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16. Rats are restrained and stimulated with a Grass Constant Voltage Stimulator with 5 trains per second of 20-ms trains of 300 Hz, 0.5-ms biphasic pulses. The current is adjusted to produce hindlimb movements without generalized convulsions, although most animals display intermittent bilateral motor activity. We noted no consistent differences in Fos staining pattern between 15 min and 1 hour of stimulation. After stimulation the animals are again returned to their cages.
17. Sections were incubated for 30 min at room temperature in 0.1*M* PB, *p*H 7.4, 0.2% Triton X-100, 0.1% bovine serum albumin, and 2% normal goat serum (PB-G). Sections were then incubated for 36 to 48 hours at 4°C with gentle agitation in primary antiserum diluted 1/100 in PB-G. After washing three times in PB for a total of 30 min, sections were processed with a Vectastain ABC Kit (Vector Labs) with secondary antiserum and ABC reagent dissolved in PB-G. Incubation times were 2 to 3 hours at room temperature. Diaminobenzidine is used as the chromagen. Adsorption controls with synthetic M peptide, 2 μg/ml, included in the incubation with primary antiserum showed no nuclear staining.
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Familial Imprinting Determines H-2 Selective Mating Preferences

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Inbred male mice typically prefer to mate with females of a different, non-self H-2 haplotype. To determine whether this natural preference is irrevocable or results from familial imprinting, a test system was used which relied on previous observations that B6 males (H-2^b) mate preferentially with congenic B6–H-2^k rather than B6 females, and B6–H-2^k males with B6 females. This preference was reversed in B6 males fostered by B6–H-2^k parents and in B6–H-2^k males fostered by B6 parents, preference in these cases favoring the same H-2 type. Thus, H-2 selective mating preference is acquired by imprinting on familial H-2 types.

INBRED MALE MICE SHOW A TENDENCY to mate with females of an H-2 type different from their own (*1*); for example, when presented with equivalent C57BL/6 (B6; H-2^b) and B6–H-2^k females in estrus, a B6 male more often selects the B6–H-2^k female and a B6–H-2^k male more often selects the B6 female (*2*), as illustrated in Table 1, group 1.

To determine whether this natural preference for the non-self H-2 haplotype is acquired during early life, we studied the mating preferences of B6 males reared by B6–H-2^k foster parents and of B6–H-2^k males reared by B6 foster parents. Although the genetic relationship is unnatural, since parents of the same homozygous H-2 genotype cannot give birth to homozygous progeny of a different H-2 type, this experimental design seemed most likely to reveal any influence that imprinting of parental H-2 types may have with respect to subsequent choice of a mate. The idea that imprinting of parental odors affects the subsequent behavior of mice is not new since it has been invoked, though not in relation to particular genes, to explain observed influences of artificial scenting of sires (*3*), removal of sires (*3*), or foster-nursing (*4*) on subsequent social or mating proclivities. Our present finding that exposure history critically determines H-2–based male mating preference implies that avoidance of mating with closely related individuals (kin) is determined neither by a direct genetic mechanism (recognition alleles) nor by use of self H-2 as a referent (*5*).

Within 16 hours of birth, entire litters were removed from their natural parents and transferred to foster parents whose own litters, born at approximately the same time, were simultaneously removed. At 21 days of age, the fostered mice were weaned and the males maintained in stock cages containing only males of the same genotype and fostering history until sexual maturity (3.5 months of age, minimum), when tests of mating preference began.

The method of testing mating preference was as described (*2*) except that the males in the present studies had been reared by foster parents. A fostered male, B6 or B6–H-2^k, vasectomized at 2 months of age to avert unwanted pregnancies and housed alone in an individually ventilated cage, was presented with two females in estrus that had been selected from congenic mouse panels, B6 and B6–H-2^k, each of 60 to 80 individually numbered age-matched females. In each test the two estrous females were selected from the panels with due attention to equality of their previous sexual experience in repeated testing of the males. The two females were placed in the male's cage and watched until successful copulation was verified by presence of a vaginal plug, or until 2 hours had elapsed. Each test was scored as valid only if the second female was shown to be in estrus and receptive to copulation, as verified by vaginal plug, with a male from a separate panel of males maintained for this purpose. The data given include only fostered males that achieved two valid tests, as defined above, within no more than three trials. The minimum interval between testing of males was 10 days.

To provide a control to verify the segregation of scent individuality with H-2, thus excluding hypothetical influences of genetic drift affecting loci other than H-2 and of any nongenetic distinguishing characteristics that might have arisen since the B6–H-2^k strain was originally derived, the B6 and B6–H-2^k strains were first rederived from a

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Table 1. Summary of 144 mating preference tests of 72 fostered B6 and B6–H-2^k males (groups 2 and 3) presented with a choice between B6 and B6–H-2^k females. Group 1 represents previous data for mating choices between congenic, unfostered B6 and B6–H-2^k mice (*2*) for comparison. These results signify that the usual preference of B6 and B6–H-2^k males for females of the non-self rather than selfsame H-2 type is reversed by fostering on parents of the non-self H-2 type. The data of groups 1 and 2 show significant mating preference favoring non-self (group 1: $\chi^2 = 13.68$, df = 2, $P < 0.01$; group 2: $\chi^2 = 6.26$, df = 2, $P < 0.05$). The data of group 3 show significant mating preference favoring self ($\chi^2 = 11.92$, df = 2, $P < 0.005$). Comparison of groups 1 and 2 shows that syngeneic foster-nursing does not significantly alter natural preference ($\chi^2 = 2.52$, df = 2, $P > 0.20$). Comparison of groups 2 and 3 shows that allogeneic foster-nursing reverses natural preferences ($\chi^2 = 10.53$, df = 2, $P < 0.02$ with Bonferroni correction for multiple comparisons).

Category of males	H-2 type of female selected in two tests		
	Same as male	Different from male	One same and one different
Group 1: Not fostered	4	20	17
Group 2: Syngeneic foster-nursing*	8	15	12
Group 3: Allogeneic foster-nursing*	18	4	15

*Data for the two comparable sets of males in group 2 (B6 males fostered on B6 parents and B6–H-2^k males fostered on B6–H-2^k parents) and in group 3 (B6 males fostered on B6–H-2^k parents and B6–H-2^k males fostered on B6 parents) are combined because they were not significantly different.

(B6 × B6–H-2^k)F_2 population. The F_2 progeny were typed for H-2 and heterozygotes were discarded. The H-2^b/H-2^b and H-2^k/H-2^k homozygous F_2 segregants, genetically equivalent to the B6 and B6–H-2^k progenitor strains, were bred to provide mice (F_3) for all purposes (mating tests, foster parents, and parents of F_4 neonates for fostering).

In previous mating tests, B6 males were shown to mate preferentially with B6–H-2^k congenic females rather than B6 females, and B6–H-2^k males with B6 females rather than B6–H-2^k females (*2*) (Table 1, group 1). As group 2 in Table 1 indicates, these preferences for the non-self H-2 haplotype were not significantly altered by fostering on syngeneic parents, which reproduces the genetic relations that obtain in the usual propagation of inbred strains.

Comparison of groups 2 and 3 in Table 1 shows that the usual mating preferences of B6 and B6–H-2^k males, noted above, were reversed by exchanging the H-2 haplotypes of the foster parents. Thus B6 males fostered by B6–H-2^k (allogeneic) parents mated preferentially with B6 females, and B6–H-2^k males fostered by B6 (allogeneic) parents mated preferentially with B6–H-2^k females. The statistical degree of this reverse preference (group 3) was not significantly different from the opposite preference of unfostered males (group 1) and of males fostered by syngeneic parents (group 2) (*6*).

Clearly the natural preference for the dissimilar H-2 type does not signify an irrevocable aversion to the self H-2 type because an equal preference for the self H-2 type can be established experimentally by exchanging the H-2 type of foster parents.

Also, since the only experimental variable introduced to reverse H-2 preference in favor of self H-2 was foster-nursing of the male, the choice of H-2 type is evidently exercised by the male, not the female. An alternative interpretation, that foster-nursing imparted to the male an altered scent that the female employed as a mating cue, was studied by determining whether males or females could be trained to distinguish between males fostered on syngeneic or allogeneic parents (as above) in the Y maze. This is a stringent test revealing odor distinctions even between mice differing genetically only by mutation of a single H-2 gene (*7*). The results were entirely negative and substantially exclude the possibility of response by females to a male scent altered by allogeneic foster-nursing.

The prime basis of H-2 selective mating preference is clearly temporal. Whichever H-2 type is experienced during the rearing period of 3 weeks becomes the less favored H-2 type. The same temporal relation was observed in a second test system in which initial perception of a given H-2 type conditioned the response to subsequent distinction of that H-2 type from another. In this pregnancy-block system, an isolated BALB/c (H-2^d) female previously mated with either a B6 or B6–H-2^k congenic male was subsequently exposed to the scent of a male of either the initial type or the unfamiliar H-2 type. The incidence of terminated pregnancy was greatly heightened by H-2 disparity between the stud and second males, regardless of whether the order of presentation was H-2^b followed by H-2^k or vice versa (*8*) and the same is true of distinction between H-2^b and the mutant H-2^{bml} in the pregnancy-block test system (*9*).

With due regard to any dispute concerning the relation of familial imprinting to post-familial memorization (*10*), the simplest view is that H-2 selective mating and pregnancy-terminating hormonal imbalance are responses conditioned by previous perception of a particular H-2 type, regardless of the previous H-2 type and of whether it was a self or non-self H-2 type.

Other reproductive factors affecting selection of H-2 in natural populations are not excluded. It might be thought that if parental and familial H-2 types were the sole determinants of subsequent H-2 mating preference then F_2 segregants of H-2 congenic crosses should not exhibit a preference since, regardless of their own H-2 types, the H-2 types of their heterozygous F_1 parents were uniformly the same. In fact such F_2 segregants do exhibit H-2 selective mating preference, although not in all cases and not to the same degree (*11*). However, the situation of F_2 H-2 homozygotes born of F_1 H-2 heterozygotes is more complex than it might seem, because the odor profile of H-2 heterozygotes, although it includes elements typical of each homozygous parent, is itself distinctive and is not simply a compound of the two homozygous parental profiles (*12*). Thus the mating preferences of congenic F_2 segregant progeny are not a simple matter of distinction between self and non-self familial H-2 haplotypes. On present evidence there is no cogent reason to invoke any agency of H-2 assortative mating other than chemosensory imprinting.

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