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**THESES OF DOCTORAL (Ph.D) DISSERTATION**

**ANALYSIS OF HORSE COAT COLOUR USING QUANTITATIVE GENETIC  
METHODS**

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**Debrecen  
2006**

## I. INTRODUCTION

Considering the appearance of phenotype, coat colour has had a significant role during the evolution of some animal species. While many wild animal species are relatively uniformly coloured, a wide variety of coat colours are found in domestic animals. Due to natural selection and human expectation, the number of colour variations has considerably been increased, thus domestic animals are proved to be an adequate research basis in studying the function and regulation process of certain genes responsible for pigmentation.

The colour, as a single exterior trait, in certain cases, is a definite characteristic and plays an important role in horse breeding and in the fields of equestrian. Nowadays the analysis of the mechanism of coat colour inheritance is significant. As the aesthetic views are becoming more important, certain coat colours earn high market value. Moreover, colour inheritance can be applied for horse identification and pedigree-control.

Coat colour of horses is determined by a pair of genes located to 6-8 loci, whose mode of inheritance and intra- and interlocal (epistatic) interactions have been revealed by molecular genetic methods. In the basic set of horse colours, several varieties, shades and states can be observed, which refer to the simultaneous effect of many genes or gene expression emerging differently in individuals. Because of allele interactions, some colours remain in the background, while others are significantly shown or altered, diluted, getting light or become colourless. In some cases, the environment can also influence colours and shades.

To treat the coat colour as a categorical trait, the subjective aspect can lead to several misinterpretations which can be eliminated by using chromameter. With the three coordinate CIE  $L^*$   $a^*$   $b^*$  (Commission International d'Eclairage) system of chromameter the intensity as well as the saturation of colour can be measured. The results of CURIK et al. (2002) firstly proved, that the method enables to quantify the coat colours of horses, with which quantitative characterization of several biological processes (such as inheritance, pigmentation) can be investigated.

Considering the quantitative and qualitative traits, it can be stated that the biochemical and genetic background of pigmentation determining the phenotypic variability is remarkably complex.

Despite the general accepted interpretation, that only major genes play role in the forming of the coat colour, the polygenic and environmental factors have similar significant effect. The evolution process of mammals presents an excellent example for this, because during acclimatization to environmental conditions, the coat colour has been determined mainly by major genes. This process can be interpreted in long term. The recent experienced large phenotypic variability clearly verifies the importance of polygenic effects.

Nowadays researches related to the coat colour apply mainly the scientific achievements of molecular genetics; while new knowledges dealing with the background of the quantitative genetics have been broaden as well. Analyses based on objective measurements enable to report new results which serve supplement in the field of the factorial genetics. Recent developments in the field of molecular genetics and statistical analyses combined with the approach presented in the current dissertation offer new perspectives for better understanding of coat colour inheritance as a trait with multiple contributing factors.

## II. AIMS OF THE RESEARCH

During a three years period (2003-2005) of our research work analysis of the horse coat colour using quantitative genetic methods was carried out. Except the investigation of the background of progressive graying in horses which presents the results of a four years period (2001-2004). This was a common project connected to the work of an international research group. For experimental work all data were collected in five Hungarian state studs (Szilvásvár, Hortobágy, Bábolna, Toponár and Marócpuszt Stud Farms) and in four foreign studs (Piber, Djakovo, Topolcanky and Lipica) as well.

The four main aims of the present dissertation were:

**1. Analyse the progressive graying process in gray horses:**

- biological background of the graying.

**2. Analyse hair melanin content in horses:**

- spectrophotometric determination of total melanin content of horse hair,
- analysis of the relationship between total melanin content of horse hair and colour traits.

**3. Analyse the relationship between instrumental colour measurements and subjective classification:**

- objective segregation of different colour classes,
- importance of colour trait variables,
- analysis of the classification according to basic colours.

**4. Analyse the genetic and environmental factors influencing the coat colour of horses**

- analysis of environmental factors influencing the coat colour,
- analysis of genetic factors influencing the coat colour,
  - analysis of the role of major genes and poligenic effects in coat colour inheritance.

### III. MATERIALS AND METHODS

Analysis of the progressive graying process was carried out in case of Lipizzan horses (n=706). During a four years period (2001-2004) repeated measurements were taken in five state studs (Table 1).

**Table 1.**

**Number of Lipizzan horses by national studs**

Country	Stud	Horse
Croatia	Djakovo	89
Austria	Piber	310
Hungary	Szilvásvár	77
Slovakia	Kistapolcsány	82
Slovenia	Lipica	148
Total		<b>706</b>

The graying dynamics was analyzed by fitting nonlinear functions (Table 2) to individual records or mean values of age groups (in years, except for year 16 which included all older horses). The following nonlinear functions were applied: general growth, Brody, logistic, Gompertz, Bertalanffy and Richards.

**Table 2.**

Function	Képlet
General growth	Graying = $a \cdot (1 - \exp(-k \cdot \text{age})/s)^{**}s$
Brody	Graying = $a \cdot (1 - s \cdot \exp(-k \cdot \text{age}))$
Logistic	Graying = $a / (1 + s \cdot \exp(-k \cdot \text{age}))$
Gompertz	Graying = $a \cdot \exp(-\exp(-k \cdot \text{age}))$
Bertalanffy	Graying = $a \cdot (1 - (\exp(-k \cdot \text{age})/3))^{**}3$
Richards	Graying = $a / ((1 + s \cdot \exp(-k \cdot \text{age}))^{**}m)$

a = asymptote, s = shape coefficient, k = growth rate; m = parameter defining relative position of the inflection point

For studying the coat colour inheritance, genetic and environmental effects influencing the colour as well as the spectrophotometric methods besides the results of Hungarian Lipizzan horses, other coloured horses from different breeds were also included (Table 3). Horses were repeatedly measured in summer and winter coat during the period of 2003-2005.

**Table 3.**

<b>Number of horses by breed</b>		
<b>Stud</b>	<b>Breed</b>	<b>Horse</b>
Bábolna	Arabian Pure Bred	32
Bábolna	Shagya Arabian	67
Toponár	Shagya Arabian	28
Szilvásvárada	Lipizzan	81
Marócpusztá	Gidran	40
Debrecen	Gidran	7
Hortobágy	Nonius	71
<b>Total</b>		<b>326</b>

By visual differentiation of colours, horses were scored by three people and grouped into 9 subjective classes: light gray, flea-bitten gray, dark gray, light-dark chestnut, bay, brown, dark brown, black. For objective measurement of coat colour the Commission Internationale de l'Eclairage, CIE L\*a\*b\* system was applied using Minolta Chromameter (Model CR-310). The values of L\* indicate light intensity and are related to the 'luminous reflectance' (quantity of reflected light weighted with the spectral response of the human eye) and take values from 0 (black) to 100 (white). The values of a\* measure the colour saturation from red to green on the scale from +60 to -60, where positive values indicate varying intensities of red; b\* measures the colour saturation from yellow to blue on the scale from +60 to -60, where positive values indicate varying intensities of yellow. The a\* and b\* coordinates were converted into polar coordinates defined as Chroma and referred to as saturation of colour,  $Chroma = [(a^*)^2 + (b^*)^2]^{1/2}$ . Chroma describes the intensity of colour, with higher Chroma indicating greater intensity. In the study of the graying process colour was measured on the right side of the body at four places (neck, shoulder belly and croup). For all other investigations measurements of three places (neck, shoulder and belly) were used.

Analysis of the total melanin content in horse hair was carried out using GENESYS 2 (Spectronic Instruments) spectrophotometer, based on the methods of OZEKI et al. (1996). For quantitative estimation of melanin 54 hair samples (six randomly chosen samples from each colour class, out of the total number of horses) were dissolved in 1 ml of a mixture of Soluene-350 : water = 9 : 1 (v/v) by heating in boiling bath for 45 minutes. The absorbance at 500 nm per 1 mg of hair (A500) is considered to reflect the total amount of eu- and pheomelanin. The relationship between total melanin content of

horse coat hair and  $L^*$  was estimated using polynomial regression functions of  $L^*$  on total melanin content.

Discriminant analysis was used to detect the relationship between objective colour measurements and subjective classification. In statistical analysis the following procedures were used: STEPDISC, DISCRIM, CLUSTER and TREE (SAS INSTITUTE, CARY, 1999-2001). Stepwise discriminant analysis was applied to select variables ( $L^*$ ,  $a^*$ ,  $b^*$  for each colour classes) and range them according to their importance.

Variance components, heritabilities, and repeatabilities for colour traits were estimated using REML from univariate animal models of the following general forms:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_1\mathbf{s} + \mathbf{Z}_2\mathbf{b} + \mathbf{Z}_3\mathbf{c} + \mathbf{Z}_4\mathbf{a} + \mathbf{Z}_5\mathbf{p} + \mathbf{e},$$

where  $\mathbf{y}$  is the vector of observations for the  $L^*$ ,  $a^*$ ,  $b^*$ , and Chroma traits;  $\boldsymbol{\beta}$  denotes the vectors of fixed effects for sex, month of year when measurements were taken, age defined as a covariate (age of horses ranged from 1 to 30 yr), and age  $\times$  mo and age  $\times$  sex interactions;  $\mathbf{s}$  defines nonlinear effects of age (if any) explained by smoothing cubic splines as outlined by Verbyla et al. (1999);  $\mathbf{b}$  is the random vector of breed effects (defined only for gray horses);  $\mathbf{c}$  is the random vector of color class effects (defined only for nongray horses);  $\mathbf{a}$  is the random vector of additive genetic effects within breed and color class;  $\mathbf{p}$  is the random vector of permanent environmental effects due to repeated measurements on the same animal;  $\mathbf{e}$  is the vector of temporary environmental effects; and  $\mathbf{X}$ ,  $\mathbf{Z}_1$ ,  $\mathbf{Z}_2$ ,  $\mathbf{Z}_3$ ,  $\mathbf{Z}_4$ , and  $\mathbf{Z}_5$ , are known incidence matrices relating  $\boldsymbol{\beta}$ ,  $\mathbf{s}$ ,  $\mathbf{b}$ ,  $\mathbf{c}$ ,  $\mathbf{a}$ , and  $\mathbf{p}$  to  $\mathbf{y}$ .

The variances of breeds, colour classes, additive genetic effects within breeds (or colour classes), permanent and temporary environment were  $\mathbf{I}^*\sigma_b^2$  (or  $\mathbf{I}^*\sigma_c^2$ ),  $\mathbf{A}^*\sigma_a^2$ ,  $\mathbf{I}^*\sigma_p^2$  and  $\mathbf{I}^*\sigma_e^2$ , respectively. Here,  $\mathbf{A}$  is the relationship matrix and  $\mathbf{I}$  is the identity matrix.

Mean values of  $L^*$ ,  $a^*$ ,  $b^*$  and Chroma for colour classes within breeds were predicted from the defined animal models as  $\mu_{age} + c$ , where,  $\mu_{age}$  is the value for particular age averaged over fixed effects and  $c$  describes the Best Linear Unbiased Prediction (BLUP) of breed and colour class combination.

The calculations of variance components were carried out using the ASREML program (GILMOUR et al., 2002) and VCE 5 (KOVAC – GROENEVELD, 2003). The following heritabilities and repeatability were calculated:

**Between breeds heritability** ( $h_b^2$ ) describes the proportion of the total phenotypic variability attributable to breeds (calculated only for gray horses), as follows.

$$h_b^2 = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_a^2 + \sigma_p^2 + \sigma_e^2}$$

**Color class heritability** ( $h_c^2$ ) describes the proportion of the total phenotypic variability attributable to genetic variability between color classes (calculated only for non-gray horses). It defines the variability due to major gene effects, as follows.

$$h_c^2 = \frac{\sigma_c^2}{\sigma_c^2 + \sigma_a^2 + \sigma_p^2 + \sigma_e^2}$$

**Within breed and color class heritability** ( $h_a^2$ ) describes the part of the variability attributable to the polygenic color effects within breeds and color classes. It is defined as the ratio of the additive genetic variance within breeds and color classes to the phenotypic variance within breeds and color classes, as follows.

$$h_a^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_p^2 + \sigma_e^2}$$

**Within breed and color class repeatability** ( $r$ ) describes the part of variability attributable to repeated measurements on the same horse and body part within breeds and color classes, as follows.

$$r = \frac{\sigma_a^2 + \sigma_p^2}{\sigma_a^2 + \sigma_p^2 + \sigma_e^2}$$

**Combined heritability** ( $h_i^2$ ) describes the proportion of total phenotypic variability attributable to the breeds, major genes and polygenic variability, as follows.

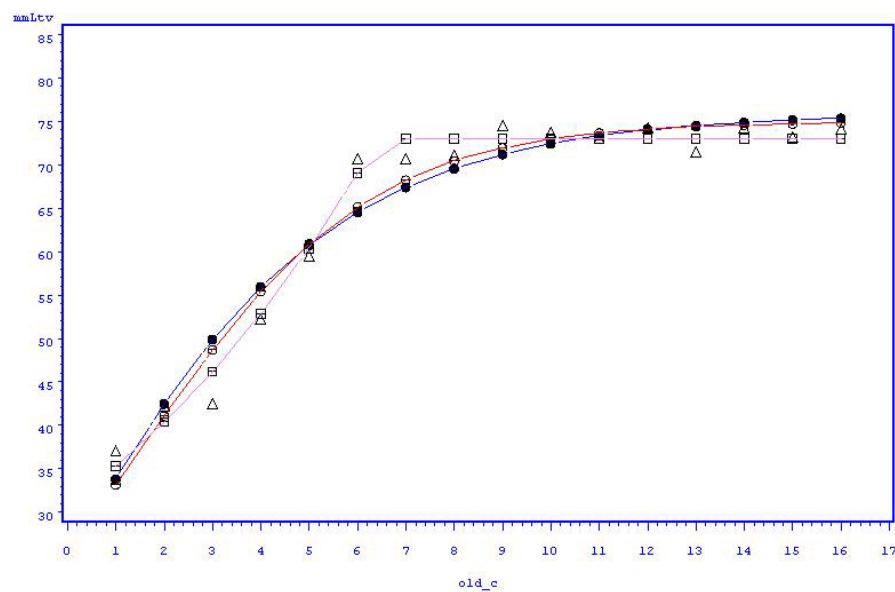
$$h_i^2 = \frac{\sigma_c^2 + \sigma_a^2}{\sigma_c^2 + \sigma_a^2 + \sigma_p^2 + \sigma_e^2}$$

## IV. RESULTS

### Analysis of the progressive graying process in horses

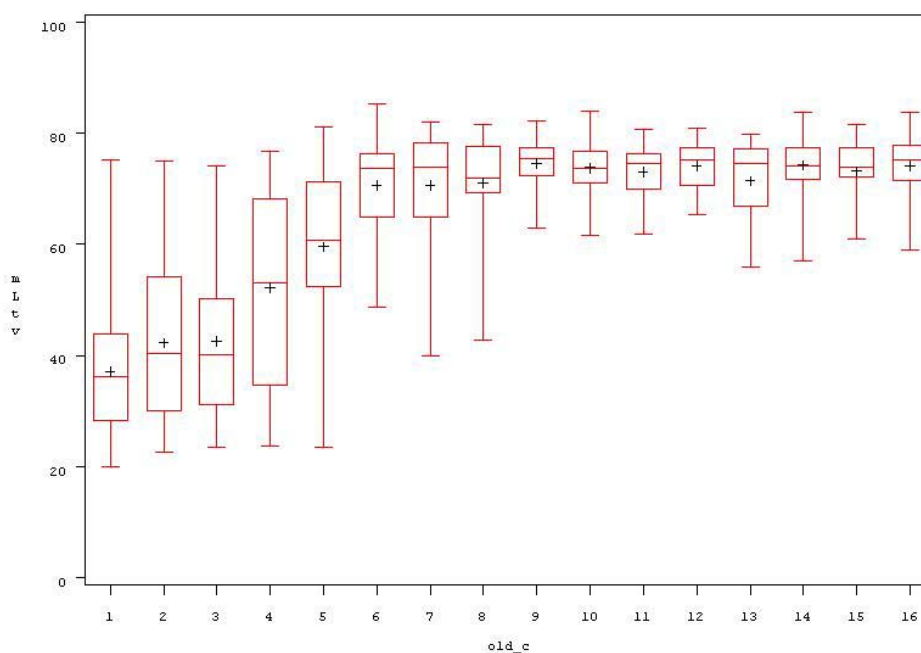
The graying dynamics was analyzed by fitting nonlinear functions to individual records or mean values of age groups (in years, except for year 16 which included all older horses). For individual values as well as for mean values, the best fit was, according to the coefficient of determination ( $R^2$ ), obtained with the Richards growth function.

Mean — triangle; General growth — dot; Logistic — circle; Richards — square



**Figure 1. Mean gray levels (mmltv) through aging (old\_c) of horses**

In case of the Richards function asymptotic value of  $L^*$  was reached at 73.34 (individual values) and 73.05 (mean values), approximately at the age of seven years. The graying dynamics of  $L^*$  through aging of Lipizzan horses is shown. [Figure 1](#) shows us the comparison of mean gray levels with values fitted by general growth, logistic and Richards function. Individual variation of the gray level presented in [Figure 2](#) demonstrates heterogeneity of variances in young versus old horses.



## 2. Figure Box-plot presentation of the individual gray levels (mltv)

Horses reached their final colour around the age of ten. Considering this fact coat colour can be defined as a threshold trait. The rate of graying process and the appearance (flea bitten or dappled; white or dark points) can vary widely among individuals-indicating involvement of genetic modifiers. The obtained results can probably link to effects of genes from the horse genom which exist and act after the birth for certain time with different expression at different age.

The graying process and the development of melanoma as well as levels of the blood metabolites are of particular interest for understanding the total complex process as well as for practical breeding reasons. SELTENHAMMER (2000) emphasizes in her thesis if results show that early graying is associated with a higher risk of melanoma development a selection procedure for early graying should be revised.

## Spectrophotometric analysis of hair melanin content

Coat colour variation is produced by genes that alter the basic pigment type (eumelanin and pheomelanin) in melanocytes, or the presence, shape, number or arrangement of pigment granules. Descriptive statistics for total melanin content of 54 randomly chosen samples are shown in [Table 4](#). Research on Arabian pure bred horses proved for

example that melanocytes in the skin produce eumelanin, while follicles of the hair produce eumelanin (WOOLF et al., 1988). In our research dark coloured horses the total melanin content of hair was 0.20 – 0.38 A500/mg. Considering the phenotypic appearance of these horses producing both brown and black melanin are of importance. Analysing the coat hair of Argentine llamas CECCHI et al. (2004) reported similar results. In our analysis from the black to the reddish brown colour the absorbance at 500nm per 1mg of hair (0.46 – 0.19 A500/mg) showed decreasing tendency.

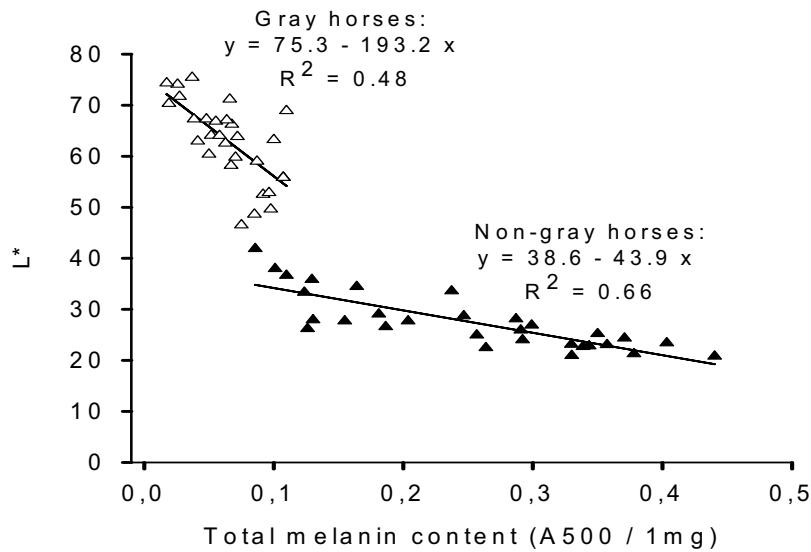
**Table 4.**

**Total melanin content of the hair samples (n=54)**

Colour class	Melanin content (A500/mg)		
	Samples	Mean	STD
Light gray	6	0.00	0.00
Dark gray	6	0.09	0.01
Flea bitten gray	6	0.06	0.02
Light chestnut	6	0.13	0.02
Dark chestnut	6	0.29	0.03
Bay	6	0.20	0.04
Brown	6	0.28	0.02
Dark brown	6	0.25	0.02
Black	6	0.38	0.05

### **Analysis of the relationship between total melanin content of horse hair and colour traits**

The current study confirmed that the spectrophotometric total melanin content of horse coat hair was strongly related to L\*. This indicates the hair melanin content is inherited through a polygenic component. Decrease of L\* corresponds to increased total melanin content and low L\* values depict less lightness and less reflectance. Since there is an obvious difference between gray and non-gray horses in L\* and melanin, these two groups of horses were analyzed separately (Figure 3). For both gray and non-gray horses, a strong negative linear relationship was detected (P < 0.01).



**Figure 3. Total melanin content of coat hair related to L\* value**

The scatter plot for nongray horses indicated a slight curvilinear relationship, but the quadratic component was not significant for this sample ( $p=0,07$ ). Also, the linear component gives an  $R^2$  of 0.66 and adding a quadratic component into the model increased the  $R^2$  only by 0.04. Note that there was larger unexplained variability for gray horses than for other non-gray horses ( $R^2 = 0.48$ ) compared to  $R^2 = 0.66$ . Considering the  $a^*$  value negative linear relationship was detected ( $p<0.05$ ). For non gray horses less variability was observed ( $R^2 = 0.32$ ) for  $a^*$  values than for gray horses ( $R^2=0.15$ ). Considering the pigmentation the  $b^*$  value hasn't shown any relationship for gray individuals ( $R^2=0.01$ ;  $p=0.55$ ) but there was significant relationship for nongray horses ( $R^2=0.17$ ;  $p=0.02$ ). Researches related to human pigmentation reported similar statements as seen above (SHIVER – PARRA, 2000).

### **Relationship between objective measurements and subjective colour classification**

According to VALVERDE et al. (1995) and WAGNER et al. (2002) the significant limitation of previous studies has been the relative lack of attention paid to defining the phenotype accurately.

The importance of colour variables was defined by SAS STEPDISC procedure. The presented results show that in separation of colours the measurements taken on the

neck, shoulder and belly are of importance. Considering the rate of the subjectively misclassified horses it can be seen that the proportion of correctly classified horses – presenting different colour classes – was 65 %. The proportion of misclassified observations of nine colour classes is shown in Table 5.

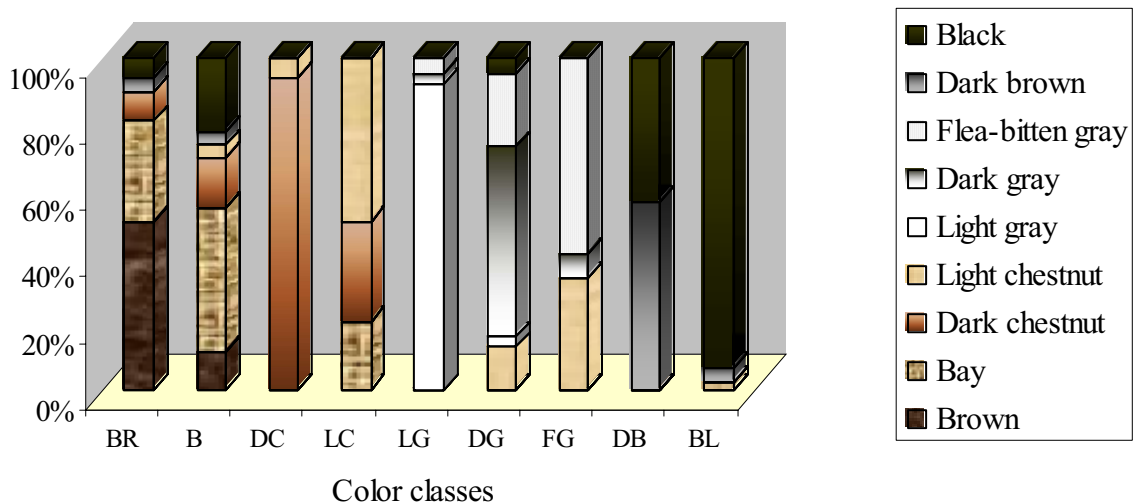
**Table 5.**

**Proportion of misclassified observations**

	Colour class <sup>a</sup>								
	LG	FG	DG	LC	DC	VP	SP	NYF	F
Prior	0.41	0.13	0.09	0.01	0.14	0.07	0.06	0.01	0.07
Rate	0.07	0.41	0.49	0.51	0.06	0.51	0.49	0.44	0.06

<sup>a</sup>LG = light gray; FG= flea bitten gray; DG= dark gray; LC = light chestnut; DC = dark chestnut; B = bay; BR = brown; DB = dark brown; BL = black

Each observation is placed in the class from which it has the smallest generalized squared distance. Figure 4 shows which colour classes belonging to the posteriori clusters. As an example the subjectively created brown cluster contains only 50 % of correctly classified horses. In addition this cluster contains at the rate of 30.43 % bay, 8.7 % dark chestnut, 4.35 % dark brown and 6.04 % black individuals. The cluster of flea bitten gray implies at the rate of 33 % the light chestnut colour class. This can be referable to the phenotype caused by the brown pigment spots on the gray horses



**Figure 4. Posteriori classification of different colour classes**

BR = brown; B = bay; DC = dark chestnut; LC = light chestnut; LG = light gray; DG= dark gray; FG= flea bitten gray; DB = dark brown; BL = black

This investigation indicated the importance of a repeated discriminant analysis, splitting the original nine colour classes into the four main colour classes (gray, chestnut, brown, black). Statistical significant differences ( $p < 0.001$ ) were detected between all four classes. Considering the proportion of the subjectively misclassified individuals in case of gray horses 6.3 %, chestnut horses 3.7 %, brown horses 18.2 % and 7.4 % of black horses was observed, respectively. This type of colour determination provides more exact and objective classification method.

### **Analysis of genetic and environmental factors influencing the colour**

Our former analysis (TÓTH et al., 2004; TÓTH, 2005) was used for testing the effect of possible non genetic factors influencing the forming of the coat colour. According to these results the age, sex, subjective colour classes and the breed influence the appearance of different colour state, shade and variant. The role of sex is justified through the different environmental effects. Considering the results of the discriminant analysis we consider to use of the nomenclature of the four main colour as mentioned above.

The current analysis indicated that the variability of horse coat colour is related to the age and sex of animals and influenced by the season of the measurements (winter or summer coat) too. During our experiment the age of stallions from gray populations were mostly above 10 years, which means they have already reached their final colour. Average values of colour traits of Arabian thoroughbred and Shagya arabian stallions were higher ( $P < 0.05$ ) than for mares. The same result ( $P < 0,01$ ) was obtained for Lipizzan horses. Flea-bitten gray stallion was not measured, the rare appearance can be related to different keeping system. Significant lower colour traits were detected only in case of Nonius stallions.

In the Gidran population no significant relationship was observed. Generally the sex has no significant effect on colour variations from biological aspects. The selection of animals is carried out based on different breeding concepts. Analysing the effect of the season of measurements significant relationships were found in case of all horse breeds except the Gidran horse.

Analysing the genetic parameters, in case of gray horses most or all of the estimated genetic variability was due to within breeds effect showing that considerable variation is inherited through a polygenic component. Similarly, a moderate heritability estimate ( $h^2 = 0.46$ ) for  $L^*$  in gray Lipizzan horses was reported by CURIK et al. (2002). Genetic parameters for  $L^*$ ,  $a^*$ ,  $b^*$  and Chroma values are shown in [Table 6](#). Considering the gray populations - included in this study - the gray colour is independent from the breed. However in the practice considerable differences can be observed in the graying process.

Definitely the gray coat colour is inherited as qualitative trait controlled by a single gray gene whose mode of inheritance is dominant and has been assigned to the chromosome 25 (ECA 25). But during analysis of the progressive graying process and gray colour varieties the effect of genes in background (possible allele combinations:  $AA$ ,  $Aa$ ,  $aa$ ,  $EE$ ,  $Ee$ ,  $ee$ ,  $Gg$ ,  $GG$ ) has to be considered. PIEBERG et al. (2005) have now developed eight new single nucleotide polymorphism (SNPs) associated with genes on ECA 25 which might result more allele combinations on Gray locus. In the current study this was the basis of our hypothesis.

Within breed heritability estimates for  $a^*$ ,  $b^*$  and Chroma were high and there was almost no variability between breeds. The repeatabilities for  $b^*$  and Chroma were lower than for  $a^*$  reflecting considerable variability between repeated measurements due to unknown temporary environmental effects.

**Table 6.****Genetic parameters for L\*, a\*, b\* and Chroma values for gray horses**

Trait <sup>1</sup>	$h^2_b$ <sup>2</sup>	$h^2_a$	r	$h^2_t$
Neck				
L*	0.00±0.00	0.49±0.17	0.81±0.03	0.49±0.17
a*	0.20±0.18	0.47±0.14	0.63±0.05	0.57±0.15
b*	0.09±0.10	0.21±0.14	0.27±0.06	0.28±0.14
Chroma	0.12±0.12	0.17±0.13	0.24±0.06	0.27±0.15
Shoulder				
L*	0.00±0.00	0.45±0.18	0.87±0.02	0.45±0.18
a*	0.15±0.15	0.48±0.15	0.68±0.04	0.56±0.15
b*	0.05±0.06	0.22±0.06	0.22±0.06	0.26±0.08
Chroma	0.07±0.08	0.20±0.06	0.20±0.06	0.25±0.09
Belly				
L*	0.00±0.00	0.49±0.18	0.88±0.02	0.49±0.18
a*	0.09±0.10	0.43±0.14	0.65±0.04	0.47±0.14
b*	0.05±0.07	0.13±0.12	0.36±0.06	0.17±0.13
Chroma	0.07±0.08	0.09±0.11	0.32±0.06	0.15±0.13
Average <sup>3</sup>				
L*	0.00±0.00	0.49±0.17	0.90±0.01	0.49±0.18
a*	0.16±0.16	0.52±0.15	0.71±0.04	0.60±0.14
b*	0.08±0.09	0.23±0.14	0.34±0.06	0.30±0.14
Chroma	0.11±0.11	0.20±0.13	0.31±0.06	0.29±0.15

<sup>1</sup> L\* = lightness, a\* = colour saturation from red to green, b\* = colour saturation from yellow to blue, Chroma = total colour saturation

<sup>2</sup>  $h^2_b$  = breed heritability,  $h^2_a$  = within breed or colour class heritability, r = repeatability

$h^2_t$  = combined heritability

<sup>3</sup> Average describes the parameters calculated from the mean values of neck, shoulder and belly

For non-gray horses, the greater part of the genetic variability of L\* values was attributable to colour class definition, which describes the effects of major genes. The estimated between colour class heritability ranged from 0.70 to 0.80 (Table 7). However, moderate within colour class heritability for the average of neck, shoulder and belly L\* values, was estimated (0.21), showing that considerable variation is inherited through a polygenic component. For non-gray horses, the estimated within colour class heritabilities for a\*, b\* and Chroma were essentially zero while colour class heritability estimates were high, ranging from 0.79 to 0.85 indicating consistent values within chestnut, bay and black coat colours.

The repeatability estimates were quite higher for the non-gray than for gray horses. This shows that due to the progressive graying, there is more variability between repeated measures in gray horses than in chestnut, bay or black horses.

**Table 7.**

**Genetic parameters for L\*, a\*, b\* and Chroma values for nongray horses**

Trait <sup>1</sup>	$h^2_c$ <sup>2</sup>	$h^2_a$	r	$h^2_t$
Neck				
L*	0.76±0.19	0.21±0.16	0.38±0.10	0.81±0.15
a*	0.83±0.19	0.00±0.00	0.45±0.09	0.83±0.14
b*	0.80±0.16	0.00±0.00	0.31±0.10	0.80±0.16
Chroma	0.82±0.15	0.00±0.00	0.38±0.10	0.82±0.15
Shoulder				
L*	0.70±0.21	0.00±0.00	0.32±0.10	0.70±0.21
a*	0.80±0.16	0.00±0.00	0.27±0.10	0.80±0.16
b*	0.79±0.17	0.00±0.00	0.30±0.10	0.79±0.17
Chroma	0.80±0.16	0.00±0.00	0.30±0.10	0.80±0.16
Belly				
L*	0.72±0.21	0.34±0.22	0.58±0.08	0.81±0.15
a*	0.81±0.16	0.00±0.00	0.42±0.09	0.81±0.16
b*	0.80±0.16	0.05±0.18	0.50±0.08	0.81±0.16
Chroma	0.81±0.15	0.00±0.00	0.50±0.08	0.81±0.15
A <sup>3</sup>				
L*	0.80±0.16	0.21±0.20	0.56±0.08	0.84±0.13
a*	0.85±0.13	0.00±0.00	0.45±0.09	0.85±0.13
b*	0.84±0.13	0.00±0.16	0.55±0.08	0.84±0.14
Chroma	0.85±0.13	0.00±0.00	0.54±0.08	0.85±0.13

<sup>1</sup> L\* = lightness, a\* = colour saturation from red to green, b\* = colour saturation from yellow to blue, Chroma = total colour saturation

<sup>2</sup>  $h^2_c$  colour class heritability,  $h^2_a$  = within- colour class heritability, r = repeatability

$h^2_t$  = combined heritability

<sup>3</sup> Average describes the parameters calculated from the mean values of neck, shoulder and belly

Shortly summarized the estimates of genetic parameters obtained for L\* in this study were high between color class and moderate within color class, indicating that major gene and polygenic effects are important. For nongray horses polygenic effects seem to be less important for chromatic parameters (a\*, b\*, and Chroma). The genetic variability was explained mostly by color class definition. On the contrary, in gray

horses polygenic components were considerable. There is quite a difference in appearance of gray horses with different patterns, such as flea-bitten or dappled. In this study those patterns were defined as unique gray color class because their inheritance is still not completely characterized. How presence, shape, and number or arrangement of pigment granules affect (RUSSEL, 1939) inherited variation captured by L\* was not studied here. Research related to human pigmentation suggests that there is a quantifiable individual variation in the number, size, and packing of melanosomes (STURM et al., 2001). It would not be a surprise if those factors are inherited and explain high heritability of L\*.

## V. NEW SCIENTIFIC RESULTS

Beside the multivarious utilization in the field of the agriculture and industry, the chromameter is a useful tool for determination of the coat colour and the quality of pigmentation in case of certain animals – like in our case the horses. Despite of the traditionally well accepted categorical approach and subjective classification, quantifying the coat colour can be carried out. In addition statistical evaluation of the results enables to report further new scientific knowledges which can contribute greatly to the enrichment of the literature resource.

From the results of the two overlapping researches the following new scientific conclusions can be drawn:

1. The graying dynamics was analyzed by fitting nonlinear functions to individual records or mean values of age groups. For individual values as well as for mean values, the best fit was, according to the coefficient of determination ( $R^2$ ), obtained with the Richards growth function. Horses had the final coat colour by the age of ten, which is characterized by high genetic variability. Presumably this can be related to the spotted or speckled (flea bitten gray) pattern of coat colour.
2. During the spectrophotometric analysis of almost acromatic gray hair samples small but comparable melanin content was measured. The dark coloured hair samples gave more melanin content (0,20 - 0,38 A500/mg). The present study confirmed that the total melanin content of horse coat hair was strongly related to  $L^*$ . For both gray and non-gray horses, a strong negative linear relationship was detected ( $P < 0.01$ ).
3. The misclassification of colour treated as a continuous variable can diminish the power of statistical testing. The instrumental colour determination proves the objectivity of identification using the main colours contrasted to use of subjective classification.

Furthermore in horse identification (e.g. draw up horse passport) it is not recommended to use nomenclature referring to state of colour, variant and shade in practice.

4. According to the colour of the short hair it is often difficult to distinguish the chestnut and bay horse colours. In our case instrumental analysis provides precise method to separate this two main colours since the chestnut shows more yellow and reddish component produced by the pheomelanin.

5. According to the results of the discriminant analysis in separation of colours the measurements taken on the neck, shoulder and belly are of great importance.

6. The current analysis indicated that the variability of horse coat colour is related to the age and sex of animals and influenced by the season of the measurements (winter or summer coat) too.

7. For gray horses, most or all of the estimated genetic variability of L\* values was due to within breeds effect ( $h^2_a=0.45 - 0.49$ ) showing that considerable variation is inherited through a polygenic component. Here the within breed heritability estimates for a\*, b\* and Chroma were moderate and there was almost no variability between breeds. For non-gray horses, the greater part of the genetic variability of L\* values was attributable to colour class definition, which describes the effects of major genes. The estimated within colour class heritabilities for a\*, b\* and Chroma were essentially zero while colour class heritability estimates were high (0.70 - 0.80), indicating consistent values within chestnut, bay and black coat colours. However, moderate within colour class heritability for the average of neck, shoulder and belly L\* values was estimated (0.21 - 0.34). These values are not greater than zero therefore no genetic variability was statistically provable.

## **VI. PRACTICAL UTILITY OF THE RESULTS**

Although the colour of horses is not connected to the performance, it is valuable for several reasons for breeders and for horsemen. Recently the increased attention on aesthetic value the research and understanding of the mechanism of coat colour inheritance is of greater significance from economical aspects as well. Individual colours coupled with excellent breeding value represent high marketable value. Knowledge of colour descende can be applied both in the fields of horse identification and pedigree-control.

The results of the dissertation show us that measurements related to the coat colour of horses allow the objective description of several biological processes. Instrumental analyses with chromameter prove the accuracy of individual identification on the basis of main colours contrasted to use of subjective colour classes. In practice it is not recommended to use nomenclature referring to state variant and shade of colour.

The results and methodology presented here open new perspectives on the various research topics (e.g. genetic diversity, physiological, morphological and behavioral traits) related to the inheritance of coat colour. Recent developments in molecular genetics and statistical analyses combined with the approach presented here offer new perspectives for better understanding of coat colour inheritance as a trait with multiple contributing factors.

## VII. SCIENTIFIC PUBLICATION LIST

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