MICROSATELLITE VARIATION IN THE BALTIC SHEEP BREEDS

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Summary. The history of sheep farming in the Baltic countries began around 2700 BC. In the modern era the western breeds have influenced heavily the improvement of the Baltic sheep breeds. Lithuanian Coarsewooled, Lithuanian Blackface, Latvian Darkheaded, Estonian Ruhnu, Estonian Whitehead, Estonian Blackhead and Estonian Saaremaa breeds were studied to evaluate the usefulness of the microsatellite markers for parentage testing in sheep breeds and to demonstrate the presence of within–population variation. 195 individuals were genotyped for 15 unlinked microsatellite markers. Allele numbers, observed (H_{obs}) and expected (H_{exp}) heterozygosities were calculated. All microsatellite loci were found to be polymorphic, with 4.4 to 10.9 alleles per locus in average. The mean number of alleles, calculated from all 15 microsatellites, varied from 3.9 (in Estonian Ruhnu) to 8.3 (in Estonian Whitehead) and supported the possible use of studied markers for paternity testing even in rare Baltic sheep breeds. The average H_{exp} values compared to the average H_{obs} values did not show big differences in the studied populations, only in Estonian Saaremaa population the observed heterozygosity was substantially lower than expected. Among studied populations, Estonian Ruhnu breed demonstrated lowest genetic diversity. Indications of recent decrease in effective population size in breeds were investigated by applying a bottleneck test with a Wilcoxon sign–rank test and a qualitative graphical method. Only Estonian Ruhnu population showed a slight mode–shift distortion in the distribution of allele frequencies, indicating possible recent reduction in effective population size.

Keywords: sheep, microsatellites, genetic diversity, genetic bottleneck.

MIKROSATELITŲ ĮVAIROVĖ PABALTIJO ŠALIŲ AVIŲ VEISLĖSE

Santrauka. Siekiant patikrinti mikrosatelitinių markerių tinkamumą tėvystės įvertinimui bei nustatyti avių vidupopuliacinę įvairovę, buvo ištirtos septynios Pabaltijo avių veislės: Lietuvos vietinė šiurkščiavilnė, Lietuvos juodgalvė, Lavijos tamsiagalvė, Estijos Ruhnu, Estijos baltagalvė, Estijos juodgalvė ir Estijos Saaremaa. 195 gyvuliai buvo genotipuoti pagal 15 tarpusavyje nesusijusių mikrosatelitinių žymeklių, o pagal genotipavimo duomenis buvo nustatytas alelių skaičius bei apskaičiuoti nustatytas (H_{obs}) ir laukiamas (H_{exp}) heterozigotiškumai. Visi mikrosatelitiniai lokusai buvo polimorfiški, o aptiktų skirtingų alelių skaičius svyravo nuo 4,4 iki 10,9 lokusui. Vidutinis alelių skaičius, apskaičiuotas veislėms, įvairavo nuo 3,9 (Estijos Ruhnu) iki 8,3 (Estijos baltagalvėje). Gauti rezultatai leidžia teigti, kad šiame darbe naudoti mikrosatelitiniai žymekliai gali būti naudojami atliekant tėvystės testą net ir vietinėms Pabaltijo avių veislėms. Lyginant laukiamo heterozigotiškumo vertes su nustatyto heterozigotiškumo vertėmis nebuvo aptikta didelio įvairavimo nei vienoje veislėje, tik Estijos Saaremaa populiacijoje nustatytas heterozigotiškumas buvo žymiai mažesnis nei laukiamas. Iš visų tirtų veislių Estijos Ruhnu avių populiacija pasižymėjo žemiausia genetine įvairove. "Butelio kaklelio efekto" testas ir kokybinis grafinis metodas buvo panaudoti efektyvaus populiacijos dydžio sumažėjimo įvertinimui. Rezultatai parodė, kad tik Estijos Ruhnu populiacijoje buvo pastebėtas alelių dažnio persislinkimas, kuris leidžia įtarti neseniai įvykusį efektyvaus šios populiacijos dydžio sumažėjimą.

Raktiniai žodžiai: avys, mikrosatelitai, genetinė įvairovė, genetinis "butelio kaklelio efektas".

Introduction. The transition to farming in the East Baltic region started in the Middle Neolithic period. Archaeological findings from Lithuanian area date the first domestic sheep–goat bones 2800 BC. It is assumed there was an exchange of products and information between the local populations and people in northern Poland. Starting from 2700 BC, farming established itself as a significant part of the economy (Daugnora and Girininkas, 1996; Milisauskas and Kruk, 2002). However, more precise data about sheep husbandry in the Baltic area is known from the 13th century, when indigenous coarsewooled sheep were kept for the private use of local people. Starting from the middle of the 19th century, local sheep were upgraded by crossing them with wool and meat type sheep of different breeds, until the desired type with good wool and meat traits, as well as good adaptation to the local environmental conditions were obtained (Zapasnikienė, 1998). In recent times, the drop in wool prices has favored the use of more energy rich feeds and altered the breeding strategy towards meat rather than wool production (Diez – Tascón et al., 2000).

The present day sheep populations are the result of mutation and genetic drift, as well as selection imposed by humans, available nutrition, endemic parasites, diseases and climate (Blott et al., 1998; Barker, 1999; Nijman, 1999). Potentially**,** there is much unrecognised beneficial genetic variation present in the rare, especially the semi managed breeds and populations, which form important reservoirs of non–exploited resources. There is a tendency for worldwide animal production to be based on a few, highly selected breeds which is causing pressure leading to a reduction in number of the local breeds

(Gill and Hughes, 1998). Maintaining genetic variation is now recognised as a crucial and international need to fulfil all the market demands and to make the progressive improvement of domestic animal populations successful also in the future (Oldenbroek, 1999).

The first step for the sustainable use of domestic animal genetic resources is the gathering of knowledge about the genetic variability in the breeds. Earlier many studies of genetic structure used polymorphism of phenotypic traits**,** such as coat and wool color, horn types, tail length or biochemical markers like blood groups and electrophoretically detected milk and blood protein variation. Nowadays increasing preference has been given to DNA markers called microsatellites, that have been proven to be useful for parentage test, linkage analysis and population studies (Diez – Tascón et al., 2000; Moazami – Goudarzi et al., 1997).

The objective of this study is to describe the native sheep breeds of the Baltic countries, to compare phenotypic and molecular variation within the breeds and to investigate if the studied microsatellite markers offer enough variability for parentage testing also in the rare breeds. We discuss factors that might lead to a loss of variation within Baltic sheep breeds.

Materials and methods.

Sampling

Blood samples were obtained from 195 individuals representing 7 Baltic sheep breeds: Lithuanian Coarsewooled (30 animals were analysed), Lithuanian Blackface (30), Latvian Darkheaded (32), Estonian Ruhnu (24), Estonian Whitehead (30), Estonian Blackhead (28) and Estonian Saaremaa (21). Sampling of close relatives was avoided when possible.

Lithuanian Coarsewooled sheep is a meat – wool type breed. It was the main breed in Lithuania at the end of the 19th and the beginning of the 20th century. The breed was developed by crossing the local coarsewooled sheep with Pomeranian, Polish long – tailed, thin – tailed and North short – tailed sheep. The improved local coarsewooled sheep have white, grey, black and greybrown wool and a thin – long tail. 80 % of rams and 20 % of ewes have horns (Table 1). The distinctive features are the thin legs, often a bare belly covered only with clothing hair, the thick skin, the narrow nose, the broad forehead and short ears. Local coarsewooled sheep are famous for non–seasonal oestrus that allows lamb dropping up to two times a year. They have an unspecified disease resistance, are undemanding as regards housing and have quick adaptation to new feeding and management conditions (Zapasnikienė, 1998; FAO, 1996).

Lithuanian Blackface sheep is a meat – wool type breed that originated in the period 1923 – 1950 as a result of Lithuanian Coarsewooled ewes mating with wool type Shropshire and meat type German Blackface rams. The animals are polled, have semifine mutton type white wool and long tails. The sheep are known for early maturity, rapid weight gains as well as quick adaptation to new feeding and management conditions (Zapasnikienė, 1998; Šveistienė, 1988).

Latvian Darkheaded sheep is a meat – wool type breed the development of which was started in the middle of the 19th century by upgrading local finewooled, semifinewooled and coarsewooled ewes with Oxford, Shropshire and Hampshire rams until locally adapted animal with uniform semifine wool and good meat traits were obtained. Sheep of the desired type have a strong constitution and fine bones. Both rams and ewes are polled and long – tailed. The head, ears and legs are covered with dark hair. The wool is uniform, white on the body, but some sheep have colored fibers. Sheep of this breed exhibit early maturity and rapid weight gains. Meat quality is further enhanced in cross – breeding with Texel, Il – de – France and German Blackface sheep (FAO, 1996).

The roots of *Estonian Ruhnu* sheep population on Ruhnu island is not clear. In 1944 a population of 300 sheep was documented, nowadays only 30 sheep are left. It is thought that Ruhnu sheep were raised for producing meat and wool. Ewes are polled, while 10 % of rams have horns. Head and legs are gray (from light to black) some animals are badgerface with light or dark pattern. The distinctive feature of this breed is the presence of one or two beads under the jaw. Animals have mainly white wool and short – tail. The sheep are undemanding as regards for housing, feeding and management. Animals of this population are well adapted to the local damp conditions and for pasturing at the seaside pastures.

Estonian Saaremaa sheep are kept on Saaremaa and Kihnu islands and are raised for producing meat and wool. The wool of animals is mainly white, sometimes black and brown. The head is covered with white or piebald hair. Rams and ewes are either horned or polled and all animals are long – tailed. The sheep of this population are undemanding as regards for housing and feeding, are well adapted to the local damp conditions and to pasturing on the seaside pastures.

The development of the *Estonian Whitehead* breed started in 1926 by crossing local white faced coarsewooled ewes with English Leicester and later with Cheviot rams, until crosses with uniform semifine wool of high quality, early maturity and good meat traits were obtained. Since 1981**,** the Ile – de – France, Finnsheep, Texel and Norwegian Dala sheep have been introduced. The sheep are unicolor – white with white head and legs. All animals are polled and long – tailed. The Estonian Whitehead sheep are well suited to the damp climate of Estonia and located countrywide (FAO, 1996).

Estonian Blackhead is a meat – wool type breed that originated as a result of a local north short – tailed sheep crossing with Shropshire and Oxford Down in 1926. Since 1980**,** German Blackface and Latvian Darkheaded sheep have been used to improve meat and wool traits. Currently**,** Oxford Down and Suffolk have been introduced from Denmark for improvement of meat performance and lamb quality. The sheep have white wool, but the head and feet are covered with black hair. The head is of medium size, the forehead broad and the nose is short. All animals are polled and long – tailed. The Estonian Blackhead sheep are well suited to the damp climate of Estonia and located countrywide (FAO, 1996).

Table 1. **Phenotypic traits of seven Baltic sheep breeds.**

Breed	Color	Wool	Horns	Tail	Weight ♂	Weight ♀	Use
Lithuanian Coarsewooled	White, grey, black,greybrown	D. coat, coarse	Polled/ horned	Long	47	35	Meat–wool
Lithuanian Blackface	White, dark head & legs	Uniform, semifine	Polled	Long	85	55	Meat–wool
Latvian Darkheaded	White, dark head & legs	Uniform, semifine	Polled	Long	95	63	Meat–wool
Estonian Ruhnu	White tan, grey,	D. coat, semifine	Polled/ horned	Short	80	50	Meat–wool
Estonian Whitehead	White	Uniform, semifine	Polled	Long	88	67	Meat–wool
Estonian Blackhead	White, dark head & legs	Uniform, semifine	Polled	Long	94	76	Meat–wool
Estonian Saaremaa	White, brown, black	Uniform, semifine	Polled/ horned	Long	60	50	Meat–wool

DNA extraction and microsatellite analyses

Blood samples of 5 – 10 ml were collected in EDTA tubes and frozen at –20 °C. DNA was isolated using the phenol – chloroform method (Miller et al., 1998). Approximately 10 – 50 ng DNA was used as template for polymerase chain reaction (PCR). All 195 sheep samples were genotyped with 15 microsatellite markers: (BM757, BM1314, BM6526, BM6506, BM8125, BM1818, BM4621, MAF48 (Bishop et al., 1994), MCM527 (Hulme et al., 1994), OarCP20 (Ede et al., 1995), CSSM31 (Moore et al., 1994), MAF65, MAF214 (Buchanan and Crawford, 1992; Buchanan et al., 1992), MAF36 (Swarbrick et al., 1991), INRA023 (Vaiman and Mercier, 1994). PCR of microsatellite loci were carried out using fluorescent – labelled primers. The amplified products were separated on 6 % denaturing polyacrylamide gel using automated laser detection (A.L.F., A.L.F. Express, Pharmacia). Genetic variants were visualised using A.L.F. win^{tm} Fragment Analyser 1.0 (Pharmacia, Uppsala, Sweden).

Data analysis

The mean number of alleles (A) per locus and population, mean observed (H_{obs}) and mean expected (H_{exp}) heterozygosities were calculated using the POP100GENE computer program (available at: http://www.ensam.inra.fr/URLB/pop100gene/pop100gene.html).

An exact probability test was conducted to test for deviations from Hardy – Weinberg equilibrium using GENEPOP version 3.3 (Raymond and Rousset, 1995). A Markov chain Monte Carlo method was applied to compute unbiased estimates of the exact probabilities (P–values). Length of chain was set to be 50 000 iterations. The critical P value was adjusted to correspond the nominal level of 0.05 on population level.

Inbreeding coefficient (F_{IS}) per locus and population was calculated according Nei and Kumar, 2000 using the formula:

$$F_{IS} = \frac{(Hexp - Hobs)}{Hexp}$$

To test for recent genetic bottleneck**,** the program BOTTLENECK (Piry et al., 1999) was used to perform a Wilcoxon sign – rank test. When a population experiences a reduction of its effective size, the allele number is reduced faster than the heterozygosity, i.e. the observed heterozygosity is larger than the heterozygosity expected from the observed allele number where the locus is at mutation – drift equilibrium (Luikart and Cornuet, 1998). The heterozygosities were obtained by 1000 iterations, assuming that the allele sizes change according to the two – phased mutation model (TPM; 95 % one–step mutations, 5 % multistep mutations with 12 variance). In addition**,** the qualitative graphical method of Luikart et al., 1998 was used to visualise the allele frequency spectra. The microsatellite alleles were classified into 10 frequency classes, which allows to check whether the distribution follow the normal L – shaped form, where alleles with the low frequencies (0.01 – 0.1) are the most abundant.

Results:

Microsatellite loci

A total of 175 alleles were detected in the 15 studied loci. All microsatellite loci were polymorphic with the number of alleles per locus ranging from 2 (CSSM31, MCM527) to 14 (BM1818). The mean number of alleles detected per microsatellite locus across all populations ranged from 4.429 (in BM8125 and MAF214) to 10.857 (in BM1818) (Table 2).

Of the 175 alleles detected, 36 alleles were unique to one breed (Table 2), but there was no correlation between the mean number of alleles and the number of unique alleles observed per locus. MAF48 was the only locus without any unique allele observed, while other microsatellite loci had from 1 to 4 unique alleles present in the populations (Table 2). On the other hand**,** all microsatellite loci had several alleles, which were present in all the studied breeds. The amount of the shared alleles did not depend on the polymorphism level of the locus and ranged from 1 to 4.

For all 15 loci the mean expected heterozygosity was 0.712, while for individual locus average H_{exp} varied from 0.500 (BM8125) to 0.857 (BM1818). In all markers mean expected heterozygosity was higher than the mean observed heterozygosity, but differences were marginal. The mean observed heterozygosities ranged between 0.460 (BM8125) and 0.857 (BM1818). Exception was

locus BM6526, where difference between H_{exp} and H_{obs} was quite large (Table 2).

In loci – population comparison, by excluding 5 loci that had H_{exp} values lower than 0.5 at least in one studied population, there was a set of 10 microsatellite markers where the H_{exp} ranged from 0.54 to 0.92.

Table 2. **Mean number of alleles across loci (A), mean observed (H_{obs}), mean expected (H_{exp}) heterozygosities, F_{IS} estimates for 15 microsatellite loci and number of unique alleles are presented. Standard deviations are given in parenthesis.**

Microsatellite locus	Mean allele number (A)	Mean H_{exp}	Mean H_{obs}	F_{IS}	Unique allele number
BM757	5.143 (1.215)	0.729 (0.059)	0.698 (0.104)	0.042	2
BM1314	7.714 (1.89)	0.743 (0.103)	0.720 (0.107)	0.031	2
BM1818	10.857 (2.794)	0.857 (0.048)	0.857 (0.036)	0.000	3
BM4621	8.429 (2.440)	0.759 (0.061)	0.763 (0.048)	–0.005	3
BM6506	5.286 (1.496)	0.565 (0.135)	0.557 (0.131)	0.014	4
BM6526	7.571 (2.370)	0.755 (0.079)	0.682 (0.172)	0.097	2
BM8125	4.429 (1.134)	0.500 (0.123)	0.460 (0.090)	0.080	1
CSSM31	9.571 (3.552)	0.785 (0.173)	0.769 (0.139)	0.020	2
INRA023	8.286 (2.290)	0.797 (0.104)	0.758 (0.190)	0.049	2
MAF214	4.429 (0.976)	0.556 (0.145)	0.537 (0.187)	0.034	4
MAF36	9.429 (2.370)	0.809 (0.050)	0.795 (0.112)	0.017	4
MAF48	5.857 (1.069)	0.709 (0.090)	0.692 (0.063)	0.024	0
MAF65	5.714 (1.113)	0.709 (0.063)	0.682 (0.110)	0.038	2
MCM527	5.714 (1.704)	0.659 (0.274)	0.592 (0.256)	0.102	2
OarCP20	6.286 (1.113)	0.742 (0.067)	0.745 (0.065)	–0.004	3
Mean	6.981 (1.943)	0.712 (0.097)	0.687 (0.104)	0.035	

Populations

There was much variation in the allele number among Baltic sheep breeds. The mean number of alleles, observed per population ranged from 3.933 in Estonian Ruhnu to 8.333 in Estonian Whitehead breed (Table 3). Latvian Darkheaded, Lithuanian Coarsewooled, Lithuanian Blackface, Estonian Blackhead and Estonian Saaremaa breeds had similar levels of allelic variation (Table 3). Estonian Ruhnu was the population with the least allelic diversity. For highly polymorphic loci only 2 – 4 alleles were detected in this breed.

The distribution of 36 unique alleles among Baltic sheep breeds was not uniform. The largest number (11) was observed in Estonian Whitehead sheep breed. 6 private alleles were detected in Lithuanian Coarewooled and Lithuanian Blackface, 5 in Estonian Saaremaa, 3 in Latvian Darkheaded and Estonian Blackhead, 2 in Estonian Ruhnu population. Frequencies of private alleles were lower than 0.1, except that of the MAF36 allele unique to Latvian Darkheaded breed, that had a frequency of 0.109, the MCM527 allele unique to Lithuanian Coarsewooled breed (0.150) and the MAF214 allele unique to Estonian Ruhnu breed (0.188).

Table 3. **Mean number of alleles across populations (A), mean observed (H_{obs}), mean expected (H_{exp}) heterozygosities and estimates of F_{IS} within seven Baltic sheep breeds are presented. Standard deviations are given in parenthesis.**

Breed	Sample size	A	H_{exp}	H_{obs}	F_{IS}
Latvian Darkheaded	32	7.400 (2.586)	0.712 (0.143)	0.698 (0.173)	0.020
Lithuanian Coarsewooled	30	6.800 (1.699)	0.743 (0.113)	0.733 (0.124)	0.013
Lithuanian Blackface	30	7.267 (2.865)	0.718 (0.165)	0.718 (0.169)	0.000
Estonian Ruhnu	24	3.933 (1.163)	0.574 (0.194)	0.600 (0.212)	–0.045
Estonian Whitehead	30	8.333 (2.845)	0.760 (0.088)	0.727 (0.098)	0.043
Estonian Blackhead	28	7.933 (2.963)	0.714 (0.159)	0.710 (0.186)	0.006
Estonian Saaremaa	21	7.200 (2.077)	0.760 (0.081)	0.625 (0.128)	0.178
Mean		6.981 (1.435)	0.712 (0.064)	0.687 (0.053)	0.035

Observed and expected heterozygosities per breed ranged from 0.600 to 0.733 and from 0.574 to 0.760 respectively (Table 3). The comparison of average H_{exp} and average H_{obs} values did not show big differences in the studied populations. The mean H_{obs} was generally lower than H_{exp}, except in Estonian Ruhnu, where the result was opposite (Table 3). Estonian Saaremaa population was less heterozygous than it was expected. Latvian Darkheaded showed lower heterozygosity than it would be expected according allele frequencies if population was in Hardy – Weinberg equilibrium (Table 3). This difference, measured with the within–breed inbreeding coefficient F_{IS}, ranged from –0.045 (Estonian Ruhnu) to 0.178 (Estonian Saaremaa). In five other

breeds a slight deficiency of observed heterozygosity was observed, as indicated by positive F_{IS} estimates, still the values were very close to zero.

Exact probability tests across populations demonstrated that out of the 105 locus – population combinations, two loci (INRA023 and MAF36) in Estonian Saaremaa population showed significant deviation from Hardy – Weinberg equilibrium after Bonferroni correction ($P<0.0033$, which corresponds with nominal P – value of 0.05).

Distribution of allele frequencies and test for bottlenecked populations

The Wilcoxon sign – rank test showed that there were no significant indications of recent reduction of effective population size in none of the studied breeds. The probability for the mutation – drift equilibrium calculated for Estonian Ruhnu breed was quite low – 0.16, where P values for the other breeds were: Latvian Darkheaded (0.96), Lithuanian Coarsewooled (0.32), Lithuanian Blackface (0.8), Estonian Whitehead (0.92), Estonian Blackhead (0.98) and Estonian Saaremaa (0.85). Slight non – significant mode – shift distortion was observed only in Estonian Ruhnu breed, where proportion of microsatellite alleles in the frequency classes 0.01 – 0.1, 0.11 – 0.2 and 0.21 – 0.3 was very similar (Figure 1). In other 6 sheep breeds allele frequency distribution followed fully the normal L – shaped form, where alleles with the lowest frequencies (0.01 – 0.1) were the most abundant.

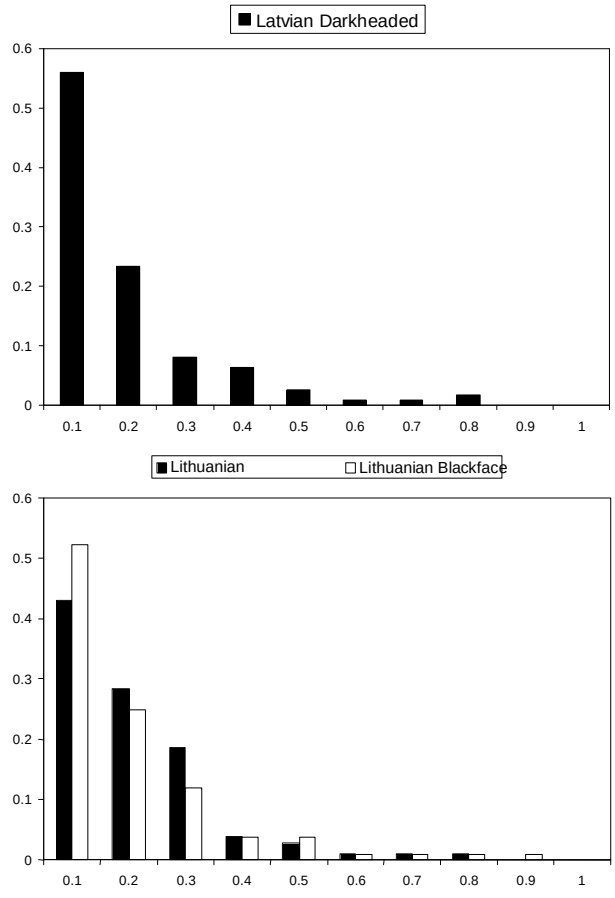

Figure 1. **Distribution in allele frequencies in seven Baltic sheep breeds. The black and white bars represent the proportion of microsatellite alleles in different frequency classes. The x axes represent the 10 frequency classes and the y axes the proportion of alleles.**

Discussion. According to the literature, microsatellite markers comprise a robust, reliable and highly effective alternative to the traditional blood group or serum protein typing methods for parentage testing and evaluation of genetic diversity (Buchanan et al., 1993; Kemp et al., 1995; Marklund et al., 1994; Bowling et al., 1997). All microsatellite loci were found to be polymorphic, but differences in microsatellite variability between breeds were observed. The rare breeds showed less polymorphism compared to the modern ones. Some loci were lacking polymorphism in Estonian Ruhnu breed, but at the same time they were moderately or highly informative in other Baltic breeds. Nevertheless**,** two thirds of loci showed consistently high diversity across all the studied populations. Out of 15 microsatellite markers analysed, four markers (MCM527, BM6526, BM8125 and MAF214) might have null alleles or selection against heterozygotes for these loci (Callen et al., 1993; Ishibashi et al., 1996). The occurrence of null alleles remaining undetected in populations may lead to incorrect inferences from genetical data (Pemberton et al., 1995), so that markers with null alleles (Jarne and Ladoga, 1996) are less usable for parentage testing. Our results support the

usefulness of microsatellite markers to determine paternity and suggests that pedigree checking with the help of microsatellite typing could be successfully applied not only in modern, but also in rare Baltic sheep breeds.

There was a significant amount of variation observed in all Baltic sheep breeds. The relatively high genetic diversity within the improved Lithuanian, Latvian and Estonian sheep populations fits to the assumption that studied populations have evolved through an admixture of breeds, belonging to different breed groups. The observed heterozygosity in Latvian Darkheaded breed was lower than it is expected under Hardy – Weinberg equilibrium (Table 3), which might be due to the breed being subjected to the high level of within flock selection and a related substructuring of the population.

Estonian Saaremaa population, in comparison with other breeds, showed evidently smaller observed than expected heterozygosities (Table 3). Only in this breed two loci showed significant deviation from the genotype frequency, expected according to the Hardy – Weinberg equilibrium. The heterozygote deficiency likely reflects a subdivided population structure (Wahlund effect) rather than selection against heterozygotes for these loci. This conclusion is supported by a high positive value of F_{IS} for Saaremaa sheep (Table 3), that is substantial evidence of fragmentation of breed and is in accordance with the knowledge that animals were sampled from numerous small flocks.

Estonian Ruhnu was found to be less heterozygous than other Baltic sheep breeds, what is in agreement with lower variation observed in exported sheep populations, which have experienced a genetic bottleneck (Tapio et al., submitted). Island breeds are expected to have lower within – breed genetic diversity as a result of founder effect, more restricted gene flow or later genetic drift, although this is not always the case (Bancroft et al., 1995). Even if fewer individuals were sampled from the local breeds (Table 3), this does not seem sufficient to explain the difference. Estonian Ruhnu is free – living, on Ruhnu island isolated breed with small population size. Free – living sheep form hierarchically structured populations, with a few large dominant males that achieve most of the matings. It might decrease the effective population size (Bancroft et al., 1995; Petit et al., 1997). In small populations**,** where a small number of breeding rams is present, allele frequency of the "ram population" might be slightly different than those of "ewe population". This might cause an outbreeding effect, which in Estonian Ruhnu would explain the negative F_{IS} value (Table 3) (Wang, 1996).

Of the studied breeds only Estonian Ruhnu showed nearly significant heterozygosity excess compared to allele numbers as well as a slightly distorted allele frequency distribution (Table 3, Figure 1). In the last 60 years this population underwent reduction of census size to a few tens of individuals, what might have resulted to the loss of alleles in the breed, but not at the same degree decreased heterozygosity (Table 3). Out of 36 unique alleles detected in Baltic breeds, only two were observed in Ruhnu population. The other sheep populations did not show signs of possible recent reduction in effective population size and**,** on the contrary**,** had relatively high amount of alleles presented, including many unique alleles.

The unique alleles might result from recent mutations or they might be indicative of distinct ancestry of the population, e.g. crossing with an exotic breed. When unique allele has a frequency below 0.1 it might be an allele that is present in several populations at low frequency and could be found also in other breeds, if greater fraction of the total population would be screened. On the other hand, the detection of unique alleles with frequency higher than 0.1 in Latvian Darkheaded, Lithuanian Coarsewooled and Estonian Ruhnu populations might reflect that some sampled individuals could be closely related, or there might also be a link between the unique alleles and some preferable functional genes. Nevertheless**,** it is difficult to know if the presence of unique alleles really increases the adaptability or survival of the population over longer evolutionary time (Allendorf and Leary, 1986; Avice, 1994; Rasplus et al., 2001).

The variation between individuals and breeds might be discerned already at the phenotypic level. Traditionally ovine breeds are classified according to their fleece characteristics, as it is assumed that such differences reflect distinct origins. The breeds we studied were semifinewooled (Lithuanian Blackface, Latvian Darkheaded, Estonian Whitehead, Estonian Blackhead, Estonian Saaremaa, Estonian Ruhnu) and coarsewooled (Lithuanian Coarsewooled). Looking in more detail into these groups we might separate breeds according to the wool color (white, brown, black), type of tail or presence/absence of horns. In the Baltic countries sheep are kept primarily for meat**,** so animals from all breeds except Lithuanian Coarsewooled have heavy body condition and are suited for meat production (Table 1). When we compare genetic data described above with the coarse information that can be obtained from the external observation of phenotypic characters of animals, it seems that phenotypic description does not provide sufficient information about the within breed variation, presented in Baltic sheep breeds. More exact evaluation of this phenotypic variability would require breeding and selection studies over generations, which is not realistic for the Baltic rare breeds at the moment. Therefore molecular studies are a good way to provide some estimates for within population variation.

For each population the breeding goal is to increase yields of primary products. For semi – managed or local breeds this task is fulfilled by retaining adaptive fitness, whereas in commercial breeds it is increasingly by improving health and welfare of animals (Bijma et al., 2002). Breeds are primarily judged by their productivity**,** whereas fitness advantage of local breeds usually goes unnoticed. To maintain genetic diversity that is still existing in rare Baltic sheep breeds and might be of crucial importance for future needs, breeding schemes that increase effective population size of rare breeds and thereby minimize the effect of genetic drift throughout the genome should be designed. If maintenance of Baltic genetic resources is not dealt properly, the changes in

farming system will lead to increased crossing of Baltic breeds with breeds of Central European and British origin. Crosses with international common breeds might result to a short – term increase of heterozygosity, but this might in long term change to loss of genetic variation when the original diversity becomes replaced. Further studies are required to investigate the long – term effects of crossing national populations on genetic variation and performance, in order to assess whether the short – term benefits outweigh the long – term costs of loss of genetic variation (Blott et al., 1998). Conservation of genetic variation in Baltic populations should be considered by breeders, in the interests of the long – term future of the breed in its native country.

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