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Genetics

Sensory distinction between *H-2*^{b} and *H-2*^{bm1} mutant mice

(major histocompatibility complex/Y maze/transfer of training/odor phenotypes/olfaction)

K. YAMAZAKI*, G. K. BEAUCHAMP*, I. K. EGOROV†, J. BARD‡, L. THOMAS‡§, AND E. A. BOYSE‡§

*Monell Chemical Senses Center, Philadelphia, Pennsylvania 19104; †The Jackson Laboratory, Bar Harbor, Maine 04609; ‡Memorial Sloan–Kettering Cancer Center, New York, New York 10021; and ‡Sloan–Kettering Division, Cornell Graduate School of Medical Sciences, New York, New York 10021

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ABSTRACT Genetic polymorphism in the *H-2:Qa:Tla* region of chromosome 17 is associated with constitutive variation of bodily odor phenotypes which permit individual olfactory recognition among mice. To determine whether known genes in the *H-2:Qa:Tla* complex are concerned in the constitution of odor phenotypes, mice were tested for their ability to sense a difference between the B6/By (*H-2*^{b}) and congeneic B6.C-*H-2*^{bm1} strains, which differ genetically by mutation of the *H-2K* gene. As in previous studies of the sensory discrimination of H-2:Qa:Tla phenotypes, mice were trained by reward in a Y maze to distinguish the odors of urine samples, and the successful distinctions of B6/By from B6.C-*H-2*^{bm1} were confirmed by transfer of training, without reward, to coded samples of urine from genetically equivalent urine donor mice which the trained mice had not previously encountered. Cosegregation of odor phenotype with *H-2*^{b} and *H-2*^{bm1} was demonstrated by transfer of training to typed H-2^b and H-2^{bm1} homozygous segregants of F_2 generations of appropriate crosses. Although it is not excluded that the differences in odor phenotype which distinguish *H-2*^{b} and *H-2*^{bm1} mice are directly related to the structure of the H-2^b and H-2^{bm1} products, it is equally possible that H-2-related odor phenotypes arise from effects of *H-2* genetic variation on metabolic pathways either directly, or indirectly through developmental polymorphism.

The *H-2:Qa:Tla* region of chromosome 17 of the mouse (1, 2) is concerned in the composition of body odors that enable mice to distinguish one another according to the constellation of alleles they carry throughout this part of the genome, which occupies about 2 centimorgans. Sensory recognition of H-2:Qa:Tla phenotypes is shown by H-2-associated mating preference (3–6), by the training of mice to distinguish body and urinary odors of H-2-dissimilar congeneic mice in a Y maze (7–10), and by the raised incidence of blockage of pregnancy or pseudopregnancy in mated females exposed to the presence or urine of a new companion whose H-2 type differs from that of the original mate (11). More than one gene of the *H-2:Qa:Tla* complex is concerned in constituting individual odor phenotypes: genetic differences in the vicinity of *H-2K* alone, and of *Qa:Tla* alone, each independently confer individuality of scent (9).

The question whether this individuality of scent can be a function of known *H-2:Qa:Tla* genes rather than of linked unknown genes can be addressed by testing whether mice can distinguish the scent of a known mutant strain from that of the otherwise genetically identical nonmutant strain. For this purpose we have studied the ability of mice to distinguish the odor of the B6/By strain from that of the B6.C-*H-2*^{bm1} congeneic mutant strain (12). The only known genetic differences between those two strains are in the *H-2K* gene, and the only known structural differences in the H-2K cell surface glycoprotein, which is the product of the *H-2K* gene, are amino acid substitutions at positions 152, 155, and 156, corresponding to DNA alterations within a sequence of 13 bases in the *H-2K* gene (13, 14).

The olfactory distinction of H-2^b from H-2^{bm1} phenotype was tested in the Y maze, as in previous studies of the relation of bodily odors to H-2:Qa:Tla phenotypes (8, 9).

MATERIALS AND METHODS

The Y Maze. The design and operation of the Y maze are described in detail elsewhere (7–9). Air is conducted through two odor chambers, containing urine samples exposed in Petri dishes, to the two arms of the maze. Gates are raised and lowered in timed sequence to permit the training or testing of each mouse in a series of up to 48 consecutive runs, the samples being changed for each run. The reward is a drop of water, the mouse having been deprived of water for 23 hr. The water dispenser in each arm of the maze is guarded by a fence, which is raised only if the mouse's choice is concordant with training.

Training. Preliminary training progressed from gross to fine distinctions, in stages, as described (8, 9). All mice were satisfactorily trained to make the final distinction between H-2^b and H-2^{bm1} phenotypes. The 10 trained mice used in the studies reported here are denoted B10.S♂1 (*H-2*^{s}), B10.S♀, B6-*H-2*^{k}♂, B6-*H-2*^{k}♀1, B6-*H-2*^{k}♀2, B10.S♂2, B10♂ (*H-2*^{b}), BALB×B6F$_1$♀ (*H-2*^{d}/*H-2*^{b}), B6♀1 (*H-2*^{b}), and B6♀2. Their performance did not significantly differ, and the data are combined in the tables.

Transfer of Training. As described fully elsewhere (8–10), the purpose of this procedure is to test new urine-donor panels without reward and thereby rule out the possibility that incidental or genetically unrelated cues are being learned and responded to; if there is no reward, there can be no learning of adventitious cues. Transfer of training is conducted with blind testing of coded samples, which is possible because no reward is called for. To maintain reinforcement (concordant response to the learned scent), the unrewarded samples from new panels are interspersed with concurrent, rewarded testing of the familiar sources to accustom the already trained mice to periodic withholding of reward. Data for transfer of training are presented in each table in two parts indicated under "test phase": (*i*) *reward*, comprising the rewarded and interspersed unrewarded trials of the familiar odor source panels, and (*ii*) *no reward*, comprising the interspersed and uniformly unrewarded trials of the new donor panels.

Urine Donor Panels. Paired panels of age-matched male mice were set up as urine donors for each of the distinctions noted in the tables. The panel mice were individually numbered so that they could be used in rotation to provide different sample pairs for each run. The strain and source of the panel mice and the numbers of mice per panel are given in the tables. All panel mice were maintained under uniform conditions in the same animal room at the Monell Chemical Senses Center. Imported

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Table 1. Distinction between B6/By (B6) and B6.C-*H-2*^{bm1} (bm1) of Egorov's colonies with transfer of training to second sets of urine donors

Trained mice	Urine donor panels*	Test phase	Trials, no.	Concordance, %
B10.S♂1 B10.S♀	First sets of B6 (reward) vs. bm1	Reward	305	79
B6-*H-2*^{k}♀1	Second sets of B6 vs. bm1	No reward†	45	73
B10.S♂2 B10♂	First sets of B6 vs. bm1 (reward)	Reward	253	81
B6♀1	Second sets of B6 vs. bm1	No reward†	37	73
Total		Reward	558	80‡
		No reward†	82	73§

*Panels of individually marked age-matched mice, numbering 25 for each B6 panel and 19 for each bm1 panel; urine samples were changed after each run.
†Transfer of training with coded samples.
‡$P < 0.0001$; P values refer to probabilities that the observed concordances differ from 50% by chance; the difference between reward and no reward phases (80% and 73%) is not statistically significant.
§$P < 0.001$.

mice were not used for at least 3 weeks, to allow for acclimatization.

Urine Samples. Urine was obtained by gentle abdominal pressure. Usually a single mouse provided enough urine (0.2–0.3 ml) to cover the bottom of a 3.5-cm-diameter Petri dish, but sometimes two (rarely more) mice were needed. Fresh samples from different donors were used for each run. As before (8–10), the samples were assigned to the left or right odor boxes of the Y maze according to a series of random numbers. On each day of testing, a given combination of two numbered donors was never repeated.

RESULTS

In the study shown in Table 1, B6/By and B6.C-*H-2*^{bm1} mice of Egorov's colonies were successfully distinguished. The trained mice also distinguished coded samples of urine, without reward, from duplicate panels of Egorov's B6/By and B6.C-*H-2*^{bm1} mice that they had not previously encountered (transfer of training).

In a similar study (Table 2), mice trained to distinguish B6/By and B6.C-*H-2*^{bm1} mice from Jackson production colonies successfully distinguished mice of the same two strains from Bailey's colonies in transfer of training tests of coded samples.

Cosegregation with *H-2* is shown by transfer of training to H-2^{b} homozygous segregants of the cross (B6 × B6-*H-2*^{k})F_2 compared with congeneic H-2^{bm1} homozygous segregants of the cross (B6.C-*H-2*^{bm1} × B6-C-*H-2*^{k})F_2 (Table 3). These particular crosses were used to test cosegregation because of the ease of typing for H-2 serologically and excluding H-2^{k} homozygous and heterozygous segregants. It was further shown, by transfer of training to coded samples, that mice trained to distinguish B6/By and B6.C-*H-2*^{bm1} mice could distinguish the (B6 × B6-*H-2*^{k})F_1 hybrids from the (B6.C-*H-2*^{bm1} × B6-*H-2*^{k})F_1 hybrids from which the F_2 segregants were bred (data not shown).

Cosegregation with *H-2* was further shown by transfer of training to H-2^{b} and H-2^{bm1} homozygous segregants of the cross (B6 × B6.C-*H-2*^{bm1})F_2 (Table 4) which were typed (Egorov) by skin grafting because they cannot readily be typed serologically.

Table 2. Distinction between B6/By and B6.C-*H-2*^{bm1} purchased from The Jackson Laboratory (B6/J vs. bm1/J) with transfer of training to mice provided by Bailey (B6/By vs. bm1/By)

Trained mice	Urine donor panels*	Test phase	Trials, no.	Concordance, %
B6-*H-2*^{k}♀2 B6-*H-2*^{k}♂	First sets of B6/J (reward) vs. bm1/J	Reward	218	78
	Second sets of B6/By vs. bm1/By	No reward†	24	79
B6♀2 BALB × B6♀	First sets of B6/J vs. bm1/J (reward)	Reward	313	81
	Second sets of B6/By vs. bm1/By	No reward†	38	74
Total		Reward	531	80‡
		No reward†	62	76§

*Panels of individually marked age-matched mice, numbering 21 for B6/J, 28 for bm1/J, 5 for B6/By, and 8 for bm1/By.
†Transfer of training with coded samples.
‡$P < 0.0001$; P values refer to probabilities that the observed concordances differ from 50% by chance; the difference between reward and no reward phases (80% and 76%) is not statistically significant.
§$P < 0.001$.

Table 3. Distinction between B6/By (B6) and B6.C-*H-2*^{bm1} (bm1) of Egorov's colonies, with transfer of training to H-2^b homozygous segregants of the cross (B6 × B6-*H-2*^{k})F_2 and H-2^{bm1} homozygous segregants of the cross (B6.C-*H-2*^{bm1} × B6-*H-2*^{k})F_2 (bF_2 vs. $bm1F_2$) typed serologically by exclusion of H-2^k-positive segregants

Trained mice	Urine donor panels*	Test phase	Trials, no.	Concordance, %
B10.S♂1 B10.S♀	First sets of B6 (reward) vs. bm1	Reward	179	88
B6-H-2^k♀1	Second sets of bF_2 vs. $bm1F_2$	No reward†	25	88
B10♂ B10.S♂2	First sets of B6 vs. bm1 (reward)	Reward	107	85
	Second sets of bF_2 vs. $bm1F_2$	No reward†	14	71
Total		Reward	286	87‡
		No reward†	39	82§

*Panels of individually marked age-matched mice, numbering 50 for B6, 38 for bm1, 8 for bF_2, and 10 for $bm1F_2$.
†Transfer of training with coded samples.
‡$P < 0.0001$; P values refer to probabilities that the observed concordances differ from 50% by chance; the difference between reward and no reward phases (87% and 82%) is not statistically significant.
§$P < 0.001$.

DISCUSSION

In a sensory communication system affected by genetic polymorphism, genetic variation might operate on the constitution of the sensory signals and on perception and response by the recipient. All work done so far on sensory recognition of H-2:Qa:Tla phenotypes, including the present report, has been directed mainly to genetically determined variation in the composition of the odor phenotypes and, to that extent, has no bearing on whether there is also genetic variation of perception and response. The latter is clearly possible and of much interest because it would relate to genes acting within the receptor–effector machinery of the olfactory or other chemosensory systems; but the main information available at present concerns the genetics of odor phenotype constitution, and this has no connection with the genetics of olfaction.

The sensory distinction of H-2^b from H-2^{bm1} mutant phenotypes shows that individuality of scent can be conferred by one of the known genes, H-2K, in the *H-2:Qa:Tla* complex. The *H-2K* gene is one of about 36 class I genes that occupy this region of chromosome 17 and may be exclusive to chromosome 17 (15). Cross-hybridization with DNA probes shows that class I genes comprise a related family, and their products, the class I cell surface glycoproteins, share a characteristic structure.

The *bm1* mutation arose in a (B6 × BALB/c)F_1 hybrid and was introduced into the B6 strain by serial backcrossing to derive the congeneic B6.C-*H-2*^{bm1} strain (12). Contamination of B6.C-*H-2*^{bm1} with BALB/c genes not linked to *H-2* need not be considered in the present study because the odor phenotypes segregate with *H-2* (Tables 3 and 4). The possibility that unknown *H-2*-linked BALB/c genes were incorporated into the chromosome 17 carrying *bm1* by recombination and were not removed by further recombination during derivation of the B6.C-*H-2*^{bm1} congeneic strain is not entirely excluded; further studies with *H-2* mutations that arose in inbred strains and thus are

Table 4. Distinction between B6/By (B6) and B6.C-*H-2*^{bm1} (bm1) of Egorov's colonies, with transfer of training to H-2^b and H-2^{bm1} homozygous segregants of the cross (B6 × B6.C-*H-2*^{bm1})F_2 (bF_2 vs. $bm1F_2$) typed by skin grafting

Trained mice	Urine donor panels*	Test phase	Trials, no.	Concordance, %
B6-*H-2*^{k}♀1 B6-*H-2*^{k}♂	First sets of B6 (reward) vs. bm1	Reward	299	86
	Second sets of bF_2 vs. $bm1F_2$	No reward†	37	68
B10♂ B6♀2	First sets of B6 vs. bm1 (reward)	Reward	275	73
	Second sets of bF_2 vs. $bm1F_2$	No reward†	22	77
Total		Reward	574	80‡
		No reward†	59	71§

*Panels of individually marked age-matched mice, numbering 35 for B6, 21 for bm1, 5 for bF_2, and 6 for $bm1F_2$.
†Transfer of training with coded samples.
‡$P < 0.0001$; P values refer to probabilities that the observed concordances differ from 50% by chance; the difference between reward and no reward phases (80% and 71%) is not statistically significant.
§$P < 0.01$.

available in the coisogeneic mode (16) are contemplated for that reason.

The sensory distinction of H-2^b and H-2^{bm1} mutant phenotypes need not imply that the odorants responsible are structural derivatives of the H-2K molecule, although that is not ruled out, nor that *H-2*^{b} and *H-2*^{bm1} mice necessarily produce structurally different odorant molecules, nor that this distinction depends on a single rather than multiple differences in odor phenotype. Genetic variation in the region of *H-2* is associated with normal variation of many biological features, such as the relative sizes of organs and cell populations (see refs. 11 and 17). The several *H-2*-associated variations in steroid metabolism are notable in that some steroid derivatives are potent odorants (18, 19). Thus one view of distinctive H-2:Qa:Tla odor phenotypes is that they are an attribute of normal metabolic variation, quantitative or qualitative, resulting from effects of genetic variation on metabolic pathways directly, or indirectly through developmental polymorphism (20, 21). The fact that the odor of H-2 heterozygotes has distinctive features in addition to features shared with parental homozygotes tends to favor the view that the constitution of odor phenotypes is not a simple function of the structure of the gene products (10).

In short, the radical functions of *H-2* and related genes are uncertain, but they surely participate in intraspecies biological polymorphism, which is likely to include constitutive metabolic variations that could account for individuality of odor phenotypes.

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