D-アミノ酸酸化酵素欠損ddY/DAO マウスの毛色遺伝子の分析とD-アミノ酸酸化酵素遺伝子と毛色遺伝子との非連鎖

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Coat color genes of D-amino acid oxidase deficient ddY/DAO$^-$ mice and nonlinkage of gene for D-amino acid oxidase to coat color genes

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ABSTRACT

Coat color genes of mutant ddY/DAO$^-$ mice that do not have D-amino acid oxidase were examined by crosses of these mice with NC (*AAbbCC*) and II TES (*aabbCCddss*) mice. All the (ddY/DAO$^-$×NC)F_1 mice had agouti coats and the (ddY/DAO$^-$× II TES)F_1 mice had black coats. These results indicate that the ddY/DAO$^-$ mice carry *aaBBccDDSS* gene.

Linkage relationship of *Dao-1* gene coding for D-amino acid oxidase to coat color genes was examined. Segregants produced from backcrosses of (ddY/DAO$^-$× II TES)F_1 with ddY/DAO$^-$ and (ddY/DAO$^-$×NC)F_1 with ddY/DAO$^-$ were examined for D-amino acid oxidase activity and coat color. F_2 segregants from a (ddY/DAO$^-$×NC) cross were also studied. These crosses showed that *Dao-1* was not linked to *a*, *b* or *c*.

1. INTRODUCTION

Almost all vertebrates have D-amino acid oxidase (DAO) [D-amino acid: O_2 oxidoreductase (deaminating), EC 1.4.3.3] in their kidneys, livers and brains (Krebs 1935; Meister 1965; Konno and Yasumura 1981; Konno *et al.* 1982). The mouse is an exceptional animal that does not have this enzyme in the liver (Shack 1943). The physiological role of this enzyme is not known since the substrates, D-amino acids, are rare in the metabolism of vertebrates.

In a survey of seven mouse strains, we found that some mice of noninbred ddY strain did not have DAO activity in their kidneys (Konno and Yasumura 1983). The mutant mouse stock (ddY/DAO$^-$) had been established. The mouse had an autosomal null allele, *Dao-1^e*. Gene dosage effect was observed for activity of this enzyme: a heterozygous mouse for this gene (*Dao-1$^+$*/*Dao-1^e*) had about half the normal activity of a wild-type homozygote (*Dao-1$^+$*/*Dao-1$^+$*). The ddY/DAO$^-$ mouse also lacked brain DAO activity. Cross experiments showed that both kidney and brain enzymes were coded by a single gene (Konno and Yasumura 1984).

In the previous studies, however, two problems remained unsettled: when the *Dao-1^e* allele arose and how it had been fixed in the population of the ddY mice. They necessitate us to examine the character and the history of this ddY mouse. Ancestral dd mice were imported into Japan before 1920 by Dr.

Sahachiro Hata. Since then, many strains have been established and the ddY strain is one of these descendants. Examination of the genetic constitution of the ddY/DAO$^-$ mouse would also reveal its history since historical records have been engraved in the genetic constitution.

In this study, we examined coat color genes of the ddY/DAO$^-$ mouse and linkage relationship of *Dao-1* gene to these genes.

2. MATERIALS AND METHODS

NC, IITES and ddY/DAO$^-$ mice were used. NC and IITES mice were kindly supplied by Dr. K. Kondo, Department of Agriculture, Nagoya University.

Mice of the ddY/DAO$^-$ stock were crossed with NC mice. Some of the hybrid F_1 mice were backcrossed with the ddY/DAO$^-$ mice and others were intercrossed to produce F_2 offspring.

Mice of the ddY/DAO$^-$ stock were also crossed with IITES mice. The hybrid F_1 mice were backcrossed with the ddY/DAO$^-$ mice.

Segregants were examined for coat color and DAO activity in their kidneys and brains. DAO activity was determined with D-alanine as the substrate by the method of Watanabe *et al.* (1978) as described before (Konno and Yasumura 1981).

3. RESULTS

Mice of ddY/DAO$^-$ stock are albino, indicating that they are *cc* homozygotes. Other coat color genes of ddY/DAO$^-$ mice were determined by cross experiments. They were crossed with NC mice (*AAbbCC*). All eight of the hybrid F_1 mice had agouti coats [*ABC*]. This result suggests that the ddY/DAO$^-$ mice carry homozygous *BB* genes. Then, ddY/DAO$^-$ mice were crossed with IITES mice (*aabbCCddss*). All nine of the hybrid F_1 mice were black [*aBCDS*], suggesting that the ddY/DAO$^-$ mice had *aaBBDDSS* genes. This result is consistent with the above result that they have *BB* genes. Therefore, we conclude that the ddY/DAO$^-$ mice carry *aaBBccDDSS* genes.

Linkage relationship of *Dao-1* gene coding for D-amino acid oxidase to mouse coat color genes was then examined. The F_1 hybrids from the prior cross (ddY/DAO$^-$×IITES) were backcrossed with the ddY/DAO$^-$ mice. If null allele *Dao-1^e* carried by the ddY/DAO$^-$ mice was linked to *c*, only two classes of segregants would be expected: black mice with intermediate DAO activity (*Dao-1$^+$/Dao-1^e*) and albino animals without DAO activity (*Dao-1^e/Dao-1^e*). However, this was not the case. Four classes of segregants were obtained (Table 1). In addition to the anticipated offspring, black mice without DAO activity and albino animals with intermediate DAO activity were obtained. The ratio was almost 1 : 1 : 1 : 1. These results indicate that *Dao-1^e* is not linked to *c*. Only two classes of progeny with respect to coat color, black and albino,

Table 1. *Coat colors and Dao-1 genotypes of segregants from a backcross of* $(ddY/DAO^- \times IITES)F_1$ *with* ddY/DAO^-

	Black [*aBCDS*]	Albino [*aBcDS*]	
$\frac{Dao\text{-}1^+}{Dao\text{-}1^e}$	11	7	18
$\frac{Dao\text{-}1^e}{Dao\text{-}1^e}$	15	11	26
	26	18	44

Table 2. *Coat colors and Dao-1 genotypes of segregants from a backcross of* $(ddY/DAO^- \times NC)F_1$ *with* ddY/DAO^-

	Agouti [*ABC*]	Black [*aBC*]	Albino [*--c*]	
$\frac{Dao\text{-}1^+}{Dao\text{-}1^e}$	2	3	9	14
$\frac{Dao\text{-}1^e}{Dao\text{-}1^e}$	2	7	9	18
	4	10	18	32

were obtained. This result is consistent with the above conclusion that the ddY/DAO$^-$ mice have *aaBBccDDSS* genes.

Then it was examined whether *Dao-1^e* was linked to *a*. The hybrid F_1 mice from the cross (ddY/DAO$^-$×NC) were backcrossed with the ddY/DAO$^-$ mice. If *Dao-1^e* was linked to *a*, neither black mice with intermediate DAO activity nor agouti animals without DAO activity would be produced. However, this was not the case. Six classes of offspring, including the above two, were obtained (Table 2). Therefore, *Dao-1^e* was concluded to be unlinked to *a*. The segregation of agouti, black and albino mice was also consistent with the above conclusion about the coat color genes of the ddY/DAO$^-$ mice.

Finally, it was examined whether *Dao-1^e* was linked to *B*. The (ddY/DAO$^-$×NC)F_1 hybrids were intercrossed to produce F_2 offspring. If *Dao-1^e* was linked to *B*, nine classes of segregants would be expected. However, this cross produced 13 classes of F_2 progeny, including additional four classes of animals (brown mice with high and intermediate DAO activity, and both agouti and cinnamon ones without DAO activity) (Table 3). These animals were not to be expected if *Dao-1^e* was linked to *B*. Therefore, these genes are considered to be unlinked. Black mice with high DAO activity and black animals without DAO activity were missing. This would be because a small

Table 3. *Coat colors and Dao-1 genotypes of F_2 segregants from a cross (ddY/DAO$^-$×NC)*

	Agouti [*ABC*]	Cinnamon [*AbC*]	Black [*aBC*]	Brown [*abC*]	Albino [*--c*]	
$\frac{Dao\text{-}1^+}{Dao\text{-}1^+}$	5	2	0	1	4	12
$\frac{Dao\text{-}1^+}{Dao\text{-}1^e}$	17	4	5	5	6	37
$\frac{Dao\text{-}1^e}{Dao\text{-}1^e}$	8	2	0	1	1	12
	30	8	5	7	11	61

number of F_2 mice were examined. The results of this cross confirmed the above conclusions that *Dao-1^e* is not linked to *a* or *c* and that the ddY/DAO$^-$ mice have *aaBBccDDSS* genes.

4. DISCUSSION

The ddY/DAO$^-$ mouse was found to carry *aaBBccDDSS* genes. These characters are different from those of mice from some inbred strains of the dd family [DD/Tbr (*AABBccSS*), DDD (*AABBccDDSS*) and DDK (*AABBccDDSS*)] (Staats, 1980). However, mice of other members of this group [DDI (*aaBBccDDSS*), DKI (*aaBBccSS*), KF (*aaBBccSS*) and 4CS (*aaBBccSS*)] have similar coat color genes (Staats 1980). The dd family is divided into two groups with respect to *A/a* gene. Yonekawa *et al.* (1982) also showed the heterogeneity in mitochondrial DNA of ddY mice and suggested the contamination of the original dd stock with Asiatic mice. Our previous result that the DD/Tbr strain did not contain D-amino acid oxidase deficient individuals (Konno and Yasumura 1983) may be consistent with the remote relationship between the ddY and DD/Tbr mice. Further examination of the genetic constitution of the ddY/DAO$^-$ mouse would reveal its origin, history and relationship to other members of this family. This approach would make it possible to trace when the *Dao-1^e* allele arose and how it had been fixed in the population of the ddY mice.

The *Dao-1^e* gene was not linked to *a*, *B* or *c*. These results are consistent with those of Seeley and Holmes (1981). They found the linkage of *Dao-1* gene to *Bcd-1* gene that codes for butyryl CoA dehydrogenase. They also showed nonlinkage of *Bcd-1* to *fz*, *ln*, *a*, *Hao-1*, *Cs*, *Adh-3*, *Pgm-1*, *wa-1*, *c^{ch}*, *d*, *se*, *Mod-1*, *v*, *wa-2*, *pe*, *s* or *ep*. The *Dao-1* and *Bcd-1* genes still remain to be assigned to a specific chromosome.

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