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Genotype-dependent participation of coat color gene loci in the behavioral traits of laboratory mice

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ABSTRACT

To evaluate if loci responsible for coat color phenotypes contribute to behavioral characteristics, we specified novel gene loci associated with social exploratory behavior and examined the effects of the frequency of each allele at distinct loci on behavioral expression. We used the F2 generation, which arose from the mating of F1 mice obtained by interbreeding DBA/2 and ICR mice. Phenotypic analysis indicated that the agouti and albino loci affect behavioral traits. A genotype-based analysis revealed that novel exploratory activity was suppressed in a manner dependent on the frequency of the dominant wild-type allele at the agouti, but not albino, locus. The allele-dependent suppression was restricted to colored mice and was not seen in albino mice. The present results suggest that the agouti locus contributes to a particular behavioral trait in the presence of a wild-type allele at the albino locus, which encodes a structural gene for tyrosinase.

1. Introduction

The domestication of animals has caused a great deal of change to mankind's way of life. The most important factor contributing to the domestication process is an understanding of the docility and environment of animals. Tameness is attributed to individual temperament, which is shaped over a lifetime, and genetic factors. Although several genetic changes may be involved, little is known about the genetic basis of the process (Rosengren Pielberg et al., 2008). However, a relationship between coat (or pelage) color and temperament, such as behavioral and emotional characteristics, has been reported for numerous species (Hemmer, 1990).

In mice, many genes are known to participate in the manifestation of coat color. A number of these genes have been cloned, and their effects on inheritance of a coat color phenotype has been elucidated (Jackson, 1994). In particular, coat color can be accounted for by a combination of genotypes in inbred strains of laboratory mice. Individually, these genes show autosomal dominant inheritance at four principal loci, which influence the synthesis of melanin, the source of pigmentation, and its transport to melanosomes in melanocytes. These loci are the agouti (*A*-: the wild type allele displays individual hairs with bands of light and dark pigment; *aa*: homozygous for the recessive allele displays non-banded eumelanotic hairs), brown (*B*-: the wild type allele produces black eumelanin; *bb*: homozygous for the recessive allele leads to production of brown eumelanin), albino (*C*-: colored; *cc*: homozygous for the recessive allele leads to albinism), and dilute (*D*-: the wild type allele; *dd*: homozygous for the recessive allele expresses a diluting effect on hair pigment, giving the coat an overall "washed-out look"). The coat color phenotype of offspring generated by the interbreeding of genetically defined strains can be surmised. Much attention has focused on the role of the agouti locus in the docility of animals, including foxes (Keeler et al., 1968), rats (Cottle and Price, 1987), and deermice (Hayssen, 1997). Findings have shown that animals homozygous for the recessive non-agouti allele (*aa*) are more docile than counterparts with the dominant wild-type agouti allele (*A*-), i.e., the wild-type coat color. Furthermore, the ectopic overexpression of agouti signaling protein (ASP), encoded by the wild-type agouti allele, resulted in changes in feeding behavior (Fan et al., 1997) and stress responsiveness (Harris et al., 2001; Bazhan et al., 2004) in specific strains of mice. In addition to the agouti locus, other loci, in particular the dilute locus (Cazala and Guenet, 1977) and albino locus (Winston et al., 1967; van Abeelen and Kroes, 1968; Tiessen et al., 1970; Rhoades and Henry, 1977; Cazala and Guenet, 1979; Katz and Doyle, 1981; Le Pape and Lassalle, 1986) exert pleiotropic effects on a number of behavioral traits. Thus, the gene loci primarily responsible for the coat color phenotype are important genetic elements influencing temperament or behavioral characteristics in individual animals. However, it remains unclear whether the genotype of individuals at each locus is reflected in behavioral expressions. In the present study, we focused on novel social exploratory behavior and determined the gene locus associated with the behavior pattern in mice. We then examined the effect of each genotype at distinct loci on

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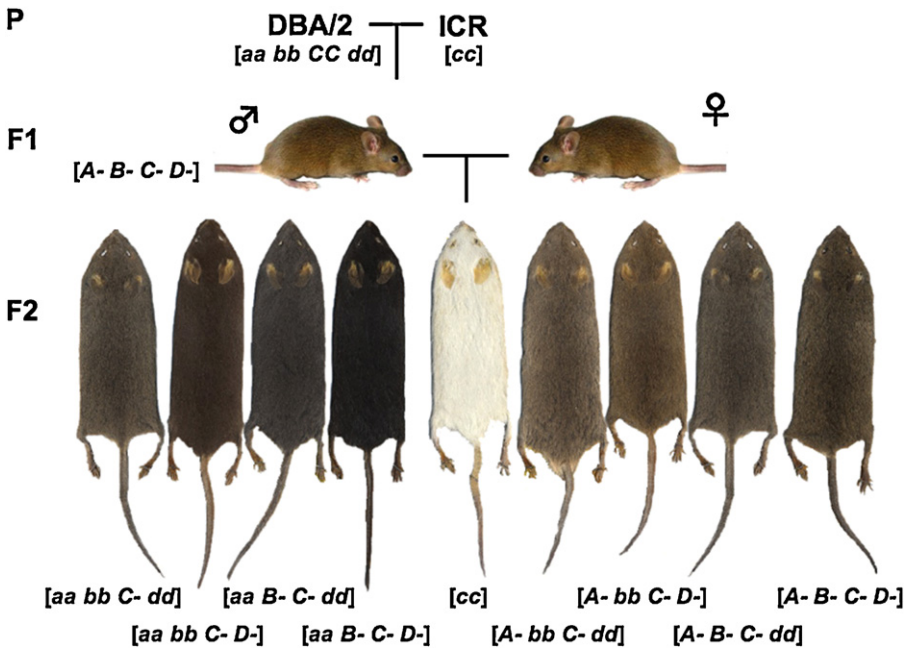

Fig. 1. Coat color phenotypes of the F2 generation, obtained by mating male and female mice with the wild-type agouti coloration from the F1 generation, a cross between inbred DBA/2 mice and a closed colony of albino ICR mice.

behavioral expression using genotyping methods established in our laboratory.

2. Materials and methods

2.1. Animals and breeding procedure

All experiments were performed in accordance with the Guidelines for Animal Experiments, College of Bioresource Sciences, Nihon University and Nihon University's Animal Care and Use Committees. Inbred male DBA/2CrSlc mice (genotype: *aa bb CC dd*) and closed colony female albino Slc:ICR mice, aged 10 weeks, were purchased from Japan SLC, Inc. (Hamamatsu, Japan). A breeding room was maintained at 23 ± 1 °C with a 14 h/10 h light/dark cycle (lights on at 06:00 h). Both strains were housed in groups (4/cage) in stainless steel cages with standard wood shaving bedding and were provided with food and water ad libitum. When they reached 12 weeks of age, female ICR mice were mated with DBA/2 males, and offspring were produced with two distinct types of coat color, agouti (*Aa*) and non-agouti (*aa*). F1 hybrid females with the agouti coat color were randomly mated with males of the same coat color phenotype at 12 weeks of age to obtain all coat color patterns, governed independently by four principal loci in the next generation. In principle, each litter was adjusted to 8 pups regardless of skin color 3 days of parturition and the offspring were weaned at 21 days of age. Consequently, we obtained 112 males from the F2 generation with 9 distinct types of coat color (Fig. 1). Siblings were group-housed (3–4/cage) until 8 weeks of age, then housed individually. The animals were next moved from the breeding room to a behavior-analyzing room and maintained with a 14 h/10 h light/dark cycle (lights off at 12:00 h) to habituate to the environment. After 1 week of habituation, the behavioral test described below was carried out between 13:00 h and 16:00 h under dim illumination.

2.2. Determination of novel-social exploratory behavior

A 60 cm × 45 cm open arena made of dark brown, opaque acrylic plates with a wall height of 40 cm was used. Placed in the center of the arena was a cylindrical tube with a diameter of 11.4 cm and a height of 20.5 cm; the lower part was made of stainless steel with many small holes and the upper part was made of a transparent acrylic plate. Each F2 male was placed alone on one side of the arena and allowed to habituate to the test environment for 3 min. A social partner, a 5- to 7-week-old castrated outbred *dd*-strain mouse from a breeding colony in our laboratory, was then placed in the cylindrical tube. Novel exploratory behavior and social interactions were recorded with a digital video camera for 5 min. The behavioral activities analyzed were as follows. Latency to start social behavior: time until the first active approach toward the social partner. Social behavior: frequency and cumulative time of following and sniffing the partner during a 5 min test. These behavioral parameters are considered to be social partner-directed activities. Exploratory rearing behavior: the frequency and cumulative time of rearing with hind legs. Self-grooming: frequency of self-grooming bouts of the nasal region by the forelegs. These behavioral parameters are considered to be non-partner-directed activities. After the recording, the mice were returned to their home cages and the test arena was immediately swept and cleaned with 35% isopropanol. Each behavioral test was conducted 6 times a day at 15–20 min intervals. One week after the last behavioral test, the whole brain of each mouse was removed, weighed and stored at −80 °C.

2.3. Preparation of genomic DNA

For genotyping by PCR, genomic DNA was extracted from 10 to 20 mg of the medulla oblongata using a Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, WI) in a final volume of 100 ng/μl of TE buffer pH 8.0, according to the manufacturer's directions with some modifications, and quantified by spectrophotometry.

2.4. Genotyping procedures for agouti and albino loci

The gene encoding mouse ASP consists of 4 exons and the loss-of-function allele (non-agouti allele) includes an 11-kb retrotransposon sequence in the intron between the first and second exons (Bultman et al., 1994). Accordingly, the following PCR primers