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GENERAL GENETICS

Morphology of Hair Pigmentation in Wild Red Foxes, Silver Foxes, and Their Hybrids

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Abstract—The effects of dominant allele A^r of locus *Agouti* on the morphology of hair pigmentation were described in foxes. The A^r allele was shown to determine the type of melanin and its content in hair with no effect on the morphology of pigment granules and their distribution throughout a hair. Using the method of electron spin resonance (ESR), the types of melanin (eumelanin and pheomelanin) and their content in the hair of red (A^rA^rEE) and silver (*aaEE*) foxes and their hybrids (A^raEE) were determined. In silver foxes, only one type of melanin (eumelanin) was found. In red foxes and their hybrids (which are phenotypically similar but darker than red foxes), both types of melanin (eu- and pheomelanin) were found. The highest melanin content was detected in the coat of silver foxes. In the hybrids, the total melanin content was lower than in silver foxes, but significantly higher than in red foxes. In red foxes, the contribution of pheomelanin to the total hair melanin content was twice as large as in the hybrids.

It is well known that loci *Agouti* (*A*) and *Extension* (*E*) control the production of yellow-and-red (pheomelanin) and brown-and-black (eumelanin) pigments in mammals. Gene *E* is expressed in melanocytes and controls the synthesis of the melanocyte-stimulating hormone receptor (MC-IR), which is placed on the plasmatic membrane of the melanocyte and participates in the regulation of pigment synthesis. Various alleles of gene *E* determine different functional states of the receptor. The expression of dominant allele *E* results in the synthesis of an active receptor; if the recessive allele *e* (which is a deletion of one nucleotide) is expressed, a functionally inactive receptor is synthesized [1].

Gene *Agouti* is expressed in dermal cells of the hair follicle, and its product (the agouti protein) is antagonistic to the MC-1R receptor. The recessive mutant allele *a* is a large insertion into this gene, and the agouti protein is not synthesized in the *aa* homozygotes [2].

A similar phenotypic effect may be caused by either a change in the level of the agouti protein or a change in the activity of the receptor controlled by gene *Extension*. Consequently, dominant mutations of gene *A* result in the same effect, the synthesis of pheomelanin, that recessive mutations of gene *E*, which produce a functionally weak or inactive receptor MC-1R. Conversely, recessive mutations of gene *Agouti* (which blocks synthesis of the agouti protein) cause synthesis of eumelanin, as well as dominant alleles of gene *E* (which cause the production of active MC1R receptor) [2]. The level of the MC-1R signal is a factor switching the pigment synthesis from pheomelanin to eumelanin: with a low level of the signal, pheomelanin is synthesized, and switching over to the synthesis of eumelanin occurs when the signal of the MC-1R receptor reaches the threshold level.

Locus *Extension* is epistatic to *Agouti*: dominant mutations of *Extension* suppress the effect of dominant alleles of *Agouti*; hence, animals carrying dominant alleles of both loci remain dark. However, the above considerations concern mice [1–3]. As for foxes, the following is known. In foxes, as well as in mice, the dark color can be determined by either recessive mutations of *Agouti* or dominant mutations of *Extension*. The recessive allele *a*, which is a deletion in the first coding exon of locus *Agouti*, is found in the common silver fox of Canadian origin (*aa)*. The color of the Alaskan silver fox is determined by a dominant mutation of *Extension*, which was termed *EA* [3] and causes synthesis of functionally active receptor. However, in contrast to mice, in foxes the *Extension* locus is not epistatic in relation to *Agouti*, and foxes carrying dominant *E* alleles can have a considerable amount of red hair: for instance, the wild red fox in which dominant alleles of both *Agouti* and *Extension* are in a homozygous state (A^rA^rEE).

Thus, a great progress has been currently observed in the study of function of color-controlling genes. Morphological effects of these genes on the nature of hair pigmentation are less clear. Many fur color varieties are known in the red fox and its hybrids. Their phenotypes and genotypes are described in published data [4–9]. It was shown that the number and color of pigment granules and their size, shape, and distribution throughout the entire hair length play a role in the deter-

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Table 1. Total melanin content in dorsal hair of red and silver foxes and their hybrids, wt %

Group	Number of animals	Awns	Down	
Red foxes	5	0.70 ± 0.07	0.85 ± 0.06	ns*
Hybrids	7	1.40 ± 0.09	1.90 ± 0.12	$P < 0.01$
Silver foxes	5	1.60 ± 0.18	1.70 ± 0.15	ns
	$P_{1–2} < 0.01$		$P_{1–2} < 0.01$	
	$P_{1–3} < 0.01$		$P_{1–3} < 0.01$	

* ns, Nonsignificant.

mination of hair color [10–14]. The types of melanin and their content in the coat of fur animals are less well studied [13–15].

This study is devoted to the effects of the dominant allele A^r of locus *Agouti* on the morphology of hair pigmentation: namely, determination of types of melanin, melanin content, morphology of pigment granules, and distribution of pigment granules throughout a hair in red and silver foxes and their hybrids.

MATERIALS AND METHODS

Animals from the Experimental Farm of the Institute of Cytology and Genetics, Russian Academy of Sciences (Novosibirsk), were used in this study. The fur melanin content in the red fox (genotype A^rA^rEE), the silver fox of Canadian origin (genotype *aaE*E), and their hybrids (A^raEE) [16] was analyzed qualitatively (detection of eumelanin and pheomelanin) and quantitatively. Awns and the down were examined. The method of electron spin resonance (ESR) of measuring the melanin content is based on the principle of direct proportionality between the intensity of signal registered by a spectrometer (which contains the fur samples) and the number of free radicals in the pigment. Total melanin content was calculated as its weight per 100 g of fur. Melanin type was qualitatively characterized based on the shape of ESR spectrum. The presence of pheomelanin in the fur was revealed based on the presence of an extra peak in the ESR spectrum, in addition to the main peak [17].

Pigment granules were isolated from the hair by hydrolysis in a strong solution of hydrochloric acid, which was followed by thorough rinsing with distilled water. Using a magnification of 90× (immersion lens), we studied the shape and color of pigment granules. Distribution of pigment throughout a hair was studied in preparations made from decolorized awns. To make the preparations, the awns were treated with a trypsin solution, rinsed with distilled water, exposed to standard histological procedures of tissue processing with alcohols and xylene, and mounted on glass in balsam. The significance of differences was determined using Student's *t* test.

RESULTS AND DISCUSSION

In the wild red fox, the fur color is yellow-and-red with a greater or lesser amount of silver hair, which are black throughout the entire hair length except a transverse white zone in the grana. The back of the animal is often colored more intensely than the belly. The tail is darker than other body parts and usually has a white tip. The neck and the upper part of the breast are almost entirely white, whereas the lower part of the breast and the belly are pale gray or red-and-gray. The legs and ears are black.

In the red foxes studied, dorsal guards are two-colored: the upper part of the hair stem is yellow; the lower part, black. The awns are of two types: most of them are entirely yellow, but some are yellow in the upper part and black near the base. The down has three color varieties: entirely light gray, entirely dark gray, and dark gray with yellow tips.

In the common silver fox, silver (black-and-white) hair is predominant. The ears and legs are entirely black, and a great amount of black hair is found on the tail. Along with the chine, the black guards and awns form a longitudinal black stripe (the belt); ventrally to the belt, the guards are not only black, but also platinum (i.e., the stem of hair is white except the black tip) and the awns are silver. Depending on the amount of platinum hair and the width of the white zone on silver awns, the coat color of silver foxes varies in darkness. The down is entirely gray, varying in intensity. The back and the sides are typically of more intense color than the belly.

The hybrids between silver and red foxes are phenotypically close to red foxes but differ from them by darkness: the hybrids have a darker color, because they bear a greater amount of silver and black hair. The main color is red-and-yellow with a dark smoky shade. Similarly to red foxes, the guards were two-colored in the hybrids: the upper part of the stem of hair was yellow, and the base of hair was black. The awns were black-and-yellow, and the down was dark gray with a small yellow tip.

As seen from Table 1, in both awns and down, the total melanin content in dorsal hair of red foxes is significantly (twice) lower than in silver and hybrid foxes.

The differences in total melanin content between awns and the down were nonsignificant in both red and silver foxes. As appears from Tables 1 and 2, in silver foxes, the differences in total melanin content between the dorsal and lateral hair (both awns and down) were nonsignificant.

In the hybrids, in contrast to silver foxes, the total melanin content in lateral hair (both awns and the down; Table 2) was significantly lower than in dorsal hair (Table 1). In addition, the lateral awns and down did not differ in the total melanin content, whereas the dorsal down contained more melanin than the dorsal awns ($P < 0.01$).

A qualitative study of melanin revealed that only one type of melanin, eumelanin, is found in silver foxes; whereas, in addition to eumelanin, pheomelanin was found in the black-and-yellow hairs of hybrid foxes, and in the yellow and black-and-yellow hairs of red foxes. In awns of hybrid foxes, pheomelanin constituted 13% of the total melanin content; whereas, in red foxes, the contribution of pheomelanin to the total hair melanin content was 31%.

Thus, taking into consideration the fact that the total melanin content in lateral hair of hybrid foxes was significantly lower than in silver foxes, we can suggest that the fur of the hybrids contains a lower amount of melanin than in silver foxes, but the total melanin content is significantly higher in the hybrids than in red foxes, and the contribution of pheomelanin to the total melanin content in the hybrids is less than half as large as in red foxes. On this basis, it becomes clear why the hybrids, which are phenotypically similar to red foxes, have darker fur. Earlier, we demonstrated that a direct relationship exists between the intensity of coat color and the amount of melanin contained in the fur [18]. On the other hand, given the same content and type of melanin, the fur color may vary [12–14] because of different morphology of pigment granules and their distribution throughout a hair. As for the phenotypes studied, we did not found any differences in either morphology of pigment granules or pigment distribution throughout a hair. In the hair of red and hybrid foxes, two types of granules were found: eumelanin and pheomelanin. In the yellow awns of red foxes and in the yellow tips of the awns of the hybrids, the granules of pheomelanin were small, round, and light (Fig. 1). They were arranged more or less densely both in the cortical layer and in the medulla, depending on the intensity of color, and were regularly distributed throughout the entire hair length (Fig. 2). In the black hair of silver foxes and the black tips of the yellow-and-black hair of red and hybrid foxes, the granules of eumelanin were large, elongated, and black (Fig. 1). They were packed very densely in both cortex and medulla and were regularly distributed throughout the entire hair length (Fig. 2). A similar pattern of pigment distribution was described by other authors [10, 11]. Thus, the phenotypic similarity between the hybrids and the red fox is realized

Table 2. Total melanin content in lateral hair of silver foxes and their hybrids, wt %

Group	Number of animals	Awns	Down
Hybrids	8	0.49 ± 0.04	0.45 ± 0.06
Silver foxes	5	1.40 ± 0.09	1.30 ± 0.19
	$P < 0.01$		$P < 0.01$

owing to the presence of pheomelanin in their hair, and a darker color of the hybrids is due to a higher total melanin content and a higher proportion of eumelanin in the total melanin content than in red foxes.

Thus, in foxes, the effect of mutation A^r on the morphology of hair pigmentation is only realized through the influence on the total melanin content and melanin type. Red foxes carrying a double dosage of this allele

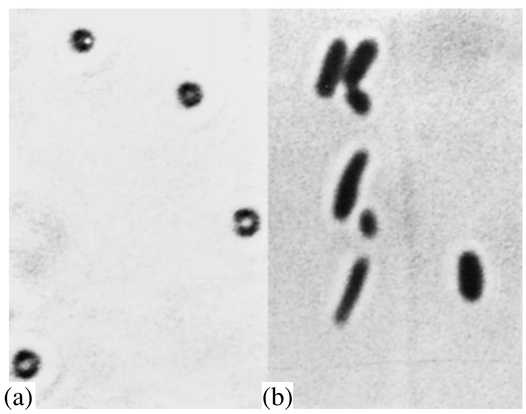

Fig. 1. Pigment granules in fox hair: (a) pheomelanin; (b) eumelanin. Magnification around ×10 000.

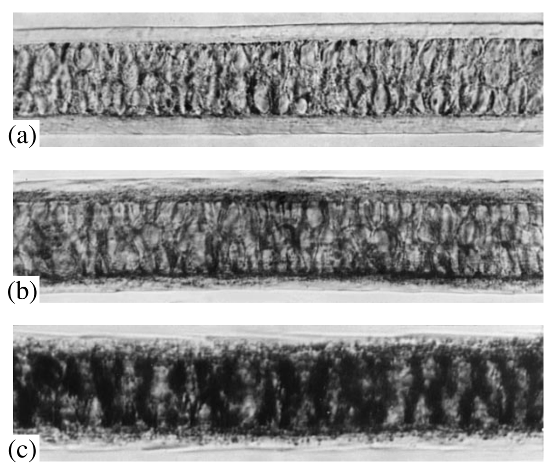

Fig. 2. Distribution of pigment throughout the awns in foxes: (a) pale yellow hair in red foxes; (b) dark yellow tip of the black-and-yellow hair in red foxes and their hybrids; (c) black hair in silver foxes. Magnification around ×300.

(A^rA^r) are characterized by a higher pheomelanin content against the background of a lower total melanin content; whereas a single dosage of this gene (A^ra), causing a slight decrease in the total melanin content (compared to silver foxes), significantly decreases the proportion of pheomelanin (compared to red foxes). In silver foxes carrying no dosage of this allele (*aa*), pheomelanin is absent, and only eumelanin is contained in their hair.

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