GENETIC ANALYSIS OF SIRE LINES AND SIRE PROGENY GROUPS IN THE OLD KLADRUB HORSE

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In 8 sire lines and 16 sire progeny groups (182 progeny) the average coefficient of inbreeding ($F_{\bar{x}}$) and average heterozygosity (H) were estimated. The average coefficient of relationship ($R_{\overline{xy}}$) and genetic distance (D) between each pair of these 16 sire progeny groups were also estimated. Analysed were the inbreeding and relationship back to the eighth parental generation and 16 systems of blood groups and biochemical polymorphism. For the whole breed these parameters were estimated: $F_{\bar{x}} = 6.72\%$, $H = 36.65\%$, $R_{\overline{xy}} = 4.92\%$ and $\overline{D} = 8.41$. The correlation coefficient between the average coefficient of inbreeding and average heterozygosity for 16 sire progeny groups was insignificant ($r = -0.11$). The correlation coefficient between the average coefficient of relationship and the genetic distance between the same 16 groups was highly significant ($r = -0.63$). The level of inbreeding does not influence the average heterozygosity in the sire progeny groups. Between higher values of the genetic distance and lower coefficient of relationship is a very close link.

Old Kladrub horse; sire lines; sire progeny groups; genetic analysis; blood groups; biochemical polymorphism

INTRODUCTION

The Old Kladrub horse is a unique breed of horses. It is adapted to a specialized niche, which is defined by the type of animal husbandry and the economic utilization. At present the breed is endangered but during the first half of the 20th century the breed size was sometimes so small that the breed was considered critical. Since the foundation at the end of the 18th and at the beginning of the 19th century the breed was kept in a limited size and more

or less inbreeding was used. Immigration of genes from related breeds was made use of the whole history of the breed.

During the past years much attention was paid to the conservation of the Old Kladrub horse. In 1992 a genetic analysis was initiated to check the inbreeding of individuals and subgroups and the relationship between pairs of individuals and sire progeny groups within the breed, calculated from pedigree and by means of biochemical polymorphism. The first results of the analysis were published by V o l e n e c et al. (1995). They estimated an average inbreeding cofficient of $F_{\bar{x}} = 7.75\%$ for the whole breed.

The knowledge of the genetic variability within a population is interesting both from the theoretical and the practical point of view. For the elucidation of this problem the blood groups and the protein polymorphisms are suitable. Information on gene frequencies of various horse breeds is available in some reviews (G l a s n á k, 1973; B o w l i n g, C l a r k, 1985). K i d d, P i r c h - n e r (1971) and G e l d e r m a n n (1972) recommended the use of gene frequencies of genetic polymorphisms for the study of relationships between populations and subgroups within populations. B l o c k h u i s and B u i s (1979) analysed the genetic distance between 12 horse breeds using 6 biochemical polymorph systems.

In a population of 176 stallions and 809 mares of various horse breeds M a y r h o f e r and D w o r a k (1983) found an average heterozygosity of 41.6–53.2%. O o m and C o t h r a n (1994) found in 77 horse breeds 65 various genetic variants at 17 loci. The same authors found only 40 genetic variants in the Sorraia breed with a high coefficient of inbreeding.

The difference in the average heterozygosity of both groups was however minimal – 37.5% versus 37.7%. They did not find a significant correlation between the average heterozygosity and the coefficient of inbreeding. B o w l i n g (1994) showed a relative invariable gene frequency of blood groups and biochemical polymorph traits for various horse populations. He found in 975 wild horses caught at 7 localities the average heterozygosity of 40.2% and in 16 breeds of domestic horses the average heterozygosity of 38.9%. He gave evidence that wild horses were derived from various domesticated breeds.

MATERIAL AND METHODS

In 1993 and 1994 a genetic analysis of the Old Kladrub breed was carried out by means of:
1. Population genetics methods.
2. Markers at the level of gene products.

The following were analysed:
182 progeny (stallions and mares) of different age and grouped into:
   7 sire lines
   16 sire progeny groups
   2 colour varieties (gray, black) – 73 gray individuals (6 sire progeny groups), 109 black individuals (10 sire progeny groups)

Population genetics methods

The coefficients of inbreeding (F_x) for all individuals and the coefficients of relationship (R_{xy}) for all pairs of individuals were estimated according to W r i g h t (1922). Analysed were the inbreeding and relationship back to the eighth parental generation.

Markers at the level of gene products

All individuals were tested for blood groups and biochemical polymorphisms (6 systems of blood groups and 10 systems of biochemical polymorphism) – Tab. I.

The parameters F_x were used for the estimation of the average coefficients of inbreeding ($F_{\bar{x}}$) for the sire groups, sire lines, for the varieties of gray and black individuals and for the whole breed. At the same time the average heterozygosity was estimated for the same genetic groups (N e i, R o y - c h o u d h u r i, 1974).

The homozygosity at the i-th locus (g_i) is

$$g_i = \sum_{j=1}^{n_i} X_{ij}^2$$

where: X_i – frequency of the j-th allele at the i-th locus
       X_{ij}^2 – frequency of the homozygotes at the i-th locus
       n_i – number of alleles at the i-th locus

The heterozygosity at the i-th locus (h_i) is

$$h_i = 1 - g_i$$

The average heterozygosity (H) is

$$H = \sum_{i=1}^{r} h_i$$

where: r = number of loci

I. Genetic polymorphism (16 systems)

System	Symbol	Factors
Blood groups		
1. A	A	a, b, c, f, –
2. C	C	a, –
D	D	a, b, c, d, e, f, g, h, i, k, l, m, n, o, –
3. K	K	a, –
4. P	P	a, b, c, –
5. Q	Q	a, b, c, –
6. U	U	a, –
Protein polymorphism		Variants
7. albumin	ALB[a]	A, B
8. transferrin	TF[a]	D, D_2, F_1, F_2, H, M, O, R
9. haemoglobin	HBA[b]	AI, AII, BI, BII
10. carbonic anhydrase	CA[b]	F, I, L, O, S
11. carboxylesterase	ES[a]	F, G, I, S
12. glucose phosphate isomerase	GPI[b]	F, I, S
13. 6-phosphogluconate dehydrogenase	PGD[b]	D, F, S
14. vitamin D binding protein	GC[a]	F, S
15. β-glycoprotein	AIB[a]	F, K, S
16. phosphoglucomutase	PGH[b]	F, S, V

a – systems of genetic variants in the serum or plasma
b – systems of genetic variants in red blood cells

The variance of H [Var (H)] is

$$\text{Var}(H) = \frac{\sum_{i=1}^{r} (h_i - H)^2}{r \cdot (r-1)}$$

The average coefficient of relationship $(R_{\overline{xy}})$ and the genetic distance were estimated between all 16 sire progeny groups

$$\left(120 \text{ pairs} - \frac{n \cdot (n-1)}{2}\right).$$

The genetic distance was estimated according to N e i (1972):

$$D = -\ln \frac{\Sigma_i \Sigma_j X_{ij} Y_{ij}}{\sqrt{(\Sigma_i \Sigma_j X_{ij}^2)(\Sigma_i \Sigma_j Y_{ij}^2)}}$$

where: X_{ij} (Y_{ij}) – frequency of the j-th allele at the i-th locus of the population X (Y)
X_{ij}^2 (Y_{ij}^2) – frequency of the homozygotes at the i-th locus of the population X (Y)

For all 16 sire progeny groups the correlation between the average heterozygosity and average coefficient of inbreeding was calculated. The correlation between the genetic distance and the average coefficient of relationship was calculated for all 120 pairs of sire progeny groups.

RESULTS AND DISCUSSION

The coefficient of inbreeding of sires is shown in Tab. II. The comparison of F_x and H for all genetic groups and the whole breed are in Tab. III. Genetic distances $(\overline{D})$ and average coefficients of relationship $(R_{\overline{xy}}$ in %) between the sire progeny groups are in Tab. IV.

II. Coefficients of inbreeding of stallions (F_x)

Number	Name	F_x (%)	
1	Generalissimus XXIX	4.4	
2	Generalissimus XXX	10.4	
3	Favory XI-K	0.9	gray
4	Rudolfo I	14.5	
5	Sacramoso XXXVII	1.7	
6	Sacramoso Aboca XL	8.9	
7	Sacramoso Eleona XXXVIII	11.3	
8	Sacramoso Xerxes XXXIX	9.5	
9	Solo Narcis IV	6.3	
10	Solo Majka VI	6.4	
11	Solo Magnifica VII	4.1	black
12	Solo Isoleta VIII	9.3	
13	Siglavi Pakra Favorina I	0.1	
14	Siglavi Pakra Barbara III	5.8	
15	Romke	9.8	
16	Romke Eleona II	0.0	

III. Comparison of $F_{\bar{x}}$ and H for the genetic groups

Number	Specification	n	$F_{\bar{x}}$ (%)	H (%)
Sire progeny groups				
1	Generalissimus XXIX	28	5.7	35.4
2	Generalissimus XXX	6	8.7	20.4
3	Favory XI-K	14	5.7	31.0
4	Rudolfo I	5	0.0	25.1
5	Sacramoso XXXVII	10	8.3	27.9
6	Sacramoso Aboca XL	10	8.4	26.6
7	Sacramoso Eleona XXXVIII	10	10.8	33.7
8	Sacramoso Xerxes XXXIX	15	8.6	35.7
9	Solo Narcis IV	5	11.3	32.8
10	Solo Majka VI	12	7.7	34.5
11	Solo Magnifica VII	17	5.7	38.4
12	Solo Isoleta VIII	14	6.7	36.8
13	Siglavi Pakra Favorina I	9	6.0	35.2
14	Siglavi Pakra Barbara III	6	8.0	33.0
15	Romke	9	0.0	38.5
16	Romke Eleona II	12	6.7	36.2
Sire lines				
1	Generalissimus	34	6.2	32.8
2	Favory	14	5.7	31.0
3	Rudolfo	5	0.0	25.1
4	Sacramoso	45	9.0	31.5
5	Solo	48	7.1	36.4
6	Siglavi Pakra	15	6.8	34.3
7	Romke	21	3.8	37.2
Varieties				
1	progeny of gray stallions	73	6.3	32.7
2	progeny of black stallions	109	7.0	39.3
	Breed total	182	6.7	36.7

IV. Genetic distance (D) and average coefficients of relationship ($R_{\overline{xy}}$ in %) between the sire progeny groups

D \ $R_{\overline{xy}}$ (%)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1		3.630	7.020	4.083	11.504	11.171	15.378	6.351	8.349	15.625	6.951	16.349	13.204	18.804	14.143	16.264
2	12.716		7.328	5.460	12.634	10.860	14.372	7.837	9.624	17.243	8.619	15.897	12.818	15.992	14.788	18.242
3	8.705	6.997		4.094	6.414	5.633	5.335	4.842	4.799	6.556	4.290	5.743	6.293	7.690	6.976	7.174
4	10.410	9.023	8.655		4.838	5.843	8.903	2.272	4.159	9.551	3.562	10.144	5.914	12.283	9.983	10.544
5	10.289	7.839	9.253	10.035		6.821	10.528	2.337	8.238	12.376	5.652	13.694	6.088	16.310	16.194	14.420
6	6.952	4.866	7.467	8.203	8.136		5.940	5.485	6.187	7.023	4.793	5.254	2.032	7.391	6.220	8.385
7	1.236	1.259	1.829	1.871	1.756	0.940		9.561	10.908	4.981	6.935	3.466	6.140	3.859	4.399	3.531
8	8.112	8.567	7.287	13.820	10.389	7.819	1.236		6.011	10.900	3.630	12.391	5.618	15.577	12.626	12.390
9	10.716	6.171	10.711	15.938	12.189	9.391	1.506	10.170		9.307	6.848	9.038	6.065	11.117	10.809	13.359
10	0.302	0.289	0.702	0.499	0.371	0.311	5.964	0.280	0.443		6.184	4.733	8.250	7.166	4.883	4.304
11	9.544	8.055	7.770	8.789	12.841	7.856	1.248	8.504	10.824	0.191		8.765	6.712	11.043	7.800	9.048
12	0.421	0.408	1.206	0.794	0.514	0.375	8.426	0.409	0.774	6.223	0.237		5.656	1.976	2.763	4.222
13	7.600	4.822	7.144	7.398	10.019	14.618	1.235	7.350	8.187	0.399	8.269	0.538		6.749	7.558	9.647
14	0.665	0.501	1.419	0.827	1.432	0.469	8.990	0.814	0.758	5.927	0.792	15.286	1.000		4.757	7.137
15	0.656	0.373	1.135	0.820	0.702	0.486	6.434	0.532	1.205	5.921	0.597	9.630	5.589	8.157		5.154
16	0.267	0.232	0.937	0.447	0.318	0.238	9.041	0.251	0.317	5.806	0.162	12.618	0.483	11.873	9.702	

The following parameters were estimated for the whole breed:
$F_{\bar{x}} = 6.72\%$, $H = 36.65\%$, $R_{\overline{xy}} = 4.92\%$ and $\overline{D} = 8.41$.

The average heterozygosity of the Old Kladrub breed is a little smaller than the same parameter estimated by M a y r h o f e r and D w o r a k (1983) – $H = 41.6$–53.2%, but comparable with the estimated parameters by B o w l - i n g (1994) – $H = 40.2\%$, resp. $H = 38.9\%$ and by O o m and C o t h r a n (1994) – $H = 37.5\%$ (77 horse breeds) and $H = 37.7\%$ (strong inbredized breed Sorraia).

The correlation between the average coefficient of inbreeding and average heterozygosity for 16 sire progeny groups was insignificant ($r = -0.11$). Neither B o w l i n g (1994) nor O o m and C o t h r a n (1994) found a significant correlation between the average heterozygosity and the coefficient of inbreeding. The level of inbreeding does not influence the average heterozygosity in the sire progeny groups. The correlation coefficient between the average coefficient of relationship and genetic distance between the same pairs of sire progeny groups was highly significant ($r = -0.63$). There is a very close link between higher values of the genetic distance and lower values of average coefficients of relationship.

CONCLUSIONS

It is very favourable for genetic analyses to combine the population parameters (F_x, R_{xy}) with the parameters based on markers (H, D). The relatively small average coefficient on inbreeding in the breed ($F_{\bar{x}} = 6.72\%$) does not influence the relatively high average heterozygosity ($H = 36.65\%$). We can support the statement of B í l e k (1955) and D u š e k (1992) that the slight intensity of inbreeding has not a negative impact on important characters (reproduction, growth). Due to the significant correlation between the average coefficient of relationship and genetic distance ($r = -0.63$) between the same pairs of sire progeny groups both parameters can be used alternatively.

The present size of breed (220 mares and 30 stallions) and the 8 sire lines allow a scheme which is known as circular group mating. Closely related animals are assigned to the same sire line. Every generation males are exchanged between groups. This system makes it possible to breed 4 generations before genes for the same group are brought in again. In principal, the mating system avoids the breeding of close relatives.

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Genetická analýza otcovských linií a skupin otcovských potomků u starokladrubských koní.

V letech 1993 a 1994 byla provedena genetická analýza u plemene starokladrubských koní pomocí jednak populačně-genetických metod a jednak markerů na úrovni genových produktů. Analyzováno bylo 182 potomků (hřebců a klisen) různého věku. Uvedení jedinci byli tříděni do 7 otcovských linií, 16 skupin otců a na dvě barevné varianty (bílá a vraná). Bílou variantu tvořilo 6 otcovských skupin a 73 jedinců.

Černá varianta se skládala z 10 otcovských skupin a 109 jedinců. Koeficienty inbrídingu (F_x) pro všechny jedince a koeficienty příbuznosti (R_{xy}) pro všechny páry jedinců byly odhadnuty podle W r i g h t a (1922). Inbríding a příbuznost byly sledovány zpětně až po 8. generaci předků. Všichni jedinci byli testováni pomocí krevních skupin a biochemického polymorfismu (6 systémů krevních skupin a 10 systémů biochemického polymorfismu). Parametry F_x byly použity pro odhad průměrných koeficientů inbrídingu ($F_{\bar{x}}$) otcovských skupin potomků, otcovských linií, obou barevných variant a celého plemene. Současně byly pro stejné genetické skupiny odhadnuty průměrná heterozygotnost (N e i , R o y c h o u d h u r i, 1974) a genetická distance (N e i , 1972). Pro celé plemeno byly odhadnuty tyto parametry: $F_{\bar{x}}$ = 6,72 %, H = 36,65 %, $R_{\overline{xy}}$ = 4,92 % a $\overline{D}$ = 8,41. Korelační koeficient mezi průměrným koeficientem inbrídingu a průměrnou heterozygotností pro 16 skupin potomků byl nevýznamný ($r = -0,11$). Korelace mezi průměrným koeficientem příbuznosti a genetickou distancí byla pro stejný počet 16 skupin vysoce významná ($r = -0,63$). U otcovských skupin potomků nemá úroveň inbrídingu vliv na průměrnou heterozygotnost. Mezi vysokými hodnotami genetické distance a nízkými hodnotami koeficientu příbuznosti existuje velmi těsný vztah.

starokladrubský kůň; otcovské linie; skupiny potomků po otcích; genetická analýza; krevní skupiny; biochemický polymorfismus

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