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Analysis of inbreeding in the genetic resource of the „Old Kladrub horse“ in the period from 1993 to 2003

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INTRODUCTION

The Old Kladrub horse is an important Czech horse breed, which fulfills most of the criteria for genetic resources. The breed existing today is a warmblood created on the basis of Old Spanish and Old Italian horses and bred continuously in the Czech Republic for more than two hundred years. The breed is a robust carriage (coach) horse, which was originally used for ceremonial purposes by the Habsburg emperors. The breeding population is located in the middle of Bohemia and in the southern parts of Moravia in two studs of the National Stud at Kladruby nad Labem (grey variety is at the stud at Kladruby nad Labem and the black one at Slatiňany) and on 100 farms of private breeders. From the very beginning it has been a population of limited number and because of this inbreeding took place over centuries and especially in the past decades. Inbreeding in its conseqequences could lead to inbreeding depression, especially in characters related to fitness (reproduction traits). This is why it is necessary, in the framework of repeated genetic analyses, to pay attention to the rate of inbreeding. VOLENEC et al. (1995) carried out a study of inbreeding rate in the Old Kladrub horse. As a result of this analysis the population was closed against gene immigration from related breeds of Spain origin.

The objective of the paper is to analyse the development and the rate of inbreeding of the Old Kladrub breed within the period from 1993 to 2003.

HISTORICAL BACKGROUND AND DEVELOPMENT OF THE BREED

Today the population is divided into 5 gray lines (GENERALE, GENERALISSIMUS, SACRAMOSO, FAVORY, RUDOLFO) and 5 black lines (SACRAMOSO, SOLO, SIGLAVY PAKRA, ROMKE, GENERALISSIMUS). The Old Kladrub horse can be traced back to the founder sires, which represent sire lines existing today (tab. 1). Originally the sire line SACRAMOSO had been black and the sire line GENERALISSIMUS had been white. Now there are stallions and mares with the opposite colour. The reason for this is the fact that the population should be bred without respect to the colour to hold the average coefficient of inbreeding as low as possible. Within the 10-year breeding period (1993-2003) the number of sires increased from 33 to 39 (tab. 2). This increment was only slight, and it was due the increase of sires in the grey variety (from 16 to 22). The number of sires in the black variety was the same (17) in the year 1993 and 2003. During the same breeding period the number of dams increased from 209 (95 grey and 114 black) to 350 (169 grey and 181 black) – tab. 3.

EFFECTIVE POPULATION SIZE

The effective population size, calculated according to FALCONER and MACKAY (1996) increased from N_e = 114.00 (1993) to N_e = 140.36 (2003). The increment of N_e during the 10 year period was 26.36 animals. From the effective population size we can see that the breed was

endangered in both years (1993 and 2003). The generation interval corresponds approximately to 10 years in the Old Kladrub horse. If random mating were applied, the rate of inbreeding with respect to the number of sires and dams would be 0.4386 % in 1993 and 0.3562 % in 2003.

RATE OF INBREEDING

Coefficients of inbreeding were calculated according to WRIGHT (1922) for all horses (stallions and mares) from the information of 5 generations of ancestors in the years 1993 and 2003.

Stallions

The tab. 2 shows average inbreeding coefficients of stallions within sire lines and both varieties in 1993 and 2003. The average coefficient of inbreeding for the whole population and in both varieties of the breeding stallions remained within the 10-year period almost unchanged despite the matings between as far as possible unrelated stallions and mares and realized circular group mating. This phenomenon can be explained by the fact, that when stallions were selected for breeding emphasis was first of all put on the results of the performance test and conformation evaluation. Within this period for all grey sire lines the decrease of $F_{\bar{x}}$ from 6.06 % to 5.20 %(-1.06 %) was recorded. The average coefficient of inbreeding increased in the black sire lines from 5.26 % to 5.94 % (0.68 %).

Mares

The tab. 3 shows average inbreeding coefficients of mares within sire lines and both varieties in 1993 and 2003. The average coefficient of inbreeding for the whole population (grey and black lines) of dams decreased from the value 7.75 % to 4.88 % (-2.87 %) within the 10-year period. The coefficients of inbreeding were reduced in all lines. The highest difference was found in the lines RUDOLFO (-5.89 %) and SACRAMOSO (-4.68 %) and the lowest difference in the lines ROMKE (-0.25 %) and FAVORY (-0.84 %). In the whole dam population the reduction of the average coefficient of inbreeding between 1993 to 2003 was remarkable from 7.75 to 4,88 % (-2,87 %). Obviously two main reasons were responsible for this decrease of the coefficient of inbreeding. The first one was the application of circular group mating. The second one was the rapid dam population growth after a bottleneck situation in 1993. Within the period of ten years the number of dams increased from 209 to 350, i.e. an increase of 141 dams (67.5 %).

Inbreeding (increase of homozygosity) results in a reduced *fitness* which is the genetic basis of the phenomenon of *inbreeding depression.* To find out if the Old Kladrub breed is endangered by the process of inbreeding the fertility of dams was analysed in the time span from 1994 to 2003.

DAM FERTILITY

The fertility of dams was analysed separately in the grey and black variety within the National stud at Kladruby and private studs from 1995 and 2003. The results of this analysis for both varieties are shown in tab. 4. The fertility rate of both varieties and both types of studs is 65.34 % and the fertility rate values are within the span of 60.23 % in 1996 and 71.84 % in 1997. The lowest fertility rate of 55.91 % was recognized in the private farms in 1996 and the highest fertility rate of 73.33 % was found in the National stud in 1997. The difference between the lowest and highest value was 17.42 %. No tendency caused by the inbreeding depression was found in the fertility rate within the period of 1995 and 2003.

POLYMORFISM OF BLOODTYPES, BIOCHEMICAL MARKERS AND MICROSATELLITES

JAKUBEC et al., 1996 and HOŘIN et al., 1998 tested stallions and mares of different age for 7 systems of bloodtypes (A, C, D,K, P, Q and U), 5 systems of genetic variants in the serum or plasma - ALB (albumin), TF (transferrin), ES (carboxylesterase), GC (vitamin D binding protein), A1B (β - glycoprotein) and 5 systems in red blood cells - HBA (haemoglobin), GPI (glucose phosphate isomerase), PGD (6-phosphogluconatedehydrogenase), PGM (phosphoglucomutase); CA (carbonic anhydrase) - only JAKUBEC et al. (1996) and Pi (protease inhibitor) – only HOŘÍN et al. (1998).

The individuals were in both cases grouped into both colour varieties (grey and black). The average heterozygosity was estimated according to NEI and ROYCHOUDHURI (1974). The result of the analysis is specified in tab. 5. HOŘÍN et al. (1998) found higher values of the average heterozygosity (0.44) at blood type and biochemical loci than JAKUBEC et al. (1996) (0,34). The average heterozygosity of microsatellites (0.65) was much higher than in the blood type and biochemical loci (0.34 resp. 0.44). The average heterozygosity in the breed was enough large and was connected with a low coefficient of inbreeding.

SUMMARY

The Old Kladrub horse is the most important genetic resource in the Czech Republic. Today the breed is divided into 5 gray a 5 black sire lines. The structure of the breed in 2003 was: 39 stallions, 350 mares and the effective population size (N_e) 140. The breed was closed against immigration in 1992. In 1993 and 2003 average coefficients of inbreeding ($F_{\bar{x}}$) were calculated from 5 parental generations for stallions and mares in the whole breed, white and black variety and sire lines within the varieties. From 1993 to 2003 decreased the $F_{\bar{x}}$ (%) in the breed for stallions from 5.65 to 5.52 and for mares from 7.75 to 4.88. No tendency caused by the inbreeding depression was found in the fertility rate within the period of 1995 and 2003. The average heterozygosity estimated on the frequencies of bloodtypes, biochemical markers and microsatellites was enough large and was connected with a low coefficient of inbreeding.

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Tab. 1. Survey of founder sires

No.	Name	Variety	Date of birth	Breed	Origin
1	Generale	Grey	1787	Old Kladrub	Kopčany stud (Moravia)
2	Generalissimus I	Grey	1797	Old Kladrub	Son of Generale (Kopčany)
3	Sacramoso	Black	1800	Old Kladrub	Kroměříž stud (Moravia)
4	Napoleone	Black	1845	Old Kladrub	Rome – line ceased in 1922
5	Solo	Black	1927	Old Kladrub	Son of Sacramoso XXIX
6	Favory	Grey	1938	Lipizzan[1]	Bábolna stud (Hungary)
7	Siglavy Pakra	Black	1946	Lipizzan[1]	Lipica stud (Slovenia)
8	Romke	Black	1966	Friesian[1]	The Netherlands
9	Rudolfo	Grey	1968	Lusitano[1]	Portugal

[1] - Breeds of Old Spanish origin

Tab. 2. Average inbreeding coefficients of stallions within sire lines and both varieties in 1993 and 2003

Year		1993		2003		Difference	Min.	Max.
Variety	Sire line	n	$F_{\bar{X}}$(%)	n	$F_{\bar{X}}$(%)	$F_{\bar{X}}$(%)	* $F_{\bar{X}}$(%)	* $F_{\bar{X}}$(%)
Grey	Generale	2	11.25	5	7.03	- 4.22	0.00	9.57
	Generalissimus	3	8.23	4	3.86	- 4.37	1.95	5.86
	Favory	6	2.42	6	4.82	2.40	1.56	7.23
	Rudolfo	2	7.25	2	2.64	- 4.61	2.53	2.73
	Sacramoso	3	6.93	5	5.47	- 1.46	3.32	7.23
	Average	16	6.06	22	5.20	-1.06	0.00	9.57
Black	Sacramoso	5	1.06	6	9.44	8.38	4.69	11.72
	Solo	6	7.83	7	5.08	- 2.75	2.34	8.98
	Siglavy Pakra	3	4.50	2	3.61	- 0.89	2.73	8.98
	Romke	3	7.90	1	0.00	- 7.90	0.00	0.00
	Generalissimus	-	-	1	1.56	1.56	1.56	1.56
	Average	17	5.26	17	5.94	0.68	0.00	11.72
Total	Average	33	5.65	39	5.52	- 0.13	0.00	11.72

* - the minimal and maximal $F_{\bar{X}}$ refer to values in 2003

Tab. 5. Average heterozygosity at blood type, biochemical and mSat loci in the Kladrub breed (HOŘÍN et al., 1998 and JAKUBEC et al. 1996)

		HOŘÍN et al., 1998				(JAKUBEC et al., 1996)	
Variety	n	Blood type loci	Biochemical loci	Average	Microsatellite loci	n	Average (Blood type and biochemical loci)
Grey	63	0.47	0.34	0.41	0.67	73	0.31
Black	73	0.53	0.42	0.48	0.64	109	0.36
Total	136	0.50	0.38	0.44	0.65	182	0.34

Tab. 3. Average inbreeding coefficients of mares within sire lines and both varieties in 1993 and 2003

Variety	Sire line	1993			2003			Diffe-rence $F_{\bar{X}}$(%)	Min. * $F_{\bar{X}}$ (%)	Max. * $F_{\bar{X}}$ (%)
		Sires n	Dams n	$F_{\bar{X}}$ (%)	Sires n	Dams n	$F_{\bar{X}}$ (%)			
Grey	Generale	2	10	8.59	2	21	6.32	- 2.27	2.34	10.55
	Generalissimus	3	28	9.84	1	17	5.89	- 3.95	2.54	10.74
	Favory	6	30	4.19	3	77	3.48	- 0.71	0.39	13.48
	Rudolfo	2	8	7.29	1	15	1.75	- 5.54	0.39	5.66
	Sacramoso	3	19	9.96	3	33	4.24	- 5.72	0.39	12.11
	Solo	-	-	-	2	3	2.21	2.21	0.00	6.64
	Siglavi Pakra	-	-	-	1	1	0.20	0.20	0.20	0.20
	Romke	-	-	-	1	2	0.00	0.00	0.00	0.00
	Average	16	95	7.29	14	169	3.99	- 3.30	0.00	13.48
Black	Sacramoso	5	24	11.31	3	63	7.26	- 4.05	2.34	17.58
	Solo	6	48	8.38	2	66	5.45	- 2.93	1.37	17.97
	Siglavi Pakra	3	14	9.21	1	14	6.33	- 2.88	2.34	15.82
	Romke	3	28	4.48	1	33	4.49	0.01	2.93	10.55
	Generalissimus	-	-	-	1	5	1.29	1.29	1.95	4.10
	Average	17	114	8.40	8	181	5.86	-2.54	1.37	17.97
Total	Average	33	209	7.75	22	350	4.88	-2.87	0.00	17.97

* - the minimal and maximal $F_{\bar{X}}$ refer to values in 2003

Tab. 4. Fertility of the grey and black Old Kladrub mares within the National stud at Kladruby and private studs

Year	National stud			Private studs			Total		
	Number of mated mares	Number of born foals	Fertility rate %	Number of mated mares	Number of born foals	Fertility rate %	Number of mated mares	Number of born foals	Fertility rate %
1995	114	76	66.67	108	69	63.89	222	145	65.32
1996	83	54	65.06	93	52	***55.91***	176	106	***60.23***
1997	105	77	**73.33**	101	71	**70.30**	206	148	**71.84**
1998	101	68	67.33	115	65	56.52	216	133	61.57
1999	104	72	69.23	102	66	64.71	206	138	66.99
2000	83	54	65.06	111	72	64.86	194	126	64.95
2001	105	69	65.71	100	70	70.00	205	139	67.80
2002	117	79	67.52	120	82	68.33	237	161	67.93
2003	83	51	***61.45***	119	71	59.66	202	122	60.40
Average	99.44	66.67	67.05	107.67	68.67	63.78	207.11	135.33	65.34

ABSTRACT

The Old Kladruber horse, which was established by the end of the 18th and at the beginning of the 19th century is the most important genetic resource in the Czech Republic. The structure of the breed in 2003 was: 39 stallions, 350 mares, effective population size (N_e)140. The breed was closed against immigration in 1992 and since that time circular group mating and mating of non-related animals within the frame of the breeding scheme were applied. In 1993 and 2003 average coefficients of inbreeding ($F_{\bar{x}}$) were calculated from 5 parental generations for stallions and mares in the whole breed, white and black variety and sire lines within the varieties. From 1993 to 2003 decreased the $F_{\bar{x}}$ (%) for stallions in the: breed from 7.16 to 5.47 , white variety from 6.06 to 5.1, black variety from 8.21 to 5.94 and for mares in the: breed from 7.9 to 5.05, white variety from 7.29 to 4.17 and black variety from 8.4 – 5.86. The variation of $F_{\bar{x}}$ (%) in the sire lines was for stallions between 2.01 and –7.9 and for mares between 2.21 and –5.72. The majority of sire lines showed a reduction in the $F_{\bar{x}}$.

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