

RECOGNITION AMONG MICE  
Evidence from the Use of a Y-Maze  
Differentially Scented by Congenic Mice of  
Different Major Histocompatibility Types\*

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The prediction that histocompatibility loci would prove to be concerned in chemosensory communication whereby animals recognize one another as individuals (1) was supported by a study in which selective mating was observed among congenic mice differing only at the major histocompatibility complex (MHC)<sup>1</sup> (2). Alternative interpretations have been excluded by observations of MHC-associated mating preference among H-2-typed F<sub>2</sub> segregants of crosses between MHC congenic strains (3). The evidence suggests that polymorphism of genes in the MHC region determines the character of the identity signal or signals, and that polymorphism of other genes in the MHC region influences the response that manifests itself in mating preference (4, 5).

Further investigation of MHC-associated sensory recognition was hampered by the arduous technicalities of the mating preference test. We therefore sought a simpler and more direct method of study that would obviate the complexities of mating. This report deals with the use of a Y-maze in which mice were trained to enter alternative chambers scented by an airflow through odor boxes occupied by MHC-congenic mice.

### Materials and Methods

*Description and Use of the Y-Maze.* The design of the Y-maze was adapted after Bowers and Alexander (6). See Fig. 1.

*Training.* Before training, the mice spent three consecutive nights in the Y-maze to become acclimatized. Reinforcement consisted in prior deprivation of water for 23 h, the reward being a drop of water for each concordant choice (Fig. 1).

Training consisted in three phases.

(a) Phase I. This required distinction between the scents cinnamon and juniper, the mice being reinforced for one or the other.

(b) Phase II. This required distinction between the inbred strains B6 (C57BL/6; H-2<sup>b</sup>) and AKR (H-2<sup>k</sup>), the mice being reinforced for one or the other.

(c) Phase III. This required distinction between the congenic strains B6 and B6-H-2<sup>k</sup>, mice

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<sup>1</sup> Abbreviation used in this paper: MHC, major histocompatibility complex.

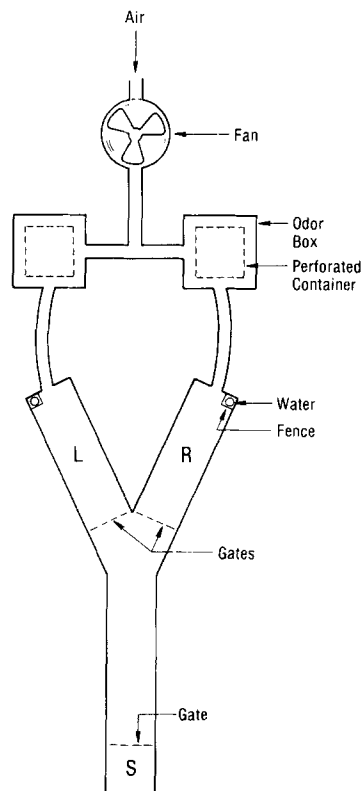


FIG. 1. Air, drawn by a fan through a pipe leading from a source near the input vent of the room (which was used solely for the Y-maze work), was conducted through the left and right odor boxes. Each odor box had a hinged lid to admit a perforated container housing the mice serving as the odor source. The air then passed to the left and right arms of the maze, both fitted with hinged transparent lids. Both arms of the maze provided access to one drop of water, available from a tube perforated at its base. The tube was guarded by a fence which was raised only if the mouse entered the arm scented by the odor concordant with its training. Each arm of the maze had a gate, which was lowered to contain the mouse once it had entered. If the choice was discordant the fence was not raised and the mouse was returned to the starting compartment (S), which also had a hinged transparent lid and gate. If the choice was concordant, the fence was raised and the mouse drank the drop of water. The time interval in the arm (with gate closed) was 30 s, which allowed time for changing the containers in the odor boxes, and replacing the drop of water (if indicated); after which, on a timed signal, all three gates were raised to commence the next run. Left-right placing was decided by a series of random numbers suited to the size of the samples (7). The stem of the maze was fitted with a hinged transparent lid. 48 perforated containers were available, for 24 consecutive runs (24 containers for odor-source mice of each genotype). At the end of each day, the containers were cleaned by scrubbing in hot water. The 24 containers for B6 occupancy were alternated each week for B6-H-2<sup>k</sup> occupancy.

reinforced for B6 in Phase II being again reinforced for B6 in Phase III, and mice reinforced for AKR (H-2<sup>k</sup>) being reinforced for B6-H-2<sup>k</sup>.

The training with mice as the odor source began with six mice per odor box, the number being reduced progressively to two as performance improved. Phases I and II of training were complete in  $\approx 1$  mo. Once the concordance of each mouse's scores reached a plateau in Phase III, all trials thereafter were included in the data presented, regardless of any daily fluctuation in performance.

*Test System.* Each trained mouse was tested up to 48 times in series on a given day. Statistical analysis showed no decline in performance during this number of trial runs in the maze. On

alternate days, each trained mouse was either rested or used in trials with urines as the odor source, which will be the subject of a future report. On rest days, including Saturdays and Sundays, the mice were supplied with water for 1 h at a time corresponding to their test period.

Odor-source mice were placed in containers and assigned to left or right odor boxes according to a series of random numbers described by Fellows (7). Unless otherwise stated, there were two mice per box. When the number of tests exceeded the number of available pairs of mice or available containers, testing was discontinued until the odor-source mice were regrouped to maximize randomization. For this purpose, mice were exchanged between odor boxes in such a way that the same pair of mice was never tested more than once with the same trained mouse on the same day.

All mice used as odor sources were males, and were serially numbered for individual identification. They were selected from groups of 77 inbred B6 mice, 83 congenic B6-H-2<sup>k</sup> mice, 30 H-2<sup>b</sup> homozygous segregants of the cross B6-H-2<sup>k</sup> × B6, and 38 H-2<sup>k</sup> homozygous segregants.

*Operation of the Maze: Blind Controls.* (See also the legend to Fig. 1.) In routine operation of the Y-maze there were two operators. The first operated the three gates, returned the trained mouse to the starting box at the set time, and did not know the placing of the odor source mice. The second operator raised the fence (if the run was concordant), recorded the result, introduced another drop of water (after concordant runs), and placed the next pair of containers with mice in the odor boxes according to the random-number sequence (7), in preparation for the next run at the set time.

Blind tests, performed periodically to monitor the objectivity of scoring, were designed to avoid any alteration of the test conditions that may detract from performance, e.g., provision of water in concordant choices should not be delayed. In periodic blind control tests there were three operators. The third, working alone in the Y-maze room, first prepared the sets of containers with mice included, marking the containers L and R (left/right), and then left the room with the coded score sheet. The other two operators then entered and conducted the tests, communicating with the third operator (in a separate room) through a miniature portable speaker system. The tests were then performed. The first operator called 1 (2, 3, etc.) as the starting gate was raised, followed by left or right immediately after the arm-gate was lowered behind the test mouse. The third operator responded yes (concordant) or no immediately, and the water fence was raised or not, accordingly.

We have seen no significant difference in concordances in blind controls as compared with routine operation of the maze.

## Results

The data below, comprising 4,855 trials exclusive of the training period, were obtained with four trained B6 (H-2<sup>b</sup>) mice, two males and two females. One of the males and one of the females were reinforced for H-2<sup>k</sup>, and the other male and female were reinforced for H-2<sup>b</sup>. These four mice were separately caged and are identified in the Tables as B6♂K, B6♀K, B6♂B, and B6♀B. The entire study lasted 10 mo, during which these four mice aged from 4 mo initially, to 14 mo. The inbred odor-source males, aged from 2 to 14 mo (B6) and 3 to 17 months (B6-H-2<sup>k</sup>), and the F<sub>2</sub> odor-source males from 2 to 10 mo during their participation in the trials.

*I. Discrimination between B6 and B6-H-2<sup>k</sup> Congenic Mice.* In this first series of tests the arms of the maze were scented by B6 and B6-H-2<sup>k</sup> congenic mice, two males in each odor box. The results in Table I show that with a high degree of statistical significance all four mice could distinguish the reinforced H-2 haplotype.

*II. Discrimination between MHC Congenic F<sub>2</sub> Segregants.* The purpose of testing F<sub>2</sub> segregants, bred from the cross B6-H-2<sup>k</sup> × B6, as sources of odor, is that these mice have shared the uniform prenatal and postnatal familial environment of their common F<sub>1</sub> hybrid parents. 2–3 wk after weaning, the F<sub>2</sub> progeny were typed for H-2 by cytotoxicity assay of cells from an excised lymph node. Heterozygotes were discarded, and the two sets of homozygotes, genotypically identical to their respective inbred

TABLE I  
*Y-Maze Tests with Inbred Congenic Strains B6 and B6-H-2<sup>k</sup>*

Trained mouse	No. of trials	Concordance %	u*
B6♂K	694	68	9.376 <i>P</i> << 0.0001
B6♂B	635	66	8.095 <i>P</i> << 0.0001
B6♀K	580	68	8.429 <i>P</i> << 0.0001
B6♀B	633	71	10.652 <i>P</i> << 0.0001
All	2,542	68	10.346 <i>P</i> << 0.0001

$$* \text{ Standardized normal deviate } u = \frac{\left| r - \frac{n}{2} \right| - \frac{1}{2}}{\sqrt{\frac{n}{4}}}$$

*r* = number of concordant responses; *n* = number of trials.

TABLE II  
*Y-Maze Tests with Typed H-2<sup>b</sup> and H-2<sup>k</sup> Homozygous F<sub>2</sub> Segregants Bred from the Cross B6-H-2<sup>k</sup> × B6*

Trained mouse	No. of trials	Concordance %	u
B6♂K	600	58	3.878 <i>P</i> = 0.0001
B6♂B	597	71	10.150 <i>P</i> << 0.0001
B6♀K	570	60	4.566 <i>P</i> < 0.0001
B6♀B	546	59	4.322 <i>P</i> < 0.0001
All	2,313	62	11.561 <i>P</i> << 0.0001

strains (except for certain rare recombinants), were caged separately. The results in Table II show that with a high degree of statistical significance all four trained mice could distinguish the reinforced haplotype when the odor sources were F<sub>2</sub> segregants.

However, the overall concordance with inbred mice is significantly higher than the overall concordance with F<sub>2</sub> segregants (68% Table I vs. 62% Table II). This might indicate that discrimination between inbred mice includes cues that are not associated with MHC haplotypes and that are randomized or eliminated by interpolation of a hybrid generation. Alternatively, the performance of the four trained mice may have declined before the series of tests with F<sub>2</sub> segregants began. Such a decline in performance might be attributable to the use of the mice in tests of other kinds not described in this report, or to age, the four trained mice having been 4–5 mo old at the time of the tests with inbred mice as odor sources, and 8–10 mo old at the time of

TABLE III  
*Concurrent Trials of Inbred and Genotypically Similar F<sub>2</sub> Segregant Mice as Odor Sources*

Trained mouse	Odor source		$\chi^2$ : difference between inbred and F <sub>2</sub>
	Inbred	F <sub>2</sub>	
	<i>Concordance % and (no. of trials)</i>		
B6♂K	69* (124)	69* (144)	0.009 (NS)
B6♂B	73* (108)	81* (141)	1.663 (NS)
B6♀K	72* (101)	77* (120)	0.351 (NS)
B6♀B	75* (105)	70* (108)	0.415 (NS)
All	72* (438)	74* (513)	0.441 (NS)

NS, not significant.

\*  $P < 0.001$  by u test (difference from 50%).

the tests with F<sub>2</sub> segregants. Therefore, it was decided to retest the four mice as follows:

*III. Concurrent Trials of Inbred and Genotypically Similar F<sub>2</sub> Segregant Mice as Odor Sources.* In this third study, inbred and F<sub>2</sub> homozygous segregant populations were used as odor sources on alternate days. Thus, each trained mouse was tested on one day with inbred mice in the odor boxes, on the next trial day with F<sub>2</sub> segregant mice in the odor boxes, on the third trial day with inbred mice again, and so on. The only modification in procedure was that four mice instead of two were placed in each odor box. Randomization was assured as before by systematically grouping the mice so that no group of four mice included more than two mice that had comprised a previously tested group of four.

In Table III the concordance for the inbred and F<sub>2</sub> populations is recorded and compared. For all four trained mice, severally and jointly, there was no significant difference in performance with inbred and F<sub>2</sub> populations. The performance of all four trained mice in this series of tests (Table III) was superior to their previous performances (Tables I and II). This is probably attributable to the more efficient dissemination of odor by four mice per box as compared with two.

### Discussion

The performance of the trained mice in the maze makes it improbable that any mode of sensory perception other than olfaction is needed for discrimination of MHC types. From the use of MHC-recombinant mice in tests of mating preference (4, 5, and further unpublished data) we suspect that several genes in the MHC region affect a range of odors. In that case, identification may involve a set of odors that is peculiar to a given MHC type because of qualitative or quantitative variation of the constituent odors.

Chemo-recognition of MHC types may have wider biological relevance than is implied by mating preference alone. This faculty may influence other features of reproduction, such as neuro-endocrine circuits affecting implantation, lactation, and suckling, that are evidently subject to genetic variation in respect both of emission and receipt of chemical signals (8, 9).

### Summary

Previous studies of mating preference signified that mice can sense one another's major histocompatibility complex (MHC) types, probably by olfaction. This conclusion has now been substantiated by the use of a Y-maze whose two arms were differentially scented with currents of air conducted through boxes occupied by B6 (H-2<sup>b</sup>) males and by B6-H-2<sup>k</sup> congenic males.

Four B6 mice, two males and two females, were successfully trained, by water deprivation and reward, to enter the arm scented by B6 or B6-H-2<sup>k</sup> males. One of the males and one of the females were trained to select the B6-scented arm; the other male and female were trained to select the B6-H-2<sup>k</sup>-scented arm.

Untrained mice showed no MHC discrimination in the maze. The performance of the trained mice in distinguishing between MHC congenic homozygous F<sub>2</sub> segregants derived from a cross of B6-H-2<sup>k</sup> with B6 was as good as their performance in distinguishing the respective inbred strains, thus essentially eliminating alternative and significant additional explanations of MHC-associated sensory discrimination. The data further indicate that chemosensory discrimination of MHC types can be entirely dissociated from sex differences and from the circumstances of mating.

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