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Genetic relationships between speed of greying, melanoma and vitiligo prevalence in grey horses

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Abstract

The quantitative inheritance of grey level, vitiligo and melanoma prevalence was analyzed on 725 Lipizzan horses born in five state studs (Austria, Croatia, Hungary, Slovakia and Slovenia). Melanoma status (levels 0-5) was recorded for 5 years, greying level (L* parameter of the CIE L*a*b system) for 4 years and vitiligo status (levels 0-3) for 2 years. Heritabilities and genetic correlations were estimated by multivariate animal model REML. As differences in speed of greying are expressed at young ages whereas melanoma and vitiligo tend to develop at later ages, data for level of greying (573 obs.) were restricted to ages of 1-6 years, whereas data on melanoma (700 obs.) and vitiligo (379 obs.) were only included for horses older than 6 years. Estimated heritabilities were 0.76±0.07 for level of greying, 0.32±0.10 for melanoma status and 0.33±0.07 for vitiligo status. The genetic correlation between speed of greying and vitiligo was high (0.66±0.10), whereas the others were very close to zero.

Introduction

Congenital and acquired proliferations of melanocytes are well recognised and are common skin tumours in humans as well as in many animal species (horse, cattle, pig, dog and mouse). Equine melanoma most commonly occur in grey horses at the age of five to six years or more (Jeglum, 1999). It has been estimated that, independently of gender, 80 % of grey horses older than 15 years develop melanoma (McMullan, 1982; Valentine, 1995; Jeglum, 1999). Other authors found 67% of Camargue (Fleury et al. 2000) and 75% of Lipizzan (Seltenhammer et al., 2003) horses older than 15 years bearing melanoma. Tumours occur most frequently underneath the tail and at high rates in the peri-anal region, lips, and eyelids (Fleury et al., 2000). The high incidence of melanoma in grey horses is thought to be related to age-associated depigmentation events (Mayr et al., 1979; Niebauer, 1980; Desser et al., 1980; Altmeyer et al., 1984; Scott, 1988; Rieder, 1999).

Melanoma in grey horses have three distinct, histopathologically unclassified clinical patterns: The first type grows slowly over many years without evidence of regional or distant metastases and is observed in the majority of cases. The second type results from malignant transformation of a benign melanoma (melanocytoma; Foley et al., 1991). The third type is malignant from the onset, but is rare (Pulley and Stannard, 1990). Although most melanoma in grey horses have benign features when they come to diagnosis, 66 % of the tumours can turn malignant later on (Gorham and Robl, 1986). In contrast to greys (Camargue, Lipizzan, etc.), solid coloured horses (chestnut, brown, bay or black horses) are rarely affected by melanotic tumours. However, melanoma in solid coloured horses can also affect very young animals, and is often highly malignant (Foley et al. 1991; Valentine 1995) with human melanoma like features.

The hereditary component of melanoma in greying horses was first studied by Rieder et al. (2000) and Seltenhammer et al. (2003). Using segregation analysis, Rieder et al. (2000) were not able to establish the mode of inheritance (single gene, polygenic inheritance, mixed inheritance), although models including a polygenic component fitted the data significantly better than a non-genetic model. The number of horses examined in this study (71) was not informative enough to provide conclusions about the mode of inheritance. On a data set including 296 grey Lipizzan horses, Seltenhammer et al. (2003) estimated a heritability of 0.36 for melanoma status.

The fact that melanoma prevalence and disease history is quite opposite in grey versus solid coloured horses indicates that the prevalence of melanoma has a hereditary component and the processes of coat colour dilution (grey horses are born black and change coat colour to white relatively early in age) and tumour development may be linked. The greying dynamics of horses was studied by Curik et al. (2002, 2004), using chromametric measurements of the level of greying with the L* parameter of the L*a*b* colour space, defined CIE (Commission Internationale de l'Eclairage, 1976). This measurement was linked to melanin content in the skin, evaluated by spectrophotometer by Toth et al. (2004). Curik et al. (2002) found in a data set of 351 horses which was extended from the data set used by Seltenhammer et al. (2003) a heritability value of 0.24±0.11 for melanoma and of 0.46±0.09 for level of greying, with a genetic correlation of 0.46±0.25.

Vitiligo is a depigmenting disorder characterized by the development of white patches in various distributions, which are due to the loss of melanocytes from the epidermis. It has been well studied in humans, and is believed to be an autoimmune disorder (Ongenae et al., 2003). It has been observed in many animal species (e.g., mouse, dog, pig, cattle, water buffalo, chicken and horse). A genetic connection of melanoma and vitiligo in grey horses, where it appears mostly in the form of patches of depigmentation around the anus and genital regions, but also in the face, has been much speculated about (Lerner and Cage, 1973; Gebhart and Niebauer, 1977; Levene, 1980; McMullan, 1982; Fleury et al., 2000; Seltenhammer, 2000). The hypothesis has not yet been tested on a sufficiently large data set.

The aim of this study is to quantify the genetic relationship between melanoma, greying and vitiligo on a comparatively large data set collected at repeated visits over a period of five years from Lippizan horses at the state studs of Austria, Croatia, Hungary, Slovakia and Slovenia. The classical approach of estimating heritabilities and genetic correlations is a first step in trying to unravel genetic mechanisms involved in the formation of all three phenomena.

Data and methods

Samples recorded

Data were recorded at five state studs breeding Lipizzan horses. These are Piber (Austria), Djakovo (Croatia), Szilvasvarad (Hungary), Topol'cianky (Slovakia) and Lipica (Slovenia). Of the major studs in Lipizzan breeding, only Monterotondo (Italy) and Fagaras (Romania) did not participate in the study. The original population screened for melanoma in 1998 and 1999 was part of an EU-Copernicus project on genetic diversity in the Lipizzan horse (e.g., Zechner et al., 2001 and 2002, Kavar et al., 2003, Curik et al., 2003, Achmann et al., 2004). The studs were repeatedly visited, the narrowest sampling frame was followed in Piber. Melanoma grades were evaluated from 1998 to 2003, greying was measured from 2001 to 2003 and vitiligo grades were determined 2002 and 2003 (Tables 1-3).

Table 1: Sampling of melanoma phenotypes

	CRO	SLO	AUT	HUN	SLK	Total
1998	-	-	X	-	-	29
1999	X	X	X	X	X	70
2001	X	-	X	X	X	382
2002	-	X	X	-	-	502
2003	X	-	X	X	X	344
N	143	162	798	92	132	1327

Table 2: Sampling of greying phenotypes

	CRO	SLO	AUT	HUN	SLK	Total
2001	X	-	X	X	X	379
2002	-	X	X	-	-	497
2003	X	-	X	X	X	343
N	128	150	741	77	123	1219

Table 3: Sampling of vitiligo phenotypes

	CRO	SLO	AUT	HUN	SLK	Total
2002	-	X	X	-	-	310
2003	X	-	X	X	X	214
N	39	150	242	51	42	524

Definition of level of greying

Coat colour was measured on four places (neck, shoulder, belly and croup) for each horse. The grey level was determined with a Minolta Chromameter CR210, using the CIE L*a*b* colour space. In this system the colour is quantified according to 3 axes: white-black (L*), red-green (a*) and yellow-blue (b*). As our intention was to quantify the grey level, the parameter L* expresses this trait. The scale reaches from 0 (black) to 100 (white). The average of the four measurements defined above was used in this analysis. The CIE L*a*b* system has already been applied in human studies related to the tanning of skin (Adhoute et al. 1994) as well as in meat science for determination of the meat colour (e.g., Hopkins and Fogarty 1998). To our knowledge, the method was not yet applied for the quantitative determination of the coat colour in animals. For an example of the variation of level of greying in foals, see Figure 1.

Figure 1: Brood mares with foals at Piber

Definition of melanoma grades

Melanoma were detected by adspection and palpation. In relation to their clinical picture, patients were classified according to a modified classification system (allowing for the intermediate marks, 0.0, 0.5 ... 4.5, 5.0) by Desser et al. (1980), see Table 4.

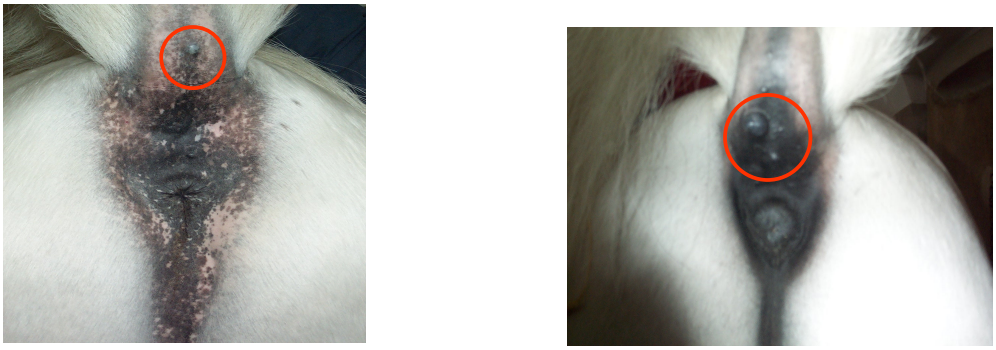
Table 4: Clinical classification and incidence of melanoma in grey horses.

Grade	Description
0	Free of melanoma.
1	Early stages of plaque-type or one solitary nodule of 0.5 cm diameter situated on typical locations.
2	Several nodules of 0.5 cm diameter or one solitary nodus of 2 cm diameter on typical locations.
3	One or several nodular melanoma of 5 cm diameter intra- and/or subcutaneous on typical locations (or lips).
4	Extensive confluent melanoma, covered with skin, signs of destruction (necrosis, ulceration) and metastasis.
5	Exophytic growth of tumours, which show wet surface and ulceration, metastasis into different organs accompanied by paraneoplastic syndromes (cachexia, fever, metabolic disorders).

Examination by adspection included sites where melanoma typically occur, such as the peri-anal and anal region, the perineal region, udder, and praeputium. The tail was bent upwards in order to discover even the smallest, plaque-like lesions. Lips and eyelids, but also parotis, peri-ocular region and ears were equally examined. Finally, the whole integument was checked for potential tumours. Pictures of horses with grades 1 and 3 are given in Figure 2.

Figure 2: Examples of horses with melanoma grades 1 and 3.

Grade 1 Grade 3



Definition of vitiligo grades

Vitiligo was graded by adspection of typical sites, such as the peri-anal and anal region, the perineal region, udder, praeputium and the face, especially around the nostrils and eyes. It was performed simultaneously with melanoma adspection, and grades from 0 (no vitiligo) to 3 (severe vitiligo) were given. In 2002, an overall grade including vitiligo patches in the perianal and the facial regions was given. In 2003, the parianal and facial regions were graded separately but averages were used for analysis. Figure 3 gives typical examples of grades 0 to 3 for the perianal region.

Figure 3: Examples of horses with vitiligo grades 0 to 3 (perianal region).

Grade 0 Grade 1 Grade 2 Grade 3

Statistical methods

Heritabilities and genetic correlation were estimated with the REML VCE package Version 4.2.5., which optimises the log likelihood by analytical gradients for covariance matrices of different sizes (Groeneveld 1998). The data were analysed with a multivariate mixed linear model including a stud x year x sex interaction and

age at measurement in years as a polynomial covariate with linear, quadratic and cubic components. Random effects included were the additive genetic animal effect and, as measurements were taken repeatedly, permanent environment. The pedigree file was extremely complete, reaching back till the 18th century (see Zechner et al., 2002). Most of the information was coming from paternal half sib groups (maximum number of paternal half sibs was 27), but some also from maternal half sib groups (maximum number of maternal half sibs was 27).
The variation of the three traits with age is very different, as visualised in Figures 4-6.

Figure 4: Means and standard deviations of the L* parameter of greying.

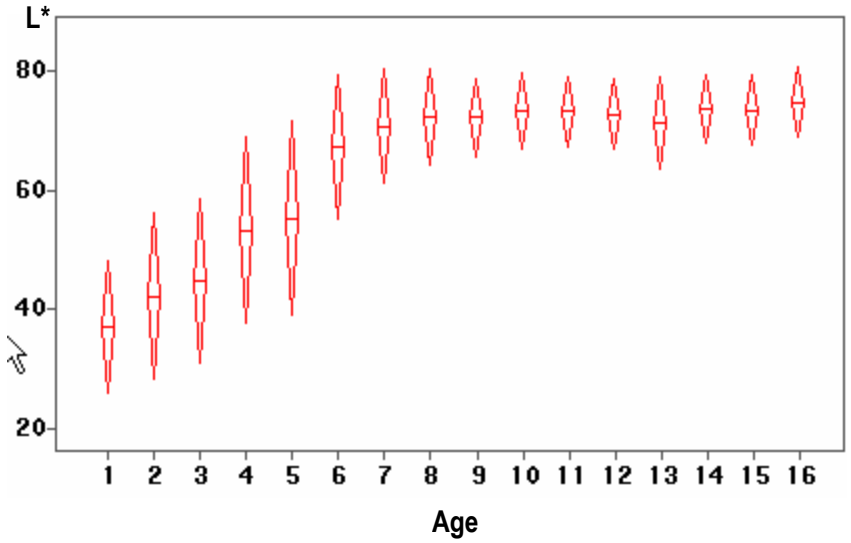


Figure 4: Means and standard deviations of melanoma grade.

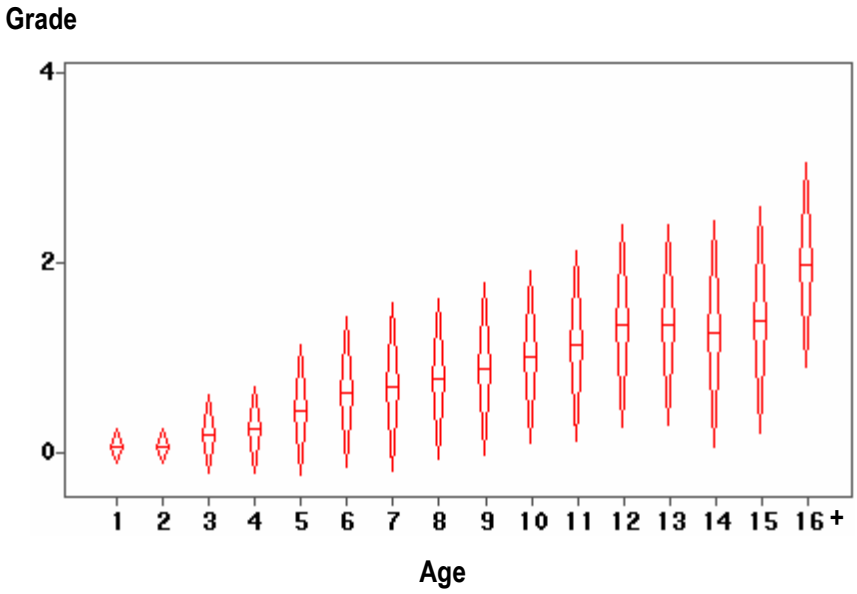
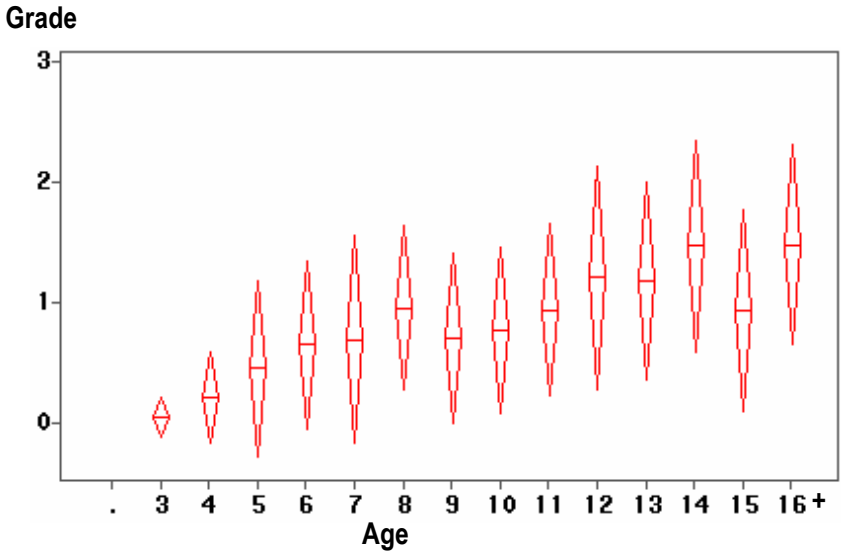


Figure 5: Means and standard deviations for vitiligo.



Greying has a large variation up to 6-10 years of age. At the age of 10, all horses have reached their final colour (see Curik et al., 2004). When studying the genetic variation in speed of greying, data have therefore to be restricted to age classes where there is still sufficient genetic variation. Melanoma and depigmentation (vitiligo) start later in life. Again, to have sufficient variation, data have to be cut. As the cut-off point is not really clear from the graphs, two different data sets were created for estimation of genetic parameters. In the first, animals younger than seven years were considered for greying and animals older than 6 years for melanoma and vitiligo. The second data set consisted of records from horses younger than 8 years (greying) and older than 4 years (melanoma and vitiligo). Table 5 provides information on the number of horses and records in the two analyses.

Table 5: Number of horses and number of records available for REL analyses with two sets of data restrictions.

	Grey < 7, Mel, Vit >6		Grey < 8, Mel, Vit >4	
	Horses	Records	Horses	Records
Greying	378	573	426	646
Melanoma	361	700	476	892
Vitiligo	316	379	397	469

Results and Discussion

Genetic parameters estimated with data constrained by the two types of age restrictions are presented in Tables 6 and 7.The heritability for the process of greying is extremely high (0.76). The estimate of Curik et al. (2002) was lower (0.46). This is related to the fact that for that analysis, horses of all ages were considered ignoring the reduction of variance for older horses, i.e. horses where the process of greying had already finished. When we performed a comparable analysis with the current data set, the heritability estimate was 0.49. The data available do not allow statements on how much of this variation is related to the genotype at the grey locus (homozygous GG versus heterozygous Gg) as genotypic information was not

available. The frequency of the g allele in the population is not very high, though, probably in the range of 0.10. Both data sets yielded similar heritabilities. Curik et al. (2004) show that there are also genetic differences in the “final” colour of horses. For a data set including only horses older than 6 years, the heritability was 0.58±0.03. This may be related to a colour phenomenon called “speckled”, “flea-bitten” or “mosquito-bitten” (Bowling, 2000, Toth et al., 2004) which is quite frequent in Lipizzan horses. This trait was recorded (again in grades from 0 to 3) from 2002 on but was not included in this analysis.

Table 6: Heritabilities (diagonal, upper row), proportion of variance explained by permanent environmental effects (diagonal, lower row), genetic correlations (off-diagonals, upper) and permanent environmental correlations (off-diagonals, lower) for the data set 1 (Grey < 7, Mel, Vit >6).

	Greying	Melanoma	Vitiligo
Greying	0.76 ± 0.07 0.13 ± 0.07	-0.03 ± 0.12	0.66 ± 0.10
Melanoma	0.69 ± 0.31	0.36 ± 0.10 0.32 ± 0.09	0.01 ± 0.20
Vitiligo	0.98 ± 0.08	0.55 ± 0.17	0.36 ± 0.07 0.33 ± 0.08

Table 7: Heritabilities (diagonal, upper row), proportion of variance explained by permanent environmental effects (diagonal, lower row), genetic correlations (off-diagonals, upper) and permanent environmental correlations (off-diagonals, lower) for the data set 2 (Grey < 8, Mel, Vit >4).

	Greying	Melanoma	Vitiligo
Greying	0.76 ± 0.05 0.10 ± 0.05	0.10 ± 0.14	0.69 ± 0.09
Melanoma	0.89± 0.15	0.19 ± 0.07 0.41 ± 0.06	-0.06 ± 0.21
Vitiligo	0.86 ± 0.17	0.54 ± 0.12	0.32 ± 0.07 0.36 ± 0.07

The heritability of melanoma grade is moderate (0.36) with a stronger restriction towards older horses and substantially lower (0.19) when including horses 5 and 6 years old. When including all horses, the heritability dropped to 0.12. The heritability implies a rather strong genetic basis for melanoma prevalence that might be explored with molecular methods. Vitiligo shows heritability similar to that of melanoma, with a lower drop when including younger horses.

The most surprising results concern the genetic correlations between traits. Melanoma is virtually uncorrelated to the level (speed) of greying and to vitiligo. This is against common but untested hypotheses (Lerner and Cage, 1973; Gebhart and Niebauer, 1977; Levene, 1980; McMullan, 1982; Fleury et al., 2000; Seltenhammer, 2000). The positive relationship between speed of greying and melanoma (0.46±0.25) shown by Curik et al. (2002) was obviously an artefact related to improper use of data of young and old horses for both traits. A similar analysis for the

current data set yielded a correlation of 0.27±0.10. Vitiligo is highly correlated with the speed of greying, a relation that has not even been speculated about.
The permanent environmental variance components and correlations are high for all traits. This is not surprising as a progressive development with age was observed for all traits. Whether spontaneous regression of melanoma is possible is topic of a separate study.

Conclusions

This paper provides the first reasonably reliable estimates of genetic parameters for speed of greying, melanoma and vitiligo in grey horses. It provides the basis for further research on genetic mechanisms involved in the expression of these traits. The most intriguing results are the very high heritability of speed of greying, the non-existence of a genetic correlation between speed of greying and melanoma and the high correlation between speed of greying and vitiligo.
All three phenomena have counterparts in human. The histopathology of human and equine vitiligo is very similar (Montes et al., 2003), implying that it is an autoimmune disorder in both species and that candidate genes will likely be homologous. The frequency of vitiligo is much higher in grey horses than in humans. This is advantageous for a genome scan. For speed of greying, there is an obvious link to human genetics. Hair depigmentation in humans is a complex process which is being more and more understood (Van Neste and Tobin, 2004). Again, the mechanisms are probably similar and familial information available in the Lipizzan horse might help to identify genetic factors responsible for premature greying of hair follicles. The fact that greying and vitiligo are closely related in grey horses adds a component not yet thought of. The dermal melanoma in grey horses are similar to blue nevi in humans which led some authors (e.g., Lerner and Cage, 1973, Sutton and Coleman, 1993) to consider melanoma in grey horses not as neoplasms but as a kind of pigment storage disorder. That this kind of melanoma is actually a true neoplasm was shown by studies on lymphocyte, polyamine and histamine levels (e.g., Desser et al., 1980, Niebauer, 1980) and histology and immunochemistry (Rieder et al., 2000, Seltenhammer, 2000). How much a genetic dissection of equine melanoma of this type will help to understand human melanoma is questionable but it will add to the general knowledge and understanding of skin diseases.
One advantage of this study is its strong longitudinal component with data collection continuing. Serum samples have also been collected for evaluation of blood metabolites involved in the pigmentation process. This offers many opportunities for future research.

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