Communal nesting patterns in mice implicate MHC genes in kin recognition

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HOUSE mice (*Mus musculus domesticus*) form communal nests and appear to nurse each other's pups indiscriminately. Communal nesting probably functions to reduce infanticide[1], but it also makes females vulnerable to exploitation if nursing partners fail to provide their fair share of care. Kinship theory predicts that females will preferentially form communal nests with relatives to minimize exploitation and further increase inclusive fitness[2-4]. Here we provide evidence from seminatural populations that females prefer communal nesting partners that share allelic forms of major histocompatibility complex genes. Such behaviour would lead to the selection of close relatives as communal nesting partners[5-7]. Although criteria for the demonstration of kin recognition are currently embroiled in controversy[8,9], this is the first vertebrate study to meet Grafen's restrictive requirements[8,10]: discrimination is based on genetic similarity at highly polymorphic loci, incidental correlations due to relatedness are experimentally controlled, and strong reasons exist for expecting the assayed behaviour to be kin-selected.

Major histocompatibility complex (MHC) gene products are cell-surface molecules that function during immune recognition[11], but they also fulfill the two major requirements for genetically based kin recognition[6,7,12-17]. These polymorphic loci strongly influence individual odour profiles[18], providing a mechanism for phenotypic discrimination, and their unprecedented genetic diversity (often over 100 alleles per locus) allows useful resolution of genetic relationships[5-7]. Consistent with this MHC-based kin recognition hypothesis are observations of MHC-disassortative mating preferences in house mice[19-21], which may function to avoid inbreeding[13,15-17,21].
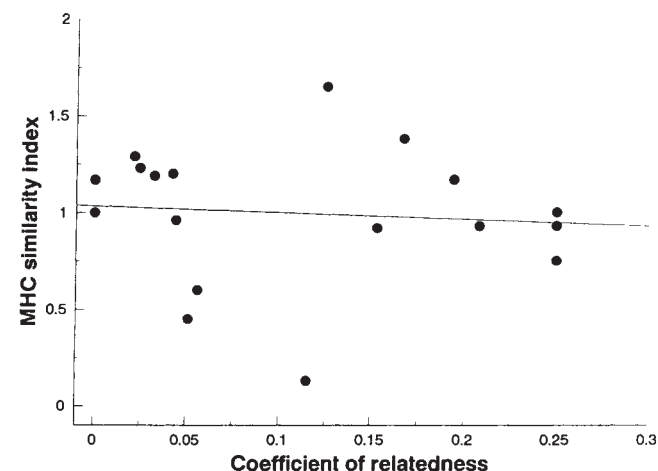

FIG. 1 Correlation between MHC similarity and relatedness. Observations of nests containing non-sibs from populations E and G–J show no correlation ($r^2 = -0.008$) between relatedness (r between communally nesting female and the mean of all nests available) and the MHC similarity index of nests available. Coefficients of relatedness (r) were calculated for all potential communal nesters by path analysis, using colony records.

To test the hypothesis that MHC plays a role in the choice of communal nesting partners, we studied six seminatural populations of *Mus*. All mice were half-wild derived but carried combinations of four MHC haplotypes derived from inbred strains (b from C57BL/6, d from BALB/c, k from B10.BR and q from DBA/1) (see ref. 21 for breeding design). In populations C and G–J (Table 1), founders had been reared to 90 days of age in cages with their same sex sibs. Founding populations had one to three sister groups of two to five individuals. Except for these cagemate sister groups, all founders were strangers. Population E consisted of offspring born in the enclosure and left to found a new population after removal of their parents and younger sibs. Table 1 provides the numbers of single and communal nests, founders and pups born for each population.

We identified all communal nesting options for each mother on the day she gave birth and calculated the MHC similarity of the mother to each of the dams in each prospective nest (see legend to Table 2). MHC similarity scores for nests chosen and nests available were compared to test the null hypothesis that nesting preferences were random with respect to MHC.

Because MHC-based kin recognition by phenotype matching could operate in several ways, eight models were created to test the range of possibilities (Table 2). These models include variations on the matching referent or template (self, nestmates or parents), whether matching is to genotype or haplotype, and whether mean or maximum similarity is used when nests consist of more than two females (see Table 2 legend). An additional variable had to be considered because sisters reared as cagemates preferentially nested together. We controlled for this variable in two ways. First, a separate analysis was done on the subset (50%) of communal nests not involving cagemates. Second, in an analysis using the entire data set, we weighted the null model (Table 2 legend) to control for the preference for cagemate sisters. This weighted data set still contained MHC similarity information when more than one nest option contained a cagemate sister or when cagemate sisters were not chosen. Table 2 shows that MHC similarity of the chosen nest was significantly greater ($P < 0.05$) than would have been achieved by random chance for all eight models when the entire data set was analysed ($n = 50$). When communal nests not involving cagemates were analysed separately ($n = 25$), six of eight models showed significant differences ($P < 0.05$) and the other two showed similar trends.

These results are consistent with the hypothesis that MHC similarity is involved in the choice of communal nesting partners. However, partners may be chosen on the basis of loci unlinked but correlated with MHC loci. Such correlations will normally exist among relatives so that preferences based on discrimination of other genes or on behavioural similarities among kin[9] could appear to be MHC-mediated. But owing to our breeding design, correlations between relatedness and MHC similarity were eliminated for non-sibs in all populations except population C (see ref. 21). Figure 1 confirms that the correlation between MHC and relatedness was abolished ($r^2 = -0.008$). In the subset of nests involving no correlation between MHC and relatedness, there is still a significant trend for MHC similarity among nesting partners (Table 2, first model; $n = 18$). These findings are inconsistent with the hypothesis that preferences based on genes unlinked to, but correlated with, MHC could explain the high MHC similarity among nesting partners. Furthermore, this data subset reveals that the coefficient of relatedness (r) for nests chosen (mean $r = 0.099$) and nests available (mean $r = 0.110$) do not differ ($n = 18$, $P = 0.46$), demonstrating that other (non-MHC) indicators of relatedness are not being used to choose communal nesting partners in these populations.

These data suggest that female house mice use an MHC-based genetic matching system in choosing communal nesting partners. In our view, kin recognition is the most likely interpretation of these findings because of expectations that kin will be preferred as nesting partners[3,4] and because this is the only hypothesis

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TABLE 1 A comparison of communal and single-mother nests in the six study populations

Population	Dates	Founders ♂	Founders ♀	Single-mother nests Number of nests	Single-mother nests Pups born	Communal nests Number of nests	Communal nests Number of litters	Communal nests Pups born
C	4/8–6/12/88	8	16	17	103	4	11	66
E	23/9–6/12/88	12	19	7	44	6	20	118
G	5/5–27/7/89	8	16	7	37	5	15	91
H	24/4–7/7/89	8	16	8	40	4	8	47
I	31/7–9/11/89	8	16	5	35	3	13	70
J	31/7–9/11/89	8	16	14	87	2	3	10
	Totals	52	99	58	346	24	70	402

Study populations: Our 9.8×4.9 m enclosures were of sufficient size and complexity to allow the formation of several male territories and social units. Unique ear punch combinations allowed identification of all individuals through binoculars. Daytime nest checks and 1–2h of behavioural observations at dusk were made 5–7 times a week. **Communal nests:** Most communal nests were formed by a mother giving birth in an already existing nest but some were combined after separate births. We quantified properties of these nests, such as the number of litters or dams already in a nest and the age of the pups. A comparison of the means for all nests available to the nest that was chosen revealed no difference for number of litters (1.26 versus 1.25 litters, $P > 0.90$) or nursing dams per nest (1.42 versus 1.48 dams, $P > 0.25$), but the age of pups in nests chosen was significantly less than the mean age of pups in all nests available (5.5 versus 6.2 days, $P = 0.009$). Although we observed 70 females who communally nested, only 50 of these females had more than one communal nest option (mean = 3.6, range 2–6). Although similar numbers of pups were born in communal and single-mother nests, these two nest types were not equally preferred. Most single-mother nests occurred when communal nesting options were not available. Ninety per cent of mothers ultimately nested communally.

TABLE 2 A comparison of MHC similarity between nests chosen and nests available

Model	N	MHC similarity to nest chosen (±s.e.)	MHC similarity to mean of nests available (±s.e.)	P
Match to self haplotype, mean MHC similarity	50	1.33 ± 0.08	1.19 ± 0.07	0.014*
(without cagemates)	25	1.18 ± 0.10	0.95 ± 0.07	0.036*
(without population C or cagemates)	18	1.20 ± 0.11	1.00 ± 0.08	0.50*
Match to self haplotype, maximum MHC similarity	50	1.45 ± 0.08	1.33 ± 0.07	0.040*
(without cagemates)	25	1.30 ± 0.09	1.06 ± 0.08	0.022*
Match to nestmate haplotype, mean MHC similarity	50	1.71 ± 0.06	1.55 ± 0.06	0.002*
(without cagemates)	25	1.49 ± 0.11	1.27 ± 0.09	0.021*
Match to nestmate haplotype, maximum MHC similarity	50	1.77 ± 0.07	1.66 ± 0.06	0.018*
(without cagemates)	25	1.58 ± 0.10	1.35 ± 0.10	0.022*
Match to nestmate genotype, mean MHC similarity	50	1.49 ± 0.11	1.33 ± 0.09	0.025*
(without cagemates)	25	1.09 ± 0.19	0.93 ± 0.12	0.148
Match to parental haplotype, mean MHC similarity	50	1.38 ± 0.08	1.25 ± 0.06	0.022*
(without cagemates)	25	1.21 ± 0.11	1.04 ± 0.06	0.064
Match to parental haplotype, maximum MHC similarity	50	1.54 ± 0.07	1.41 ± 0.06	0.014*
(without cagemates)	25	1.40 ± 0.11	1.17 ± 0.09	0.027*
Match to parental genotype, mean MHC similarity	50	0.97 ± 0.13	0.78 ± 0.09	0.012*
(without cagemates)	25	0.78 ± 0.18	0.45 ± 0.07	0.013*

Models: Work by Yamazaki et al.[25] suggests that mice use parents as referents for mate choice. But because fathers are not generally present in the nest in nature and because communal nesting may cause confusion about maternity, the efficacy of such a system is doubtful. Accordingly, we have modelled all reasonable possibilities. Match to self assumes self is the odour referent to whom matching is done. Match to nestmates assumes all nestmates and both parents can serve as referents. Match to parents

assumes both parents are referents. The set of loci that comprise the MHC are tightly linked, allowing the haplotype to be treated as the unit of inheritance. Matching was analysed at both the genotypic and haplotypic levels. A genotypic match requires identical genotypes. Haplotype matching can be complete (identical genotypes) or partial (matching one haplotype). Mean MHC similarity score for each nest is the mean of the scores for all dams in the nest. Mean MHC similarity index score for all nests available is the average of all nest means. Maximum MHC similarity scores are derived by taking only the score of the female in the nest with the highest similarity score. For mean of nests available, maximum scores of individual nests are averaged. Mean MHC similarity scores provide a way for a female to evaluate her relatedness to the nest as a whole. Thus a nest with non-relatives in addition to relatives is proportionately devalued. Maximum scores assume that a female choosing a nest only cares about the one individual in that nest that is most MHC similar. **MHC similarity index:** MHC similarity for matching to self models is scored as 0 if all four haplotypes of two individuals are different, 1 for two heterozygotes with one haplotype in common (such as bd and bk), 1.5 for a heterozygote with one haplotype matching both haplotypes of a homozygote (such as bd and bb), and 2 for either identical homozygotes (bb and bb) or heterozygotes (bd and bd). Identical homozygotes or heterozygotes (score of 2) would also be scored as a genotype match. When the matching referent is nestmates or parents there are several complicated ways a partial match can occur. Accordingly, the MHC similarity index is simplified as 0 = no match, 1 = partial match, 2 = complete or genotype match. **Nesting options:** A nest was considered a communal nesting option if it contained pups no more than 17 days of age or if the dam(s) had lost a litter within three days. These pupless dams always demonstrated normal communal nesting behaviour toward the new litter. **Weighting of the models:** The null model assumes that females will choose randomly with respect to MHC. But because females chose a nest containing a former cagemate 92% of the time one was available, nest choice was not random with respect to former cagemates. To control for this we conditioned the model on the probability that a female will choose to nest with a cagemate and nesting options were weighted in the following way. For the five populations containing only familiar sib cagemates or unfamiliar non-cagemates, an estimate of the likelihood that a female would choose a cagemate nest whenever both a cagemate (type i) and a non-cagemate (type j) option were available was calculated as $P_i = X_i/N_{i+j}$ where X_i is the number of times a cagemate nest (type i) was chosen and N_{i+j} is the number of times both cagemate nest and non-cagemate nests were available. Mean MHC similarity of all nests available to each dam on the day she gave birth was computed as $M_d = \sum_j d_j P_{dj}$ where M_{dj} denotes MHC similarity between dam d and nest options j. Although the weighting process eliminated much of the power of the data involving cagemates, additional information is gained because females tended to choose a cagemate sister with a higher MHC similarity index when more than one such nest was available. Also, on the rare occasions when females did not choose a nest containing a cagemate, they tended to choose nests with high MHC similarity indices. **Statistic:** A single sample t test was used to test the null hypothesis that the difference between MHC similarity to nest chosen and the mean MHC similarity of nests available was zero.

  * $P < 0.05$.

predicting both MHC-assortative preferences during coopera-tive behaviour and MHC-disassortative preferences during mat-ing[19-21]. (A putative kin recognition system in the tunicate *Botryl-lus schlosseri* shows remarkable similarities, where a highly polymorphic gene influences preferences during both mating and cooperative behaviour[14,22,23].) But these findings are also consistent with the hypothesis that females with MHC-familiar odours are preferred because long-term associations between 'group members' are favoured[9,24]. If so, the observed preferences for unfamiliar but MHC-similar partners would represent a case of mistaken 'group' identity. In either case, to our knowledge this is the first demonstration in a vertebrate of specific genes used to target individuals for differential treatment during cooperative behaviour. □

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The perception of heading during eye movements

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WHEN a person walks through a rigid environment while holding eyes and head fixed, the pattern of retinal motion flows radially away from a point, the focus of expansion (Fig. 1a)[1,2]. Under such conditions of translation, heading corresponds to the focus of expansion and people identify it readily[3]. But when making an eye/head movement to track an object off to the side, retinal motion is no longer radial (Fig. 1b)[4]. Heading perception in such situations has been modelled in two ways. Extra-retinal models monitor the velocity of rotational movements through proprioceptive or efference information from the extraocular and neck muscles and use that information to discount rotation effects[5]. Retinal-image models determine (and eliminate) rotational components from the retinal image alone[6-12]. These models have been tested[13,14] by measuring heading perception under two conditions. First, obser-vers judged heading while tracking a point on a simulated ground plane. Second, they fixated a stationary point and the flow field simulated the effects of a tracking eye movement. Extra-retinal models[5] predict poorer performance in the simulated condition because the eyes do not move. Retinal-image models[6-12] predict no difference in performance because the two conditions produce identical patterns of retinal motion. Warren and Hannon[13,14] observed similar performance and concluded that people do not require extra-retinal information to judge heading with eye/head movements present, but they used extremely slow tracking eye movements of 0.2–1.2 deg s^{-1}; a moving observer frequently tracks objects at much higher rates (L. Stark, personal communication). Here we examine heading judgements at higher, more typical eye movement velocities and find that people require extra-retinal information about eye position[15] to perceive heading accurately under many viewing conditions.

Experiment 1 reproduced the conditions of Warren and Han-non[13] except we used constant, faster rotation rates (actual or simulated) from 0 to 5 deg s^{-1} and a vertical axis of rotation. In addition, the fixation point was positioned slightly above the horizon and moved independently of the ground plane. At the end of a simulus presentation, seven vertical lines appeared and the observers indicated the one that corresponded most closely to the perceived heading. As in the experiments of Warren and Hannon[13,14], real and simulated eye movement conditions pro-duced identical patterns of retinal image motion, so retinal-image models predict similar performance in the two conditions.

Figure 2 shows that both observers responded very differently

FIG. 1 Optical flow fields for an observer moving across a ground plane. *a*, Flow field for a translational movement; the observer has moved forward while holding the eye and head fixed. The circle marks the focus of expansion, which corresponds to the obser-ver's direction of motion. *b*, Flow field for translation plus rotation; the observer has again moved forward while tracking an object moving from left to right. As before, the circle marks the observer's heading. In the real eye movement condition of experiment 1, the flow field on the display screen resembled the one in *a* and the flow field on the retina resembled the one in *b*; in the simulated eye movement condition, the flow fields on the display screen and retina resembled the one in *b*.
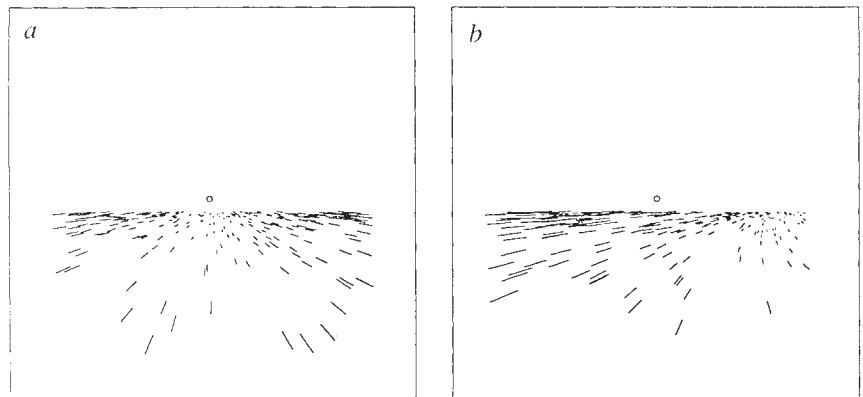
Of mice and the MHC

Alan Grafen

Do mice recognize their kin? They are known to discriminate between conspecifics through the products encoded by the highly polymorphic loci of the major histocompatibility complex (MHC)[1]; the MHC is not only involved in immune recognition, but also affects an individual's odour profile. There is, however, a general methodological reluctance to accept such discrimination as evidence of kin recognition[2]. It was shown last year[3] that female mice prefer mating with males that differ from them at the MHC locus and now, on page 581 of this issue[4], the same authors (C. J. Manning, E. K. Wakeland and W. K. Potts) show that females nest communally with other females that are similar at the MHC. Smelling MHC differences seems to be important in the social lives of mice.

The technique of Manning and colleagues is to put the geneticist's knowledge of inbred strains, breeding designs and MHC-typing to the service of behavioural ecology. Seminatural conditions allow freedom of mating and communal nesting, and individual tags mean that the mice can be observed without disruption.

Around the time a female mouse gives birth, she usually finds a nestmate who has just given birth. The two females (sometimes more) suckle the pups indiscriminately. Manning *et al.* compared the nestmate that was chosen by each female with the other females that could have been chosen instead. They found that the MHC similarity was significantly greater with the chosen nestmate. The genetic manipulations allowed relatedness and MHC similarity to be uncorrelated in some of the groups, so the preference for MHC could not be explained by females choosing relatives according to a general background of genetic similarity. The MHC locus stood out as the focus of choice. These results should encourage workers on kin recognition in other species to investigate the importance of genetic discrimination in natural conditions.

We have one well-documented example of kin recognition in the ascidian *Botryllus schlosseri*, larvae of which tend to settle with histocompatible individuals[5]. Can mice be taken as a second example? Certainly Manning *et al.* have shown that the discrimination is used in nature, and this in itself is very important. It is still worth asking whether it counts as kin recognition, the essential question being whether mice choose nestmates according to MHC alleles in order to help relatives.

Manning *et al.* refer to "expectations that kin will be preferred as nesting partners". In a recent theoretical study[6], Giraldeau and Caraco have tackled the issue of whether kin selection should lead to kin groups, and the answer is subtle. There is no general presumption. Some concepts can be introduced with some starkly simplified examples based on the nesting of mice.

(1) Two sets of two sisters give birth on the same day, and nests are big enough for only two females. If success in rearing pups is unaffected by the

Suckling cousins? A single female mouse nurses the broods of her nestmates.

relatedness of nestmates, then there is no kin-selective reason why the sisters should pair up.

(2) Suppose there are two sets of two sisters and one extra female. There is a disadvantage to nesting alone, and two sisters should pair up if this makes it less likely that either of them will end up as the odd one out.

(3) Suppose there are two sisterhoods of three and four. A quick-counting member of the three would try to pair up with one of the four. When the others realized what was happening, and paired up with sisters, it would be one of the original four that was the odd one out.

These examples are meant to show that any warm cosy feeling animals may gain from grouping with kin is not enough. A kin-selective advantage must come about through differential effects on number of offspring of the options of pairing with kin and not doing so. They are also meant to show that in some circumstances it may actually be advantageous to group with non-kin[6].

Returning to the real world, should mice really prefer to nest with kin? It does seem likely that, in general, nesting with kin would reduce the chance that kin end up nesting alone. To this extent it would be churlish to deny mice parity with *B. schlosseri*. But this tentative acceptance as kin recognition would be placed in doubt if it were shown that helping kin was not the major selective force. For instance, a possible reason for preferring similarity at the MHC locus is that females may discriminate between their own pups using the MHC. A female may prefer to belong to a nest in which both nestmates find it more difficult to distinguish their own pups, as this may increase effective cooperation. That would be an interesting selective force, but not a kin-selective one.

A point not explicitly made by the authors can be drawn tentatively from their Table 2 (see page 582). A female seems to match the MHC of potential nestmates against a referent that is derived from the haplotypes present in her own natal nest, and not from her own genotype. More direct evidence of this would be welcome. For one thing, the workings of the recognition system are of interest in themselves. Further, if females choose in an evolutionary sense to use a natal nest referent rather than their own MHC alleles, this may reflect on the selective forces involved. Specifically, is natal-nest MHC similarity really the best cue females have for kinship?

One technical reservation concerns the breeding scheme used to derive the experimental mice in which relatedness and MHC similarity were uncorrelated. Only a few generations elapsed between the founders and the experimental animals. So far as I can see, it follows that a large stretch of chromosome on either side of the MHC locus is likely to share similarity with the MHC locus. Not enough crossovers have had time to occur to restrict the conclusions definitively to that locus rather than one nearby, but this point does not threaten the rejection of general genetic similarity across all chromosomes as the effective variable.

Recognition systems are harder to study in nature than in the laboratory. But they are much more informative too. □

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