A Study of Biodiversity of Russian Local Sheep Breeds Based on Pattern of Runs of Homozygosity †

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Abstract: A rapid spreading of cosmopolite breeds leads to decrease of population sizes of Russian local sheep that consequently might result in their biodiversity loss. Estimation of the runs of homozygosity (ROH) in local sheep genomes is an informative tool to address their current genetic state. In this work, we aimed to address the distribution of the ROH and to estimate genome inbreeding in Russian local sheep breeds based on SNP-genotyping. Medium-density SNP-genotypes of twenty-three local sheep breeds (n = 332) were obtained in our previous study. We used a consecutive runs method implemented in the R package “detectRUNS” to calculate ROH which were estimated for each animal and then categorized in the ROH length classes (1–2 Mb, 2–4 Mb, 4–8 Mb, 8–16 Mb, >16 Mb). The frequency of short ROH segments (≤2 Mb) were the highest in all studied breeds (63.15–93.10%). The longest segments (>16 Mb) were the least frequent and were missing in four breeds. The genomic coefficients based on ROH estimation varied from medium (0.114) to low (0.035). Thus, we found that Russian local sheep breeds are characterized by a low level of genomic inbreeding.

Keywords: sheep; genetic diversity; SNPs; genomic inbreeding

1. Introduction

Genetic resources of Russian local sheep include breeds, which were specifically selected for wool and combined (wool and meat) production, and aboriginal breeds, which are adapted to extreme environments and from which all types of sheep products are used by local smallholders [1]. A rapid spreading of cosmopolite breeds leads to decrease of population sizes of Russian local sheep that consequently might result in their biodiversity loss.

Runs of homozygosity (ROH) are numbers of homozygous loci that inbred progeny inherit from parents which originated from a common ancestor. Calculation of the length of ROH segments allows to find out whether inbreeding in the populations was recent or ancient [2,3]. Evaluation of genomic inbreeding coefficients based on ROH calculation is not determined by allelic frequencies, sampling procedures, and is considered as a robust measure for describing levels of inbreeding in the populations with missing pedigree information [4]. In this aspect, estimation of runs of homozygosity in genomes of livestock species is an informative tool to address their current genetic state [5].
In this work, we aimed to address the distribution of the ROH and to estimate genome inbreeding in Russian local sheep breeds based on medium-density SNP-genotyping data.

2. Experiments

2.1. Materials and Methods

Materials for this study included SNP-genotypes of twenty-two local sheep breeds (n = 332) which were generated with OvineSNP50 BeadChip (Illumina, San Diego, CA, USA) in our previous research [6]. The SNP-profiles of the Romanov, Kuchugur and Baikal fine-fleeced breeds were not analyzed in the present study. The list of the studied breeds and sample sizes are presented in Table 1.

| Group             | n ¹ | F_{ROH}_Mean ² | F_{ROH}_Min ³ | F_{ROH}_Max ⁴ |
|-------------------|-----|----------------|---------------|---------------|
| Altay Mountain    | 12  | 0.05 ± 0.003   | 0.04          | 0.07          |
| Andean Black      | 16  | 0.077 ± 0.008  | 0.03          | 0.15          |
| Buubei            | 17  | 0.05 ± 0.007   | 0.03          | 0.15          |
| Dagestan Mountain | 16  | 0.064 ± 0.003  | 0.05          | 0.09          |
| Edilbai           | 17  | 0.046 ± 0.002  | 0.04          | 0.06          |
| Groznensk         | 13  | 0.049 ± 0.002  | 0.04          | 0.06          |
| Kalmyk            | 18  | 0.047 ± 0.004  | 0.03          | 0.11          |
| Karachaev         | 16  | 0.049 ± 0.002  | 0.03          | 0.06          |
| Karakul           | 21  | 0.054 ± 0.002  | 0.04          | 0.07          |
| Kuibyshev         | 15  | 0.061 ± 0.004  | 0.05          | 0.1           |
| Kulundin          | 13  | 0.07 ± 0.003   | 0.06          | 0.09          |
| Lezgin            | 13  | 0.035 ± 0.002  | 0.03          | 0.05          |
| Manych Merino     | 16  | 0.057 ± 0.002  | 0.04          | 0.08          |
| North Caucasian   | 16  | 0.082 ± 0.003  | 0.06          | 0.11          |
| Russian Longhaired| 13  | 0.114 ± 0.007  | 0.08          | 0.18          |
| Salsk             | 16  | 0.063 ± 0.003  | 0.04          | 0.09          |
| Soviet Merino     | 14  | 0.056 ± 0.001  | 0.05          | 0.06          |
| Stavropol         | 15  | 0.069 ± 0.008  | 0.04          | 0.16          |
| Tsigai            | 16  | 0.05 ± 0.003   | 0.03          | 0.09          |
| Tushin            | 11  | 0.05 ± 0.005   | 0.03          | 0.08          |
| Tuva              | 13  | 0.043 ± 0.002  | 0.03          | 0.06          |
| Volgograd         | 15  | 0.071 ± 0.002  | 0.06          | 0.08          |

¹ n – sample number; ² F_{ROH}_Mean – inbreeding coefficient calculated based on ROH with a minimum length of 1 Mb; ³ F_{ROH}_Min – minimum values of F_{ROH} estimations observed in individual animals within breeds; ⁴ F_{ROH}_Max – maximum values of F_{ROH} estimations observed in individual animals within breeds.

2.2. Data Processing

For ROH calculation, we used a window-free method for consecutive SNP-based detection [7] implemented in the R package “detectRUNS” [8]. One SNP with missing genotype and up to one possible heterozygous genotype was allowed in the run. The minimum ROH length was 1000 kb.

ROH were estimated for each animal and then categorized in the corresponding ROH length classes: (1–2 Mb, 2–4 Mb, 4–8 Mb, 8–16 Mb, >16 Mb). The total number of identified ROH was calculated for each length category in each of the individuals of each breed. The
The mean sum of ROH was computed by adding up the length of all ROH for each individual in the sheep populations and then the results were averaged per breed population.

The genomic inbreeding coefficient based on ROH ($F_{ROH}$) was estimated as the sum of the length of all ROH per sheep as a proportion of the total autosomal SNP coverage (2.44 Gb).

3. Results

ROH were found on all autosomes in all Russian local sheep breeds. The genome coverage by ROH was higher on OAR1 (7.88–12.10%), OAR 2 (9.03–11.94%) and OAR3 (6.28–11.42%), and lower on OAR20 (1.05–2.44%) and OAR26 (1.06–2.24%). ROH length and ROH number varied from 93.73 ± 4.29 Mb and 68 in the Lezgin breed to 301.69 ± 19.71 Mb and 117 in the Russian Longhaired breed. The individual minimum was found in the Lezgin breed (68.16 Mb and 54), while maximum was detected in the Russian Longhaired breed (469.99 Mb and 138).

Figure 1 shows the distribution of the ROH segments in length classes in Russian local sheep breeds. The frequency of short ROH segments (≤2 Mb) was the highest in all studied breeds and varied from 63.15% in the Russian Longhaired breed to 93.10% in the Lezgin breed. The Tushin breed had the minimum of the ROH segments of 2–4 Mb length class (6.09%), while the Russian Longhaired breed had the maximum (24.62%). The frequencies of the ROH segments within length class (4–8 Mb) varied from 0.57% in the Lezgin breed to 10.01% in the Russian Longhaired breed. Long ROH segments (8–16 Mb) ranged from 0.19% in the Tuva breed to 3.46% in the Andean breed. The longest ROH segments (>16 Mb) were the least frequent (0.07–1.22%) and were missing in four breeds.

![Figure 1](image_url). Distribution of the runs of homozygosity in length classes (1–2 Mb, 2–4 Mb, 4–8 Mb, 8–16 Mb, >16 Mb) in Russian local sheep breeds.

Estimates of inbreeding coefficient calculated based on ROH in Russian local breeds are given in Table 1. The values of genomic inbreeding coefficient varied from medium to...
low. The minimum $F_{ROH}$ was calculated in the Lezgin breed ($F_{ROH} = 0.035$) and the maximum was detected in the Russian Longhaired breed ($F_{ROH} = 0.114$). The range of individual $F_{ROH}$ variability was more noticeable in several breeds including Andean Black ($F_{ROH}$ from 0.03 to 0.15), Buubei ($F_{ROH}$ from 0.03 to 0.15), Kalmyk ($F_{ROH}$ from 0.03 to 0.11), North Caucasian ($F_{ROH}$ from 0.06 to 0.11), Russian Longhaired ($F_{ROH}$ from 0.08 to 0.18), and Stavropol ($F_{ROH}$ from 0.04 to 0.16).

4. Discussion

There are several approaches to address biodiversity and its dynamics in the populations of livestock species: effective population size, heterozygosity and runs of homozygosity [9]. In our previous study, we calculated and analyzed effective population sizes and heterozygosity to unlock current state of genetic diversity in Russian local sheep breeds [6]. Nonetheless, estimation of runs of homozygosity is a useful tool to reveal the presence of long-term inbreeding in livestock populations [3].

A prevalence of short ROH segments revealed in Russian local sheep breeds are compatible with the relevant patterns detected in Swiss [4] and Italian local sheep breeds [10] as well as in commercial sheep breeds including Charollais, Suffolk, Texel [11], Border Leicester, Merino, and Poll Dorset [9].

The average values of ROH length calculated in our study were higher than those obtained in commercial sheep breeds (93.73–301.69 Mb versus 92.61–128.31 Mb [11]/94.88–126.06 Mb [9], respectively). Maximum individual ROH length values estimated in our study (400 and 469.99 Mb) were close to those obtained in Australian populations of Border Leicester, Merino, and Poll Dorset breeds (427.2, 410.5 and 396.45 Mb) [9].

Mean $F_{ROH}$ varied from 0.016 to 0.099 in Italian local breeds including Valle del Belice and Comisana [10] and from 0.022 to 0.153 in Swiss local breeds [4]. These estimates are compatible with the $F_{ROH}$ variation detected in Russia local sheep breeds.

The highest values of genome coverage by ROH segments and the maximum inbreeding coefficient calculated based on ROH, which were found in the Russian Longhaired breed, might correspond to the small population size (1400 heads at the end of 2019) [12] and to using of a limited number of sires.

5. Conclusions

Here, we presented a detailed analysis of pattern of distribution of the runs of homozygosity in major Russian local sheep breeds based on 50K SNP profiles. Our findings provide an evidence of a low genomic inbreeding in local sheep populations (except for the Russian Longhaired). The study results provide useful information to design conservation programs for local genetic resources of sheep.

Author Contributions: N.Z., G.B. and T.D. conceived and designed the experiments; T.D. and H.R. performed the experiments; A.D. and H.R. analyzed the data; M.S. and K.W. contributed reagents/materials/analysis tools; T.D. wrote the paper.

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Abbreviations

The following abbreviations are used in this manuscript:

- **SNP** Single Nucleotide Polymorphisms
- **ROH** Runs of homozygosity
- **FROH_Mean** inbreeding coefficient calculated based on ROH with a minimum length of 1 Mb

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