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Preliminary Results Regarding the Genetic History of a Native Horse Breed- Genetic Relationship between Sire Stallions and Broodmares

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ABSTRACT

Background: This study is a part of an ample research concerning the genetic history of Hucul horse breed. The genetic analysis studies are part of animal genetic resources Management because just start of them we elaborate the strategies for inbreeding management. The itself genetic relationship of a population is the average genetic relationship of all individuals between them, existing at a time. Therefore, this parameter can be described three components: the genetic relationship between stallions, between mother mares themselves and the genetic relationship between stallions and mares. The last component is most important because of the average population inbreeding increases in successive generations.

Methods: The research was carried out during 2012-2020. The material are represented by genealogical information from reproductive nucleus of Hucul breed from Lucina studfarm. The sample extracted from population was represented by seven sire stallions and seventy-three broodmares. The method are based on A matrix coefficients. All analysis were performed in the profile laboratories of Animal Sciences Faculties from Bucharest and Timisoara.

Result: Between individual average genetic relationship values of stallions with mares, is found significant differences, highlighted by the global test of homogeneity test (F = 4.891). These differences allow us to assert that, through stallions selection, is made a reproductive discrimination much more intense than in mares case. The average genetic relationship of the population was calculated at 0.1941±0.0053.

Key words: Genetics, Genotype, Horse, Inbreeding, Phenotype, Relationship.

INTRODUCTION

The genetic analysis studies are part of Animal Genetic Resources Management because just start of them we elaborate the strategies for inbreeding management (Maftei *et. al.,* 2011, 2022).

The itself genetic relationship of a population is the average genetic relationship of all individuals between them, existing at a time. Therefore, this parameter can be described three components: the genetic relationship between stallions, between mother mares themselves and the genetic relationship between stallions and mares (Falconer and Mackay, 1997). The last component is most important because increase the average population inbreeding at successive generations. The phenomenon of the existence, in reproductive nucleus, of several overlapping generations has a double importance for the controlled evolution of domestic animals populations: increasing of genetic variability, with direct effect on the adaptability of the population and increase the generations interval (Metzger, *et al.* 2015). The two consequences affecting the work of breeding (selection effect) in contrary directions: on one hand playing field is created for artificial selection and the selection differential increases, but at the same time, annual response to selection decreases over time because for the long time needed to change a generation to another (Popa *et al.*, 2004).

The average value of genetic relationship between males and females from reproductive nucleus, is a very important indicator in management of genetic resources, because it influences directly, the average increase of homozygot, despite consequences (Marginean *et al.*, 2005). The value of this parameter is required to be determined at any time of population evolution, since it is an indicator of the principles to be observed in small populations, under continuous threat of inbreeding (Marginean *et al.*, 2012).

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MATERIALS AND METHODS

In order to achieve the proposed objectives, the biological material used in this experiment is represented by a sample of 80 individuals (7 sire stallions and 73 broodmares), from 5 bloodlines. The research and analysis were performed in the laboratories of the University of Agronomic Sciences and Veterinary Medicine, Animal Sciences Faculties from Bucharest and Timisoara. The method that was used is the *numerator relationship matrix* elaborated by Henderson and Cunningham (Grosu 1997, Popa 2009). To highlight the existence of differences we applied a Fisher test.The research was carried out during 2012 - 2020. The research and analysis were performed in the profile laboratories of Animal Sciences Faculties from Bucharest and Timisoara, Romania.

RESULTS AND DISCUSSION

The genetic diversity can be highlighted by the determination of kinship stallions-mares from the years of birth by age structure of groups born in different years (Maftei, 2011), the overall heterogeneity testing using Fisher's exact test (F = 1.538), which revealed the existence of significant differences between the values of relationship coefficients (Table 1, Fig 1).

Analyzing the graph it is obvious that there are some moments, in the evolution of population, in which genetic structure it was disturbed (marked on the chart with the brace). The red horizontal line marks the average relatedness in the population, establishing the line between the fields of action of two breeding factors: crossbreeding and inbreeding.

Table 1: Genetic relationship between stallions and mares on birth years from age structure and on groups born in different years.

Stallion / Mares	1987 (Ouşor IX)	1993 (Goral XXI)	1995 (Ouşor X, Pietrosu XI)	1997 (Goral XX)	2000 (Hroby XXIV, Prislop XI)
1990	0.1365	0.1195	0.1636	0.1376	0.1378
1991	0.1110	0.2584	0.1244	0.1668	0.2503
1992	0.1337	0.1890	0.1380	0.1521	0.1726
1993	0.1310	0.1472	0.1658	0.1236	0.1350
1994	0.3393	0.1457	0.2600	0.1471	0.1313
1995	0.1181	0.1339	0.1822	0.1337	0.1510
1996	0.3227	0.1132	0.2386	0.1229	0.1344
1998	0.1235	0.2380	0.1599	0.1721	0.1848
1999	0.2604	0.1600	0.2098	0.1402	0.1831
2000	0.1621	0.1341	0.2220	0.1738	0.1710
2001	0.3563	0.1353	0.2602	0.1271	0.1309
2002	0.4197	0.1678	0.3142	0.1344	0.1472
2003	0.3659	0.1478	0.2116	0.1278	0.1557
2004	0.5698	0.1991	0.2820	0.1467	0.1432
2005	0.3186	0.1870	0.1886	0.1600	0.1976
2006	0.2950	0.1716	0.2500	0.1342	0.1849
2007	0.1897	0.1532	0.2350	0.2392	0.2051

F = 1.538***

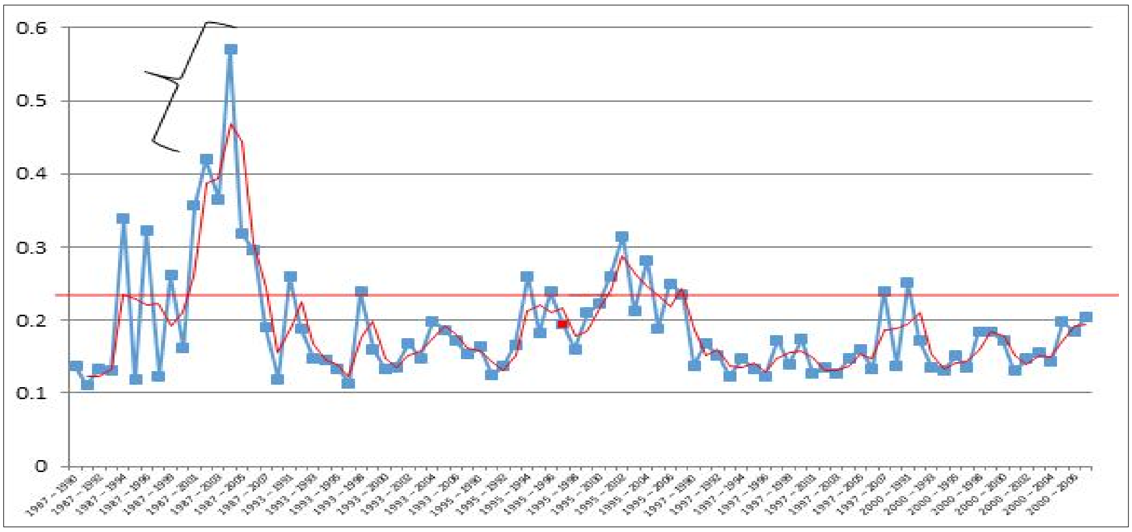

Fig 1: Evolution of genetic relationship between stallions and mares on birth years.

Table 2: The difference between genetic relationship stallions-mares of different years of age structure and average genetic relationship of the total population.

Average genetic relationship stallions-mares by years of birth			Observed differences: r_{A-1}/years-$r_{pop.}$		Difference limit	Difference limit	Difference limit
Birth year ♂ ♀	No of combination	r_{A-1}/years	$r_{pop.}$ = 0.1941 525 combinations	Observations	$DL=t_{0.05}.s_d$	$DL=t_{0.01}.s_d$	$DL=t_{0.01}.s_d$
1987-1990	1	0.1365	-0.0576***	Crossbreeding	0	0	0
1987-1991	4	0.1110	-0.0831***		0.0488	0.0641	0.0819
1987-1992	3	0.1337	-0.0604^{NS}	Reproduction in endogamy	0.093	0.1223	0.1562
1987-1993	5	0.1310	-0.0631^{NS}	without disturbing genetic	0.0639	0.084	0.1073
1987-1994	2	0.3393	0.1452^{NS}	structure	0.4703	0.618	0.7895
1987-1995	3	0.1181	-0.076*	Crossbreeding	0.0656	0.0862	0.1101
1987-1996	2	0.3227	0.1286^{NS}	Reproduction in endogamy	0.4507	0.5923	0.7566
				without disturbing genetic structure			
1987-1998	3	0.1235	-0.0706*	Crossbreeding	0.067	0.0881	0.1126
1987-1999	3	0.2604	0.0663^{NS}	Reproduction in endogamy	0.3212	0.4221	0.5392
1987-2000	4	0.1621	-0.032^{NS}	without disturbing genetic	0.1288	0.1693	0.2163
1987-2001	2	0.3563	0.1622^{NS}	structure	0.4126	0.5423	0.6927
1987-2002	3	0.4197	0.2256**	Inbreeding	0.1688	0.2219	0.2834
1987-2003	4	0.3659	0.1718^{NS}	Reproduction in endogamy	0.2327	0.3058	0.3907
				without disturbing genetic structure			
1987-2004	1	0.5698	0.3757***	Inbreeding	0.0488	0.0641	0.0819
1987-2005	7	0.3186	0.1245^{NS}	Reproduction in endogamy	0.2037	0.2677	0.342
1987-2006	14	0.2950	0.1009^{NS}	without disturbing genetic	0.124	0.1629	0.2081
1987-2007	14	0.1897	-0.0044^{NS}	structure	0.0808	0.1062	0.1357
1993-1990	1	0.1195	-0.0746***	Crossbreeding	0	0	0
1993-1991	4	0.2584	0.0643^{NS}	Reproduction in endogamy	0.1121	0.1473	0.1882
1993-1992	3	0.1890	-0.0051^{NS}	without disturbing	0.1469	0.1931	0.2467
1993-1993	5	0.1472	-0.0469^{NS}	genetic structure	0.0626	0.0823	0.1051
1993-1994	2	0.1457	-0.0484^{NS}		0.0578	0.0760	0.0971
1993-1995	3	0.1339	-0.0602*	Crossbreeding	0.0488	0.0641	0.0819
1993-1996	2	0.1132	-0.0809*		0.0632	0.0831	0.1061
1993-1998	3	0.2380	0.0439^{NS}	Reproduction in endogamy	0.1521	0.1999	0.2553
1993-1999	3	0.1600	-0.0341^{NS}	without disturbing	0.0594	0.0781	0.0998
1993-2000	4	0.1341	-0.0600^{NS}	genetic structure	0.0777	0.1021	0.1304
1993-2001	2	0.1353	-0.0588^{NS}		0.0670	0.0881	0.1126
1993-2002	3	0.1678	-0.0263^{NS}		0.0614	0.0806	0.1030
1993-2003	4	0.1478	-0.0463^{NS}		0.0610	0.0802	0.1025
199 -2004	1	0.1991	0.0050NS		0.0488	0.0641	0.0819
1993 -2005	7	0.1870	-0.0071NS		0.0656	0.0862	0.1101

Table 2: Continue...

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1993-2006	14	0.1716	-0.0225^{NS}		0.0542	0.0712	0.0910
1993-2007	14	0.1532	-0.0409^{NS}		0.0537	0.0705	0.0901
1995-1990	2	0.1636	-0.0305^{NS}		0.0488	0.0641	0.0819
1995-1991	8	0.1244	-0.0697**	Crossbreeding	0.0518	0.0680	0.0869
1995-1992	6	0.1380	-0.0561*	Reproduction in endogamy	0.0504	0.0663	0.0847
1995-1993	10	0.1658	-0.0283^{NS}	without disturbing genetic structure	0.0598	0.0786	0.1003
1995-1994	4	0.2600	0.0659^{NS}		0.1262	0.1658	0.2118
1995-1995	6	0.1822	-0.0119^{NS}		0.0801	0.1053	0.1345
1995-1996	6	0.2386	0.0445^{NS}		0.1247	0.1638	0.2093
1995-1998	6	0.1599	-0.0342^{NS}		0.0813	0.1068	0.1365
1995-1999	6	0.2098	0.0157^{NS}		0.0923	0.1214	0.1550
1995-2000	8	0.2220	0.0279^{NS}		0.1187	0.1560	0.1993
1995-2001	4	0.2602	0.0661^{NS}		0.1082	0.1421	0.1816
1995-2002	6	0.3142	0.1201^{NS}		0.1777	0.2335	0.2983
1995-2003	8	0.2116	0.0175^{NS}		0.0758	0.0996	0.1272
1995- 2004	2	0.2820	0.0879^{NS}		0.1546	0.2031	0.2595
1995-2005	14	0.1886	-0.0055^{NS}		0.0693	0.091	0.1163
1995-2006	28	0.2500	0.0559^{NS}		0.0789	0.1037	0.1324
1995-2007	28	0.2350	0.0409^{NS}		0.0757	0.0994	0.1270
1997-1990	1	0.1376	-0.0565*	Crossbreeding	0.0488	0.0641	0.0819
1997-1991	4	0.1668	-0.0273^{NS}	Reproduction in endogamy	0.0649	0.0852	0.1089
1997-1992	3	0.1521	-0.042^{NS}	without disturbing genetic structure	0.0789	0.1037	0.1324
1997-1993	5	0.1236	-0.0705*	crossbreeding	0.0568	0.0747	0.0954
1997-1994	2	0.1471	-0.0470^{NS}	Reproduction in endogamy without disturbing genetic structure	0.0685	0.09	0.1149
1997-1995	3	0.1337	-0.0604*	Crossbreeding	0.0499	0.0656	0.838
1997-1996	2	0.1229	-0.0712**		0.0501	0.0659	0.0841
1997-1998	3	0.1721	-0.0220^{NS}	Reproduction in endogamy without disturbing genetic structure	0.1003	0.1318	0.1684
1997-1999	3	0.1402	-0.0539*	crossbreeding	0.0521	0.0685	0.0875
1997-2000	4	0.1738	-0.0203^{NS}	Reproduction in endogamy without disturbing genetic structure	0.1219	0.1602	0.2047
1997-2001	2	0.1271	-0.0670*	Crossbreeding	0.0570	0.0749	0.0957
1997-2002	3	0.1344	-0.0597*		0.0515	0.0677	0.0864
1997-2003	4	0.1278	-0.0663*		0.0531	0.0698	0.0892
1997-2004	1	0.1467	-0.0474^{NS}	Reproduction in endogamy 0.0488	0.0641	0.0819	
1997-2005	7	0.1600	-0.0341^{NS}	without disturbing genetic structure	0.0767	0.1008	0.1287
1997-2006	14	0.1342	-0.0599*	Crossbreeding	0.0502	0.0660	0.0843

Table 2: Continue...

Table 2: Continue...

1997-2007	14	0.2392	0.0451NS	Reproduction in endogamy	0.1116	0.1466	0.1873
2000-1990	2	0.1378	-0.0563NS	without disturbing genetic structure	0.0583	0.0766	0.0978
2000-1991	8	0.2503	0.0562NS		0.1041	0.1368	0.1747
2000-1992	6	0.1726	-0.0215NS		0.0685	0.0900	0.1149
2000-1993	10	0.1350	-0.0591*	Crossbreeding	0.0509	0.0669	0.0855
2000-1994	4	0.1313	-0.0628*		0.0506	0.0665	0.0850
2000 -1995	6	0.1510	-0.0431NS	Reproduction in endogamy without disturbing genetic structure	0.0601	0.0790	0.1009
2000-1996	4	0.1344	-0.0597*	Crossbreeding	0.0522	0.0686	0.0877
2000-1998	6	0.1848	-0.0093NS	Reproduction in endogamy	0.0621	0.0817	0.1043
2000-1999	6	0.1831	-0.0110NS	without disturbing genetic structure	0.0881	0.1158	0.1479
2000-2000	8	0.1710	-0.0231NS		0.0685	0.0900	0.1149
2000-2001	4	0.1309	-0.0632*	Crossbreeding	0.0488	0.0641	0.0819
2000-2002	6	0.1472	-0.0469NS	Reproduction in endogamy	0.0572	0.0752	0.0960
2000-2003	8	0.1557	-0.0384NS	without disturbing genetic structure	0.0711	0.0935	0.1194
2000-2004	2	0.1432	-0.0509*	Crossbreeding	0.0488	0.0641	0.0819
2000-2005	14	0.1976	0.0035NS	Reproduction in endogamy	0.0702	0.0922	0.1178
2000-2006	28	0.1849	-0.0092NS	without disturbing genetic structure	0.0584	0.0768	0.0981
2000-2007	28	0.2051	0.0110NS		0.0729	0.0958	0.1223

To highlight important moments in the evolution of population is necessary to determine the difference between genetic relationship stallions-mares of different years of age structure and average affinity of the total population. The differences who are statistically significant underlines the action either crossbreeding or to inbreeding.

Defined, inbreeding is the mating of individuals close related than the average genetic relationship of the population, while the reverse is the crossbreeding (Todd, *et al*., 2020). In light of this idea, the data presented in Table 2, reveal very clearly that, because of overlapping generations, the population is fragmented into three distinct groups, the improvement factors acting in different proportions: a group with a significant share of crossbreeding, another in which act the inbreeding and a third, all with large weight, in which act the process of reproduction in endogamy without "disturbing" genetic structure.

The times when the difference of relationship stallions -mares and average relatedness in the population has no statistical significance, that moments of inbreeding or crossbreeding weak in intensity, represents population reproduction in endogamy or the return of population after interfamilial crossing, means after application of the matching mating system, specific for horse studs to avoid increasing the homozygous.

Analyzing the results fromTable 2, we can observe two situations where significant differences limit points: inbreeding that may occur as a result of deliberate actions for increasing similarity with a remarkable ancestor or because of errors in matching mating, crossing that may arise because immigration, or as a consequence of matching mating system characteristic for small populations to avoid in breeding.

The first situation, regarding the inbreeding, is generated by the combination of birth year groups 1987 for stallions (Ousor IX) and 2002 for mares (3 heads) and combination of birth year groups 1987 for stallions (Ousor IX) and 2004 (a mare) for mares. Inbreeding is caused, most likely, by the errors in matching pairs, whereas previous analyzes showed clearly that in Hucul horse population from Lucina stud, was missing the desire to maintain genetic similarity with some outstanding ancestors.

The important share of crossbreeding (Table 2) is generated by compliance of matching pairs system, based on the principle of rotation, the only way to reduce inbreeding in small populations by doubling the effective size. For Hucul horse population from Lucina studfarm, the data presented reveals a gladdening fact, that the principle of interfamily rotating crossing ensuring a population normal evolutionary way, without danger of slippage in genetic drift. The share of crossbreeding definitely cannot be attributed to immigration whereas previous analyzes, we can observe clearly the absence of immigration (foreign genetic material infusion).

Between individual average genetic relationship values of stallions with mares, is found significant differences, highlighted by the global test of homogeneity test (F = 4.891 ***) - Table 3 and Fig 2.

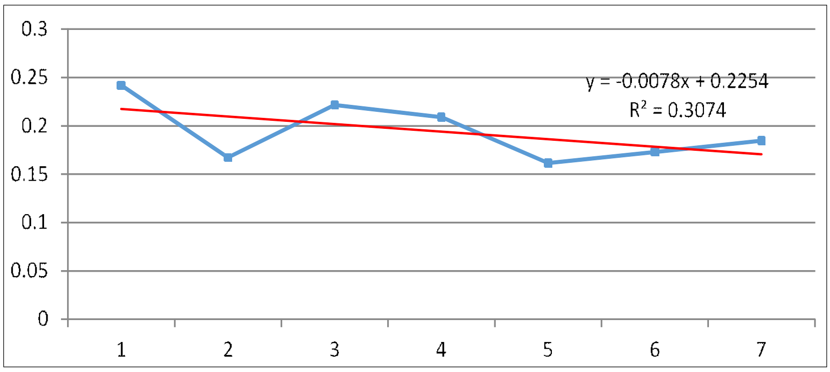

Fig 2: The average individual kinship of the stallions with the broodmares.

Table 3: Individual average genetic relationship between sire stallions and mares.

No. Crr.	Stallions (code)	Individual average relationship sires-mares	$\hat{F}$
1	Ouşor IX (220)	0.2418	4.891***
2	Goral XXI (329)	0.1670	
3	Ouşor X (252)	0.2216	
4	Pietrosu XI (274)	0.2090	
5	Goral XX (328)	0.1615	
6	Hroby XXIV (327)	0.1729	
7	Prislop XI (330)	0.1848	

CONCLUSION

Analysis of individual average genetic relationship of stallions with mares reveals some problems. Activation of two stallions, father and son (Ousor IX, born in 1987 and Ousor X, born 1995), for reproduction, is a serious error because it results in inbreeding. Also, long reproductive exploitation for a long time, for this two stallions, which translates into a large number of descendants, may be generating inbreeding. The same discussion is valid for Pietrosu XI (born in 1995). In other words, problems arise where the average genetic relationship mares-stallion, outweighs the average genetic relationship in the population (Pietrosu and Ousor lines situation, when, over time, has been promoted, in reproductive nucleus, a large number of mares of these two lines, with high affinity with the two stallions). The issue is not valid for Goral XXI, who, although born in 1993, working as a sire stallion for about 2 years. Solution for Ousor line is to select one candidate from each of the two stallions and creating two “blood current “ in the same line.

Between individual average genetic relationship values of stallions with mares, is found significant differences, highlighted by the global test of homogeneity test (F = 4.891 ***). These differences allow us to assert that, through stallions selection, is made a reproductive discrimination much more intense than in mares case. The average genetic relationship of the population, based on genetic relationship coefficients matrix was calculated at 0.1941±0.0053.

Conflict of Interest: None.

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