

Features of coat color and markings and impact of dun factor on Vyatka horse breed

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Abstract. The predominant coat colors in Vyatka horse breed are bay-brown (69.6 %) and mousey (20.8 %). Among the genotyped livestock, three genotypes of the base bay coat color (EE/AA, EE/Aa, Ee/AA) and two genotypes of the base solid blackcock (EE/a/a, Ee/aa) have been detected. The proportion of horses with Cr allele is 2.1 %. In Vyatka horse breed, three isabelline-brown horses (Cr/Cr) have been recorded and the presence of W20n allele was detected. Among the horses genotyped, 35.5 % are DD homozygous, 61.3 % are heterozygous (Dd1, Dd2), 3.2 % have the nd2/nd2 genotype. Allele d2 against the background of D does not always cause the presence of “wild” markings, unlike D/D. The influence of Dun-factor on the depigmentation area has not been detected. 39.9 % of horses have white markings (including 30 % of stallions), which are mainly facial markings (59.8 %), less often they are leg markings (21.6 %) or both facial and leg markings (18, 6 %).

1 Introduction

Coat color and markings are characteristic genetic markers of the horse breed, which play an important role in their identification and are an important selection criterion, the action of which is aimed at consolidating the phenotypic originality of the breed, which is crucial in maintaining the gene pool of rare and endangered indigenous breeds and horse populations. Particular importance is attached to coat color in breeding amateur-class horses, as horses with an unusual coat are currently in great demand, which, in particular, are horses of domestic indigenous breeds [1].

The coat color of the northern forest horse have been set for a long time by natural selection, as a result of which the zonal indigenous coat colors have always dominated in horses of this type, due to the action of the dominant lightening Dun gene (D) [2–4]. The action of Dun gene is characterized by lightening of the covering hair on the horse's body, which does not affect the head, distal limb sections, mane and tail [3, 4]. The Dun gene lightens the areas stained with both eumelanin (black pigment) and pheomelanin (red pigment), which, along with the genetic diversity of coat colors in Vyatka breed horses, results in the widest variation of shades: from light beige or light gray, almost white to dark brown and dark gray, almost black [3, 5].

At all times, the most common coat color in Vyatka horses was bay-brown. In recent years, the proportion of bay-brown coat color in Vyatka breed horses has significantly decreased in favor of a great deal more popular mousey coat (solid blackcock and brown) [2, 3].

At the same time, the chestnut (red and brown) coat color has become rather rare in the breed. Representatives of non-brown coat (that do not have D gene) have not been used in Vyatka horses selection for many years, but sometimes (rarely) cases of birth of non-brown foals are observed. Coats determined by the admixture of white hair (gray, roan, piebald, mottled) have not been observed in purebred Vyatka horses [3].

Vyatka breed horses, carrying the Cream (Cr) allele in the genotype, which causes the light-bay and light-gray coat color, have always been rare. In past years, light-bay and light-gray working horses were rejected due to their tendency to soil easily. To date, consumer demand for elegant light-bay and light-gray horses has led to an increase in their number in the Vyatka breed [3].

An increase in the proportion of horses, which are used in breeding, with Cr allele, led to the birth of foals with isabelline-brown coats, carriers of two Cr / Cr alleles [2, 3]. The isabelline coat, apart from being unusual, beautiful and rare, is associated by practical horse breeders with some undesirable qualities, such as a pampered body composition, decreased fertility and vitality, vulnerability to sunlight and predisposition to sunburn of the nose and sometimes skin cancer.

In this regard, it is not recommended to widely use representatives of the isabelline coat in the aboriginal breeds selection, and Vyatka breed in particular [2]. In Russian zootechnical terminology, all homozygous carriers of the Cream gene are described as horses with isabelline coat, but in the world practice, isabelline horses are described in more detail: “cremello” – with

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the action of 2 copies of the gene Cr on the red coat, “perlino” – on the bay one and “ashy-blackcock” (or “smoky cream”) – on the black one [1, 4]. It is very difficult from the point of view of phenotype to identify the above-mentioned colors even in breeds that do not have the Dun factor in the genotype. Genotyping of Akhal-Teke and Orlov Trotter horse breeds revealed errors in the visual identification of coat colors with Cr allele [1, 6].

Manifestation of the Dun gene action on the coat color is accompanied by the presence of “wild” (or primitive) markings. Using the terminology, proposed by V.A. Kurskaya [6], the following “wild” markings can be distinguished in Vyatka breed: “An eel stripe” (a dark strip with clear boundaries, 0.5–3 cm wide, running along the spine from withers to tail stump); “zebra markings” (dark short transverse strips on legs, located in the wrist and / or hock area); “a zipper” (light, sometimes almost white integumentary hair on the backside of the lower part of the leg); “wings” or “color touches” (dark area on the neck, withers, shoulders and shoulder blade); “a mask” (the dark lower part of the horse’s head, sometimes capturing the entire head); “a frost” (dirty yellow, fawn or white hair locks in the mane, which are concentrated along its edges); “a web” (a net of dark lines converging in the center of the forehead and resembling a web in shape); a dark edging along the perimeter of the ear, with clear boundaries, often the back ear surface is half or completely dark; white ear tips. Among all “wild” markings listed above, only “an eel stripe” is required for brown horses. Other markings are found in different combinations and may be poorly visible [6].

The researchers from the University of California have found that Dun gene causes a radially asymmetric deposition of pigment in the growing hair, which is controlled by the expression of TBX3 gene in the hair follicles. The lack of lightening (a more peripheral distribution of pigment in the hair) is the result of a deletion of 1617 base pairs of DNA, which reduces the expression of the TBX3 gene in the hair follicle. Different manifestations of wild markings are associated with the presence of SNP-variants. It has been shown that three alleles are associated with various phenotypic manifestations of the Dun gene: D (expresses itself as lightning and the presence of “wild” markings), nd1 (no lightning, “wild” markings are present, but their manifestation is various), nd2 (deletion of 1617 base pairs of DNA, no lightning, no “wild” markings). Speaking about interaction options, it should be noted that variant D dominates nd1 and nd2, and nd1 dominates nd2. The action of the recessive alleles nd1 / nd1 and nd1 / nd2 allows the presence of “wild” markings; horses with nd2 / nd2 genotype have no “wild” markings [7].

White markings as a sign of domestication in Vyatka breed horses are undesirable [4]. According to Yanova’s data, in stud horse breeds, a certain correlation was detected between the size of the white area on the head and the size of the white markings on legs, in addition, a high degree of reliability of the positive relationship between these signs was noted [8]. One of the identified

genes that influence the presence of white markings in stud horse breeds was W20 known since 2007 [5].

Genes that determine the coat color of the horse have been known for a long time, therefore, at present, the detection of functional alleles at the DNA level has become quite logical. This approach allows reducing the number of errors in determining the coat color of an animal and opens up new possibilities in breeding [9]. For the first time in Russia, the genetic determination of coat colors, including, but not limited to, the identification of rare unique genes and mutations, has been studied in domestic horse breeds: Akhal-Teke, Vladimir, Orlov Trotter, Soviet Heavy Draft and some others horse breeds [6, 9–11]. Genotyping of coat colors of Vyatka breed horses has been carried out for the first time.

2 Objective of the study

Evaluation of the genetic determination of the coat color and markings in Vyatka breed horses as one of the main breeding traits. To achieve this goal, the following tasks were set and solved: the diversity and distribution of coat colors in Vyatka breed horses has been analyzed; the genetic determination of coat colors and the influence of Dun factor on coat color and the appearance of characteristic markings have been studied; the incidence of white markings has been specified.

3 Material and methods

The phenotypic determination of coat color was carried out visually according to the established rules used in horse breeding. The study subjects were Vyatka breed horses born in 1998–2018, totaling 1903 livestock units, including 888 stallions and 1015 mares.

Our own graphic descriptions of horses were made according to the established sectorial rules were employed as material for the study of markings and distinguishing features. This study subject were Vyatka breed stallions from different breeding regions of different origin and age, totaling 326 livestock units, including 120 stud horses.

In order to characterize more fully and reliably the coat color, which is an important breeding trait of Vyatka breed, the genotypes of the main coat colors in stud horses were identified. Samples of hair follicles, taken from 31 Vyatka breed horses with various coat colors, were employed as study material. The biomaterial was sampled from horses of different ages, sexes, originating from different geographical areas. The phenotypic determination of coat colors, a graphic description of markings and distinguishing features of the genotyped horses was carried out according to the rules adopted in horse breeding. DNA-genotyping of the coat color was carried out at the Independent Research Laboratory affiliated with Vladimir Horse Breeding Society according to 4 main loci (Extension, Agouti, Dun and Cream) for 30 livestock units, and for 1 livestock unit it was carried at the Etalon Diagnostics laboratory (USA, California, with the participation of Dr.

M. Wikinson) according to 17 loci that determine the coat color (A, E, IP, G, Ch, Cr, Dun, Prl, Z, DW, LWO, LP, PATN1, SW (MITF, PAX3), SB1, TO).

4 Results and subsequent deliberations

The most common coat color in Vyatka horses turned out to be bay-brown, specified in the documents as brown. The proportion of horses of this color in Vyatka breed reaches almost 70 % of the total number of livestock (Table 1).

Table 1. Distribution of Vyatka horses by coat color

Coat color	Total		Stallions		Mares	
	No.	%	No.	%	No.	%
Brown	1324	69.6	618	69.6	706	69.5
Mousey	396	20.8	184	20.7	212	20.9
Chestnut	52	2.7	26	2.90	26	2.6
Light-bay and brown	32	1.7	16	1.80	16	1.6
Light-bay	3	0.16	1	0.10	2	0.2
Isabelline and brown	3	0.16	3	0.30	–	–
Light-gray and brown	1	0.05	–	–	1	0.1
Red	21	1.10	12	1.40	9	0.9
Bay	41	2.15	18	2.00	23	2.3
Solid blackcock	24	1.26	8	0.90	16	1.6
Dark-red	1	0.05	–	–	1	0.1
Black-brown	5	0.27	2	0.20	3	0.3
TOTAL	1903	100	888	100	1015	100

A coat color is an important breeding trait when working with Vyatka breed. The study of the hereditary nature of the coat color of horses is carried out in conjunction with genetic researches, since it is visually impossible to distinguish horses of the same color, but with different genotypes, while horses that are phenotypically identical as for coat color can give the crop with various coat colors. Only knowing the genetic formula of the coat color, horses can be easily matched to get a crop with a given desired coat color. The results of genetic studies of Vyatka breed horses are presented in Table 2.

The most common coat color of Vyatka horses is bay-brown. In the group of genotyped horses, three of the four possible genotypes of the basic bay coat color were detected (EE / AA, EE / Aa, Ee / AA) which are influenced by DD and Dd2 genes, causing bay-brown coat color (Table 2). The Dd1 allele was not found in the group of studied bay-brown horses.

The more required mousey coat color among horses born over the past 20 years makes up a little more than 20 %. In the genotyped horses, both genotypes of the base blackcock coat color (EE / a / a, Ee / aa) were detected with the action of all three studied genes which cause mousey (blackcock-brown) coat color DD, Dd2, Dd1 (Table 2).

The proportion of chestnut (red-brown) coat color in the livestock of modern Vyatka horses makes up only 2.7 % (Table 1). Chestnut stud horses are removed from

nuclear stock because chestnut coat color is highly heritable, less demanded by customers, and also because of the high probability of the appearance of undesirable in this breed white markings, associated with this coat color. The same reasons explain the extremely rare incidence in Vyatka breed of the light-gray (light-gray and brown) coat color, carrying the demanded Cr allele on the non-demanded base red (chestnut) coat color. It is noteworthy that the identical genotype ee / aa / DD was detected in all 4 genotyped chestnut horses, which causes the birth of brown foals by horses of any color (Table 2).

Table 2. Distribution of the genotyped livestock of Vyatka horses by coat color and genotype

Coat colors, genotypes	No.	Caused by the Dun gene (livestock units)			
		D/D	D/d1	D/d2	d2/d2
Bay-brown in total, including:	9	2	–	7	–
E/E, A/A	2	–	–	2	–
E/e, a/a	5	1	–	4	–
E/e, a/a	2	1	–	1	–
Mousey in total, including:	9	3	2	4	–
E/e, a/a	5	3	–	2	–
E/e, a/a	4	–	2	2	–
Chestnut in total, including:	4	4	–	–	–
e/e, a/a	4	4	–	–	–
Light-bay and brown, including:	4	1	2	1	–
E/E, A/A, C/c	2	–	1	1	–
E/E, A/A, C/c	1	1	–	–	–
E/E, A/A, C/c	1	–	1	–	–
Smoky (ashy) mousey in total, including:	3	1	–	2	–
E/e, a/a, Cr/cr	1	–	–	1	–
E/e, a/a, Cr/cr	2	1	–	1	–
Isabelline-brown (perlino) in total, including:	1	–	1	–	–
E/e, a/a, Cr/cr	1	–	1	–	–
Solid blackcock, including:	1	–	–	–	1
E/e, a/a	1	–	–	–	1
Total horses, units	31	11	5	14	1
%	100	35.5	16.1	45.2	3.2

The total proportion of Vyatka breed horses carrying the Cream (Cr) allele in the genotype which causes the light-bay and light-gray coat color makes up only 2.1 % (Table 1). Moreover, the coat colors of the light-bay group are the only ones in which Vyatka breeders discount the fact of presence or absence of D allele – phenotypically brown coat color. Obviously, the number of Cr carriers in Vyatka breed will increase due to the interest of breeders in such stud horses.

The simultaneous action of D and Cr alleles makes the phenotypic identification of a number of coat colors almost impossible. The results of the genotyping carried out detected three smoky-mousey horses (in another terminology – ashy brown), having both base blackcock genotypes EE / aa and Ee / aa, due to the action of Cr /

cr, DD and Dd2 genes (Table 2). Visually, these light-bay and mousey horses are a cross between light-bay and brown and light-mousey ones and are difficult to distinguish.

Over the past decade, the appearance of three representatives of a rare isabelline-brown coat color, carriers of two Cr / Cr alleles, was recorded in Vyatka breed. The stallion of this color, included in the study, phenotypically has a characteristic “milky” body color, light-beige protective hair and clearly distinguishable zonal markings: an eel stripe, zebra markings, color touches, dark ear edgings. Its genotype Ee / Aa / CrCr / Dd1 (Table 2) causes the appearance of the isabelline-brown coat color on the bay one (“perlino”).

The representatives of non-brown coat colors (which do not have D gene) are not used in Vyatka breed selection, with the exception of the aforementioned isolated cases of generating a light-bay coat color in a horse breed. The total proportion of registered non-brown Vyatka horses, born over the past 20 years, makes up 5 % of the total livestock (Table 1). The appearance of non-brown foals is due to the presence of recessive nd1 (non-dun1) and nd2 (non-dun2) genes in Vyatka breed. Only one genotype nd2 / nd2 of the three known groups of recessive alleles of this group was detected in one Vyatka stallion among the horses subject to our study. Phenotypically, against the background of its blackcock coat, the reddish ends of the protective hair are observed, apart from this, the stallion is visualized completely black.

In general, in the group of genotyped Vyatka horses, 11 livestock units (35.5 %) are homozygous DD, 19 livestock units (61.3 %) are heterozygous, of which 5 livestock units have Dd1 gene and 14 livestock units have Dd2 gene and 1 livestock unit (3.2 %) has the nd2 / nd2 genotype (Table 2). We have studied the effect of alleles DD, D / nd1, Dnd2, d2 / d2 on the coat color using three phenotypic criteria: the shade of the coat color, as the degree of its lightening by means of the Dun factor (classic, light, dark); the degree of manifestation of “wild” markings (“bright”, “medium”, “feebly-marked”, “not present”); the presence and size of white markings as an alleged sign of domestication (large, not significant, not present). The results of our study are given in Table 3.

All horses from the tested group that possessed two copies of the dominant DD gene had a medium strong coat color shade. Among horses with Dd1 gene, 2 animals had a light shade of coat color and 3 animals had a medium one. Among the animals carrying Dd2 gene, 2 horses had a pronounced dark shade of coat color (registered as dark-brown), 5 horses had a mostly dark shade of coat color, 6 horses had a medium one and 1 horse had a light one (Table 3). A stallion with a light shade of a light-mousey coat color should be considered separately, because it is the only one in the studied group that was tested according to 17 loci causing the coat color. Its identified genotype EE / aa / Dd2 / W20n reveals the presence in Vyatka breed of the unique Dominant White gene, two copies of which cause the white coat color. The manifestation of W20n allele in representatives of Vyatka breed is to be studied in future,

but it can be assumed that it is this allele which makes mousey coat color very light, since d2 allele causes insufficient lightening. Along with this factor, the action of W20n gene caused the presence of small white markings on the head of this stallion (a small star) and on the left hind leg (up to fetlock joint). The presence of other studied loci (IP, G, Ch, Cr, Pr1, Z, LWO, LP, PATN1, SW (MITF, PAX3), SB1, TO) was not detected in this stallion.

Table 3. Effect of the Dun gene on the degree of lightening of the coat color and the presence of “wild” and white markings

Coat color shade and markings	Total livestock units	Caused by the Dun gene (livestock units)			
		D/D	D/d1	D/d2	d2/d2
Coat color					
"Classic" (common)	20	11	3	6	–
Dark or mostly dark	8	–	–	7	1
Light or mostly light	3	–	2	1	–
Manifestation of "wild" markings					
Bright	15	5	4	6	–
Medium	6	4	–	2	–
Feebly-marked	7	2	1	4	–
Not present	3	–	–	2	1
White markings					
Not present	17	4	4	9	–
Insignificant	11	5	1	4	1
Large	3	2	–	1	–

Among the genotyped Vyatka horses with phenotypically pronounced “wild” markings (zebra markings and color touches), 6 animals (40.0 %) had D / d2 allele, 4 animals (26.7 %) had D / d1 allele and 5 animals (33 , 3 %) had homozygous dominant D / D allele. “Wild” markings of medium degree of manifestation were mostly observed in carriers of two DD copies (67.7 %), and weakly expressed primitive markings prevailed in horses with D / d2 genotype (57.1 %).

The absence of “wild” markings was revealed in carriers of D / d2 and d2 / d2 alleles. Our results confirm the hypothesis that the action of d2 allele, even in combination with the dominant D allele, cannot always cause the phenotypic presence of “wild” markings, while the presence of two copies of D / D allele causes the presence of zebra markings and color touches of various degrees of manifestation in horses. The absence of white markings slightly prevailed in horses with D / d2 genotype (52.9 %), and their presence was distributed approximately identically between homozygous and heterozygous animals carrying D allele, with a slight bias towards the first ones (Table 3).

White markings on the head and legs of purebred Vyatka horses are relatively rare. White markings are a very common phenomenon in crossbred animals (F1, F2, F3). Selection in Vyatka breed is aimed at the elimination of any white markings. However, in spite of a rather strict selection of the breeding stock according to this criterion, horses with various kinds of depigmented areas can be observed, they make up

39.9 % of the total number of stallions analyzed, including 30 % of the breeding stock (Table 4).

The analysis of the studied factual material showed that the stallions of the studied sample group had the largest proportion of white markings on their heads – 59.8 %, including 21.5 % of markings which look like a small star. At the same time, various kinds of stripes are not present on the heads of horses of the breeding stock.

To a greater extent, white markings on legs are undesirable for the breed, however, white areas on legs are found in 21.6 % of the livestock. The combination of white areas on the head and limbs is present in 18.6 % of cases. It should be noted a combination of a large star and white area with a large capture of legs surface in 5 livestock units. At the same time, minor markings in the head area are not combined with white areas on legs.

Table 4. Distribution of Vyatka breed stallions by the presence of white markings

The presence of white markings and their localization	Number of stallions		Including stud horses	
	No.	%	No.	%
No white markings	196	60.1	84	70.0
With white markings in total, including	130	39.9	36	30.0
Only head, including:	78	59.8	20	55.6
Graying	17	13	6	16.7
A small star	28	21.5	9	25.0
A star	15	11.5	4	11.1
Graying, a small flesh mark between the nostrils	6	4.6	1	2.8
A star (a small star) and white area between the nostrils	3	2.3	–	–
A narrow, short stripe	1	0.8	–	–
A star (a small star) with a narrow stripe	7	5.3	–	–
A large star with a large stripe	1	0.8	–	–
The left hind leg only, including:	17	13.1	6	16.6
Below the fetlock joint	3	2.3	2	5.5
Up to fetlock joint	10	7.7	4	11.1
Above fetlock joint, but not higher than half hind cannon	4	3.1	–	–
The right hind leg only, including:	3	2.3		
Below the fetlock joint	2	1.5	–	–
Up to fetlock joint	1	0.8	–	–
Both hind legs only, including:	6	4.6	2	5.6
Below the fetlock joint	5	3.8	1	2.8
The right hind leg above fetlock joint, the left one at half fetlock joint	1	0.8	1	2.8
One front leg and two hind legs:	2	1.6		
The right front leg and both hind legs not higher than fetlock joint	1	0.8	–	–
The left front leg and the right hind leg below fetlock joint, the left hind leg above fetlock joint	1	0.8	–	–
The head and the left hind leg, including:	11	8.5	3	8.3
A small star, the left hind leg not higher than half fetlock joint	5	3.8	2	5.5
A star (a small star), the left hind leg above fetlock joint	3	2.3	–	–
Graying, a stripe, the left hind leg at half fetlock joint	1	0.8	–	–
A star, a stripe, the left hind leg up to fetlock joint.	1	0.8	–	–
A large star, a narrow dash stripe, a flesh mark between the nostrils, the left hind leg at half hind cannon	1	0.8	1	2.8
The head and the right hind leg, including:	7	5.5	1	2.8
A small star, the right hind leg at half fetlock joint	3	2.3	1	2.8
Graying, the right hind leg including fetlock joint	1	0.8	–	–
A star, the right hind leg including fetlock joint	1	0.8	–	–
A small star, the right hind leg at half hind cannon	1	0.8	–	–
A star, a flesh mark between the nostrils, the right hind leg including fetlock joint	1	0.8	–	–
The head and both hind legs, including:	4	3	2	5.6
A small star, the hind legs not higher than up to fetlock joint	2	1.5	1	2.8
A star, a stripe, the hind legs including fetlock joint	2	1.5	1	2.8
The head, the left front leg and both hind legs, including:	2	1.6	2	5.6
A small star, the left front leg and the hind legs not higher than fetlock joint	1	0.8	1	2.8
A star, the left front leg at 2/3 of the pastern, the hind legs at half hind cannon	1	0.8	1	2.8
TOTAL	326	100	120	100

5 Conclusion

The results of the studies confirmed that the main coat color of Vyatka breed horses is bay-brown, the proportion of it makes up almost 70 % of the total number of Vyatka horses. Mousey color, which is more

demanding and popular in the market, makes up a little more than 20 %, the proportion of chestnut coat color (red-brown) makes up 2.7 %. In the genotyped horses group, three genotypes of the base bay color out of 4 possible (EE/AA, EE/Aa, Ee/AA) and both genotypes of the base solid blackcock coat color (EE/a/a, Ee/aa) have been detected. The total proportion of Vyatka breed

horses, carrying the Cream (Cr) allele in the genotype, which causes the light-bay and light-gray coat colors, makes up 2.1 %. Three representatives of the rare isabelline-brown coat color, carriers of two Cr / Cr alleles, were recorded in the breed. The presence of a unique W20n allele was detected in Vyatka breed, which presumably causes the presence of white markings and also influence the lightening of the coat color shade.

In the group of genotyped Vyatka horses, 11 livestock units (35.5 %) are DD homozygous, 19 livestock units (61.3 %) are heterozygous, 5 livestock units of them have Dd1 gene and 14 livestock units have Dd2 gene, and 1 livestock unit (3.2 %) has nd2 / nd2 genotype (Table 2). The analysis of the obtained data confirmed the hypothesis that the action of d2 allele, even in combination with the dominant D allele, cannot always cause the phenotypic presence of “wild” markings, while the presence of two copies of the D / D allele causes the presence in horses of zebra markings and color touches of various degrees of manifestation.

In 39.9 % of horses of the analyzed livestock, various white markings were detected, including in 30 % of stud stallions. The largest proportion of white markings is found on the head – 59.8 %, including in the form of a small star in 21.5 % of animals, white areas on the legs are observed in 21.6 % of animals. A combination of white areas on the head and limbs is present in 18.6 % of cases. The influence of Dun factor on the depigmentation of body parts was not detected.

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