Russian Journal of Genetics, Vol. 41, No. 11, 2005, pp. 1281–1286. Translated from Genetika, Vol. 41, No. 11, 2005, pp. 1552–1558.

ANIMAL GENETICS

Inheritance of Coat Color in the Mole Vole (*Ellobius talpinus* Pallas)

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Received January 21, 2005; in final form, May 26, 2005

Abstract—Based on the ecological features of the mole vole, family analysis of the inheritance of coat color was performed with the use of material collected in a wild population. Analysis of coat color in parents and offspring has demonstrated that the offspring segregation into black and nonblack animals after crosses of different types agrees with the hypothesis on the monogenic inheritance of these color variations. Black mole voles are homozygous for the recessive allele (genotype *aa*). Homozygotes for the dominant allele (*AA*) are brown. Heterozygotes (*Aa*) may be brown or have transitional color. The mean frequency of brown coat color in heterozygotes is 0.509 and is very variable. The higher the color intensity in black elements of parent coat color, the more is the offspring coat color saturated with these elements.

INTRODUCTION

The mole vole *Ellobius talpinus* Pallas is polymorphic with respect to coat color: there are brown, black, and transitional forms [1]. The color polymorphism is especially pronounced in southern Ural and southern Trans-Ural populations. The frequency of color morphs may vary in different years in the same population and in different populations in the same year [2]. These differences seem to be adaptive, because different color morphs have been demonstrated to have specific physiological characteristics [3, 4]. The polymorphism of this character, which is probably correlated with components of adaptation, makes the study of the inheritance of coat color in mole voles an important current problem.

Certainly, genetic analysis of coat color requires that that the animals are kept in a vivarium, because controlled crosses should be performed in at least two generations. However, many cases are known when preliminary data on the mode of inheritance of various characters were obtained by collecting family material in wild populations, if the researchers were able to use the advantage of some ecological and biological specificities of the species studied [5].

The purpose of this study was to determine the mode of inheritance of coat color in the mole vole with the use of the results of observations in a wild population.

MATERIALS AND METHODS

During several years (1985–1999), mole voles were captured and tagged in a polymorphic population from the Kurtamysh raion of the Kurgan oblast [2]. The population comprised mole voles with a black (Bl), transitional (T), and brown (Br) coat colors. We divided the T color into five variants arranged in the order of decreasing the intensity of the black color (from the coat color close to Bl to that close to Br).

T1. Black hair was predominant; there was a brown stripe on the side or on the belly.

T2. The back was black; the sides and the belly were brown (the so-called saddlecloth color pattern). This variant of coat color was observed in approximately two-thirds of animals with T coat color.

T3. The belly was black; the back was brown.

T4. Black hairs were sparsely and almost evenly distributed among brown hairs (the general color was dark brown).

T5. The coat color pattern was similar to T4, but black hair contained less pigment, and the coat color had a gray shade (the general color was gray-brown or dark-gray).

As a rule, mole voles live in relatively stable family groups. In each family, only one pair is usually involved in reproduction for as long as five years [2]. Long-term observations of tagged animals made it possible to take into account migration between families.

The female that was involved in reproduction was identified due to the traces of lactation and nourishing the young.

The reproducing male was identified on the basis of the body length and weight and the state of the reproduction system. Usually, this was the largest and oldest of the males. In this male, well-developed testes were usually observable when the experimenter stretched the skin around the pelvic girdle. The animals constituting the family pair often kept together and were trapped one after the other. Our experience of breeding mole

1022-7954/05/4111-1281

voles in captivity showed that the weight of animals younger than one year was sometimes greater than the weight of adult animals. In this cases, age was the decisive criterion.

In addition, we found that mole voles formed stable family pairs that broke up only when one of the partners died. The life together with reproductively active animals suppressed the sexual maturation of the young (under one year of age). Both members of the family pair displayed a negative, aggressive attitude towards other mature animals appearing in their territory and often attacked them together. These characteristic features were taken into account when determining paternity on the basis of field records. This was done taking into account the age, body weight, and history of the formation of the family group. In most cases, paternity was determined unambiguously.

Sometimes, however, we had to make a choice. For example, the black female no. 110 participated in reproduction in family 36 in 1996–1998. The brown male no. 74 was identified as the sire; it had the largest body weight among the males of this family, including males of the same age (i.e., the oldest ones) in that period. In 1990–1992, the transitional-color female no. 80 participated in reproduction in family 4. The transitional-color male no. 79 was identified as the sire; it was born in 1989 and had the largest body weight among males; in addition, the two animals were captured one after the other.

We performed family analysis of coat color; in each family, the colors of the parents and their offspring were recorded.

We used the χ^2 test [6] for the statistical analysis of the uniformity of contingency and conformity tables; in the case of low expected values, Fisher's exact test for $R \times C$ tables was used (the RCEXACT software package; http://www2.qimr.edu.au/davidD/davidd.html) [7]. The integral uniformity of independent contingency tables was tested by combining Fisher's P values (Fisher's combination test, FCT) [8].

RESULTS AND DISCUSSION

We tested the uniformity of segregation in both sexes separately in each of 76 litters from all families where coat-color segregation was observed in the offspring. Significant differences (at the 5% significance level) were found in only one case. The FCT for all litters was $P = 0.99$. Since the segregations for females and males were similar, subsequent analysis was performed for a pooled sample of both sexes.

In 46 families, segregation was observed in different numbers of litters (one, two, three, four, and five litters in 16, 12, 8, 9, and 1 families, respectively). The uniformity of segregations in different litters of the same family was analyzed. Only in two families were litters nonuniform at the 5% significance level; the combination of P values yielded 0.11. Therefore, we pooled data on different litters within each family in subsequent analysis and analyzed the segregations in 46 families.

In the offspring of 13 pairs of black parents, all of the 230 offspring were black; hence, black mole voles were homozygous.

Table 1 shows the family segregations with respect to coat color in the offspring of parents differing from each other in coat color. We found that the genetic determination of coat color in mole voles did not correspond to the standard simple mode; therefore, for the clarity of the subsequent description and discussion of the results, let us first formulate the genetic hypothesis and then prove it by analyzing the observed segregations.

The hypothesis on the genetic control of coat color. The differences in coat color in the wild population of mole voles are determined monogenically. Black animals are recessive homozygotes *aa*. Animals with transitional color are heterozygotes *Aa*. Brown animals may be either dominant homozygotes *AA* or heterozygotes *Aa*. Modifiers and/or environmental factors determine which phenotype (the transitional or brown coat color) is expressed by the heterozygote *Aa*.

Analysis of segregations. Crosses between mole voles with transitional coat color (T × T) in families 1–4 resulted in the segregation of black animals; in family 5, there were no black offspring; however, the sample size was small (four animals). The segregations in different families were nonuniform ($P = 0.0002$). However, families were uniform ($P = 0.57$) with respect to the black : nonblack segregation and nonuniform ($P = 1.8 \times 10^{-5}$) with respect to the transitional : brown segregation. In total for all five families, the observed ratio (12 black : 40 nonblack animals) agreed with the hypothesis on monogenic segregation (1 : 3) (3 ($P = 0.75$). Thus, animals with black coat color were recessive homozygotes (*aa*) and those with transitional coat color were heterozygotes (*Aa*); however, the transitional-to-brown animal ratio did not correspond to the regular 2 : 1 segregation.

In all families (6–15), black offspring segregated after crosses between transitional and black animals (T × Bl and Bl × T). In four families (6–8 and 15), brown offspring appeared. If black animals were actually *aa* homozygotes and animals with transitional color were *Aa* heterozygotes, the appearance of brown offspring meant that *Aa* heterozygotes could have either transitional or brown coat color. The segregations in T × Bl families were uniform ($P = 0.42$), and the observed ratio 13 black : 20 nonblack animals agreed with the expected 1 : 1 ratio (1 ($P = 0.22$). Note that, in this case, the transitional-to-brown animal ratios in different families were uniform ($P = 0.20$). The segregations in Bl × T families were nonuniform ($P = 0.003$). They were uniform with respect to the black-to-nonblack ratio ($P = 0.15$), and the observed segregation 24 black : 18 nonblack animals agreed with the expected 1 : 1 ratio ($P = 0.35$). However, they were non-

Table 1. Coat-color segregation of the offsprings of parents with different phenotypes

Family no.	Parental phenotypes, female × male	Parental genotype		Offspring phenotype			
		female	male	black	transitional	brown	total
1	T × T	*Aa*	*Aa*	3	1	13	17
2				2	3	0	5
3				3	8	1	12
4				4	6	4	14
5				0	4	0	4
6	T × Bl	*Aa*	*aa*	2	1	4	7
7				9	8	3	20
8				1	2	1	4
9				1	1	0	2
10	Bl × T	*aa*	*Aa*	2	5	0	7
11				6	4	0	10
12				4	1	0	5
13				5	3	0	8
14				6	1	0	7
15				1	0	4	5
16	T × Br	*Aa*	*Aa*	5	0	13	18
17				3	6	8	17
18				1	2	4	7
19				5	1	5	11
20				2	3	8	13
21				1	0	4	5
22		*Aa*	*AA*	0	7	23	30
23	Br × T	*Aa*	*Aa*	5	5	10	20
24				1	1	2	4
25				4	5	10	19
26				4	1	1	6
27		*AA* or *Aa*	*Aa*	0	4	4	8
28	Br × Bl	*Aa*	*aa*	2	0	2	4
29				8	1	9	18
30				10	0	9	19
31				4	0	7	11
32				10	5	11	26
33				3	1	0	4
34		*AA*	*aa*	0	16	6	22
35				0	7	11	18
36	Bl × Br	*aa*	*Aa*	7	7	3	17
37				10	0	7	17
38				4	0	10	14
39				1	0	3	4
40		*aa*	*Aa* or *AA*	0	0	3	3
41	Br × Br	*Aa*	*Aa*	1	0	2	3
42				5	0	11	16
43				2	5	6	13
44		One parent is *Aa*; the other is either *AA* or *Aa*		0	2	1	3
45				0	5	2	7
46		Both parents are either *AA* or *Aa* or one is *AA* and the other is *Aa*		0	0	8	8

uniform with respect to the transitional-to-brown ratio ($P = 0.0013$).

In the offspring of transitional-color and brown animals (T × Br and Br × T), the segregations in families 16–21 and 23–26 was in line with that observed for T × T crosses: all offsprings contained black animals. The segregations in T × Br families being uniform ($P = 0.18$), the summary data (17 black : 12 transitional-color : 42 brown animals) did not correspond to the 1 : 2 : 1 ratio altogether ($P < 1 \times 10^{-4}$), whereas the black-to-nonblack animal ratio corresponded to 1 : 3 ($P = 0.84$), and the transitional-to-brown ratio did not correspond to 2 : 1 ($P < 1 \times 10^{-4}$). The segregations in Br × T families being uniform ($P = 0.61$), the summary data (14 black : 12 transitional-color : 23 brown animals) also did not correspond to the 1 : 2 : 1 ratio ($P = 3 \times 10^{-4}$), whereas the black-to-nonblack animal ratio corresponded to 1 : 3 ($P = 0.56$), and, again, the transitional-to-brown ratio did not correspond to 2 : 1 ($P < 1 \times 10^{-4}$). Thus, it is conceivable that the parents in these T × Br and Br × T families were *Aa* heterozygotes.

There were no black offsprings in families 22 (T × Br) and 27 (Br × T). If we nevertheless assume that the parents were heterozygous in both cases, the probability of this event is $P = 1.8 \times 10^{-4}$ in the former case and $P = 0.10$ in the latter case. Since one parent in family 22 has a transitional coat color (an *Aa* heterozygote), then the other one (brown) was obviously a dominant homozygote (*AA*). In family 27, the brown parent may have been either an *Aa* heterozygote or an *AA* homozygote.

In the offsprings of three families (41–43) of brown animals (Br × Br), there were black animals. The segregations in these three families were uniform ($P = 0.06$, and the summary ratio (8 black : 5 transitional-color : 19 brown animals) did not correspond to the expected 1 : 2 : 1 ratio ($P < 1 \times 10^{-4}$), with the black-to-nonblack ratio corresponding to the expected 1 : 3, and the transitional-to-brown ratio was opposite to the expected ratio 2 : 1 ($P < 1 \times 10^{-4}$). Hence, both parents were heterozygous (*Aa*).

In three Br × Br families (44–46), there were no black offspring. However, there were transitional-color offspring in families 44 and 45. Therefore, in these families, one of the parents was an *Aa* heterozygote, and the other was either an *AA* homozygote or an *Aa* heterozygote, because the probability that black offspring would not be segregated if both parents were heterozygous was $P = 0.42$ in family 44 and $P = 0.13$ in family 45. In family 46, there were only eight brown offspring; therefore, both parents may have been *AA* homozygotes. It is also possible that both parents were *Aa* heterozygotes (then, the probability of nonsegregation of black offspring was $P = 0.10$) or one of the parents was an *AA* homozygote and the other was an *Aa* heterozygote.

In the crosses between black and brown parents (Br × Bl and Bl × Br; families 28–33 and 36–39), black offspring appeared; therefore, all brown parents were *Aa* heterozygotes. However, brown offspring appeared in all families except one (family 37), which confirmed that *Aa* heterozygotes may have a brown coat color. The segregations in families 28–33 were uniform ($P = 0.31$); the ratio 37 black : 45 nonblack animals corresponded to the 1 : 1 segregation ($P = 0.38$); transitional-to-brown animal ratios in different families were also uniform ($P = 0.07$). In Bl × Br crosses, the black-to-nonblack ratios in families 36–39 were similar ($P = 0.34$), and the segregation in the pooled sample (22 black : 30 nonblack animals) corresponded to the theoretical 1 : 1 ratio ($P = 0.27$).

For families 34 and 35, the probabilities that a brown female was an *Aa* heterozygote were 2.4×10^{-7} and 3.8×10^{-6}, respectively; therefore, these females were *AA* homozygotes. In family 40, a brown male may have been either an *AA* homozygote or an *Aa* heterozygote ($P = 0.125$).

Thus, the results of all types of crosses agree with the hypothesis formulated above.

Phenotypes determined by genotype Aa. The results of our study allowed us to estimate the probability (P) that an *Aa* heterozygote would have a brown coat color.

We obtained the following estimates of p:

$$\text{for } Aa \times Aa \text{ crosses, } p = 2 \times [h(\text{br}) - h(\text{bl})];$$

$$\text{for } Aa \times aa \text{ crosses, } p = 2 \times h(\text{br});$$

$$\text{for } Aa \times AA \text{ crosses, } p = 2 \times [h(\text{br}) - 0.5]; \text{ and}$$

$$\text{for } AA \times aa \text{ crosses, } p = h(\text{br}),$$

where h(br) and h(bl) are the frequencies of brown and black mole voles, respectively.

The number of offspring in a family was usually small, and the variation of p was wide. Therefore, we estimated p for a pooled sample of all types of crosses (T × T, T × Bl, …, Br × Br) in families 22, 34, and 35 (Table 2). For these ten subsamples, the median of P values was 0.509, and the 95% confidence interval was wide (from 0.282 to 0.664) [6]. Note that the probability that heterozygous offspring (*Aa*) would have a brown coat color depended on the parental phenotype: it was lower (about 0.3) if heterozygous parents had a transitional coat color (crosses T × T, T × Bl, and Bl × T), higher (about 0.8) if *Aa* parents were brown (Br × Br, Br × Bl, and Bl × Br), and intermediate (about 0.5) if both parents were heterozygous, one of them being brown and the other having a transitional color. The causative relationships in this case are a complicated problem and requires special investigation.

Thus, the *Aa* genotype was characterized not only by a variable dominance mode (complete in the case of the brown coat color and incomplete in the case of the transitional color), but also by a considerable variation of the frequencies of dominance modes in different families. This may have been accounted for by both genotypic environment (the absence or presence of different modifiers in different genotypes) and some external environmental factors.

Table 2. The probability for *Aa* heterozygous offspring to have a brown coat color as dependent on the parental phenotype

Family no.	Parental phenotypes, female × male	Parental genotype		Probability (*P*)
		female	male	
1–5	T × T	*Aa*	*Aa*	0.231
6–9	T × Bl	*Aa*	*aa*	0.485
10–15	Bl × T	*aa*	*Aa*	0.190
16–21	T × Br	*Aa*	*Aa*	0.704
22		*Aa*	*AA*	0.533
23–26	Br × T	*Aa*	*Aa*	0.367
28–33	Br × Bl	*Aa*	*aa*	0.927
34, 35		*AA*	*aa*	0.425
36–39	Bl × Br	*aa*	*Aa*	0.885
41–43	Br × Br	*Aa*	*Aa*	0.688

Table 3. The relationship between the parental coat color and the expression of the transitional coat color in the offspring

Parental phenotype	Offspring phenotype		Total
	T1, T2	T3, T4, T5	
One is Bl and the other is T1 or T2; both are T1 or T2	26	0	26
One is Bl, T1, or T2 and the other is T3, T4, T5, or Br	37	11	48
Both are T3, T4, T5, or Br	12	12	24
T o t a l	75	23	98

Expressivity of the transitional coat color. As noted above, we subdivided the transitional coat color into five types ordered in the direction from black to brown. We compared the phenotypes of the parents (the black, all transitional, and brown coat colors) and the types of the transitional coat color in the offspring. The contingency table for the offspring (Table 3) does not include the phenotypes of the black and brown coat colors, because they were distinctly related to segregation depending on the type of crossing. As can be seen from Table 3, the characters of the parents and offspring were correlated with each other: the more intense the black elements of the parental coat color, the more intense were they in the offspring ($P = 1 \times 10^{-4}$). The corrected contingency coefficient [9] was 0.39.

Earlier, Gershenzon [10–12] noted the evolutionary importance of dominant genes with variable penetrance and expressivity. It should be emphasized that we speak of different degrees of expression and intensity of the character in animals genetically identical with respect to the given locus (*Aa*). Incomplete penetrance of coat-color characters is common in rodents. A 0.97 penetrance of allele a^e (*extreme non-agouti*) has been observed in heterozygous (Aa^e) *Arvicola terrestris* L. [13]; and a 0.86 penetrance of allele *h* (*houded*), in heterozygous (*Hh*) wild *Rattus norvegicus* L. [14].

Further studies on the inheritance of coat color in the mole vole *E. talpinus* are required, both under field conditions (analysis of migrations between families) and under laboratory conditions (controlled crosses).

ACKNOWLEDGMENTS

We are grateful to N.N. Khromov-Borisov for valuable consultations and the help with the statistical treatment of the data.

This study was supported by the Russian Foundation for Basic Research (project nos. 03-04-49 776, 04-04-48 352, and 04-04-96 100 r2004ural) and the Mari State University (the task of the Ministry of Education of the Russian Federation).

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