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Characterization of greying, melanoma, and vitiligo quantitative inheritance in Old Kladruber horses

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ABSTRACT: The paper deals with the greying process, melanoma, and vitiligo depigmentation occurrence in Old Kladruber horses (OKH). The study includes 376 animals of both sexes at the age of 1–25 years. The evaluation was performed repeatedly during 4 consecutive years. The greying status was measured spectrophotometrically on four body parts using $L^*a^*b^*$ colour system. Melanoma and vitiligo were detected visually and by palpation in all the animals and classified using 5 (3) grade scale. The GLM procedure of SAS package was used to examine the influence of the effects of line, age, sex, stud, and year of evaluation. The GLM analysis confirmed a significant impact of age on greying. Parameter L^* showed progressive increase until 10 years of age when all horses reached final grey level. Subsequent analyses suggested the influence of sex, i.e. a notably higher level of greying in mares. The incidence of melanoma was confirmed. Melanoma in OKH most frequently occurs on the bottom of the tail or at the anal and perineal area. Probably only benign forms of melanoma have been detected. The global occurrence of melanoma in OKH (ca. 13%) is substantially lower than in Lipizzan and Camargue horses. Overall incidence of melanoma in horses at the age of 15 and older reached 68%. Genetic parameters were estimated for melanoma grade, grey level, and vitiligo. Greying is strictly influenced by age (h^2 = 0.52 ± 0.07). Prevalence of melanoma progresses with age (h^2 = 0.07 ± 0.04). Vitiligos of both facial and anal parts are influenced by the effects studied (h^2 = 0.20 ± 0.05 and 0.34 ± 0.06 for vitiligo A and vitiligo F, respectively).

Keywords: pigmentation; skin neoplasm; heritability; genetic correlations; spectrophotometry

INTRODUCTION

The Old Kladruber horse (OKH) is an autochthonous Czech heavy warmblood created on the basis of Old Spanish and Old Italian horses at the end of the 18th century. Originally this breed was used as a carriage horse for ceremonial purposes. The breed is nowadays included in the genetic resources of the Czech Republic and the population has been fully closed since 2002. As the population is small (effective population size N_e = 201), its genetic diversity is continuously monitored (Horin et al. 1998; Jakubec et al. 2009; Vostry et al. 2011; Janova et al. 2013). The population is bred in two coat colours – grey (6 sire lines) and black (4 sire lines).

The inheritance mode of grey coat colour is autosomal dominant. The locus for greying process is epistatic against all loci in coat colour determination involved. Foals of grey horses are born with fully pigmented coat (black, brown, chestnut) and become grey with ageing. The final grey (“white”) colour is reached at the age of 6–7 years, but in some greys the greying process is never completed. The skin of greys is always dark pigmented with the exception of white markings on head and legs. Sometimes depigmented areas on skin (so called vitiligo) occur with ageing (Henner 2002). Quantitative parameters of horse pigmentation studied Toth et al. (2006) suggesting that polygenic component of greying is dependent on melanin content.

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The locus for greying was mapped at ECA25 (Henner 2002; Henner et al. 2002; Locke et al. 2002; Swinburne et al. 2002). Pielberg et al. (2008) have detected a 4.6 kb duplication in intron 6 of the gene *STX17*, which was in all cases associated with greying phenotypes. The speed and level of greying is quite variable, there are substantial differences among breeds and even horses within a breed showed high variability in greying level. It is possible the greying process is influenced by modifying genes, which probably can cause flea-bitten or dappled grey (Swinburne et al. 2002). A higher level and quicker process of greying was noticed in homozygote (*GG*) in comparison to heterozygote (*Gg*) animals (Pielberg et al. 2008). The quantitative characteristics of greying process in Lipizzan horses are described by Curik et al. (2004). This study showed high phenotypic variability in greying in Lipizzans at the age until 6 years, final coat colour is reached at the age of 10 years. There is a number of grey OKH showing prolonged greying and speckling caused by unknown complex genetic background which corresponds with the results of a thorough study of horse greying in Curik et al. (2013). This study at Lipizzans covering greying, vitiligo, melanoma, and speckling is indicating a polygenic component of greying with strong pleiotropic effects of a grey mutation and presents heritabilities for parameters studied.

The higher susceptibility of dermal melanoma was recognized in greys (Fleury et al. 2000a, b; Heizerling et al. 2001). The melanoma is usually noticed at greys at the age of 5–6 years (Jeglum 1999). Very rarely, cases of the early melanoma stadium were noticed at the age of 3 years (Rodriguez et al. 1997). Some papers showed the prevalence of skin melanoma in grey populations in 80% of horses at the age over 16–18 years (Valentine 1995; Jeglum 1999). However, the greying cannot be a direct cause of melanoma because there is a number of very old grey horses without skin melanoma (Rieder et al. 2000). Hereditary effects on melanoma prevalence in grey OKH also suggest Futas et al. (2012).

The places, where melanoma typically occurs, are the peri-anal and anal region, perineal region, praeputium, udder. It could be also noticed on lips, eyelids, and ears (Fleury et al. 2000a). The thorough characteristics of skin melanoma in grey horses have been unknown yet, supposing it is mainly benign tumour with the possibility of change to malignant stage later on (Solkner et al. 2004). Due to skin melanoma some physiological activities (urination, defecation, mating) could be affected, with negative influence on the welfare and exploitation of the animals (Rodriguez et al. 1997; Swinburne et al. 2002).

A higher incidence of melanoma at greys is in some papers related to skin depigmentation called vitiligo, which appears at higher age on the same body parts where melanoma occurs (Rieder 1999). Vitiligo is a depigmenting disorder creating white patches due to the loss of melanocytes in epidermis (Solkner et al. 2004). There is a current incidence of vitiligo in Old Kladruber grey horses without a visible negative influence.

The aim of this work was to analyze the possible genetic aspects of skin melanoma prevalence, greying process, and vitiligo occurrence in OKH and to estimate genetic parameters for traits studied.

MATERIAL AND METHODS

Experimental design. The study included a total of 376 grey horses of Old Kladruber breed aged from 1 to 25 years. Due to a low number, horses older than 21 years were merged to one group for statistical analysis. By repeated measurements in four consecutive years 702 records were obtained (1–4 records from each individual, 160 horses were evaluated once, 122 horses twice, 78 horses three times, at 16 horses all four records were obtained). Out of 376 horses, there were 61% (230) females and 39% (146) males. The representation of lines (21% Generale, 22% Generale-Generalissimus, 10% Favory-Generalissimus, 20% Favory, 20% Sacramoso, 7% Rudolfo) corresponds to the line affiliation in the population. Most of the collected data come from young horses, aged 2–3 years. This data structure is advantageous for the evaluation of greying process. Evaluation of the incidence of melanoma at horses aged at least 6 years is more valuable because in younger individuals the melanoma is virtually absent. Besides the horses bred at the National Stud Kladruby nad Labem, other animals from smaller private breeders have been included to the evaluation as well.

Coat colour of each individual was recorded by Konica Minolta Spectrophotometer CM-2500d (Konica Minolta, Tokyo, Japan) in accordance with Toth et al. (2006). The measured parameter of greying level is L^* – the colour at the axis: black = 0, white = 100. The higher value of this parameter means the higher

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Table 1. Classification of melanoma occurrence[1]

Grade of melanoma	Description
0	free of melanoma
1	early stages of plaque type or nodule of 0.5 cm in diameter
2	several nodules of 0.5 cm or one nodus of 2 cm in diameter
3	one or several nodules of 5 cm in diameter, or subcutaneous melanoma
4	extensive melanoma covered with skin, signs of destruction, metastasis
5	exophytic growth of tumours, vet surface, cachexia, health disorders

[1]after Solkner et al. 2004 and Curik et al. 2013

level of greying. Measurements were performed for each individual at four body parts (neck, shoulders, abdomen, and back). Each recorded value was an average of three consecutive measurements.

Along with spectrophotometrical measurements of the colouring, the incidence of melanoma was assessed visually and by palpation. Evaluation was done according to the scale published by Solkner et al. (2004) and in agreement with Curik et al. (2013) (Table 1). In the evaluation of melanoma incidence a special attention was paid to the occurrence of unpigmented areas on the skin (so called vitiligo). Quantification was performed subjectively by classifying an animal into one of the groups according to Solkner et al. (2004) and Curik et al. (2013) (Table 2). The incidence of vitiligos was assessed separately in the head part (vitiligo F) and back parts – anal and perineal area and the external genitals (vitiligo A). All horses were inspected by a single person.

Statistical analysis. Fixed effects influencing all the traits were analyzed by a general linear model using the GLM procedure of SAS (Statistical Analysis System, Version 9.1, 2004). The statistical models comprised the following effects:

Table 2. Classification of vitiligo occurrence[1]

Grade of vitiligo	Description
0	full pigmentation
1	small depigmented patches
2	greater continuous depigmented areas of skin
3	extensive depigmented areas of skin

[1]after Solkner et al. (2004) and Curik et al. (2013)

$$L_{ijklm} = \mu + b_1\,AGE_i + b_2\,AGE_i^2 + LINE_j + SEX_k + YEAR_l + STUD_m + e_{ijklm} \quad (1)$$

$$MELANOMA_{ijklm} = \mu + b_1\,AGE_i + b_2\,AGE_i^2 + b_3\,L + LINE_j + SEX_k + YEAR_l + STUD_m + e_{ijklm} \quad (2)$$

$$VITILA_{ijklm} = \mu + b_1\,AGE_i + b_3\,L + LINE_j + SEX_k + YEAR_l + STUD_m + e_{ijklm} \quad (3)$$

$$VITILF_{ijklm} = \mu + b_1\,AGE_i + b_3\,L + LINE_j + SEX_k + YEAR_l + STUD_m + e_{ijklm} \quad (4)$$

where:

μ = overall mean
$b_1\,AGE_i$ = fixed linear regression on age at evaluation (i = 1, 2,..., 21)
$b_2\,AGE_i^2$ = fixed quadratic regression on age at evaluation (i = 1, 2,..., 21)
$b_3\,L$ = fixed linear regression on level of greying
$LINE_j$ = fixed effect of line (j = 1–6)
SEX_k = fixed effect of sex (k = male, female)
$YEAR_l$ = fixed effect of evaluation year (l = 2005–2008)
$STUD_m$ = fixed effect of stud (m = Kladruby nad Labem, Favory Benice, Bartak Nahoruby, Others)
e_{ijklm} = residual error

Due to a small number of evaluated animals the sex-stud-year effect cannot be used, but only separate fixed effects.

Taking into account selected fixed effects, variances of genetic and non-genetic effects in the studied traits were estimated by the REML method using the VCE program (Version 5.1, 2002) (Kovac et al. 2002). The fixed effect of line was included in the models (1–4) by breeders' proposal due to its routine use in practice. However the effect of line is not a suitable indicator for grouping animals according to relationship, because the additive genetic value is theoretically the average of the additive value of parents plus a random Mendelian sampling (Falconer and Mackay 1996). For this reason, the relationship matrix was used in the mixed model for further analysis. Due to a small number of horses and a large number of traits, genetic correlations among traits were estimated by a two-trait animal model:

$$Y = \mathbf{X}b + \mathbf{Z}_1 a + \mathbf{Z}_2 pe + e \quad (5)$$

where:

Y = vector of observation (L^*, melanoma, vitiligo A, vitiligo F)
b = vector of fixed effects (linear and quadratic regressions on age at evaluation, effect of sex, effect of evaluation year, effect of stud)

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a = random direct genetic effect of an individual
pe = random effect of permanent environment of an individual
e = residual error
X, $\mathbf{Z}_1$, $\mathbf{Z}_2$ = incidence matrices of fixed and random effects

It was assumed that random effects are independent and that the effects show a normal random distribution with a mean of zero and variances $\mathbf{A}\sigma_a^2$, $\mathbf{I}\sigma_{pe}^2$, and $\mathbf{I}\sigma_e^2$, where σ_a^2 = additive genetic variance of direct genetic effect, σ_{pe}^2 = variance of the effect of the permanent environment of an individual horse, σ_e^2 = variance of the effect of residual error, **A** = relationship matrix, and **I** = identity matrix.

Based on the estimated variance-covariance components, genotype and phenotype variance-covariance matrices were constructed and subjected to the weighted bending method (Jorjani et al. 2003).

Coefficients of phenotypic correlations were estimated using the CORR procedure of SAS (Statistical Analysis System, Version 9.1, 2004), using the Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Greying process. Results of the survey in 4 consecutive years proved compliance with the common knowledge about the loss of pigmentation of greying horses with their age (Sponenberg 1996; Rieder et al. 2000 and others). Lower age groups, especially foals after birth, show basic coat colours (black, brown, very rarely chestnut), which corresponds with low L^* values (< 50) and relatively high variability of the parameter (Figure 1). Greying process and its progression can be noticed after each hair exchange (moult). With the loss of pigment and increasing proportion of white hairs, the value of L^* parameter increases and the variability of L^* is decreased. Approximately at the age of 10 years, animals reach the final stage of greying and the values of L^* remain unchanged. These results are in a very good agreement with those obtained in Lipizzan horses by Curik et al. (2004, 2013).

In addition to determining the effect of age, other factors may affect the greying process (Table 3). The GLM analysis confirmed a significant influence of age; another examined factor that reached statistically significant level was sex. Mares exhibit higher levels of greying in comparison to stallions (average L^* value for females 65.3 vs 63.0 for males). However, a potential influence of sex hormones should be further experimentally tested.

The incidence of melanoma. The study confirmed the occurrence of cutaneous melanoma in Old Kladruber grey horses, which corresponds to the incidence in breeds that exhibit the same colour according to the study on horses of Camargue breed (Fleury et al. 2000a, b) and the study on Lipizzan horses (Curik et al. 2002; Seltenhammer et al. 2003; Solkner et al. 2004; Curik et al. 2013). Cutaneous melanoma is a black pigmented, hemispherical tumour of solid consistency with prevailing incidence at anal and perineal area. In particular, it frequently occurs on the bottom of the tail. These findings are in full compliance with Fleury et al. (2000a) and Seltenhammer et al. (2004).

Out of total 376 horses evaluated, 47 cases (12.5%) of melanoma were diagnosed (regardless of the grade). In the case of repeated evaluation the record obtained in the highest age of the horse was considered. This low incidence is due to the large number of young horses in the dataset. Until four years of age no melanoma occurs. The first case of early stage (grade 1) was recognized at a four-year-old horse. The occurrence of melanoma in different age groups was as follows: 6% in horses between 6 and 10 years, 32% in horses between 11 and 15 years, 60% in horses

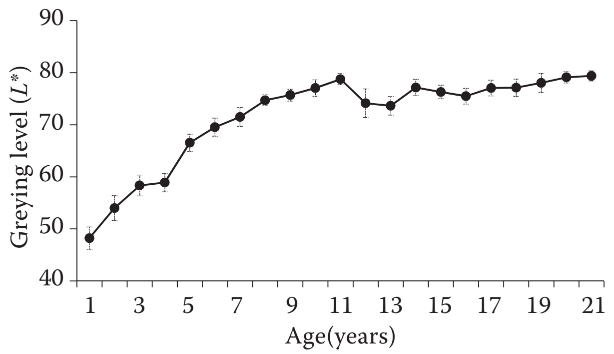

Figure 1. The age and greying level relationship

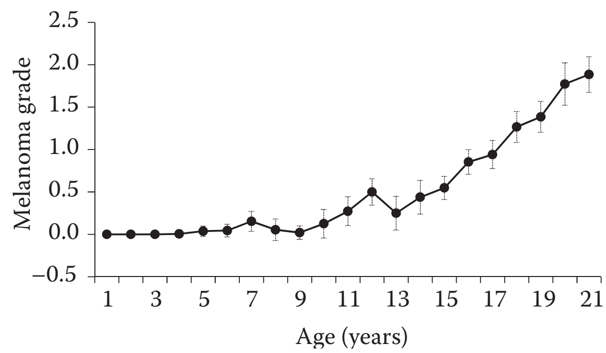

Figure 2. The age and melanoma grade relationship

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Table 3. Analysis of possible effects on observed variables (P-values)

	Greying (L^*)	Melanoma	Vitiligo A	Vitiligo F
Age	< 0.0001	0.0002	< 0.0001	< 0.0001
Age^2	< 0.0001	< 0.0001	–	–
Line	0.3083	0.0043	0.0027	< 0.0001
Sex	0.0312	0.6516	0.4002	0.0353
L^*	–	0.1451	0.0395	< 0.0001
Year	0.7920	0.6182	0.3621	< 0.0001
Stud	0.0748	0.1541	0.2072	< 0.0001

between 16 and 20 years, 83% in horses older than 21 years. Considering repeated evaluation, 113 positive cases from 702 observations were recorded, i.e. 16%. Mean values of melanoma grade in different age groups are shown in Figure 2.

At OKH probably only a benign form has been detected, no case with external malign symptoms was revealed. Horses with melanoma exhibited considerably low grades (1 or 2) without any impact on health status. No grade 5 melanoma was detected among the OKH population under study, i.e. there was no horse showing signs of melanoma with metastasis followed by disorders like apathia, loss of weight, cachexia, colics and peripheral oedema, nostrils bleeding, breathlessness, ataxia. The keepers of horses with light melanoma did not mention any problems in horse urination or defecation. According to Bertone (2006), in the case of metastasis external symptoms may not be necessarily manifested. Because the studied animals did not undergo a histological test, the possibility of malignancy cannot be excluded.

The first incidence of early melanoma stage has been reported in an individual aged 4 years. Rare occurrence in young horses was confirmed by Rodriguez et al. (1997). Jeglum (1999) argues that melanoma occurs in grey horses of 5–6 years of age. In the age group of 6 years and older, melanoma in Old Kladruber grey horse occurred in 33% of cases, which represents significantly lower incidence in comparison to the data published in Lipizzan horses (Curik et al. 2002; Seltenhammer et al. 2003). In the category of 15 years and older, the incidence of melanoma increases in OKH population up to 68%, which is in line with data reported at Lipizzan horses (Seltenhammer et al. 2003) and French breed Camargue (Fleury et al. 2000b). The group of individuals included a few high-age horses (25 years) lacking phenotypic occurrence of cutaneous melanoma. This fact corresponds with the opinion of Rieder et al. (2000), that greying cannot be considered as a direct cause of melanoma. No cases of spontaneous regression of melanoma were observed.

The analysis of potential factors influencing the formation and development of cutaneous melanoma confirmed the effect of age (Figure 2). Besides that, the line affiliation also reached statistical significance during the verification of factors, which suggests an influence of hereditary factors (Table 3). Out of 6 evaluated lines, the Favory-Generalissimus, Rudolfo, and Generale-Generalissimus show the greatest rate of melanoma incidence. The lowest level of reported melanoma was observed at line Favory. Differences between lines are shown in Table 5.

The influence of sex and herd (stud) is not statistically significant. The conclusion that sex is not influencing a prevalence of melanoma was published by Smith et al. (2002). In the Camargue breed Fleury et al. (2000b) found higher prevalence of melanoma in geldings when compared with mares and stallions, but the differences were not statistically significant.

Table 4. Estimated genetic parameters (h^2, r_p, r_G)

	L^*	Melanoma	Vitiligo A	Vitiligo F
Greying level (L^*)	0.5182 ± 0.0747	0.0247 ± 0.2129	0.6727 ± 0.1135	0.5332 ± 0.1015
Melanoma	0.2947	0.0724 ± 0.0384	0.3774 ± 0.1485	0.0113 ± 0.1620
Vitiligo A	0.3663	0.4060	0.2019 ± 0.0534	0.5422 ± 0.0763
Vitiligo F	0.3725	0.1710	0.3103	0.3459 ± 0.0644

heritabilities (h^2) on the diagonal, phenotypic correlations (r_p) below, and genetic correlation (r_G)above the diagonal

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Table 5. Melanoma grade in lines

Line	G	G-Gss	F-Gss	F	S	R
G	0.1734 ± 0.0500	0.0870	0.1908*	0.0781	0.0438	0.1048
G-Gss		0.2605 ± 0.0473	0.1073	0.1652**	0.0433	0.0177
F-Gss			0.3642 ± 0.0728	0.2689**	0.1470*	0.0860
F				0.0953 ± 0.0450	0.1219*	0.1829*
S					0.2172 ± 0.0533	0.0610
R						0.2782 ± 0.0756

G = Generale, G-Gss = Generale-Generalissimus, F-Gss = Favory-Generalissimus, F = Favory, S = Sacramoso, R = Rudolfo mean values on the diagonal, differences between lines above the diagonal
*significant difference, **highly significant difference

Also, the parameter of L^* value did not reach the statistical significance. This fact supports the argument that the loss of pigment may not be the direct cause of cutaneous melanoma (Rieder et al. 2000).

The incidence of vitiligo. Skin depigmentation known also as vitiligo was assessed separately for the head (vitiligo F) and back parts – the area under the tail, anal and perianal area, udder, foreskin (vitiligo A). Vitiligo F overall incidence was 67% (31% grade 1, 23% grade 2, 13% grade 3) being higher than that of vitiligo A with 26% (16% grade 1, 7% grade 2, 2% grade 3). As shown in Figures 3 and 4, the level of vitiligo A increases with age, while that of vitiligo F does not. There is only a certain increase starting at the age of 5 years. The level of vitiligo F also shows a higher variability in comparison with vitiligo A.

The analysis of potential factors responsible for vitiligo A incidence (Table 3) suggests a significant influence of age and parameter L^*, i.e. the level of greying, which confirms the connection between vitiligos and an overall loss of hair pigmentation. Also, the influence of line affiliation has been proved. The highest level of vitiligo incidence was detected in the line Generale and the lowest in Favory-Generalissimus line. Differences between the lines are shown in Table 6.

The analysis of factors affecting vitiligo F incidence (Table 3) shows a statistical significance of all considered effects (age, L^* – level of greying, sex, line, year of evaluation, stud). With respect to our knowledge about greying and vitiligo A, the relevance of the influence of line (Table 7), age, and L^* parameter can be well explained because a clear relationship of all those to the loss of pigment has been proved. The relevance of the influence of sex, year, and breed on the unpigmented areas in the head area appears rather difficult to explain. The influence of stud can be explained by a period, during which horses from the National Stud were bred separately from other studs and different sires were used for each herd.

Estimate of genetic parameters. The estimated heritability for the melanoma level (h^2 = 0.07 ± 0.0384) (Table 4) is very low in comparison to the data from available literature (Solkner et al. 2004, Curik et al. 2013). Such discrepancy might be explained by the fact that all age groups of OKH were used for the estimation, including young horses that do not exhibit any melanoma.

When excluding data of young horses, a higher value of heritability coefficient could be assumed in accordance with literature. Solkner et al. (2004) reported h^2 of 0.36 for Lipizzan horses over 6 years

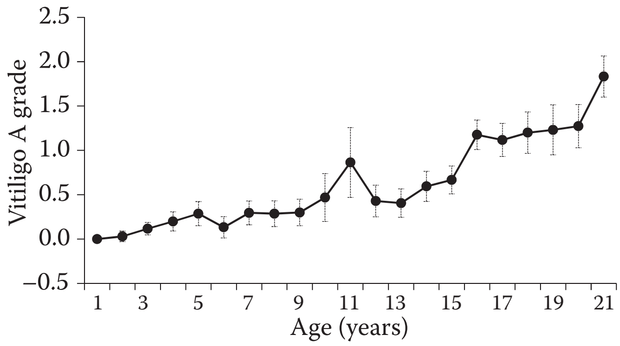

Figure 3. The age and vitiligo A relationship

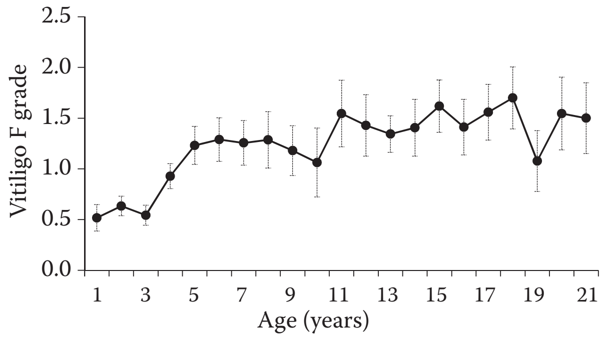

Figure 4. The age and vitiligo F relationship

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Table 6. Vitiligo A grade in lines

Line	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

G = Generale, G-Gss = Generale-Generalissimus, F-Gss = Favory-Generalissimus, F = Favory, S = Sacramoso, R = Rudolfo
mean values on the diagonal, differences between lines above the diagonal
*significant difference, **highly significant difference

of age, while the dataset enrolling also young animals gave h^2 of only 0.12. Seltenhammer et al. (2003) estimated a heritability of 0.36. Curik et al. (2013) stated a narrow sense total heritability h^2 = 0.37. In the threshold model by Vostry et al. (2012) low heritability (h^2 = 0.11 ± 0.05) for melanoma in OKH was estimated.

Despite the high variability of greying level, the L^* parameter reaches a mean value of heritability coefficient (h^2 = 0.52 ± 0.07), which corresponds to a significant genotype effect on the loss of pigment.

The depigmentation of head (vitiligo F) showed a mean value of heritability coefficient (h^2 = 0.35 ± 0.06), which supports the claim about a different determination of head depigmentation compared to that in back body parts (vitiligo A), which is characterized by a low value of heritability coefficient (h^2 = 0.20 ± 0.05). It is not possible to directly compare the estimated heritability coefficients with those previously published by Curik et al. (2002, 2004, 2013) and Solkner et al. (2004), because the authors did not assess depigmentations of head and back parts separately.

Phenotypic correlations between the variables melanoma, greying level (L^*), vitiligo A, and vitiligo F were estimated using Pearson's correlation coefficient. Relations between the monitored indicators are given by a mean value for vitiligo A and vitiligo F (r_P = 0.31), vitiligo A and melanoma (r_P = 0.41), for the relationship between melanoma and vitiligo F the value is very low (r_P = 0.17). As indicated by the values of phenotypic correlations, there is a clear positive correlation between the vitiligo A depigmentation and the grade of melanoma, i.e. in horses with higher levels of vitiligo in the back parts a higher level of melanoma can be anticipated. The level of the relationship between head depigmentation (vitiligo F) and back parts depigmentation (vitiligo A) is surprisingly low. This fact together with low correlation values between depigmentation on the head and the melanoma grade suggests a possibility of different determination of depigmentation in the head and depigmentation of back parts of the body.

Compared to the generally accepted thesis on the relationship between the level of greying and the incidence of melanoma (Fleury et al. 2000a, b; Seltenhammer et al. 2003), the value of genetic correlation in OKH is very low (r_G = 0.02 ± 0.21). This finding is completely consistent with the third scenario results (covering *GG* genotype horses

Table 7. Vitiligo F grade in lines

Line	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

G = Generale, G-Gss = Generale-Generalissimus, F-Gss = Favory-Generalissimus, F = Favory, S = Sacramoso, R = Rudolfo
mean values on the diagonal, differences between lines above the diagonal
*significant difference, **highly significant difference

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only) of Curik et al. (2013) who show the incidence of melanoma does not correlate with the level of greying. The relationship between vitiligo incidence and melanoma is clearly genetically conditioned in the case of vitiligo A (r_G = 0.38 ± 0.15), whereas in the case of vitiligo F the relationship has not been confirmed (r_G = 0.01 ± 0.16). This result also suggests that the depigmentation of head and back possesses a different character. A closer relationship between the incidence of melanoma and vitiligo A is also given by the fact that both occur at identical locations of the animal body. Values of genetic correlation (r_G = 0.67 ± 0.11 and 0.53 ± 0.10, respectively) confirm a genotype conditioned relationship between greying levels (L^*) and both vitiligo types. The genetic correlation between vitiligo on the head and vitiligo in the back part is characterized by a mean value (r_G = 0.54 ± 0.08).

Despite the fact that our results well correspond with those of the cumulative study by Curik et al. (2013), especially with their third scenario covering *GG* genotype horses only, all Old Kladruber grey horses under our study are surely not homozygous. Out of the 50 breeding mares tested for *STX17* mutation, 26% were heterozygous (*GG*), moreover, some sires must be heterozygous, too, because non greying foals are rarely born.

CONCLUSION

The prevalence of dermal melanoma in OKH population was confirmed and its extent and grade is significantly dependent on the effects of age and line. In the affected horses the lowest grades of melanoma (grade 1 and/or 2) predominated without any negative influence on their health status. In the population under study, neither the highest grade (5), nor the malignant state of melanoma were detected. A practical recommendation is that the breeders should monitor and record any melanoma occurrence in horse population, it does not seem necessary to restrict the grey coat colour or to favour other coat colours. The case of occurrence of high grade melanoma in young stallion or mare is the reason to restrict their exploitation in reproduction or select them out of the breeding plans totally.

The relationship between the level of greying and the grade of melanoma was not proved. The level of greying showed moderate heritability, that is why breeding horses with a high level of greying can be recommended.

The occurrence of vitiligo in greys is a common condition. The analysis in the OKH population showed a positive genetic correlation between vitiligo in the anal parts and the grade of melanoma, on the contrary, the relationship between facial vitiligo and melanoma was not proved. Different values of genetic parameters for both facial and anal vitiligo could assume the possibility of different determination for vitiligo in different body parts. This assumption should be checked by further studies.

REFERENCES

Bertone J.J. (2006): Equine Geriatric Medicine and Surgery. Elsevier, St. Louis, USA.

Curik I., Seltenhammer M., Solkner J. (2002): Quantitative genetic analysis of melanoma and grey level in Lipizzan horses. In: Proc. 7th World Congress on Genetics Applied to Livestock Production, Montpellier, France, 19–23.

Curik I., Seltenhammer M., Toth S., Niebauer G., Solkner J. (2004): Quantitative inheritance of the coat greying process in horse. In: Proc. 52th Annu. Mtg., European Association for Animal Production (EAAP), Bled, Slovenia, 316.

Curik I., Druml T., Seltenhammer M., Sundstrom E., Pielberg G.R., Andersson L., Solkner J. (2013): Complex inheritance of melanoma and pigmentation of coat and skin in grey horses. PLoS Genetics, 9, e1003248.

Falconer D.S., Mackay T.F.C. (1996): Introduction into Quantitative Genetics. Longman, Harlow, UK.

Fleury C., Berard F., Balme B., Thomas L. (2000a): The study of cutaneous melanomas in Camarque-type gray skinned horses (1): clinical-pathological characterization. Pigment Cell Research, 13, 39–46.

Fleury C., Berard F., Leblond A., Faure C., Ganem N., Thomas L. (2000b): The study of cutaneous melanomas in Camargue-type gray skinned horses (2): Epidemiological survey. Pigment Cell Research, 13, 47–51.

Futas J., Vychodilova L., Hofmanova B., Vranova M., Putnova L., Muzik J., Vyskocil M., Vrtkova I., Dusek L., Majzlik I., Horin P. (2012): Genomic analysis of resistance/susceptibility to melanoma in Old Kladruber greying horses. Tissue Antigens, 79, 247–248.

Heizerling L.M., Feige K., Rieder S., Akens M.K., Dummer R., Stranzinger R., Moelling K. (2001): Tumor regression induced by intratumoral injection of DNA coding for human interleukin 12 into melanoma metastase in grey horses. Journal of Molecular Medicine, 78, 692–702.

Henner J.E. (2002): Molecular genetic studies on the coat colour inheritance in horses with special emphasis on Freiberger breed. PhD Diss. Zürich Univ. 119 p. Available from: http://aleph-www.ub.fu-berlin.de. (in German)

doi: 10.17221/8524-CJAS

Henner J.E., Poncet P.A., Guerin G., Hagger C., Stranzinger G., Rieder S. (2002): Genetic mapping of the G-locus, responsible for the coat color phenotype "progressive greying with age" in horses (Equus caballus). Mammalian Genome, 13, 535–537.

Horin P., Cothran E.G., Trtkova K., Marti E., Glasnak V., Henney P., Vyskocil M., Lazary S. (1998): Polymorphism of Old Kladruber horses, a surviving but endangered baroque breed. European Journal of Immunogenetics, 25, 357–363.

Jakubec V., Vostry L., Schlote W., Majzlik I., Mach K. (2009): Selection in the genetic resource: genetic variation of the linear described type traits in the Old Kladrub horse. Archiv Tierzucht, 52, 343–355.

Janova E., Futas J., Klumplerova M., Putnova L., Vrtkova I., Vyskocil M., Frolkova P., Horin P. (2013): Genetic diversity and conservation in a small endangered horse population. Journal of Applied Genetics, 54, 285–292.

Jeglum K.A. (1999): Melanomas. In: Robinson N.E. (ed.): Current Therapy in Equine Medicine. W.B. Saunders Company, Philadelphia, USA, 399–400.

Jorjani H., Klei L., Emanuelin U. (2003): A simple method for weighted bending of genetic (co)variance matrix. Journal of Dairy Science, 86, 677–679.

Kovac M., Groeneveld E., Garcia-Cortes L.A. (2002): VCE 5, a package for the estimation of dispersion parameters. In: Proc. 7th World Congress on Genetics Applied to Livestock Production. INRA, Castanet-Tolosan, France, 33, 741–742.

Locke M.M., Penedo M.C.T., Bricker S.J., Millon L.V., Murray J.D. (2002): Linkage of the grey coat colour locus to microsatellites on horse chromosome 25. Animal Genetics, 33, 329–337.

Pielberg G.R., Golovko A., Sundstrom E., Curik I., Lennartsson J., Seltenhammer M.H., Druml T., Binns M., Fitzimmons C., Lindgren G., Sandberg K., Baumung R., Vetterlein M., Stromberg S., Grabbher M., Wade C., Lindblad-Toh K., Ponten F., Heldin C.H., Solkner J., Andersson L. (2008): A cis-acting regulatory mutation causes premature hair graying and susceptibility to melanoma in the horse. Nature Genetics, 40, 1004–1009.

Rieder S. (1999): Applied comparative genetics of melanoma using the horse as an example. PhD Diss. Zürich Univ. 132 p. Available from: https://e-collection.library.ethz.ch/view/eth:22891. (in German)

Rieder S., Stricker C., Joerg H., Dummer R., Stranzinger G. (2000): A comparative genetic approach for the investigation of aging grey horse melanoma. Journal of Animal Breeding and Genetics, 117, 73–82.

Rodriguez M., Garcia-Barona V., Pena L., Castano M., Rodriguez A. (1997): Grey horse melanotic condition: a pigmentary disorder. Journal of Equine Veterinary Science, 17, 677–681.

Seltenhammer M.H., Simhofer H., Scherzer S., Zechner P., Curik I., Solkner J., Brandt S.M., Jansen B., Pehamberger H., Eisenmenger E. (2003): Equine melanoma in a population of 296 grey Lipizzan horses. Equine Veterinary Journal, 35, 153–157.

Seltenhammer M.H., Herre-Ress E., Brandt S., Druml T., Jansen B., Pehamberger H., Niebauer G.W. (2004): Comparative histopathology of grey-horse melanoma and human malignant melanoma. Pigment Cell Research, 17, 674–681.

Smith S.H., Goldschmidt M.H., McManus P.M. (2002): A comparative review of melanocytic neoplasm. Veterinary Pathology, 39, 651–678.

Solkner J., Seltenhammer M., Curik I., Niebauer G. (2004): Genetic relationship between speed of greying, melanoma and vitiligo prevalence in grey horses. In: Proc. 52th Annu. Mtg., European Association for Animal Production (EAAP), Bled, Slovenia, 83.

Sponenberg P. (1996): Equine Color Genetics. Iowa State University Press, Ames, USA.

Swinburne J.E., Hopkins A., Binns M.M. (2002): Assignment of the horse grey coat colour gene to ECA25 using whole genome scanning. Animal Genetics, 33, 338–342.

Toth Z., Kaps M., Solkner J., Bodo I., Curik I. (2006): Quantitative genetic aspects of coat color in horses. Journal of Animal Science, 84, 2623–2628.

Valentine B.A. (1995): Equine melanotic tumors: a retrospective study of 53 horses (1988–1991). Journal of Veterinary Internal Medicine, 9, 291–297.

Vostry L., Kracikova O., Hofmanova B., Czernekova V., Kott T., Pribyl J. (2011): Intra-line and inter-line genetic diversity in sire lines of the Old Kladruber horse based on microsatellite analysis of DNA. Czech Journal of Animal Science, 56, 163–175.

Vostry L., Hofmanova B., Vostra Vydrova H., Pribyl J., Majzlik I. (2012): Estimation of genetic parameters for melanoma in the Old Kladruber horse. Czech Journal of Animal Science, 57, 75–82.

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