Genetic Analysis of Greying Process in Old Kladruber Horse

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Introduction

The grey coat color in horse is a result of the progressive greying - the loss of pigment in hairs. This known phenomenon is typical for some breeds or lines but it is not exclusive. The greys are born dark coloured (brown or black) and turning grey as the ageing causes a loss of pigment in hair although the skin remains pigmented (Toth et al. (2006)). In older greys, the loss of pigment called skin vitiligo of distal body parts (Sölkner et al. (2004)) could be even seen. The Old Kladruber Horse has been bred in Bohemia for nearly 450 years. Being originally bred as a baroque coach horse for Astro-Hungarian Imperial Court in Vienna, it belongs among those grey breeds like Lipizzan horse, Camargue horse etc. The greying process has been thoroughly studied in Lipizzan horse up to this time (Curik et al. (2002); Sölkner et al. (2004)).

Materials and Methods

The data collection was carried out in grey variety of Old Kladruber breed in stud Kladruby nad Labem, stud Benice and others. Data were collected after detailed inspecting and measuring 376 horses repeatedly during four consecutive years (1 to 4 observations per horse, total number of records used for statistical analysis was 702). The greying status was measured using Spectrophotometer Minolta 2500D. The coat color was measured on four parts (neck, shoulder, belly, croup) using L*a*b* colour system, in which colour is quantified to three axis: white-black (L* parameter), red-green (a* parameter), yellow-blue (b* parameter). To quantify the grey level, our analysis is related to the parameter L* only. The higher values L*, the more grey is coat colour of a horse. Every value used is a mean of three consecutive measuring of the same place. Depigmented areas, so called vitiliga, were identified on each animal. Vitiliga were assesed by adspection of typical parts peri-anal and anal region (vitiligo A) and the face (vitiligo F). During the process of quantification, all horses were devided into four groups according to Sölkner et al. (2004). This subjective quantification process was carried out by a single person.

In statistical analysis the GLM procedures of SAS package were used.
The influence of effects line, age, sex, parameter L, year of evaluation and stud on speed of greying, vitiligo A and vitiligo F was analyzed.
The following models were applied:

$$\mathbf{L_{ijklm} = \mu + b_1\, AGE_m + LINE_l + SEX_k + YEAR_j + STUD_i + rep + a + e_{ijklm}}$$

$$\mathbf{VITILA_{ijklm} = \mu + b_1\, AGE_m + LINE_l + SEX_k + YEAR_j + STUD_i + rep + a + e_{ijklm}}$$

$$\mathbf{VITILF_{ijklm} = \mu + b_1\, AGE_m + LINE_l + SEX_k + YEAR_j + STUD_i + rep + a + e_{ijklm}}$$

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where: $\boldsymbol{\mu}$ – overall mean
$\mathbf{b_1\ AGE_m}$ – fixed linear regression on age at evaluation (m = 1,2,…,21)
$\mathbf{LINE_l}$ – fixed effect of the line (l = 1, 2, 3, 4, 5, 6)
$\mathbf{SEX_k}$ – fixed effect of the sex (k = 1, 2)
$\mathbf{YEAR_j}$ – fixed effect of the evaluation year (j = 2005, 2006, 2007, 2008)
$\mathbf{STUD_i}$ – fixed effect of the stud (i = 1, 2, 3, 4)
rep – random effect of the permanent environment of the individual horse
a – random effect of the individual horse
$\mathbf{e_{ijklm}}$ – random residual effect of the individual horse

The phenotypic correlations were estimated with SAS package, procedure CORR. Heritabilities and genetic correlations were estimated using REML VCE 5 (Kovac et al. (2002)).

Results and discussion

The speed of greying assesed by parameter L* showed progressive growing with the age of the horse (Figure 1). All horses reached the highest value at the age of 10 years and this L* value is not changing. The results of our study in Old Kladruber horse breed are confirming all knowledge on greying and its speed known in another horse breeds (Curik et al. (2004); Sölkner et al. (2004)). The GLM analysis confirmed the significant impact of the age on the greying. Of all another effects studied reached the statistical significance the effect sex only. The subsequent analysis suggests notably higher level of greying in mares of Old Kladruber horse compared to sires. For studying of the genetic variation in greying or speed of greying, data could be restricted to age classes where still sufficient level of genetic variation persist.

The vitiligo skin depigmentation of the Old Kladruber horse occurs normally without any adverse effects. The results of observation show that the degree of vitiligo observed in the rear parts of the body (vitil A) intensifies with aging (Figure 2). The vitiligo in the head part (vitil F) does not show a similar trend however, an obvious vitiligo‘s increase has been noticed starting at the age of approximately 5 years (Figure 3). Analysis of potential factors responsible for the occurrence of vitiligo A shows a conclusive influence of a line affiliation. The highest level of vitiligo A was proved in the line Generale, the lowest level then in line Favory-Generalissimus (Table 1). The connection of the greying and vitiligo is confirmed by significant values of the L*parameter and the age of the horse.
A thorough analysis of the head part depigmentation proved significance for all six factors considered. Regarding the knowledge of the process of greying and vitiligo A creation, easily could be explained the significance of the line, age, parameter L* respectively because these effects are related to the loss of the pigment. The statistic significance of effects sex, year of search and stud on vitiligo F is difficult to explain. The influence of the stud could be explained by period of separated breeding of Kladruber stud and the studs of private owners which is characterized by using of very different sires.

Correlation analysis proved significant phenotypic correlation between vitiligo A and vitiligo F (r_p= 0.31). Genetic correlations are confirming expected relation between greying and both types of vitiligo. Heritabilities for all parameters under the study are in good agreement with papers concerning the greying of the horse (Table 2).

Table 1: Vitiligo A grade in lines

	G	G-Gss	F-Gss	F	S	R
G	**0.4152** ±0.0571	0.2055**	0.2131*	0.0245	0.0440	0.1982*
G-Gss		**0.2097** ±0.0534	0.0076	0.1810**	0.1615*	0.0073
F-Gss			**0.2021** ±0.0838	0.1886*	0.1691*	0.0149
F				**0.3907** ±0.0517	0.0195	0.1737
S					**0.3712** ±0.0612	0.1542
R						**0.2170** ±0.0869

Sire lines: G=Generale, G-Gss=Generale-Generalissimus, F-Gss=Favory-Generalissimus, F=Favory, S=Sacramoso, R=Rudolfo

Mean values diagonal, differences between lines above the diagonal

*significant difference, **highly significant difference

Table 2: Estimated genetic parameters (h^2, r_P, r_G)

	L	VITILA	VITILF
L	0.5182±0.0747	0.6727±0.1135	0.5332±0.1015
VITILA	0.3663	0.2019±0.0534	0.5422±0.0763
VITILF	0.3725	0.3103	0.3459±0.0644

Heritabilities on the diagonal, phenotypic correlations below and genetic correlation above the diagonal.

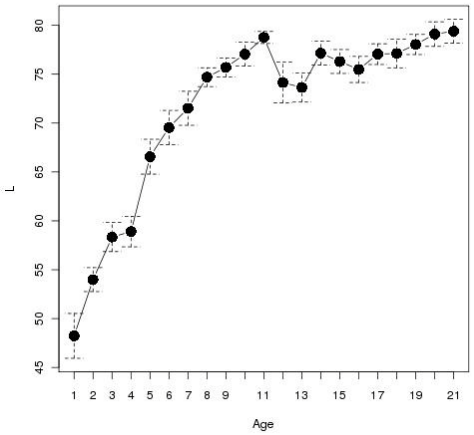

Fig. 1: Relation between the age and greying level

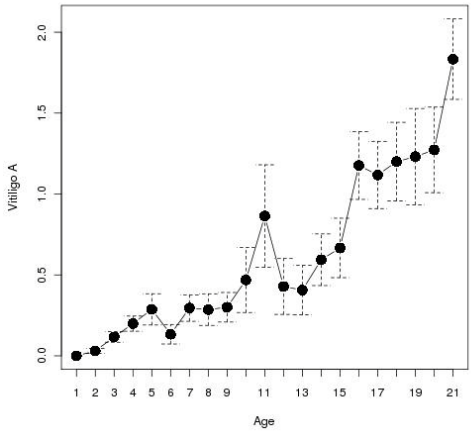

Fig. 2: The age and vitiligo A relation

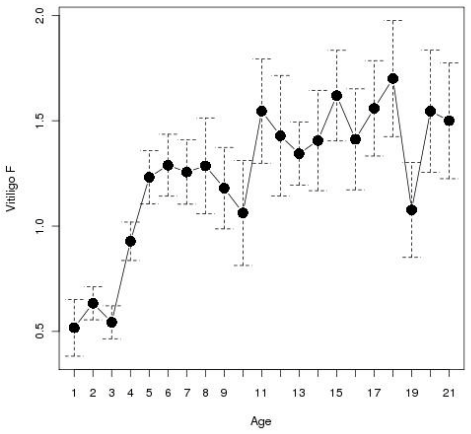

Fig. 3: The age and vitiligo F relation

Conclusion

The paper is studying the process of greying and depigmentation characters in gene resource Old Kladruber Horse. The greying was studied in 376 horses of both sexes at the age 1-25 years during 4 consecutive years. The speed of greying was measured with spectrophotometer and expressed by parameter L*, the occurrence and grade of depigmentation as vitiligo was detected by grading system. The GLM model (SAS package) was used to examine the influence of effects line, age, sex, year of evaluation and stud on traits under study. The genetic parameters were estimated for greying (L*), vitiligo A and vitiligo F. The greying is significantly influenced by age and line affiliation (h^2= 0.52). Vitiligo of both facial and anal part are influenced by trait studied, h^2= 0.2 for vitiligo A and 0.34 for vitiligo F respectively. The genetic correlations showed significant relations between greying and both types of vitiligo.

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