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MOLECULAR GENETICS METHODS, AS TOOL OF SHEEP BREEDING

METODELE GENETICII MOLECULARE CA INSTRUMENTE PENTRU AMELIORAREA OVINELOR

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The authors have summarized shortly their research projects from the last 10ys in the field of sheep molecular genetics.

1. Investigation of melatonin receptor 1a (Mel_{1A}) as a candidate gene influencing reproductive seasonality in sheep

Seasonality is a common feature among various mammalian species. Out of the breeding season the ovulatory activity of ewes is inhibited (acyclic ovarian function, syn. seasonal anoestrus). Great variability exists in sheep between breeds and within breeds in terms of the presence, time and duration of anoestrus (Roche et al, 1985; Quirke and Hanrahan, 1985; Notter, 1992).

Two melatonin receptor subtypes have been cloned and characterized in mammals: Mel1a (or MT1) and Mel1b (or MT2) (Reppert et al, 1994, 1995, 1996), but only one, melatonin-receptor 1a (Mel1a), has been reported to be present in small ruminants (Migaud et al, 2002). The Mel1a gene, composed of two exons separated by a large intron, was investigated by several research groups and different single nucleotide polymorphisms (SNPs) were found in exon II of the gene.

In Hungary most of the sheep breeds have cyclic ovarian activity from August till January. It means that the milk production, and producing of milk products, is not continuous during the year even though the market would require it. Therefore use of a polymorphic genetic marker in the selection program for continuous milk production would be particularly advantageous in the intensive dairy

sheep farms. Our aims were to determine if a similar relationship exists in the Awassi population concerning the RsaI and MnII RFLP sites to those reported previously, and to search new polymorphisms in exonII of Mel1a gene using three breeds: Awassi (a dairy breed from the Mediterranean areas), Tsigai (a native breed from the continental area) and Hungarian Prolific Merino (selected for 3 times lambing within two years).

Blood samples were collected from 340 Awassi ewes for genotyping of the two RFLP sites (RsaI and MnII) and milk samples were collected from the same animals for progesterone (P4) measurement. For sequencing of the exonII of Mel1a gene blood samples were collected from 28 Hungarian Prolific Merino, 23 Tsigai ewes and 124 Awassi sheep (ewes and rams).

DNA was extracted from blood as described by Zsolnai and Orban (1999). Primers (Applied Biosystems, USA), PCR conditions for amplification of exon II of the Mel1a gene and the restriction conditions for each Mel1a RFLP (RsaI and MnII) test were used as described by Messer et al. (1997). HotStar Taq DNA polymerase (Qiagen, USA) was used for PCR. In case of both RFLP sites allele was called „A" if the PCR product was not cut by the enzyme and called „B" if it was.

PCR products were purified by Montage PCR plate system (Millipore Corp., Bedford, MA). Sequencing was performed on an ABI Prism 3730 sequencer (Applied Biosystems) using ABI PRISM Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer, Foster City, CA). Sequencer Software 5.2 (Foster City, CA) was used to assemble the sequences and Phred, Phrap, PolyPhred and Consed programs were used for SNP identification.

Positions of the mutations were determined according to a reference sequence with GeneBank No: U14109. Altogether 16 SNPs were identified in the three breeds. Six of the 16 were not previously reported. Four of the new SNPs cause amino acid changes in the protein sequence and two of them are silent. The SNP 712 and 959 were present only in Awassi, the mutation at position 874 was present in Awassi and Tsigai but not in the Hungarian Prolific Merino.

Concerning the ovarian function, 36% of the Awassi ewes had cyclic ovarian activity with 10-11 weeks after lambing in spring. In case of RsaI and MnII polymorphisms, 37% and 42% of the homozygous BB ewes, 31% and 34% of the heterozygous (AB) and 64% and 31% of the homozygous AA animals had cyclic ovarian function out of the breeding season, respectively (Figure 1.)

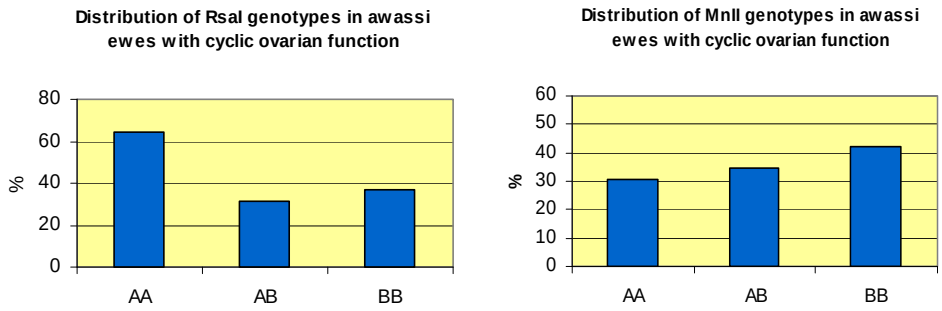

Figure. 1. Distribution of RsaI and MnII genotypes in Awassi ewes with cyclic ovarian activity

The distribution of ewes having or not having cyclic ovarian activity (in out of season) within the three MnII genotypes (AA, AB and BB) may suggests an association between the genotype and phenotype but not in the case of RsaI polymorphisms. Similar results were reported by Pelletier et al. (2000) and Notter et al. (2003). Since ovulatory activity is influenced by many factors, such as age, year or number of lactation, we can not draw any final conclusion based on these results. An adequate statistical analysis will be done when the data collection of the influencing factors will be completed.

Exon II of Mel1a is highly polymorphic. Sixteen SNPs were found in an 824 bases long sequence in the three examined breeds. Six of the 16 SNPs cause amino acid changes in the protein which can be supposed to cause changes in the function and/or construction of the melatonin receptor giving inspiration for further investigation of Mel1a. On the other hand, three of the functional mutations were found to be breed-specific. This can firm the suggestion of Hernandez et al (2005), that the influence of the MnII polymorphisms (which is indirect and the SNP or QTL linked to the MnII RFLP sites has the real effect) on the regulation of seasonal function is dependent upon the breed and/or environment.

We continue our study with screening the three breeds for the functional mutations, constructing haplotypes and making association study between the haplotypes and the ovarian activity in out of season.

2. Case study of a Hungarian breeding program using imported Booroola rams

Profitability of sheep breeding is mainly determined by litter size. Selection for prolificacy based on phenotype has a low genetic gain (Safari and Fogarty, 2003). The first major gene for prolificacy identified in sheep was the Booroola (FecB) gene. After the recognition of the existence of the single gene, Booroola Merino has spread all over the world. In Europe, Hungary was the first country that imported Booroola Merino rams and ewes (Veress, 1983). A new breed, called

Hungarian Prolific Merino, was established based on the crossing of Booroola Merino rams and Hungarian Merino ewes, and was acknowledged in 1992. The aim of the breeders was to create and maintain a homozygous flock for the FecB locus and use it in cross breeding programmes (Veress et al., 1987). To date, the carriers of the Fec^B allele were identified on the basis of ovulation rate records in the case of ewes, and extensive progeny testing in the case of rams. This method is time and labour consuming, which hinders its practical application. The only way to determine the FecB genotypes has been the measurement of the ovulation rate over a long period. In 2001, the Booroola mutation was identified. Mutation on the bone morphogenetic protein receptor – 1B gene was found to be associated with the increased ovulation rate in the Booroola Merino ewes. 138 ewes and 46 rams in the Hungarian Prolific Merino population were tested for this mutation by PCR-RFLP and their FecB genotypes were determined. One copy of the Fec^B allele increased ($P<0,05$) the ovulation rate by 0.89 ova and two copies increased by an average of 2.27 ova. Effectiveness of the FecB genotype estimation based on phenotype measurement was also compared to the results of direct DNA testing, and was found to have up to 80% accuracy.

The aim of this study was to demonstrate that the high ovulation rate in the Hungarian Prolific Merino is caused by the mutation in the BMPR-1B receptor gene and to compare the effectiveness of the genotyping method based on the ovulation rate to the results of the direct gene test.

By using the direct gene test, 139 ewes and 46 rams altogether were genotyped (involving the 90 ewes and 25 rams, which had estimated FecB genotypes and further 49 young ewes and 21 rams, which had no estimated FecB genotypes) for the Booroola mutation in 2003.

The PCR-RFLP technique using primers and restriction enzyme (AvaII) as described by Wilson et al. (2001) was used for the detection of the mutation in the bone morphogenetic receptor type 1 B (BMPR-1B) gene. DNA was isolated from blood and semen samples as reported by Zsolnai and Fésüs (1996).

Least Square Means of the OR in the three groups of FecB genotype ($Fec^{B}Fec^{B}$; $Fec^{B}Fec^{+}$; $Fec^{+}Fec^{+}$) were calculated. Mixed Model Least-Squares and Maximum Likelihood Computer Programme PC-2 were used for the ANOVA (Harvey, 1999). The FecB genotype determined by direct DNA test, the year and the age of ewes at the time of OR measurement were involved into the model as fix effects. The ovulation rate data (115 measurement altogether) of 64 pedigree ewes were involved in the analyses of variance because of the availability of the fix effects data.

The accuracy of the estimation of FecB genotypes was calculated by comparing the estimated genotypes to the genotypes determined by the direct gene test. The correspondence was expressed in percentage.

Mutation in the BMPR-1B receptor gene is proved to result in the high ovulation rate in the Hungarian Prolific Merino population on the basis of the results of the one-way ANOVA between ovulation rate and FecB genotypes. Although, it was published that the Booroola mutation has an additive effect on ovulation rate

by Piper et al. (1985), different results were obtained in our experiment. The ovulation rate of homozygous carriers had more than twice as much as the non-carriers. This phenomenon could be explained by the allele-allele interaction between the wild type (Fec^{+}) and the Fec^{B} allele. Similar case was observed in the crosses of Inverdale and Booroola Merino by Davis et al. (1999). When the 2 genes, the FecI and FecB, were in combination the OR was higher than the sum of the effects of each gene alone.

Distribution of the three FecB genotypes in the female and the male populations reflects that the aim of the breeders has not yet been fulfilled, since less than half of the whole population is homozygous $Fec^{B}Fec^{B}$. In this study great differences were found in the results of determination of FecB genotype by direct DNA test and estimation of the genotype based on ovulation rate in the female population. FecB genotype was overestimated by 20% by breeders. This explains, partly, the high frequency of the $Fec^{B}Fec^{+}$ heterozygous and $Fec^{+}Fec^{+}$ non-carriers in the Hungarian Prolific Merino population. Moreover, FecB genotypes of the rams were also estimated improperly based on their progeny test. The accuracy of the estimation of FecB genotype was different depending on the PMSG treatment in the progeny test. Classification was found to be more accurate if it was based on the daughters' OR results which were measured in normal oestrus cycle, comparing to the methods wherein PMSG treatment was taken. Although several research groups used previously the PMSG treatment in the progeny tests to estimate the FecB genotype (Cleverdon and Hart, 1981; Davis and Kelly, 1983; Oldham et al., 1984; Davis and Johnstone, 1985), this methods was proved to be not reliable in our experiment. Rams with genotype of $Fec^{+}Fec^{+}$ or $Fec^{B}Fec^{+}$ were used for a long time because of the missed classification. This also gives a good explanation for the high frequency of the non-carriers, and the heterozygous ($Fec^{B}Fec^{+}$) animals in the present population. No other publications were found to report data similar to those presented here.

In conclusion, results support that the high rate of ovulation is caused by the mutation in the BMPR-1B receptor gene in the Hungarian Prolific Merino. Efficiency of the breeding programme to increase the frequency of the Fec^{B} allele in this population was hindered by the previously applied improper genotype classification.

Application of the direct DNA test will accelerate the spread of the Fec^{B} allele in the flock. At present an experiment is under way for exploiting the connection between endocrinologycal processes (e.g. IGF, leptin level) and seasonality and litter size, respectively.

3. Estimation of Genetic Difference between different Tsigai and Zackel type of sheep in East- Middle and South Europe

Hungary is one of the first countries where is recognized the preservation of domestic animal species, the conservation of the entire genetic variancy, and the regulation of function and relation of different genotypes is a very important task in

cultural and technical aspects too. In Hungary the Racka, Tsigai and Cikta cultures were almost entirely liquidated in the previous decades, and the headcount of Racka and Tsigai flocks began to grow only in the last decade. The Tsigai got into Hungary around 1700. The claim of the blaize-factories inspired the farmers in Transylvania to change the rough woolly Curkan to Tsigai, which produce more softer wool (Rodiczky, 1904; cit.: Gáspárdy, 2002). Many Tsigai variation is bred in East-and Central European countries and other regions. These variations differ in bodysize, bodyweight, growth and colour too. In Serbia on the region of Bácska and Bánáti the more extensive Cokanski and the heavier, better milking Zomborsky type is bred. The home representative of the latter is the Lédeci of sorts flock (Dunka, 1997). The Transylvanian Tsigai has more chest width, relatively shorter legs. Covasna variant has brown head and legs (Gáspárdy, 2001). In Bulgaria it has also two types, the North-West and the South-Bulgarian one. (Dimov, 2000; cit.: Kukovics and Jávor, 2002). Nowdays we distinguish two types of Tsigai in Hungary, the autochthonous and the –selected for milk production- Zomborski variant. Between these there are several transitional types (Kukovics et al, 2003). Earlier the blood type and protein polimorphism was examined to demonstrate genetic distinctions (Fésüs, 1974). Nowadays the usage of genetic markers became general to estimate genetic distance, because it is more efficient and has more sureness.

Our research was done from blood sample of Pál Gábor'(selfemployed person) Milking Zomborski, Transylvanian Rusty and from the Farm of University Debrecen Centre of Agriculture Sciences Department of Animal Breeding and Nutrition Cokanski Tsigai flock (n=252), while all the other case we had hair samples (n=1253) Collecting of the hair and blood samples were started in 2004, because the sampling was numerous and included 8 (Hungary, Romania, Albania, Bulgaria, Croatia, Turkey, Slovakia, Serbia-Montenegro) countries and it required a long preliminary preparation (Table 1.). The samples were stored on -20^{o}C till next step. The isolation of genomical DNA from blood was done following the Zsolnai and Orbán (1999) method. The taking of hair samples was done by picking and the samples were taken into nylon or paper bags. The extraction of the genomical DNA was followed the worked out method of FAO (2004). The genomical DNA samples were stored on -20^{o}C for further steps.

Table 1

Used population and their main character

Country	Population	Group	Number	Label
Hungary	Autochthonous	Tsigai	53	HU-SMA-AC
			40	HU-KMKK-AC
			39	HU-KMNP-AC
			53	HU-SZIC-AC
			45	HU-MRD-TAC
	Cokanski	Tsigai	125	HU-DE-CSC
	Zomborski	Tsigai	77	HU-PG-ZC
			39	HU-LB-TCZ
			42	HU-OJ-TC
	Rusty (from Jucu)	Tsigai	50	HU-PG-TRC
Romania	Romanian Ruda		40	RO-RUDA
	Rusty (from Turda)	Tsigai	40	RO-RUST-TS
Albania	Albanian Tsigai	Tsigai	39	AL-TS
	Albanian Ruda	Zackel	37	AL-RUDA
	Bardhoke	Zackel	31	AL-BARDH
Bulgaria	Patched Faced Maritza	Zackel	39	BU-PFMAR
	Pleveny Blackhead sheep	Zackel	35	BU-PLBH
	Rodopski Tsigai	Tsigai	30	BU-ROD-TS
	Staroplaninski Tsigai	Tsigai	42	BU-STAR-TS
	White Maritza sheep	Zackel	41	BU-WFMAR
Croatia	Croatian Tsigai	Tsigai	50	CR-TS
Turkey	Sakiz	Zackel	49	TR-SAKIZ
	Gokceada	Zackel	42	TR-GOKCE
	Kivircik (Marmara region)	Tsigai	46	TR-KIV-MAR
	Kivircik (Trakya region)	Tsigai	53	TR-KIV-TRA
Slovakia	Handel	Tsigai	25	SL-HAN-TS
	Jugat	Tsigai	22	SL-JUG-TS
	Kamo	Tsigai	19	SL-KAO-TS
	Sirig	Tsigai	22	SL-SIR-TS
	Vojin	Tsigai	5	SL-VOJN-TS
	Jurbis	Tsigai	24	SL-JUR-TS
	Kamendin	Tsigai	16	SL-KAM-TS
	Olymp	Tsigai	5	SL-OLYM-TS
	Ondrej	Tsigai	16	SL-OND-TS
	Rybar	Tsigai	16	SL-RYB-TS
	Vancover	Tsigai	15	SL-VAN-TS
	Brend	Tsigai	10	SL-BREN-TS
Serbia-Montenegro	Zomborski	Tsigai	41	SM-ZP-TS
	Cokanski	Tsigai	12	SM-CS-TS
	Svrljiska Zackel Pramenka	Zackel	48	SM-SVR-PR
	Krivovirska Zackel Pramenka	Zackel	32	SM-KRI-PR

Our research were done with the following microsatellites:

BM 6506 (1); OarFCB 20 (2); MAF 70 (4); MCM 527 (5); INRA 127 (8); ILSTS 11 (9); TGLA 53 (12); TGLA 357 (14); MAF 65 (15); Oar CP 49 (17); Oar AE 119 (19); Oar CP 20 (21); BM 1314 (22); MAF 35 (23); MCMA 7 (25); CSSM 43 (26).

We used ABI 9700, ABI2700 and MJ Research Thermocycler programable PCR (DNA Thermal Cycler) for PCR reactions. The detection and examination of alleles were done with ABIPRISM 3100 Genetic Analyzer. The collection of data was done by the help of GeneScan software (Applied Biosystems). The evaluation of informations was done by Genographer software. In the course of evaluation of statistic data we used the POPULATIONS, GENEPOP, MICROSAT, PHYLIP and ARLEQUIN version 2.0 softwares.

The genetic distance research, which covered 41 Tsigai and Zackel flock in 8 countries, was fulfiled by the help of microsatellite markers. On the examined locus we determined totally 384 alleles. The least number of alleles (11) were found on locus MAF35, the most larger number of alleles (35) were found on locus MAF70. The avarage number of alleles is ranged between 4.1 (MAF35) and 10.4 (MAF70).

The expected mean value of heterozygosity was on the 16 locus 0,716. The least number was (0,614) in BM6506, the most higher value was (0,812) on locus BM1314. In case of every markers the expected heterozygosity value was higher than the observed value. The mean observed heterozygosity value was 0,525. We have got the least number in case of MAF65 (0,3190), the largest one in case of locus BM1314.

The mean heterozygosity value among examined population was between 0,356-0,629, the expected value is changing between 0,640-0,843. All examined populations were less heterozygous than it was expected. The absence of heterozygous individuals were the largest in the Serbian Zomborski population (SM-ZP-TS), the least in one of the Hungarian autochthonous population (HU-SMA-AC). According to inbreeding coefficient the inbreeding failure is higher in the Serbian Zomborski population (52,7%), least in Croatian Tsigai population (12,8%) among examined populations. Based on the mean number of alleles, the Hungarian Cokanski population, HU-DE-CSC (8.8) is the most diverse, the least diverse population is the Serbian Zomborski, SM-ZP-TS (2.3).

On the examined locus we determined 50 population specific alleles in 21 populations. Preservation of these alleles require special attention, because they are typify only definite population. The most informative markers are TGLA357 and OarCP49. Population specific alleles were determined in the following population (with the number of specific alleles in the parenthesis): HU-KMKK-AC (2,) HU-PG-ZC (2), HU-LB-TZC (1), HU-SZIC-AC (1), HU-SMA-AC (5), HU-DE-CSC (3), HU-OJ-TC (1), BU-STAR-TS (4), BU-ROD-TS (1), BU-PFMAR (1), BU-WFMAR (5), BU-PLBH (3), RO-RUST-TS (2), RO-RUDA (1), AL-TS (1), AL-RUDA (1), AL-BARDH (2), SL-HAN-TS (1), SL-VAN-TS (1), SL-JUR-TS (3),

SL-RYB-TS (1), SL-SIR-TS (1), TR-KIV-TRA (3), SM-CS-TS (2), SM-KRI-PR (1), SM-SVR-PR (1).

We constracted genetic distance matrix - on the grounds of Nei standard genetic distance (D_S) and minimum genetic distance values (D_M) – in order to identify the genetic connection among examined population. This short thesis is not contain the matrix because of its size.

These genetic relationships are reflected on the phylogenetic tree, constructed from genetic distance (D_M) data using the UPGMA algorithm (Figure 2). The Serbian Zomborski and the Croatian Tsigai differ from all the examined populations. The two Bulgarian Tsigai (BU-STAR-TS and BU-ROD-TS) are in very close relation and they next to the Turkish Sakiz (TR-SAKIZ), which belongs to the Zackel type. The Kivircik populations (TR-KIV-TRA and TR-KIV-MAR from two Turkish regions) are close to each other and on the phylogenetic tree they belong to the same branch with the Bulgarian Pleveny Blackhead sheep (BU-PLBH), two Bulgarian Maritza (Patch Faced Maritza (BU-PFMAR), White Faced Maritza (BU-WFMAR) and three Slovakian Tsigai (SL-KAO-TS, SL-KAM and SL-OND-TS). Among Rumanian populations, the Rumanian Ruda (RO-RUDA) has the closest relation to Rumanian Rusty Tsigai (from Turda-RO-RUST-TS). The Hungarian examined Tsigai populations are on two branches of tree. On one of them two smaller branches can be distinguish, where the two autothchtonous populations (HU-KMKK-AC and HU-KMNP-AC) –from Kőrös-Maros National Park-, and HU-SZIC-AC population and the population from Makó-Rákos (HU-MRD-TAC) are found. The Albanian Tsigai (AL-TS) is teh closest relation with them. The other branch,which contains the Hungarian flocks, also devides into two sub-branches containing two-two populations. Population from Soltszentimre (HU-SMA-AC) and Akasztó (HU-OJ-TC) are close relationship although the previous as a autothchtonous one, the latter as a milking one according to the official register. The Rusty Tsigai (HU-PG-TRC), which was crossed with Merino; has the closest relation to the Milking population from Cegléd (HU-LB-TCZ). Population from Csóka (HU-DE-CSC) is closeset to an other Milking population (HU-PG-ZC). In Hungary the population from Cegléd is regarded as the most typical Milking type, which were developed with Serbian (Zomborski and Cokanski) bucks in the last 15 years. Among Slovakian population on one branch of the tree there are the SL-JUR-TS and SL-VAN-TS, which are genetically near to the SL-OLYM-TS. On the other branch of the tree there are the SL-JUG, SL-HAN-TS and SL-RYB-TS, SL-BREN-TS populations. The SL-SIR-TS is in the nearest connection with SL-JUG and SL-HAN-TS. The Albanian Bardhoke sheep (AL-BARDH) is near to these, which belongs to the Zackel type. Far from these population there is an other Slovakian group. SL-KAO-TS, SL-KAM-TS and SL-OND-TS belong to this group. The SL-VOJN-TS differs not only from the other examined Slovakian, but from all examined population.

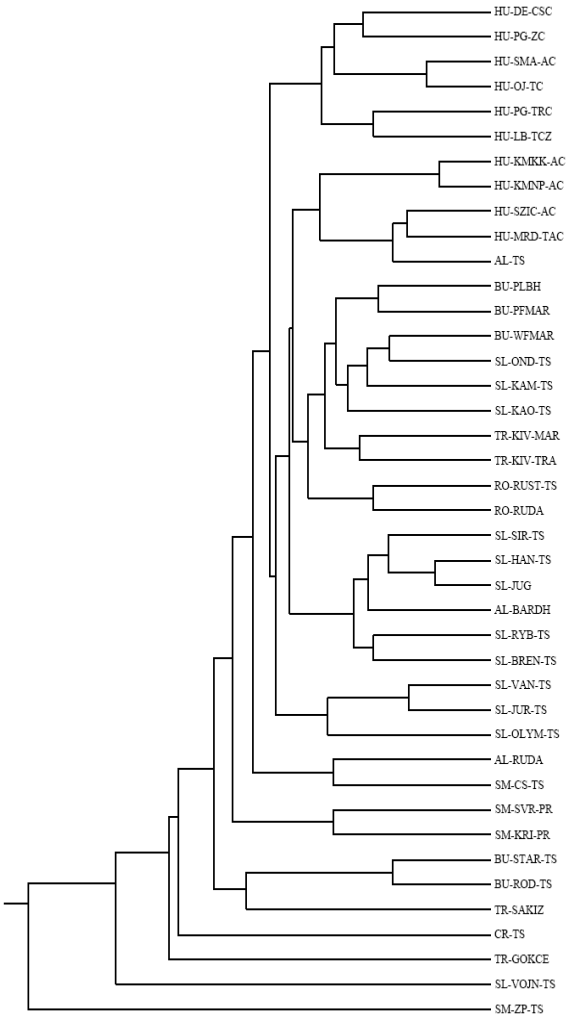

Figure 2: UPGMA phylogenetic tree of the examined populations

4. In our Institute there are the following running projects at this moment:

Mapping quantitative trait loci for milk production on chromosome 6 in Awassi sheep breed

Aim: to find markers linked to a QTL or major gene influencing milk production, and use the information in a Marker Assisted Selection program in an Awassi population in Hungary. (genotyping of half sib-families for 12 microsatellite loci covering the whole chromosome, using „QTL express" for QTL mapping; measured traits: milk yield, milk protein kg, milk fat kg, somatic sell account).

Polymorphism of milk protein genes in Tsigai sheep breed

Aim: to determine ß-LG, κ-CN, α-s1-CN genotype of Milking and gene reserve Tsigai population and looking for association between genotype and milk composition, cheese producing parameters.

Determination of the Callipyge genotypes in the Hungarian Suffolk population

Aim: animal carrying the Callipyge mutation can be used in the cross breeding programme to improve the meat production. The callipyge gene could prove beneficial in breeding because it enables the sheep to convert food into muscle 30% more efficiently than normal sheep. The DNA test is very important for detection the mutation carriers sine it is inherited in polar overdominance, which means that the only sheep with the abnormal muscling are those which are inherited a normal copy of a specific gene from their mother, but a mutated copy of that same gene from their father. Our aim is using heterozygous rams in a cross breeding program to investigate the effect of the callipyge mutation on meat production of the Hungarian Merino and native Racka sheep.

Wool sheep crossing for hair sheep

Long sheep tails are docked and producing wool of no profit means a handicap, as well. The creation of short tailed hairy Hungarian sheep breeds is planned from three such genotypes: the Mouflon, well adapted to our climate, and two African breeds, the polled Somali and the prolific Cameroon Sheep. The listed genotypes are at our proposal, or are available from Hungarian sources in required numbers. Creation of the polled „Muso" breed is planned from Mouflon x Somali, and that of the polled and prolific „Musoka" from the (Mouflon x Somali) x Cameroon crossings. The wooly domestic sheep would be approved to short tailed hairy by repeated crossings with Mouflon, then with Muso, or Musoka Sheep.

Bibliography

1. **Cleverdon, J.M., Hart, D.S.:** Oestrus and ovarian activity of Booroola Merino crossbred ewe hoggets. Proceeding N. Z. Soc. Anim. Prod. 41 (1981), 189-192
2. **Davis, G.H., Dodds, K.G., Bruce, G.D**.: Combined effect of the Inverdale and Booroola prolificacy genes on ovulation rate in sheep. Proc. of the 13th Conf. Assoc. for the Advancement of Animal Breeding and Genetics (1999), 74-77.
3. **Davis, G.H., Johnstone, P.D.:** Ovulation response to pregnant mares serum gonadotrophin in prepubertal lambs of different Booroola genotypes. Anim. Repr. Sci. 9 (1985), 145-151
4. **Davis, G.H., Kelly, R.W**.: Segregation of a major gene influencing ovulation rate in progeny of Booroola sheep in commercial and research flock. Proceeding N. Z. Society Animal Production 43 (1983), 192-197
5. **Dunka, B.** (1997): A cigája és a cikta. Állattenyésztők Lapja.7:7.
6. **Harvey, L.R.:** Mixed Model Least-Squares and Maximum Likelihood Computer Programme PC-2. Manual (1999)

7. **FAO** Handbook of laboratory Exercise (2004): FAO/IAEA Inter-regional Training Course on Molecular Methods in Livestock Genetics and Breeding. Seibersdorf. Austria.18.
8. **Fésüs, L.** (1974): A juh vércsoportjai I. Az első hazai vizsgálatok eredménye. Állattenyésztés. 5:83-88
9. **Gáspárdy, A.** (2001): A cigája. Magyar Állattenyésztők Lapja. 29:7.
10. **Gáspárdy, A.** (2002): Cigája (Berke). In: Génmegőrzés; kutatási eredmények régi háziállatfajták értékeiről (edited:Jávor,A.- Mihók,S.) Debrecen, 2002.október 16; 153-161.
11. **Hernandez, X., Bodin, L., Chesneau, D, Guillome, D., Chemineau, P., Malpaux, B., Migaud, M.** (2005): Relationship between MTI melatonin receptor gene polymorphism and seasonal physiological responses in Île-de-France ewes. Reprod. Nutr. Dev. 45: 151-162.
12. **Kukovics, S.- Jávor, A.** (2002): The Tsigai sheep, CAB International, London.1-36.
13. **Kukovics, S., Molnár, A., Jávor, A., Gáspárdi, A., Dani, Z**.(2003): A hazai cigája juhállományok változatai és termelési különbségei. Magyar Juhászat. Magyar Mezőgazdaság melléklet.6:2-6.
14. **Messer, L.A., Wang, L., Tuggle, A.K., Yerle, M., Chardon, P., Pomp, D., Womack, J.E., Barendse, W., Crawford, A.M., Notter, D.R., Rothschild, M.F.:** Mapping of the melatonin receptor 1a (MTNR1A) gene in pigs, sheep and cattle, Mammalian genome, 1997; 8:368-370
15. **Notter DR, Cockett, NE and Hadfield TS:** Evaluation of melatonin receptor 1a as a candidate gene influencing reproduction in an autumn-lambing sheep flock, J. Anim.Sci. 2003; 81: 912-917
16. **Notter, D.** (1992): Genetic improvement of out-of-season breeding through selection. In: Proceedings of the Out-of-Season Breeding Symposium, 19-20 June 1992, Ames, Iowa, USA, pp. 55-81
17. **Oldham, C.M., Gray, S.J., Poindon, P., Bindon, B.M**.: Progeny testing for F gene using prepubertal ewe lambs. In: Lindsay, D. R. and Pearce, D. T. (eds) Reproduction in Sheep, Cambridge Univ. Press. (1982), 260-264
18. **Pelletier, J., Bodin, L., Hanocq, E., Malpaux, B., Teyssier, J., Thimonier, J., Chemineau, P**.: Association between expression of reproductive seasonality and alleles of the gene Mel_{1a} receptor in the ewe. Biol. Reprod. 2000; 62: 1096-1101
19. **Piper, L.R., Bindon, B.M., Davis, G.H.:** The single gene inheritance of the high litter size of Booroola Merino, In: R.B. Land, D.W. Robinson (eds.), Genetics of Reproduction in Sheep, London:Buterworth (1985), 115-125
20. **Quirke, J.F., Hanrahen, J.P.:** Breeding differences in the breeding season in sheep. In: Ellendorf, F., Elsaesser, F. (eds.): Endocrine causes of seasonal and lactational anoestrus in farm animals. Martinus Nijhoff Publishers, Dordrecht, The Netherlands, 1985; pp. 29-43

21. **Reppert S M, Weaver D R and Gondson C**: Melatonin receptors step into the light: cloning and classification of subtypes. Trends Pharmacol Sci. 1996; 17(3):100-2
22. **Roche, J.F., Hanrahan, J.P., Quirke, J.F., Ronayne, E.:** Effects of melatonin on the time of onset of the breeding season in different breeds of sheep. In: Ellendorf, F., Elsaesser, F. (eds.): Endocrine causes of seasonal and lactational anoestrus in farm animals. Martinus Nijhoff Publishers, Dordrecht, The Netherlands, 1985; pp. 55-65
23. **Safari, A., Fogarty, N.M.:** Genetic Parameters for Sheep Production Traits: Estimates from the Literature. Technical Bulletin. NSW Agriculture. Orange. Australia 49 (2003)
24. **Veress, L.:** Data for the breeding programme of the Booroola Merino (Adatok a booroola merinó tenyésztési programjához). Hungarian Journal of Animal Production. 32 (1983) 4,329-334
25. **Veress, L., Végh, J., Horváth,** Vné: Breeding possibilities of the Booroola Merino in Hungary (A booroola merinó tenyésztési lehetőségei Magyarországon). Hungarian Journal of Animal Production. 36 (1987) 1, 53-61
26. **Wilson, T., Wu, X.Y., Juengel, J.L., Ross, I.K., Lumsden, J.M., Lord, E.A., Dodds, K.G., Walling, G.A., McEwan, J.C., O'Conell, A.R., McNeilly, K.P.,** Montgomery, G.M.: Highly Prolific Booroola Sheep Have a Mutation in the Intracellular Kinase Domain of Bone Morphogenetic Protein IB Receptor (ALK-6) that in Both Oocyte and Granulose Cells. Biology of Reproduction. 64 (2001), 1225-1235
27. **Zsolnai, A., Fésüs, L**.: Simultaneous analysis of bovine kappa-casein and BLAD alleles by multiplex PCR followed by parallel digestion with two restriction enzymes. Animal Genetics 3 (1996), 207-209
28. **Zsolnai, A., Orbán, L.:** Accelerated separation of random complex DNA patterns in gels: comparing the performance of discontinuous and continuous buffers. Electrophoresis, 1999; 7: 1462-1468

METODELE GENETICII MOLECULARE CA INSTRUMENTE PENTRU AMELIORAREA OVINELOR

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Autorii au prezentat pe scurt proiectele lor de cercetare din ultimii 10 ani în domeniul geneticii moleculare la oaie. S-au realizat investigaţii asupra receptorului de melatonină 1a (Mel_{1A}) ca genă candidat care influenţează sezonalitatea reproducţiei la oaie. Cercetările s-au efectuat pe trei rase, şi anume Awassi, Merinos prolific maghiar şi Ţigaie. La aceste rase s-au determinat poziţiile mutaţiilor genelor în funcţie de secvenţa d referinţă a GeneBank nr. U14109. În total, la cele trei rase s-au identificat un număr de 16 polimorfisme nucleotidice singulare (SNP). Exonul II a genei Mel1A este puternic polimorfic. Şase din SNP identificaţi produc modificări ale aminoacizilor din proteina care poate fi cauza modificărilor funcţiei şi/sau construcţiei receptorului de melatonină. Studiul se va continua pentru a investiga cele trei rase pentru mutaţiile funcţionale, construirea de haplotipuri şi asocierile dintre haplotipuri şi activitatea ovariană extrasezon. O altă abordare o reprezintă studiul de caz a programului maghiar de ameliorare folosind berbeci importanţi din rasa Booroola. În Europa, Ungaria a fost prima ţară care a importat berbeci şi oi din rasa Merinos Booroola, pe baza cărora s-a format o nou rasă Merinos Prolific Maghiar, recunoscută în 1992. În concluzia studiilor de până acum, rezultatele au arătat că rata ridicată de ovulaţie la castă rasă este produsă de mutaţia receptorului genei BMPR-1B. Eficienţa programului de ameliorare dea creşte frecvenţa alelei Fec^B în această populaţie de ovine a fost întârziată de aplicarea anterioară a metodelor improprii de clasificare genotipică. Un proiect mai amplu al echipei de cercetători este acela de a estima diferenţele genetice dintre variatele tipuri de oi Ţigaie şi Ţurcană din Estul, Centrul şi Sudul Europei. În prezent, în cadrul institutului se derulează următoarele proiecte de cercetare: cartografierea locilor însuşirilor cantitative pentru producţia de lapte din cromozomul 6 la rasa Awassi; polimorfismul genelor proteinei din lapte la rasa Ţigaie; determinarea genotipurilor Callipyge la populaţia Suffolk Maghiar;încrucişarea raselor de ovine de lână pentru a produce ovine pentru păr.

Cuvinte cheie: metode genetice moleculare, ovine, ameliorare genetică.