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Pink-eyed Dilution, a Coat Color Mutation in the Japanese Field Vole (*Microtus montebelli*)

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Inheritance of dilute coat color with pink eye in the Japanese field vole (*Microtus montebelli*) was investigated by mating of the dilute mutant with a normal agouti vole and a white vole. As the results, it was cleared that an autosomal recessive gene *p* is responsible for the pink-eyed dilution in *M. montebelli*. — KEY WORDS : coat color, microtus, pink-eyed dilution

The vole (genus *Microtus*) is one of the small herbivorous rodents having multichambered stomach, so it has possibility to be a useful experimental animal alternating to herbivorous domestic animals. From the viewpoint to develop the vole as an experimental animal, we have reared the Japanese field vole (*Microtus montebelli*) in our laboratory [6, 7]. Original coat color of the wild vole is normal agouti with dark grey or brown on dorsum and ash-colored on abdomen (Fig. 1).

In general, coat color mutations of a wild rodent have been detected frequently in course of its breeding in the laboratory. Regarding *Microtus montebelli*, Saito [16] detected a white vole (varying from white to cream in coat color), and succeeded in its breeding (Fig. 2). He estimated that the mutant of this coat color was controlled by an autosomal recessive gene. Pink-eyed dilution (Fig. 3), another coat color mutation of *M. montebelli*, was detected in the breeding colony at the Forestry and Forest Products Research Institute. Both mutants of white and dilute coat colors were introduced into our laboratory and proliferated. In the present investigation, we describe the inheritance of pink-eye dilution in *M. montebelli*.

Mating experiments were carried out using three kinds of Japanese field voles of different coat color, i. e., normal agouti, white, and pink-eyed dilution (or shortened as 'dilute'),

all of which were maintained at the National Institute of Animal Health. Number of offspring were 47 heads (male 23, female 24) in the mating experiment between agouti and dilute, and 85 heads (male 44, female 41) between white and dilute. Coat color was judged by the naked eye.

Throughout the experiments, all the animals were raised in a room conditioned to a temperature of 23–24°C and a humidity of 50–60%. The voles were fed on commercial pellets for mice and lucerne cubed hay after their weaning. They took food and water *ad libitum*.

According to the morphological observation of the vole under the laboratory husbandry [12], hair appears to grow about 4 days of age, though not seen at birth. Eyelid opening averages 7.9 days of age. Since the appearance time of these features in the dilute vole is the same as that in the normal agouti, the dilution with pink eye can be identified at 8 days of age. A young dilute vole can be easily discriminated from others by its agouti silver coat color (Fig. 4).

Table 1 shows the number of offspring classified by coat color in F₁ hybrid (agouti × dilute) and in BCF₁ (backcross of F₁ to dilute). The mating between normal agouti and dilute voles produced F₁ young of normal agouti. When a few F₁ animals, which might be heterozygous for dilute on marker gene, were back-

Table 1. Number of voles classified by coat color in (agouti $\times$ pink-eyed dilution)- F_1 and backcross BCF_1

Dam $\times$ Sire	No. of litter	Sex	Coat color in offsprings		X^2 -test**
			agouti	dilute*	
agouti $\times$ dilute	4	Male	10	0	
		Female	11	0	
		Total	21	0	
F_1 $\times$ dilute	5	Male	5	8	$0.25 < P < 0.50$
		Female	6	7	$0.50 < P < 0.75$
		Total	11	15	$0.50 < P < 0.76$

* pink-eyed dilution ** Null hypothesis ; an autosomal recessive gene p is responsible for pink-eyed dilution. The same as in the following tables.

Table 2. Number of voles classified by coat color in (white $\times$ dilute) - F_1 and backcross BCF_1

Dam $\times$ Sire	No. of litter	Sex	Coat color in offsprings		X^2 -test
			agouti	dilute	
white $\times$ dilute	3	Male	4	0	
		Female	6	0	
		Total	10	0	
F_1 $\times$ dilute	6	Male	6	10	$0.10 < P < 0.25$
		Female	7	8	$0.50 < P < 0.75$
		Total	13	18	$0.25 < P < 0.50$

Table 3. Number of voles classified by coat color in (white $\times$ dilute)- F_2

Dam $\times$ Sire	No. of litter	Sex	Coat color in offsprings			X^2 -test
			agouti	white	dilute	
F_1 $\times$ F_1	10	Male	17	5	2	$0.25 < P < 0.50$
		Female	12	3	5	$0.25 < P < 0.50$
		Total	29	8	7	$0.25 < P < 0.50$

crossed to dilute mutants, BCF_1 were 11 normal agouti and 15 dilutes. The result deviates slightly from expected 1 : 1 backcross ratio ($X^2 = 0.346$, $0.50 < p < 0.75$).

Table 2 shows the result of the mating between white and dilute voles. In *M. montebelli* white in coat color is controlled by an autosomal recessive gene. We express the gene by a symbol $+^{crm}$ tentatively, depending on its indeed coat color rather cream than white, and its allelic non-white gene (exhibiting agouti)

by a symbol $+^{crm}$. As seen in the Table 2, all F_1 animals were agouti in color. In BCF_1 ($F_1 \times$ dilute), agouti (13 heads) and dilute (18 heads) were seen at the ratio of nearly 1 : 1 ($X^2 = 0.516$, $0.25 < p < 0.50$). The mating between F_1 hybrids produced 44 F_2 voles ; consisting of 29 normal agouti, 8 white, and 7 dilute (Table 3). This is statistically within a deviation from the ratio of 9 agouti : 3 white : 3 dilute : 1 white with pink eye that would be expected if pink-eyed dilution (gene symbolized as p) is a

Table 4. Color aberrations in *Microtus* species

Color	Species	Inheritance	Researchers
Albino	<i>M. arvalis</i>	recessive	Frank und Zimmermann ⁵⁾
	<i>M. pennsylvanicus</i>	recessive	Barrett ¹⁾
	<i>M. pennsylvanicus</i>		Sillman & Wellwood ¹⁸⁾
	<i>M. montanus</i>	recessive	Jannett ⁹⁾
	<i>M. montebelli</i>	recessive ?	Watanabe ²¹⁾
Black	<i>M. arvalis</i>	recessive	Frank und Zimmermann ⁵⁾
	<i>M. ochrogaster</i>	recessive ?	Semeonoff ¹⁷⁾
Melanism	<i>M. pennsylvanicus</i>		Blossom ³⁾
Light brown	<i>M. pinetorum</i>		Owen & Shackelford ¹³⁾
	<i>M. pennsylvanicus</i>		Owen & Shackelford ¹³⁾
Yellow	<i>M. pennsylvanicus</i>		Owen & Shackelford ¹³⁾
	<i>M. pinetorum</i>		Owen & Shackelford ¹³⁾
Creamy	<i>M. pennsylvanicus</i>	recessive	Clark ⁴⁾
Silver	<i>M. arvalis</i>	recessive	Frank und Zimmermann ⁵⁾
Ivory	<i>M. arvalis</i>	recessive	Frank und Zimmermann ⁵⁾
Chinchilla	<i>M. arvalis</i>	recessive	Frank und Zimmermann ⁵⁾
White	<i>M. montebelli</i>	recessive	Saito ¹⁶⁾
Smoky	<i>M. arvalis</i>		Frank und Zimmermann ⁵⁾
	<i>M. ochrogaster</i>		Pinter & Negus ¹⁴⁾
Dilute	<i>M. ochrogaster</i>	recessive	Semeonoff ¹⁷⁾
Pale cinnamon	<i>M. pennsylvanicus</i>	recessive ?	Kutz and Smith ¹¹⁾
Pink-eyed dilution	<i>M. arvalis</i>	recessive ?	Frank und Zimmermann ⁵⁾
	<i>M. pennsylvanicus</i>	recessive	Owen & Shackelford ¹³⁾
	<i>M. schermans</i>		Owen & Shackelford ¹³⁾
Extreme dilute	<i>M. pennsylvanicus</i>	dominant	Barrett ²⁾
Piebald	<i>M. pennsylvanicus</i>		Clark ⁴⁾
Dominant spotting	<i>M. montanus</i>	dominant	Pinter ¹⁵⁾
	<i>M. arvalis</i>	dominant	Sugawara <i>et al.</i> ²⁰⁾
White spotting	<i>M. ochrogaster</i>	recessive	Hartke <i>et al.</i> ⁸⁾
	<i>M. pennsylvanicus</i>		Owen & Shackelford ¹³⁾
Recessive spotting	<i>M. arvalis</i>	recessive	Frank und Zimmermann ⁵⁾

simple Mendelian unit character ($X^2 = 3.677$, $0.25 < p < 0.50$).

Based on the literatures, color aberrations in genus *Microtus*, are summarized as in Table 4. As for *M. montebelli*, two variants of coat color, albino [10, 21] and white [16], were reported. Only white voles are maintained in the laboratory [16].

Coat color inheritance in wild rodents is generally referred to that in laboratory mice. In mice, color of pelage depends upon *B-b* and *A-a* locus. The former controls the synthesis of melanin pigment, while the latter controls the pigment distribution in each hair. Combination of *A-a* and *B-b* reveals four basic coat colors, i. e., agouti, black, cinnamon and chocolate. In addition, there is *C-c* locus which is responsible

for the presence of the pigment. Mice having a genotype *c/c* result in albino, regardless of genotype of loci *A-a* and *B-b*. And autosomal recessive gene is responsible for the dilution with pink eye [19].

Also in genus *Microtus*, four basic coat colors were observed as well as in laboratory mice (Table 4). The mode of coat color inheritance in *Microtus* seemed to be similar to that in the mice. However, we cannot confirm whether the inheritance of coat color in *M. montebelli* is the same as laboratory mice, because no coat color variants of them have not been bred in the laboratory except for normal agouti and white.

In the present study, we hypothesized *crm* as a symbol of white gene and *+^{crm}* as its allelic

non-white gene in *M. montebelli*, and mated dilute voles with agouti, homozygous for $+^{crm}$, and with white, homozygous for crm . As the results, it was cleared that dilution with pink eye in *M. montebelli* depends upon an autosomal recessive gene located in the different locus from crm for its expression. We named the gene as pink-eyed dilution expressed by a symbol p and its allelic gene by $+^p$. So the genotypes of three kinds of the voles are represented by $+^{crm}/+^{crm}$; $+^p/+^p$ for agouti, crm/crm ; $+^p/+^p$ for white, and $+^{crm}/+^{crm}$; p/p for dilute.

In F_2 (descendent of white $\times$ dilute), white vole with pink eye (crm/crm ; p/p) was not found, although it would be expected at the ratio of 1/16 theoretically. Recently we have detected 4 white voles with pink eye in the offspring between dilutes ($+^{crm}/crm$; $p/p \times +^{crm}/crm$; p/p). However, neither agouti with pink eye nor dilute with normal dark eye were found in our colony. These facts support that the gene p is responsible for the pink-eyed dilution in *M. montebelli*.

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ハタネズミ (*Microtus montebelli*) における pink-eyed dilution の遺伝

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本邦産ハタネズミ (*Microtus montebelli*) に出現した紅眼を伴う淡色毛 (pink-eyed dilution) の遺伝様式を、野生色および白色ハタネズミと交配することによ

て調べた。その結果、この形質は常染色体劣性遺伝子によって支配されていることが明らかになった。この遺伝子を pink-eyed dilution, 遺伝子記号を p と命名した。

Explanation of Figures

- Fig. 1. Normal agouti vole
Fig. 2. White vole
Fig. 3. Dilute vole with pink eye

- Fig. 4. Young voles of pink-eyed dilution. One vole shows agouti silver pelage which appears only in its infancy.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.