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ANIMAL GENETICS

The Pleiotropic Effect of Selection for Behavior on Coat Color in Grey Rats (*Rattus norvegicus*)

L. A. Prasolova, I. N. Oskina, and I. Z. Plyusnina

Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, Novosibirsk, 630090 Russia;
e-mail: pras@bionet.nsc.ru

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Abstract—The effects of selection for a type of behavior relative to humans (tame and aggressive) on the intensity of coat color in agouti rats with the *AAHH* genotype were studied. Animals that were not under selection for behavior (wild animals) were used as the control. Morphometric analysis of the hair parameters that influence the intensity of coat color demonstrated that, on the one hand, polymorphism in the main coat color exists in the population of wild agouti rats, that is, both light and dark agouti animals exist. On the other hand, it was demonstrated that selection for a type of behavior in rats is accompanied by selection of animals that differ in the intensity of the main genetically identical coat color. Dark-colored animals are more prevelent among the aggressive animals, while light-colored animals prevail among tame animals. The association of the effects of selection for behavior with the modification of coat color is probably caused by the presence of common neurohormonal mechanisms for the regulation of these processes.

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INTRODUCTION

It is known that coat color has a protective function in some animal species in the wild, but it also can be associated with functional and behavioral peculiarities. Animals that are genetically and phenotypically different in color, which is not always protective, frequently exist in natural populations of even a single species, but animals with different color morphs can differ in behavioral or functional features, which have adaptive significance [1–4]. Thus, along with brown and grey animals, black animals are also found in a single population among rodents, whose color is determined by the agouti gene. It is noted that melanists (black animals) frequently have an advantage in reproduction and survival under stress conditions, since they are more aggressive, more sexually active, and are more resistant to stress and diseases [5–7]. Domestication of animals, i.e., selection for tame behavior, under conditions controlled by humans was accompanied by a loss of the protective function of coat color. The most complete data on the effects of selection for behavioral properties on genetic systems of pigment genesis and coat color were obtained in experiments on the artificial domestication of different species (silver fox, American mink, wild agouti rats) that were carried out at the Institute of Cytology and Genetics (Russian Academy of Sciences, Siberian Branch) [8–12]. One of the first consequences of domestication is the appearance of white piebalds in the coat and the emergence of new color mutations, which influence some behavioral properties and/or hormonal, reproductive, and morphological features. Lightening of the tone of the main hair color was observed among the consequences of artificial selection for domesticed behavior [9, 11]. This relates to *platinum* and *white-faced* pigmentation mutations in fox and a number of mutations in the American mink (*sapphire, Aleutian*, et al.) [13, 14]. The *agouti* and *extintion* genes affect attenuators of the main color in rodents, since they determine the amount and distribution of eumelanin and pheomelanin in hair. While genetic, behavioral and functional peculiarities of the carriers of the *agouti* gene mutations that are associated with selection for behavior have already been quite completely studied [8, 15, 16], morphological analysis of the main genetically identical agouti coat color in animals with different types of behavior is lacking. The present study is devoted to a phenotypic analysis of morphological variations of the main agouti coat color (the *AAHH* genotype) in grey rats that were selected for tame and aggressive behaviors towards humans.

MATERIALS AND METHODS

The study was conducted on adult agouti colored rats (males) from a wild population that were not under selection for behavior (the control group, $n = 15$), on agouti colored rats (the *AAHH* genotype) at the age of 5 days after birth ($n = 15$), and on adult males of the same color genotype of the 68th generation of selection for tame (domesticated) and aggressive behavior relative to humans ($n = 20$ and $n = 16$, respectively). Aggressive rats show a pronounced aggressive defensive reaction with humans, while tame

rats do not show such reaction and can be easily taken in a person's hands. All animals were bred via outbreeding. Animals were kept in metal cages with free access to the food and water. Samples of awns from the dorsal side of the body were taken for an objective estimation of the tone of the main coat color in the agouti rats. The parameters of the hairs that influence the intensity of the main color to the greatest degree (total length of the awn, length of the top black tip, and length of yellow band that lies below the top) were measured and analyzed. Differences in the intensity of the color of the dark juvenile hairs of 5-day-old rats were estimated according to the differences in the length of their top pigment-free (white) tip, whose length was measured under the microscope. Statistical treatment of the results was performed using the Student's criterion.

RESULTS

Alternation of black and yellow regions in the top portion of awns is a peculiarity of the agouti hair color in rats. A black pigment (eumelanin) is synthesized in melanocytes of hair bulbs at the beginning of the agouti awns growth, then, with the growth of hair, the melanocytes switch on the synthesis of the yellow pigment pheomelanin. A transition zone, in which both types of pigment granules are present, is located between the eumelanin and pheomelanin zones. Eumelanin is again synthesized in the lower (basal) part of the hair. The intensity of the main coat color depends on the ratio of the length of the top black region of the awn to the length of subapical yellow band. The higher this ratio is, the darker the main color of the coat is. Morphometric analysis of the agouti hair color according to the indicated parameters in rats from a wild (recently caught) population demonstrated the presence of polymorphism in the color intensity of the main coat; "light" and "dark" animals and animals that can be attributed to an intermediate group according to indicators of hair color are present in the natural population. In "light" rats, the total length of hairs is greater and the length of the top black tip of the awn is less than in "dark" rats (although the difference is not significant); but at the same time, the length of the light (pheomelanin) part of the hair is significantly greater in "light" rats ($p < 0.01$). As a result, the ratio of the dark part of the hair to the light part is lower in "light" rats as compared with "dark" rats (Fig. 1). Analysis of the agouti color intensity for the same parameters in rats that were selected for tame and aggressive behaviors demonstrated that indicators of the agouti color intensity in rats with the tame type of behavior are very similar to those in "light" rats from the wild population. In contrast, the indicators of hair-color intensity in rats with the aggressive type of behavior were similar to the indicators of "dark" rats from the wild population (Fig. 2). It should be noted that as opposed to the control group, the length of overhairs in the tame rats is significantly larger ($p < 0.001$) and the length of the eumelanin portion is significantly less ($p < 0.001$) than in aggressive rats.

It is known that the pigment genesis system is active from the earliest stages of development. We were interested in the question of how early differences in hair color in agouti rats that were selected for different types of behavior develop in ontogenesis. Young rats are born without hairs, which begin to appear on the surface of the skin from the second day of postnatal development. By the fifth day after birth, young rats are covered by dark hairs with light tips (their total length is approximately 1 mm). Analysis of the total length and length of the light tips of juvenile hairs in young rats that were born to mothers with different types of behavior demonstrated that even at this age the hairs are lighter in young rats from tame mothers (as well as in adults with tame behavior) than in young rats from aggressive mothers, since they have a significantly larger length of pigment-free tips ($p < 0.001$, Fig. 3).

DISCUSSION

The agouti gene is widespread among rodents and belongs to the category of genes that are variable and have incomplete penetrance. Morphometric analysis of hair pigmentation in agouti rats demonstrated the presence of polymorphism in the main color in the population of wild animals, which are not under selection for behavior. Among wild agouti rats, darker and lighter rats, as well as rats that are intermediate in their main color are found. Little is known about the genetic nature of variability in the coat color within a single genotype; however, the large evolutionary importance of dominant genes that are variable and that are maintained by natural selection for the origination of adaptive polymorphisms in the population has been mentioned in literature [17]. The individual variability in coat color in genetically identical animals from populations with different types of behavior is most likely due to behavioral or morphofunctional peculiarities, which provide an advantage in reproduction or survival in stressful environmental conditions, as is observed in genetically different color morphs in a single population of wild animals. To answer questions about genetics and the role of genes with incomplete dominance in populations, it is necessary to study multiple adaptive systems using different methods on laboratory and wild animals. The individual variability of the main agouti color in the wild population can be caused by the presence of alternative promoters in the agouti gene, which has been described for variants of dark dorsal and light ventral sides in mice [18]. But it also can be associated with other mutations in major genes that regulate the synthesis of eu- and pheomelanin in different ratios, namely in melanocortin receptors or in the agouti protein, as

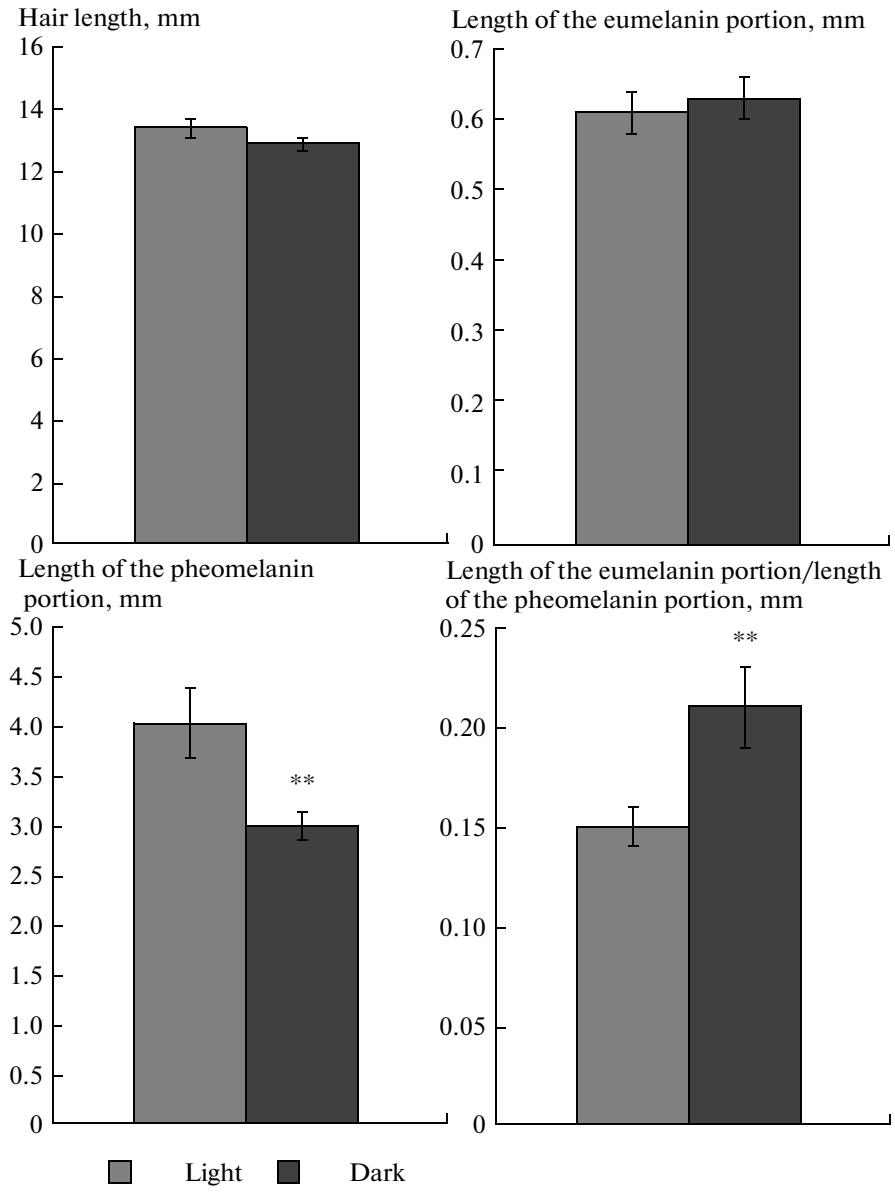

Fig. 1. Morphometric parameters of awns in wild rats with light and dark agouti colors. ** $p < 0.01$.

well as in tyrosinase, which regulates the rate of melanin synthesis [3].

The results we obtained indicate unidirectional selection for behavior and the intensity of the main coat color in agouti rats in selected populations. Selection of animals with different behaviors is accompanied by selection of extreme variants for coat color. Based on morphometric estimation of hairs, dark agouti account for 70% of the aggressive rats and only 30% of the tame rats (the remaining rats are light). Since differences in the intensity of the agouti color in rats with different behaviors are determined by a different ratio of the length of the eumelanin part of the hair to the length of the subapical pheomelanin part, it means that differences occur in the time intervals of the synthesis of these pigments and the switch from eumelanin to pheomelanin and back to eumelanin with the growth of the hair.

Behavioral studies on the objects of artificial selection have demonstrated that the correlation of coat pigmentation with the peculiarities of behavioral reactions in animals is caused by the presence of common neurohormonal mechanisms of the regulation of behavior and melanogenesis, among which a large role belongs to catecholamines. Brain catecholamines participate in the synthesis of pigments, the regulation of emotional reactions, and the activity of the pituitary–adrenal system [19, 20]. Selection for domesticated behavior in wild gray rats correlates not only with attenuation of the main agouti color, but also with a

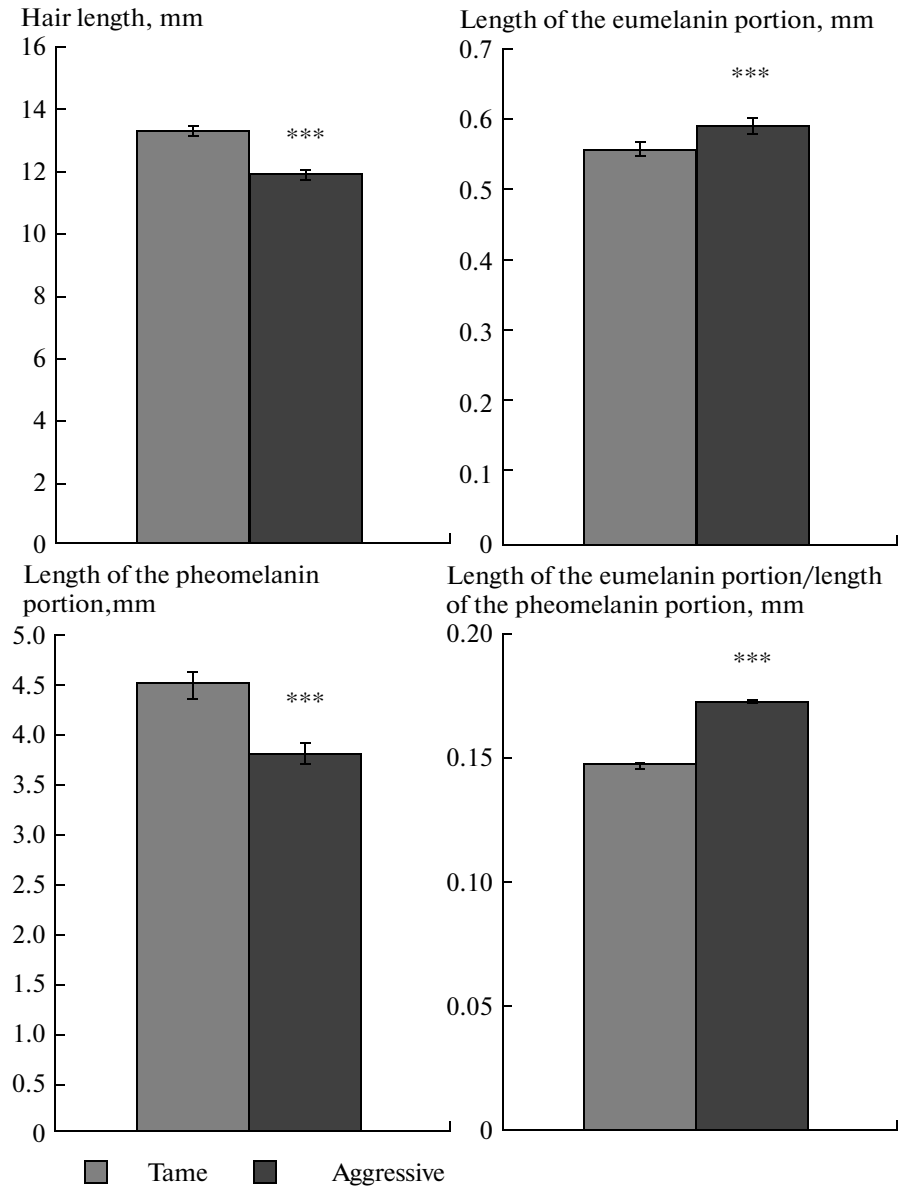

Fig. 2. Morphometric parameters of awns in adult rats with tame and aggressive types of behavior. *** $p < 0.001$.

decrease in their defensive reaction and an increase in searching behavior as compared with aggressive behavior [21].

In studies on different domestication models it has been demonstrated that the hypothalamic–pituitary–adrenal system (HPAS), a system for adaptation and stress, is one of the first hormonal systems that respond to selection for behavior. It has been shown that this system also actively participates in the regulation of behavior and pigment genesis, beginning from the earliest stages of embryonic development [22, 23]. Studies have demonstrated that the activity of the HPAS is decreased in tame rats as compared with aggressive rats. The basal level of glucocorticoids (GC) is lower in the agouti rats that were selected for tame behavior than in aggressive rats. On the other hand, it was demonstrated that selection for domesticated behavior in the silver fox is accompanied by a decrease in the expression of the proopiomelanocortin (POMC) gene in hypophysis, from which melanocyte-stimulating hormone (MSH), which regulates melanogenesis, is produced as a result of processing [2]. The data also indicate that differences in the intensity of coat color in rats with different types of behavior are manifested from the earliest days of postnatal development. We do not know the genotype of the juvenile hair color; it is possible that it is one of the agouti alleles. Differences in the lengths of the pigment-free tips of juvenile hairs can be also associated with different tyrosinase activity or with some factors

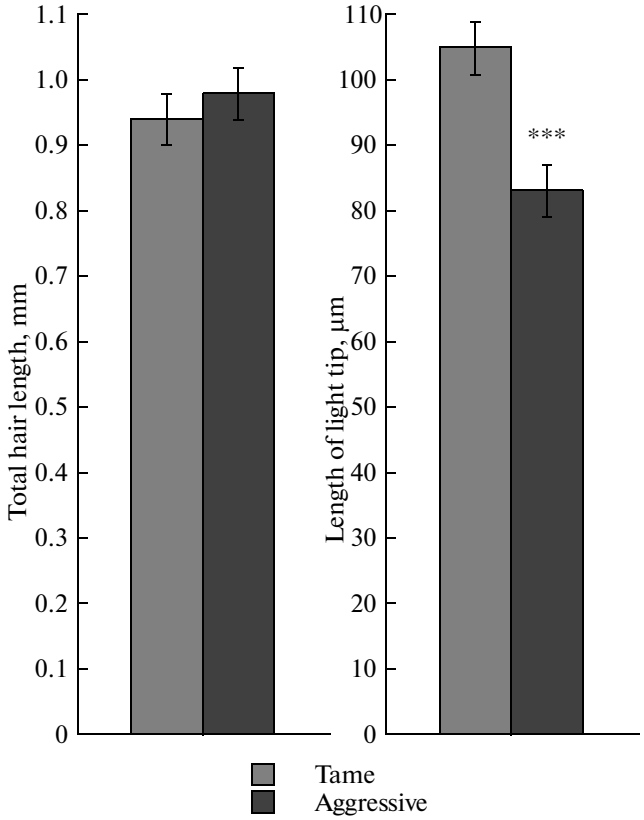

Fig. 3. The morphometric parameters of juvenile hairs in young rats born to mothers with different types of behavior. *** $p < 0.001$.

of the external or internal environment. More than 100 alleles of this enzyme are known, which are characterized by different activities and can give a wide range of coat color (from very light to dark) [3]. It is obvious that in this case variability in the main tone of the black hair color is not associated with the genes that affect some structural or functional characteristics of melanocytes (as is observed in the case of many color mutations in mink and foxes), since the histological picture of hair pigmentation is not impaired in length and in layers. Recently, much attention has been given to studies that are related to epigenetic changes in coat color as a reflection of the increased expression of the agouti gene as a result of an increase in DNA methylation with a visual phenotypic effect. A special role is assigned to the influence of the maternal embryonic environment. Experiments with methyl additions in the food of pregnant yellow mice (the *Avy/a* genotype) indicate that darkening of the coat color is observed in some of the descendants of such mothers that received a methyl diet [24]. It is difficult to say whether a change in DNA methylation occurred in our experimental animals, but it is known that DNA methylation is regulated by hormones and that glucocorticoids can change gene expression; on the other hand, methylation can control a hormonal signal [25]. It is possible that differences in the intensity in the agouti color in rats with different behaviors in our experiments are due to differences in the level of their glucocorticoid hormones.

Thus, morphometric analysis of the hair pigmentation in the agouti rats demonstrated the presence of polymorphism in the main coat color in the population of wild rats that were not under selective presure for behavior, while differences in the main coat color in genetically identical animals that were selected for tame or aggressive behaviors, are consequences of their selection for a type of behavior. Selection of grey agouti rats for the presence or intensification of aggressive behavior towards humans is accompanied by selection of animals that differ in the phenotype of their main coat color. Significantly more animals with a dark tone of the main color were observed in the population of aggressive rats than in the population of tame rats. It is possible that selection for behavior correlates not only with coat color, but also with behavioral and morphofunctional peculiarities, which can play an adaptive role under certain environmental conditions. The correlation between the effects of selection for the characteristics of behavior with coat pigmentation may be caused by common neurohormonal mechanisms for the regulation of these processes.

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REFERENCES

1. Ducrest, A., Keller, L., and Roulin, A., Pleiotropy in the Melanocortin System Coloration and Behavioural Syndromes, *Trends Ecol. Evol.*, 2008, no. 9, pp. 502–510.
2. Gulevich, R.G., Oskina, I.N., Shikhevich, S.G., et al., Effect of Selection for Behavior on Pituitary-Adrenal Axis and Proopiomelanocortin Gene Expression on Silver Foxes (*Vulpes vulpes*), *Physiol. Behav.*, 2004, vol. 82, pp. 513–518.
3. Hoekstra, H.E., Genetics Development and Evolution of Adaptive Pigmentation in Vertebrates, *Heredity*, 2006, vol. 97, pp. 222–234.
4. Price, T. and Pavelka, M., Evolution of a Color Pattern: History Development and Selection, *J. Evol. Biol.*, 1996, vol. 9, pp. 451–470.
5. Bazhan, N.M., Polymorphism of the Fur Pigmentation Genes and the Hormonal Adaptation System in the Water Vole (*Arvicola terrestris*), *Izv. Akad. Nauk SSSR, Ser. Biol.*, 1991, no. 3, pp. 363–367.
6. Keeler, C.E., The Association of the Black (*nonagouti*) Gene with Behavior in the Norway Rat, *J. Hered.*, 1942, vol. 33, no. 12, pp. 371–384.

7. Wilcock, J., Gene Action and Behavior on Evolution of Major Gene Pleiotropism, *Physiol. Bull.*, 1969, vol. 72, no. 1, pp. 1–29.
8. Trut, L.N., Plyusnina, I.Z., and Prasolova, L.A., Mutations *hooded* and *nonagouti* among Wild Norway Rats (*Rattus norvegicus*): Selection at Behavior and Photoperiod, *Russ. J. Genet.*, 2000, vol. 30, no. 6, pp. 813–822.
9. Belyaev, D., Ruvinsky, A., and Trut, L., Inherited Activation—Inactivation of the *Star* Gene in Foxes, *J. Hered.*, 1981, vol. 72, no. 4, pp. 264–274.
10. Gulevich, R.G., Plyusnina, I.Z., Prasolova, L.A., et al., White Spotting in Norway Rats Selected for Tame Behavior, *J. Zool.*, 2010, vol. 280, pp. 264–270.
11. Trapezov, O., *Black crystal* a Novel Color Mutant American Mink (*Mustela vison* Schr.), *J. Hered.*, 1997, vol. 88, no. 2, pp. 164–166.
12. Trut, L., Early Canid Domestication: The Farm-Fox Experiment, *Am. Sci.*, 1999, vol. 87, no. 2, pp. 160–169.
13. Belyaev, D.K., Trut, L.N., and Ruvinskii, A.E., Allelic Relationships between the *Georgian white, platinum* and *white face* Mutations in Foxes, *Genetika* (Moscow), 1973, vol. 9, no. 10, pp. 71–77.
14. Zvereva, L.P., Belyaev, D.K., and Privalova, G.N., Phenogenetic Analysis of Pigmentation in American Mink (*Mustela vison* Schr.) Mutants: 2. Effect of *Aleutian* Mutation and Interaction of the Aleutian and Silver-Blue Color in the Sapphire Mink Genotype: Influence of "*stuart*" Factor on the Hair Pigmentation, *Genetika* (Moscow), 1976, vol. 2, no. 2, pp. 104–109.
15. Plyusnina, I.Z., Trut, L.N., Karpushkeeva, N.I., et al., Some Behavioral and Physiological Characteristics of *nonagouti* Mutation in Gray Rats during Selection for Aggressiveness, *Zh. Vyssh. Nervn. Deyat. im. I.P. Pavlova*, 2003, vol. 53, no. 5, pp. 613–621.
16. Trut, L.N., Plyusnina, I.Z., Prasolova, L.A., and Kim, A.A., The *hooded* Allele and Selection of Wild Norway Rats *Rattus norvegicus* for Behavior, *Russ. J. Genet.*, 1997, vol. 33, no. 8, pp. 983–989.
17. Gershenzon, M.S., Genetic Polymorphism in an Animal Population and Its Evolutional Value, *Zh. Obshch. Biol.*, 1974, vol. 35, no. 5, pp. 678–684.
18. Klungland, H. and Vage, D.I., Molecular Genetics of Pigmentation in Domestic Animals, *Curr. Genomics*, 2000, vol. 1, pp. 223–242.
19. Haussen, V., Gunawardhana, S., and Meyer, J., The *agouti coat*—Color Locus May Influence Brain Catecholamines Regional Differences in Norepinerphine and Dopamine in the Brains of Two Color-Morphs of Deer Mice (*Peromyscus maniculatus*), *Comp. Biochem. Physiol.*, 1994, vol. 107, pp. 51–55.
20. Naumenko, E.V., Popova, N.K., Nikulina, E.M., et al., Behavior Adrenocortical Activity and Brain Monoamines in Norway Rats Selected for Aggressiveness Towards Man, *Pharmacol. Biochem. Behav.,* 1989, no. 33, pp. 85–91.
21. Plyusnina, I.Z. and Os'kina, I.N., Ontogenesis of Behavior and the Selection for Domestication of Wild Norway Rats *Rattus norvegicus*, *Sovremennye kontseptsii evolyutsionnoi genetiki* (Modern Concepts of Evolutionary Genetics), Novosibirsk: Institut tsitologii i genetiki Sibirskoe Otd. Ross. Akad. Nauk, 2000, pp. 341–349.
22. Slominski, A., Tobin, D., Shibahara, S., and Wortsman, J., Melanin Pigmentation in Mammalian Skin and Its Hormonal Regulation, *J. Physiol. Rev.*, 2004, vol. 84, pp. 1156–1228.
23. Os'kina, I.N. and Plyusnina, I.Z., Pituitary-Adrenal System under Selection for Domestication Behavior, *Sovremennye kontseptsii evolyutsionnoi genetiki* (Modern Concepts of Evolutionary Genetics), Novosibirsk: Inst. Tsitol. Genet. Sib. Otd. Ross. Akad. Nauk, 2000, pp. 327–334.
24. Wolf, G.L., Kodell, R.L., Moore, S.R., and Cooney, G.A., Maternal Epigenetics and Methyl Supplements Affect *agouti* Gene Expression in *Avy/a* Mice, *FASEB*, 1998, pp. 949–957.
25. Vanyushin, B.F., DNA Methylation and Epigenetics, *Russ. J. Genet.*, 2006, vol. 42, no. 9, pp. 1186–1199.