1.1 program [45]. The identity state matrices (IBS, identical-by-state) for principal component analysis (PCA) were constructed in PLINK v1.07. R version 3.2.3 was used for creating input files and visualization [46]. Population structure was analyzed in STRUCTURE 2.3.4 [47] using burn-in period of 50,000, Markov chain Monte Carlo (MCMC) of 50000 repetitions, 10 iterations. The calculation was carried out for the coefficient k = 2 {the number of expected populations). For each of the groups, the average value of the membership ratio Q in the i-th cluster was determined for the total number of clusters k ($Q_{i/k}$).

Results. Loci BLT001B and CSAP36 were excluded from the STR set because of the lack of information in 70 % OAM animals. The final STR set included 9 loci. Primary analysis of SNP profiles in the parents showed the presence of 45155 polymorphic SNP in Romanov sheep and 9816 polymorphic SNP in argali. After MAF, GENO, and HWE filtering, 9002 polymorphic SNPs common for both groups were selected, which were supplemented with 231 unique monomorphic SNPs for each group. The resulting set consisted of 9233 markers. As a result of quality control throughout the sample using the GENO filter and the LD test, 61 and 581 SNPs were excluded. The final set of SNPs included 8591 markers.

Regardless of the type of DNA markers, a higher genetic diversity based on H_o and Ar was established in ROM compared to OAM (Table). Crossing led to an increase in this index in hybrids F_1. In both backcrosss groups (BC_1 and BC_2), the H_o values calculated for STR and SNP were higher than those in the parents. The Ar value calculated for SNP decreased in groups BC_1 and BC_2 compared to that in F_1 animals and was intermediate relative to those of parents. For STR, there were no clear changes in Ar in BC_1 and BC_2.

Parameters of genetic diversity of parental and interspecific hybrid forms of the genus *Ovis* on STR and SNP-markers

Marker	Groups	*n*	H_o	H_e	F_{IS}	Ar
STR	OAM	10	0.544±0.107	0.683±0.058	0.266	1.719±0.061
	ROM	35	0.656±0.040	0.775±0.028	0.154	1.778±0.147
	F_1	1	0.778			1.786±0.028
	BC_1	38	0.837±0.026	0.680±0.020	–0.242	1.689±0.021
	BC_2	14	0.745±0.074	0.757±0.025	0.027	1.785±0.027
SNP	OAM	10	0.270±0.002	0.280±0.002	0.038	1.296±0.002
	ROM	35	0.369±0.002	0.368±0.001	–0.004	1.458±0.005
	F_1	1	0.458			1.373±0.001
	BC_1	38	0.418±0.002	0.357±0.002	–0.172	1.362±0.002
	BC_2	14	0.433±0.003	0.344±0.002	–0.260	1.357±0.002

N o t e. H_o — observed heterozygosity, H_e — expected heterozygosity, F_{IS} — fixation index, Ar — rarified allelic richness; the values of H_o and Ar for SNP markers were significant at $p > 0.999$. OAM — argali (*Ovis ammon polii*), ROM — Romanov sheep (*O. aries*), F_1 — hybrid male from crossing of Romanov sheep and argali (50 % argali blood), BC_1 and BC_2 — backcrosses of Romanov gimmers to hybrid F_1 males (25 % argali blood) and BC_1 (12.5 % argali blood), respectively. The data for F_1 are not given, since the F_1 group was represented by one individual.

Similar trends for STR and SNP markers were noted when assessing the dynamics of heterozygotes in the generations. Thus, in the OAM, a heterozygote

deficiency was found which was notable for STR markers (13.9 %) and small for SNP markers (1.0 %). The ROM group was practically in equilibrium for SNP markers, while the analysis by STR showed a decrease in the heterozygote deficit to 11.9 %. Both types of markers indicated an excess of heterozygotes in BC_1 with reverse trends in BC_2 (a heterozygote deficiency estimated by STR and an increase in the excess of heterozygotes for SNP).

The PCA (Fig. 1) of DNA profiles for two types of genetic markers showed a more objective distribution of the animals according to their origin when using SNP markers. In summary, the first two components (PC1 and PC2) accounted for 25.8 7% of the variability of SNP markers and only for 12.46 % of the variability of STR markers. The third component (PC3) allowed to explain 6.16 and 4.45% variability, respectively.

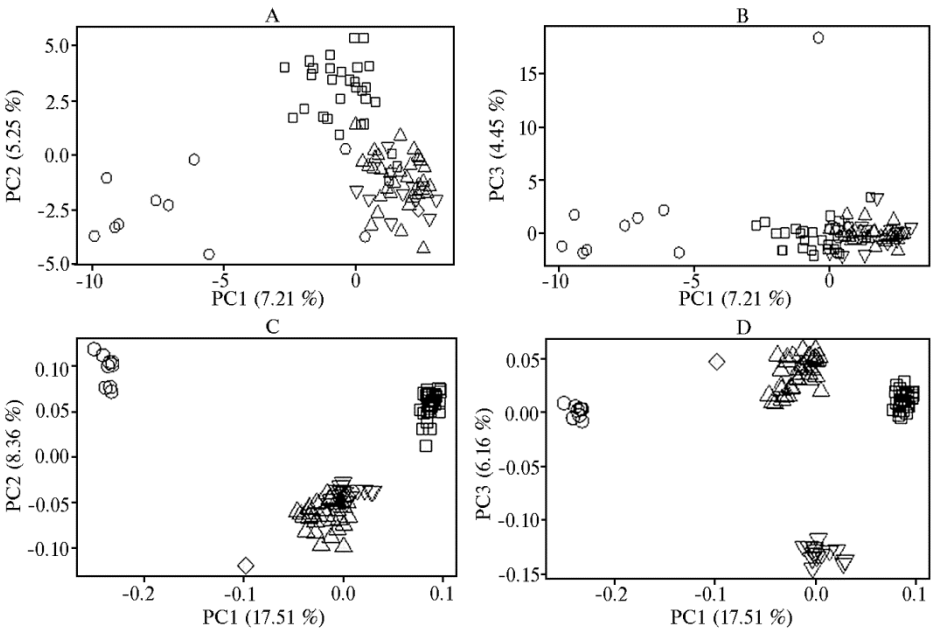

Fig. 1. PCA analysis for 9 STR loci (A, B) **and 8591 SNP** (B, D) **loci in the space of the components 1 and 2** (A, B)**, and1 and 3** (B , D) **for parents and their interspecific hybrids of the genus *Ovis***: ○ — OAM, argali (*Ovis ammon polii*), □ — ROM, Romanov sheep (*O. aries*), ◇ — F_1, hybrid male from crossing the Romanov sheep and argali (50 % argali blood), △ and ▽ — BC_1 and BC_2, backcrosses of Romanov gimmers with hybrid F_1 males (25 % argali blood) and BC_1 (12.5 % argali blood).

STR-based clusterization was fuzzy, and there were animals localized in the "alien" cluster (see Fig. 1, A, B). With SNP, already PC1 allowed to clearly differentiate OAM, ROM, F_1 and BC_1 + BC_2 (see Fig. 1, B). The equidistant position of the F_1 hybrid from the two parent forms was interesting and fully corresponding to its origin. PC3 made it possible to clearly separate the BC_1 and BC_2 (see Fig. 1, D).

When using SNP, a comparative analysis of genetic differentiation in the groups by F_{ST} and D_N showed a clear correspondence between the nature of genetic connections and the origin of animals. The maximum differences were observed between the parent species (F_{ST} = 0.280, D_N = 0.250). In hybrid individuals, as the blood content of the argali decreased, the genetic differences with respect to argali increased (F_{ST} = 0.223, D_N = 0.174 for BC_1, FS_T = 0.272, D_N = 0.218 for BC_2) which brought them closer to the domestic form. The nature of genetic linkages between groups, established by STR markers, did not correspond to the origin of the animals. Perhaps this is due to the insufficient information capacity of nine STR loci.

To date, many researchers [29, 47-49] consider the cluster analysis of admix models for multilocus marker in the STRUCTURE program as the most effective in detecting hybrids. In our work, the grouping of the studied individuals into clusters based on STR profiles did not correspond to their origin both for k = 2 (Fig. 2, A) and k = 3 (data not shown). This is due to two main reasons. First, the microsatellites used are discovered and recommended for the genetic analysis of domestic sheep, rather than argali. Secondly, the information capacity of nine markers could not be enough for such studies. So, according to J.P. Vaha and C.R. Primmer [29], at least 48 loci are required to adequately separate the backcrosses from purebred parental specimens.

STRUCTURE analysis for 8591 selected SNP marker showed that the clusters corresponded to the actual origin of individuals (see Fig. 2, B). The parents (wild and domestic forms) were grouped into two strictly consolidated clusters. The membership in the own cluster for argali was $Q_{1/2} = 0.94 \pm 0.04$, for Romanov sheep — $Q_{2/2} = 0.99 \pm 0.01$. Hybrid F_1 with 50 % of wild and domestic blood was approximately equal to the membership values in each of the two clusters ($Q_{1/2} = 0.54$ and $Q_{2/2} = 0.46$). In the BC generations the percentage of cluster membership specific for OAR decreased ($Q_{1/2} = 0.27 \pm 0.05$ in BC_1 and $Q_{1/2} = 0.22 \pm 0.04$ in BC_2) while that specific for ROM increased ($Q_{2/2} = 0.73 \pm 0.05$ in the BC_1 and $Q_{2/2} = 0.78 \pm 0.04$ in the BC_2).

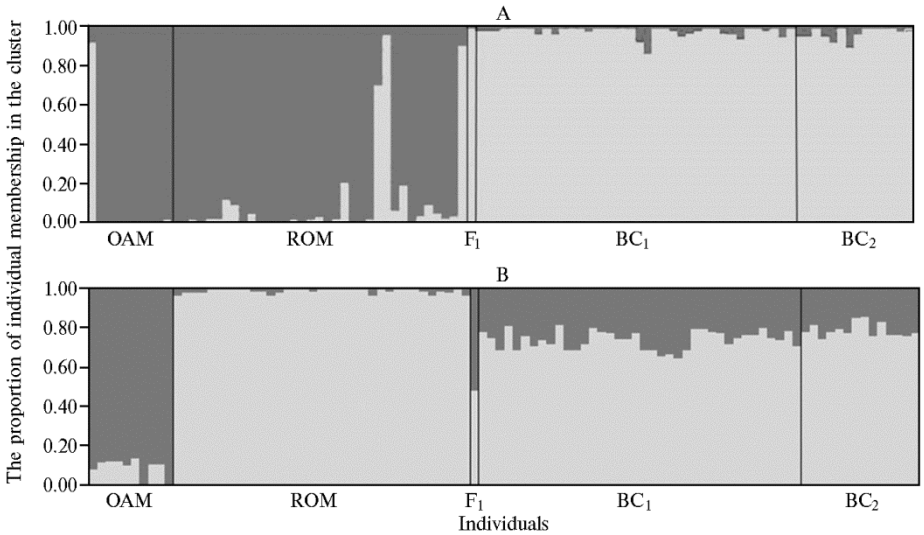

Fig. 2. Population assignment for parental and interspecific hybrids of the genus *Ovis* based on 9 STR loci (A) **and 8591 SNP marker** (B) **in the STRUCTURE program for k = 2:** OAM — argali (*Ovis ammon polii*), ROM — Romanov sheep (*O. aries*), F_1 — hybrid male from crossing of Romanov sheep and argali (50 % argali blood), BC_1 and BC_2 — backcrosses of Romanov gimmers with hybrid F_1 males (25 % argali blood) and BC_1 (12.5 % argali blood).

Thus, the change in genetic diversity in generations of interspecific hybrids due to the introgression of argali can be determined by both STR and SNP markers. Nevertheless, the significant advantage of using multiple SNP markers for differentiating hybrid animals from parental forms has been revealed. In the future, a more detailed search is planned to detect the diagnostic SNP loci required for a detailed study of introgression in generations more distant from the wild ancestor.

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