ÁLLATTENYÉSZTÉS ÉS TAKARMÁNYOZÁS, 2006. 55. 4. 313–322. 313

DNA MICROSATELLITE TEST OF THOROUGHBRED AND TROTTER HORSES IN HUNGARY

JÓZSA, CSILLA Ms. — HUSVÉTH, FERENC — BÁN, BEÁTA Ms. — TAKÁCS, ERZSÉBET Ms.

SUMMARY

DNA microsatellite test of Thoroughbred (n=58) and Trotter horses (n=52) was carried out to prove the applicability of this method in the parentage control. The evaluation was made by means of ABI 310 automatic genetic analyser.

Among the 12 microsatellites tested the frequence of VHL20, HMS7, ASB2, HTG10 and HMS3 showed strong ($P<0,001$), while microsatellites AHT4 and HTG7 exhibited somewhat less pronounced ($P<0,01$) significant differences in gene frequence between Thoroughbred and Trotter horses. Comparising the two breeds, however, no significant differences were detected in the frequency of microsatellites HTG4, AHT5, HTG6 and HMS2.

In most cases the distribution of alleles in microsatellites also showed significant differences ($P<0,001$; $P<0,01$; $P<0,05$) between the two breeds tested.

The results prove that by DNA microsatellites can be used for the comparison of herds and for the estimation of genetic distance or similarity.

ÖSSZEFOGALALÁS

Józsa Cs. – Husvéth F. – Bán B. – Takács E.: A TELIVÉR ÉS AZ ÜGETŐ LÓFAJTÁK DNS-MIKROSZATELLIT VIZSGÁLATA MAGYARORSZÁGON

A vizsgálatok során két lófajta az angol telivér és az amerikai ügető tenyészállatainak (n=110) DNS-mikroszatellit vizsgálatát végezték el a szerzők, származásellenőrzés céljából. 12 mikroszatellitet használtak fel az elemzés során a következőkben felsoroltak szerint: VHL20, HTG4, AHT4, HMS7, HTG6, AHT5, HMS6, HMS3, ASB2, HTG10, HTG7, HMS2. Az értékelést ABI 310 automata genetikai analizátor segítségével végezték el.

A kapott eredmények alapján a két fajta között egyes mikroszatellitek esetében jelentős (ASB2, HMS7, HMS3), más mikroszatellitek esetében (AHT5, HTG4, HTG6) viszont nem mutatkozott különbség a két fajta között. A legnagyobb eltérések a VHL20, a HMS7, a HMS6, az ASB2, a HTG10, a HMS3, az AHT4 és a HTG7 mikroszatellitekben (($P<0,001$; $P<0,01$) voltak. A mikroszatellitek alléljainak százalékos megoszlását figyelve, egyes allélok kiugróan magas gyakorisággal fordultak elő.

Eredményeik igazolják, hogy a fajtákon belül végbemenő genetikai valtozások, a különböző rokoni kapcsolatok, a beltenyésztettség is figyelemmel kísérhetőek.

INTRODUCTION

The DNA molecule, as carrier of hereditary traits, is the nucleus of microsatellite tests. From the aspect of our study, out of the polymorphisms the so called variable number of tandem repeats (VNTR) is remarkable. The rather strong polymorphism of VNTRs is due to the different number of tandem repeating DNA sequences that can be found in the eucaryota genome. Depending on the length of repetitive stages, satellites, minisatellites and microsatellites are distinguished (*Bowling,* 1996).

Microsatellites (STR: Short Tandem Repeat) are efficient tools for genome mapping. By their application, animals can be identified easily and accurately (*Bowling,* 1996). They belong to the repetitive sequence family in which very simple 1–4 basis and sequence repeat themselves in one stage, however, at the same time the duplicate number shows a significant variability.

The relatively equal distribution of microsatellites in the genome makes the above mentioned gene mapping possible, while the considerable marker activity could be effectively utilized both in the individual breed identification and the genetic comparisons (*Takahashi et al.,* 1998; *Domján et al.,* 1998; *Zhou and Lamont,* 1999; *Romanov and Weigend,* 2001). Their typifying is based on the numerical variability of the repetition of some base pairs, that can be found between two sequence sections typical of only a given genome section (*Weber and May,* 1989).

Microsatellites proved to be the most up-to-date and usable markers for controlling the parentage of horses (*Bowling,* 1996).

On the basis of the examination of genetic markers by microsatellite investigation, numerous calculations can be performed. In addition to the establishment of their frequency, additional information may be obtained on the breed and for evaluation carried out within the herd (e.g. heterozygosity, inbreeding, presence of within-microsatellite alleles). Consequently, microsatellites can be used for the comparison of herds and for the estimation of genetic distance or similarity (*Nei,* 1972, *Domján et al.,* 1998).

MATERIALS AND METHODS

Sample collection and preparation

Blood samples from each of two Hungarian herds of Thoroughbred and Trotter horses (two-two populations) were used for the test. The principle of randomisation was used to select the horses. Tubes containing EDTA were used to take blood samples from the jugular vein for the DNA microsatellite determination. Blood samples were stored at –20 °C until the analyses.

Laboratory analysis

The collected samples were subjected to PCR and microsatellite analysis. The examinations were carried out in the DNA department of the Immune-Genetic Laboratory of the National Institute for Agricultural Quality Control.

Twelve microsatellites, recommended for the parentage control of horses by the International Society for Animal Genetics (ISAG), were determined (*Table 1*).

Table 1.

List of the 12 horse DNA microsatellites examined

Locus(1)	Primary sequence (5'→ 3')(2)	Allele length (bp)(3)	Reference(4)
VHL20	(FAM)-CAAGTCCTCTTACTTGAAGACTAG AACTCAGGGAGAATCTTCCTCAG	86–105	*vanHaeringen et al.* (1994)
HTG4	(FAM)-CTATCTCAGTCTTGATTGCAGGAC CTCCCTCCCTCCCTCTGTTCTC	120–140	*Ellegren et al.* (1992)
AHT4	(FAM)-AACCGCCTGAGCAAGGAAGT GCTCCCAGAGAGTTTACCCT	146–170	*Binns et al.* (1995)
HMS7	(FAM)-CAGGAAACTCATGTTGATACCATC TGTTGTTGAAACATACCTTGACTGT	170–188	*Guerin et al.* (1994)
AHT5	(JOE)-ACGGACACATCCCTGCCTGC GCAGGCTAAGGGGGCTCAGC	129–149	*Binns et al.* (1995)
HMS6	(JOE)-GAAGCTGCCAGTATTCAACCATTG CTCCATCTTGTGAAGTGTAACTCA	157–171	*Guérin et al.* (1994)
HTG6	(JOE)-CCTGCTTGGAGGCTGTGATAAGAT GTTCACTGAATGTCAAATTCTGCT	80–107	*Ellegren et al.* (1992)
ASB2	(JOE)-CCACTAAGTGTCGTTTCAGAAGG CACAACTGAGTTCTCTGATAGG	240–270	*Breen et al.* (1997)
HTG10	(TAM)-CAATTCCCGCCCCACCCCCGGCA TTTTTATTCTGATCTGTCACATTT	92–112	*Marklund et al.* (1994)
HTG7	(TAM)-CCTGAAGCAGAACATCCCTCCTTG ATAAAGTGTCTGGGCAGAGCTGCT	118–130	*Marklund et al.* (1994)
HMS3	(TAM)-CCAACTCTTTGTCACATAACAAGA CCATCCTCACTTTTTCACTTTGTT	149–172	*Guérin et al.* (1994)
HMS2	(TAM)-CTTGCAGTCGAATGTGTATTAAAT ACGGTGGCAACTGCCAAGGAAG	218–238	*Guérin et al.* (1994)

1. táblázat: A lovak vizsgált 12 DNS mikroszatellitje
mikroszatellit(1), elsődleges szekvencia(2), allél hosszúság(3), hivatkozás(4)

Genomic DNA was isolated from lymphocytes by the method described by *Marklund et al.* (1994). 40 µl from blood samples thawed at room temperature was poured into Eppendorf tubes, 600 µl T10E1 cleansing linquid was added and the tubes were shaken on a laboratory equipment. Thereafter the samples were ultracentrifuged (13,000 rpm, 2 min, at 4 °C), the supernatant was decanted and 600 µl wash liquid was pipetted to the precipitate. This was followed by vortexed for 10–30 sec to suspend the precipitate. The above two steps were repeated twice and then the content of tubes was ultracentrifuged (13,000 rpm, 2 min). The supernatant was decanted and the tubes were laid over dry paper cloth to have drops of wash liquid strained off before starting the lysis. One hundred µl lysis solution (Proteinaze K solution + lysis buffer) was added to the dried precipitate, then the samples were vortexed for 30 sec. Subsequently the samples were put in water-bath of 56 °C for 45 minutes, then into a water-bath of 94 °C for additional 10 minutes. After having been cooled to room temperature the samples were ultra-centrifuged (13,500 rpm, 1 sec).

After isolation of the DNA, the product was amplified (*Panaccio et al.*, 1993). To carry out polymerase chain reaction (PCR), the kit of Applied Biosystems StockMarks® developed for horses was used.

The total volume of PCR reaction compounds used in the analyses was 15 µl by sample. Of this volume, 1 µl was a purified genomic DNA and 14 µl included a solution prepared from StockMarks PCR buffer, dNTP Mix, primary mix, AmpliTaq Gold® polymerase (Applied Biosystems, USA) and distilled water.

Reproduction of the DNA sequences being present in the samples was performed by means of GeneAmp PCR System 9700 (Applied Biosystems, USA) apparatus.

The PCR thermocycler program was as follows: 1 cycle at 95 °C for 10 minutes, then 30 cycles at 95 °C for 30 sec, at 60 °C for 30 sec and at 72 °C for 1 minute and 1 cycle at 72 °C for 60 minutes (*Bozzini et al.*, 1997).

Subsequently, 11.5 µl DI formamide and 0.5 µl GS500 Rox standard (Applied Biosystems, USA, 2001) were added to the PCR product. DNA denaturation was carried out on a covered tray at 95 °C for 3 minutes, then the samples were analysed with the ABI PRISM™ 310 Genetic Analyzer. Genotyper Software (Applied Biosystems, USA, 2001) was used for the evaluation of analyses.

Statistical analysis

Statistical testing of the degree of differences and similarities was performed by homogeneity examination. χ^2-tests were used for the case number tables (*Prichner,* 1983; *Andersson,* 1985). A test was performed for some alleles / factors, as well as for the distributions of differences between lines. The χ^2-tests indicate the level of significance of the genetic "distance" of breeds. Moreover, the degree of similarity was expressed in an empirical, plainer percentage index number as the complementary of the Cramer-index (CI) formed from the χ^2 value.

RESULTS

Out of the factors of microsatellite VHL20 the alleles I, L, M and N were present in Thoroughbred while alleles I, L, M, N, P and R in Trotter horses. Alleles L and M showed the most significant difference between the two breeds. Many times the allele frequency values of VHL20-L in Trotter horses exceeded those present in Thoroughbreds (*Table 2*). For allele VHL20-M an inverse pattern was found in allele frequency, as the values found in Thoroughbred exceeded many times the values measured in Trotter horses. Some alleles found in Trotter were not present in Thoroughbred. These were the alleles VHL20-P and VHL20-R. The greatest similarity between the breeds was found in the frequency of allele VHL20-N. A significant difference was found between the breeds in the allele of microsatellite VHL20 ($P<0.001$; *Table 3*).

However, no significant difference was found in microsatellites HTG4, HTG6, AHT5 and HMS2 ($P>0.05$). Nevertheless, among the alleles of the four microsatellites mentioned above there were several significant differences ($P<0.001$) between Thoroughbred and Trotter horses.

Table 2.

DNA microsatellite allele frequency values in Thoroughbred and Trotter horses

L	B	Microsatellites (allele frequency values)(1)											
		VHL20	HTG4	AHT4	HMS7	AHT5	HMS6	HTG6	ASB2	HTG10	HTG7	HMS3	HMS2
A	T												
	S												
B	T								0.1316				
	S												
C	T								0.0526				
	S												
D	T												
	S												
E	T												
	S												
F	T												
	S												
G	T							0.4342					
	S							0.5					
H	T			0.171									0.0395
	S			0.12									0.05
I	T	0.2763		0.0131						0.4079		0.5263	
	S	0,14		0.06				0.01		0.70		0.14	
J	T			0.2895	0.2237	0.2368		0.3816	0.0264				0.0658
	S			0.21	0.06	0.15		0.47	0.16				0.05
K	T		0.4474	0.079		0.25	0.1184		0.3553	0.1579	0.1053		0.2368
	S		0.42		0.05	0.31	0.18		0.08		0.07		0.1
L	T	0.0658	0.2368		0.1974		0.1054			0.1053			0.5263
	S	0.33	0.22		0.51		0.09			0.12			0.59
M	T	0.2632	0.2368		0.1447	0.1842	0.3026	0.0526	0.0921	0.2368		0.0921	0.1316
	S	0.06	0.17			0.26	0.27		0 16	0.07	0.08		0.07
N	T	0.3947	0.0264		0.1184	0.2632	0.2368		0.0789		0.4079	0.0526	
	S	0.36	0.14	0.01	0.38	0.28		0.01	0.09		0.18	0.22	
O	T			0.4474	0.3158	0.0658		0.1053	0.0526	0.0526	0.4868	0.1842	
	S		0.05	0.46			0.25	0.01	0.36	0.05	0.67		0.06
P	T		0.0526				0.2368					0.1448	
	S	0.01		0 14			0.21			0.02		0.48	0.06
Q	T								0.1184				
	S								0.04			0.06	
R	T							0.0263	0.0921	0.0131			
	S	0.1							0.11	0.04		0.1	0.02
S	T									0.0264			
	S												
T	T												
	S												
U	T												
	S												

L: locus(2), B: breed(3), T: Thoroughbred(4), S: Trotter(5)

2. táblázat: DNS mikroszatellit allélfrekvencia értékek telivér és ügető fajtákban

Vizsgált mikroszatellitek (allélfrekvencia értékek)(1), L: allélok(2), B: fajták(3), T: angol telivér(4), S: amerikai ügető(5)

Table 3.

Statistical comparison of breeds (n=110)

L	Microsatellites (χ^2 value per allele)(1)											
	VHL20	HTG4	AHT4	HMS7	AHT5	HMS6	HTG6	ASB2	HTG10	HTG7	HMS3	HMS2
A												
B								13.9 ***				
C								5.4 *				
D												
E												
F												
G							0.8 NS					
H			0.9 NS									0.1 NS
I	5.04 *		2.5 NS				0.8 NS		15.1 ***		30.3 ***	
J			1.5 NS	10.2 **	2.1 NS		1.4 NS	8.4 **				0.2 NS
K		0.1 NS	8.2 **	3.9 *	0.8 NS	1.3 NS		20.5 ***	16.9 ***	0.7 NS		6.04 *
L	17.8 ***	0.07 NS		18 ***		0.1 NS			0.09 NS			0.7 NS
M	14.2 ***	1.2 NS		15.4 ***	1.4 NS	0.2 NS	5.4 *	1.8 NS	9.9 **	6.4 *	9.6 **	1.9 NS
N	0.2 NS	6.8 **	0.8 NS	15.1 ***	0.06 NS	26.4 ***	0.8 NS	0.07 NS		11.2 ***	9.6 **	
O		3.9 *	0.03 NS	36.6 ***	6.8 **	22.2 ***	8.1 **	23.2 ***	0.006 NS	5.9 *	20.01 ***	4.7 *
P	0.8 NS	5.4 *	11.6 ***			0.2 NS			1.5 NS		21.8 ***	4.7 *
Q								3.9 *			4.7 *	
R	8.1 **						2.7 NS	0.2 NS	1.1 NS		8.1 **	1.5 NS
S									2.7 NS			
T												
U												
Stat.	38.6*** SI: 28%	16.3ns SI: 49%	23.6** SI: 36%	81*** SI: 10%	9.9ns SI: 58%	44.2*** SI: 25%	18.3ns SI: 46%	65.7*** SI: 15%	36*** SI: 30%	17.2** SI: 47%	83.5*** SI: 10%	18ns SI: 46%

*P<0.05, **P<0.01, ***P<0.001, L: locus, Stat.: Comparison of breeds on the basis of χ^2 value between distributions, SI: similarity index(2)

3. táblázat: A fajták statisztikai összehasonlítása (n=110)
vizsgált mikroszatellitek (allélenkénti χ^2-érték alapján)(1), *P<0,05, **P<0,01, ***P<0,001, L: allélok, Stat.: a fajták összehasonlítása megoszlások közötti χ^2-érték alapján, S: a hasonlósági index(2)

Five alleles (K, L, M, N, P) of microsatellite HTG4 were present in Thoroughbred and similarly five (K, L, M, N, O) in Trotter. The frequency value of allele HTG4-P was 0.0526 in Thoroughbred and that of HTG4-O was 0.05 in Trotter. Allele HTG4 had the highest frequency value in both breeds. No significant difference was found between the two breeds in the alleles of microsatellite HTG4-K (P>0.05).

Alleles G, J, M, O and R of microsatellite HTG6 were present in Thoroughbred and G, I, J, N and O in Trotter. Representing a value above 0.45 frequency, alleles HTG6-G and HTG6-J dominated in Trotter. Allele HTG6-G showed the highest frequency value also in the Thoroughbred (0.45). The greatest difference between the two breeds in allele frequency was found in allele HTG6-O, which had a frequency of 0.1053 in Thoroughbred and 0.01 in Trotter.

Five alleles (J, K, M, N, O) of microsatellite AHT5 were present in Thoroughbred and four (J, K, M, N) in Trotter. In the case of allele AHT5-O, a significant difference was found between the two breeds studied. That allele had a frequency of 0.0658 in Thoroughbred while it was not present in Trotter. No significant difference was found in the distribution of other alleles (P>0.05).

In microsatellite HMS2 alleles H, J, K, L, and M were present in Thoroughbred and H, J, K, L, M, R, O and P in Trotter. The allele frequency of HMS2-O, HMS2-P and HMS2-R proved to be 0.06, 0.06 and 0.02, respectively, in the Trotter. However, these alleles could not be detected in the Thoroughbred. Allele HMS2-L showed dominance in both breeds.

The allele of microsatellite AHT4 appeared in only one of the breeds tested. While AHT4-K could be detected only in the Thoroughbred, AHT4-N and AHT4-P were found only in Trotters. The greatest difference was shown in allele P, where the allele frequency value of Thoroughbred was 0.00 while that of the Trotter was 0.14. Allele AHT4-O dominated in both breeds.

In microsatellite HMS7 five alleles (J, L, M, N, O) were present in Thoroughbred and four (J, K, L, N) in Trotter. The allele frequency value of HMS7-M and HMS7-O was 0.1447 and 0.3158, respectively, in the Thoroughbred, while these alleles could not be detected in the other breed tested. In the latter allele a remarkable significant difference (P<0.001) was found between the two breeds.

In microsatellite HMS6, allele HMS6-L showed the most considerable similarity.

The frequency values of all alleles in microsatellite HMS3 differed significantly (P<0.05, 0.01, 0.001) between the two breeds. Alleles I, M, N, O and P of microsatellite HMS3 were present in Trotter and I, N, P Q and R in Thoroughbred horses. In Thoroughbred horses, the allele frequency values of HMS3-M and HMS3-O were 0.0921 and 0.1842, respectively. Of all the alleles present in Thoroughbreds, allele HMS3-I had the highest frequency value. However, in Trotters P was the dominant allele.

In microsatellite ASB2 the alleles B and C were detected only in the Thoroughbred and were not present in Trotter horses. The greatest breed differences (P<0.001) were found in alleles ASB2-K and ASB2-O. Allele K was dominant in Thoroughbred (0.3553) while O in Trotter horses (0.36).

In microsatellite HTG10 seven alleles (I, K, L, M, O, R, S) were present in Thoroughbred and six (I, L, M, O, P, R) in Trotter. Allele HTG10-I was found to be dominant both in Thoroughbred (0.4079) and Trotter (0.7). In microsatellite HTG10 some alleles, present only in one of the breeds tested, could also be detected. Alleles that were present only in one breed could also be found in this microsatellite. Alleles K and S were found in the Thoroughbred while allele P in Trotters.

Significant differences ($P<0.01$) were shown in the distribution of alleles of microsatellite HTG7. Allele HTG7-O was dominant in both breeds. Allele HTG7-M could be found only in Trotters.

DISCUSSION

Compared to blood typing and analysis of biochemical polymorphisms used in parentage control up to the present, DNA markers, due to their extremely large number and the more definite polymorphism of some loci, can be used on a higher level and more efficiently in numerous fields of animal breeding, such as analysis of the genetic structure of populations, test of homozygosity, estimation of the degree of inbreeding within populations, maintenance of autochthonous populations (gene reserve), parentage control, estimation of genetic distance between populations and breeds, planning of crossing programs (heterosis breeding), and clarification of litigious matters regarding the unlawful appropriation of animals (*Bowling et al.*, 1997; *Luikart et al.*, 1999). It is not negligible that the object of analysis (DNA) can be obtained from several tissues such as blood, hair bulbs and semen (*Ellegren et al.*, 1992; *Bowling*, 1996).

Based on the results of Trotter and Thoroughbred horses (two-two populations) tested in our trials, the genetic differences or similarities of breeds can be clearly determined. In the case of breeds and populations in the evolution of which other breeds were continuously and significantly involved, the gene flow can be followed by DNA tests. Of the two breeds studied in this work, in the Trotter this could be followed to the end, as the Thoroughbred, similarly tested, played a significant role in its evolution. As a consequence, the observed allele number was higher in the former breed.

In the case of microsatellite VHL20 the alleles I, L, M and N were present in Thoroughbred and J, L, M, N, P and R in Trotter. Van *Haeringen et al.* (1994) obtained the same result for the aforementioned two breeds. *Ellegren et al.* (1992) detected allele G from microsatellite HTG6 in both breeds. The same results were obtained in this research. The greatest differences were found in microsatellites VHL20, HMS7, ASB2, HTG10, HMS3, AHT4 and HTG7. Observing the percentage distribution of the alleles of microsatellites, the incidence of some alleles was remarkably high. Several possibilities may be responsible for producing these cases. The cause of this phenomenon may be associated with the use of stallions.

It is characteristic of Hungarian horse breeding that only few stallions are used for covering as compared to the number of horses in the given stock. However, this technology is not adequate, because in this way the frequency of alleles that can be found in the stallion himself will remarkably increase. As a consequence, this involves the danger of inbreeding in the given stock. There is the possibility to use a higher number of stallions; however, they have similar or identical alleles. In this case the same problem arises as outlined above.

Of the microsatellite alleles of the Trotter and Thoroughbred stallions tested, the approximately 70% allele frequency of HTG10-I in the Trotter stock was indicative of the problems raised above. After having carefully examined the covering book of the stallion, it became evident that the aforementioned remarkable allele frequency was due to the small number of stallions used for covering in the stock.

A remarkably high allele frequency value, such as the 35.53% frequency of allele K of microsatellite ASB2, was also present in the Thoroughbred stock. In this case the number of service stallions compared to the size of the stock was more favourable, but the identical alleles of stallions could lead to the high allele frequency values.

Compared to the total allele number of the microsatellites tested, a low number of alleles can be observed both in Thoroughbred and Trotter in Hungary. Microsatellite ASB2 proved to be an exception to this, presenting 9 alleles in Thoroughbred and 7 alleles in Trotter out of the total of 12. Norwegian and Korean research workers obtained similar results. In the case of Thoroughbred, *Bjornstad et al.* (2000) also found 9 alleles, whereas *Cho* (2002) detected 10 alleles in the aforementioned microsatellite.

Considering the allele frequency values, there is a high similarity between our results and those of the Korean research team, despite of the significant differences. The greatest similarities between the results of the two research groups were found for the following alleles: VHL20-I, AHT4-J, HTG4-N, HMS3-I, HMS3-P. However, in the case of alleles HTG4-L, AHT5-N, HMS3-P and ASB2-R significant differences were observed. With the exception of microsatellite AHT4, the number of alleles observed in the Korean Thoroughbred stock was higher than the number found in the Hungarian stock (*Cho*, 2002).

REFERENCES

Andersson, L.(1985): The estimation of blood group gene frequencies· a note on the allocation methods. Anim. Blood Groups Biochem. Genet., 16. 1–7.

Applied Biosystems(2001): StockMarks®, Horse and Cattle Paternity PCR Typing Kits (Protocol)

Binns, M.M. – Holmes, N.G. – Holliman, A. – Scott, A.M.(1995): The identification of polymorphic microsatellite loci in the horse and their use in Thoroughbred parentage testing. Br. Vet. J., 151. 9–15.

Bjornstad, G. – Gunby, E. – Roed, K.H.(2000): Genetic structure of Norwegian horse breeds. J. Anim. Breed. Genet., 117. 307–317.

Bowling, A.T.(1996): Horse Genetics. CAB International, Wallingford

Bowling, A.T. – Eggleston-Scott, M.L. – Byrns, G – Clark, R.S. – Dileanis, S. – Wictum, E.(1997): Validation of microsatellite markers for routine horse parentage testing. Anim. Genet., 28. 247–252.

Bozzini, M. – Fantin, D. – Ziegle, J.S. – Van Haeringen, H. – Jacobs, W. – Ketcham, M. – Spencer, M. – Bates, S.(1997): Automated Equine Paternity Testing. PE Appl. Biosys., 1.

Breen, M. – Lindgren, G. – Binns, M.M. – Norman, J. – Irvin, Z. – Bell, K. – Sandberg, K. – Ellegren, H.(1997): Genetical and physical assignment of equine microsatellites – first integration of anchored markers in horse genome mapping. Mammal. Genome, 8. 267–273.

Cho, G.J.(2002): Microsatellite DNA polymorphism of Thoroughbred horses in Korea. Korean J. Genet., 24 2. 177–182.

Domján, E. – Zsolnai, A. – Fésüs, L.(1998a): A DNS vizsgálatok jelentősége a lovak származásellenőrzésében. XIV. Állat-biotechnológiai Kerekasztal Konferencia, Hódmezővásárhely

Domján, E. – Zsolnai, A. – Fésüs, L.(1998b): Automatizált származásellenőrzési teszt lovaknál, mikroszatellit markerek segítségével. XXVII. Óvári Tudományos Napok, Mosonmagyaróvár

Ellegren, H. – Johasson, M. – Sandberg, K. – Andersson, L.(1992): Cloning of highly polymorphic microsatellites in the horse. Anim. Genet., 23. 133–142.

Guérin, G. – Bertaud, M. – Amigues, Y.(1994): Characterization of seven new horse microsatellites: HMS1, HMS2, HMS3, HMS5, HMS6, HMS7 and HMS8. Anim. Genet., 25. 62.

Luikart, G. – Biju-Duval, M.P. – Ertugrul, O. – Zagdsuren, Y. – Maudet, C. – Taberlet, P.(1999): Power of 22 microsatellite markers in fluorescent multiplexes for parentage testing in goats (*Capra hircus*). Anim. Genet., 30. 431–438.

Marklund, S. – Ellegren, H. – Eriksson, S. – Sandberg, K. – Andersson, L.(1994): Parentage testing and linkage analysis in the horse using a set of highly polymorphic microsatellites Anim Genet 25. 19–23.

Nei, M.(1972): Genetic distance between populations. Am. Nat., 106 283–292

Panaccio, M. – Georgesz, M. – Lew, A.M.(1993): FoLT PCR: a simple PCR protocol for amplifying DNA from whole blood. Biotechniques, 14. 238–243.

Prichner, F.(1983): Population Genetics in Animal Breeding. Freeman Company. San Francisco

Romanov, M.N. – Weigend, S.(2001): Analysis of genetic relationships between various populations of domestic and jungle fowl using microsatellite markers. Poult. Sci., 80. 1057–1063

Takahashi, H.K. – Nirasawa, K. – Nagamine, Y. – Tsudzuki, M. – Yamamoto, Y.(1998): Genetic relationships among Japanese native breeds of chicken based on microsatellite DNA polymorphism. J. Hered., 89. 543–546.

Van Haeringen, H. – Bowling, A T. – Stott, M.L. – Lenstra, J.A. – Zwaagstra, K.A.(1994) A highly polymorphic horse microsatellite locus: VHL20. Anim. Genet., 25. 207.

Weber, J.L. – May, P.E.(1989): Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am. J. Human Genet., 44. 388–396.

Zhou, H.J. – Lamont, S.J.(1999). Genetic characterization of biodiversity in highly inbred chicken lines by microsatellite markers. Anim. Genet., 30. 256–264.

Érkezett: 2005.

Szerzők címe: *Józsa, Cs. – Húsvét, F.*: VE, Georgikon Mezőgazdaságtudományi Kar
Authors' address: University of Veszprém, Georgikon Faculty of Agriculture
H-8360 Keszthely, Deák F. u. 16.
Bán, B. – Takács, E.: Országos Mezőgazdasági Minősítő Intézet
National Institute for Agricultural Quality Control
H-1021 Budapest, Keleti K. u. 24.