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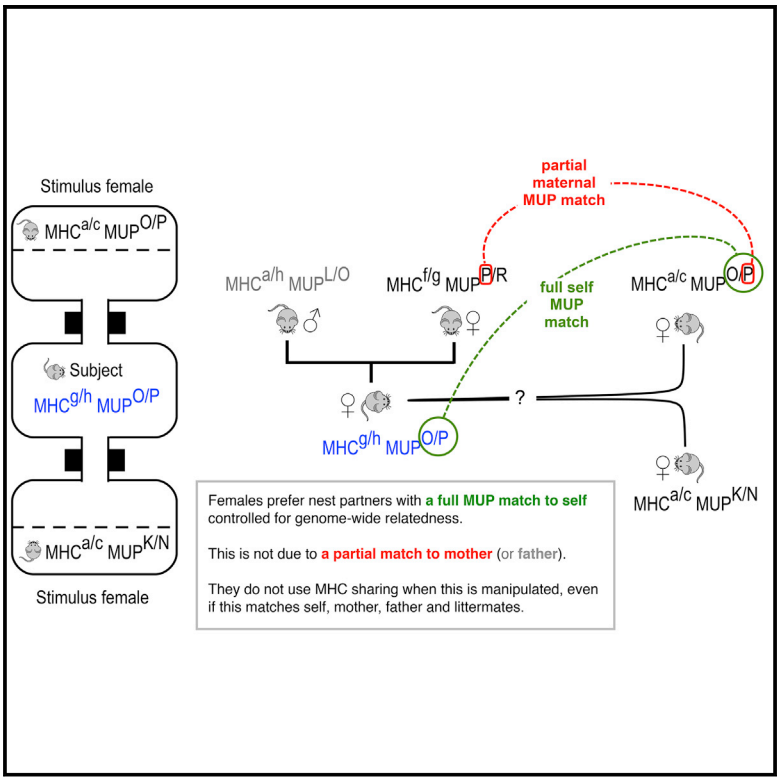
Current Biology

The Genetic Basis of Kin Recognition in a Cooperatively Breeding Mammal

Graphical Abstract



Highlights

- Female house mice use genetic markers to choose closely related nesting partners
- They strongly prefer partners sharing their own major urinary protein (MUP) genotype
- Without MUP sharing, partners sharing multiple loci across the genome are preferred
- MHC sharing is not used; instead, MUP provides a species-specific kinship marker

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In Brief

Female house mice can breed cooperatively and usually select related nest partners, but the genetic markers they use to recognize kin are unknown. Green et al. show that mice prefer partners that match their own major urinary protein (MUP) genotype, a species-specific kinship marker. Contrary to widespread assumption, MHC sharing is not involved.

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The Genetic Basis of Kin Recognition in a Cooperatively Breeding Mammal

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SUMMARY

Cooperation between relatives yields important fitness benefits, but genetic loci that allow recognition of unfamiliar kin have proven elusive. Sharing of kinship markers must correlate strongly with genome-wide similarity, creating a special challenge to identify specific loci used independently of other shared loci. Two highly polymorphic gene complexes, detected through scent, have been implicated in vertebrates: the major histocompatibility complex (MHC), which could be vertebrate wide, and the major urinary protein (MUP) cluster, which is species specific. Here we use a new approach to independently manipulate sharing of putative genetic kin recognition markers, with the animal itself or known family members, while genome-wide relatedness is controlled. This was applied to wild-stock outbred female house mice, which nest socially and often rear offspring cooperatively with preferred nest partners. Females preferred to nest with sisters, regardless of prior familiarity, confirming the use of phenotype matching. Among unfamiliar relatives, females strongly preferred nest partners that shared their own MUP genotype, though not those with only a partial (single-haplotype) MUP match to themselves or known family. In the absence of MUP sharing, females preferred related partners that shared multiple loci across the genome to unrelated females. However, MHC sharing was not used, even when MHC type completely matched their own or that of known relatives. Our study provides empirical evidence that highly polymorphic species-specific kinship markers can evolve where reliable recognition of close relatives is an advantage. This highlights the potential for identifying other genetic kinship markers in cooperative species and calls for better evidence that MHC can play this role.

INTRODUCTION

In cooperatively breeding species, individuals can gain indirect fitness benefits by helping kin to reproduce [1], but reliable mechanisms are needed to distinguish close kin. Discrimination could be achieved by matching phenotypes encoded by highly polymorphic genetic loci in other individuals [2, 3], allowing recognition of relatives carrying genetic markers regardless of prior familiarity. To be useful, though, kinship markers must normally correlate strongly with sharing across the rest of the genome. This creates a special challenge for identification of the specific loci used for kin recognition, as tests of putative kinship markers must fully control for matching at any other loci that could play a role [3–6]. Indeed, among vertebrates, genetic markers used to assess kinship have yet to be definitively identified, particularly in the context of cooperative behavior. However, two highly polymorphic gene complexes have been implicated as putative kinship markers, both of which influence individual scent cues.

Odors associated with the highly polymorphic major histocompatibility complex (MHC) are the textbook example of a putative kinship marker, with the potential to apply across all vertebrates [7, 8]. In fact, evidence is surprisingly scarce from studies that properly control sharing at other loci across the genome. When equally related siblings (sibs) are tested, juvenile arctic char (*Salvelinus alpinus*) prefer waterborne odor from those sharing their MHC IIb genotype [4, 9], and African clawed toad tadpoles (*Xenopus laevis*) preferentially shoal with those of the same MHC genotype [5, 10]. In both cases, though, preference for shared MHC type does not extend to unfamiliar non-sibs [9, 11]. If MHC-based discrimination occurs only between sibs, this would not function as a genetic kinship marker. The main evidence that MHC type directly influences odor-mediated discrimination comes from inbred strains of laboratory mice in which MHC type is the only difference between individuals [12–14]. Some strains of inbred male mice prefer mates of a different MHC type from their mother due to familial imprinting on parents (but not littermates) during rearing [13, 15, 16]. However, this model tests only for discrimination against those genetically identical to a familiar parent (thus, parent recognition). It does not test the crucial requirement that a specific kinship marker is recognized in other genetically distinct

individuals through phenotype matching. To address more naturalistic scenarios, early studies crossed MHC types from laboratory mice onto a semi-wild genetic background to provide heterogeneous animals with a restricted set of MHC haplotypes [17–19]. Correlations in these experiments between MHC sharing and kin-biased behavior (mate selection or communal nursing between females) are consistent with the hypothesis that MHC acts as a genetic marker of kinship. Crucially, though, as in studies of non-model species in natural populations [20], correlations with other loci shared through kinship are not controlled.

Another highly polymorphic cluster of at least 21 functional genes on mouse chromosome 4 encodes the major urinary proteins (MUPs) [21, 22], inherited independently of MHC. These specialized communication proteins are present at high concentration in mouse urine. The patterns of MUP isoforms expressed by genetically heterogeneous house mice (*Mus musculus domesticus*) are used for individual recognition [23–25] and to assess genetic heterozygosity [26]. Like MHC, the MUP region is inherited as a haplotype of tightly linked genes. Mice inheriting the same MUP genotype on heterogeneous backgrounds express similar phenotypes, evident in females (Figure S1) as well as in males [23, 27]. Thus, MUPs also have strong potential for providing a genetic kinship marker in mouse urine. An initial test assessed whether sharing MUP and/or MHC haplotypes influenced mating preferences when background relatedness was controlled among wild-stock mice breeding freely in large semi-natural enclosures [3]. Consistent with use of MUP type as a kinship marker to avoid inbreeding, there was a substantial deficit of mating between those of the same MUP genotype. By contrast, mating was not reduced when male MHC haplotypes matched the female or her mother. However, disassortative mate preferences could also arise from heterozygous advantage at the putative marker itself (for example, improved immunity for MHC and individual and/or heterozygosity signaling for MUP) rather than signifying use of a kinship marker to avoid inbreeding across the genome. Such large-scale naturalistic approaches also provide very limited evidence concerning the mechanisms involved and cannot test the full range of phenotype-matching templates that could be used. This requires the ability to manipulate the specific rearing experiences and genetic inheritance of individual animals while simultaneously controlling for experience of matching at all other loci.

Here we develop a different approach to solve this longstanding problem to establish the genetic markers and recognition templates used for recognition of unfamiliar close kin among normal, genetically heterogeneous animals. Using a carefully designed captive breeding program, we generated family lines of outbred wild-stock house mice. This provided a large selection of unfamiliar individuals with different parents that were all equally related to each other within a family line (coefficient of relatedness, $r = 0.19$ or 0.25) to control for genome-wide sharing. Each individual carried different random combinations of MHC and MUP haplotypes, tracked by descent through family pedigrees; in utero and during rearing, they also experienced different sets of haplotypes from their mothers and littermate sibs for potential familial imprinting. Thus, responses could be tested toward unfamiliar kin that differed in their match to the individual subject at one of the two putative kinship markers, while we controlled for any match at the other marker and across the genome. Different matches could be assessed either to the subject itself or to haplotypes that the subject had experienced during rearing (but did not carry itself) to test for any familial imprinting.

We use this approach to test whether female wild-stock house mice (*Mus musculus domesticus*) use genetic kinship markers based on shared MHC haplotypes, MUP haplotypes, and/or other genes to preferentially establish cooperative associations with related females. House mice live in family-based social groups, but mixing between relatives and nonrelatives is extensive. Females nest socially and often cooperate to rear offspring in communal nests, where each breeding female provides milk and other care to the communal litter [28]. Prior familiarity between females is a major factor influencing the success of communal nests [28]. Communal nursing partnerships are established with nest sharing before females reproduce, with females choosing to share nest sites with preferred partners [29, 30]. In free-ranging environments, females prefer to nest and communally rear offspring with close kin such as sisters [18, 31, 32], but relatedness and prior familiarity are conflated in such studies. Familiar close kin could be recognized using learned individual-specific cues rather than genetic kinship markers, so our first experiment established that females prefer to form nest alliances with close kin (sisters) over unrelated females using genetic kinship markers, regardless of prior familiarity. We then tested the specific genetic markers and recognition templates that they use.

RESULTS

Recognition of Kinship Does Not Require Familiarity

To test partner preferences with genetic sharing and prior familiarity manipulated independently, we gave wild-stock female house mice a choice between a sister and an unrelated female. Stimulus animals were either (1) both unfamiliar (a non-littermate sister versus age-matched nonrelative from other cages) or (2) both familiar cagemates (littermate sisters cohabiting from conception were housed with age-matched nonrelatives from weaning to reflect the mixing of unrelated animals once independent). Thus, females could recognize familiar sisters through individual-specific cues learned during rearing and by phenotype matching of genetic markers, but they could use only phenotype matching of genetic markers to recognize unfamiliar sisters. We established an assay in which each subject female could move freely between two potential nest partners or a neutral cage to assess the independent preferences of subject females (Figure 1A). Nest partner preferences were assessed over a 72 hr test period to ensure that choices were consistent over time and reflected a real preference for nesting with another female rather than simple investigation of scents.

Females showed a strong preference to spend time with a sister versus an equivalent age-matched nonrelative ($p = 0.001$) and, overall, prior familiarity had no influence on preference for kin ($p = 0.48$; Figure 1B). To check that this association preference reflected a choice to nest with a sister, we broke down behavior into the inactive light and active dark phases of the light cycle. This confirmed that in the light, when females were largely inactive and resting, there was strong preference to nest with a

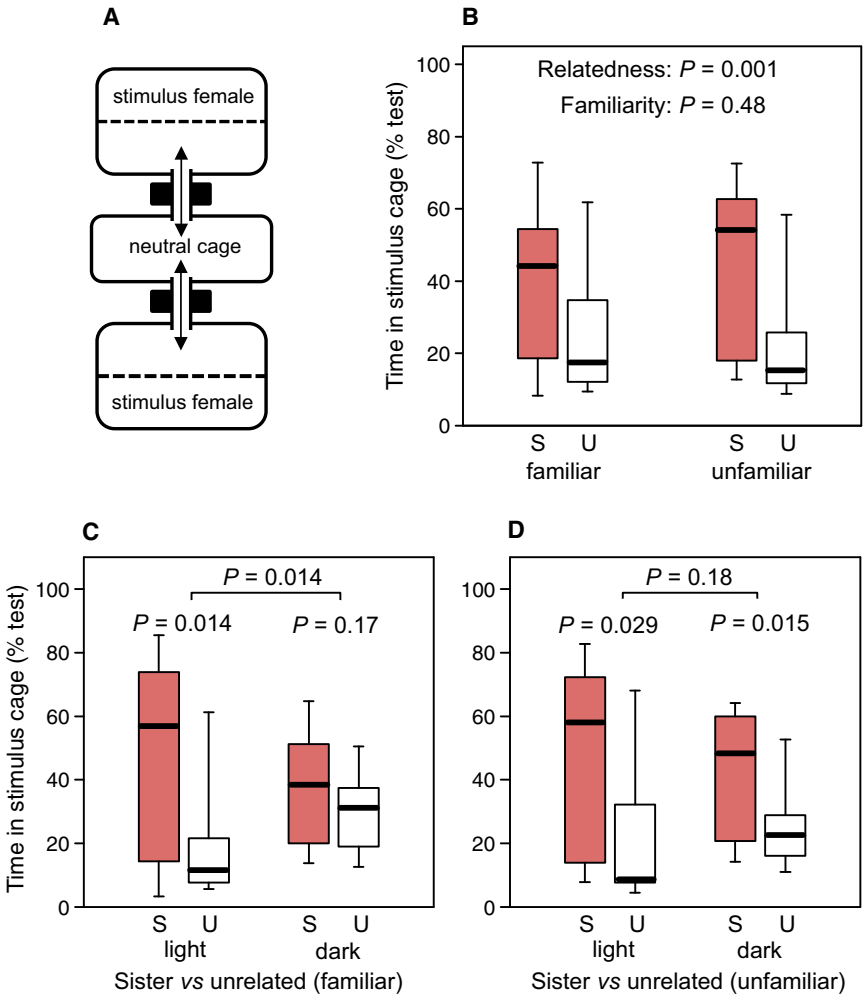

Figure 1. Females Prefer to Associate with Sisters over Unrelated Females

(A) Subject females could move between cages housing a sister, an unrelated female or a neutral cage, with their direction of movement through the linking tunnels being monitored continuously (black boxes).

(B–D) Percentage of total trial time (72 hr) in the sister (S, red fill) versus unrelated (U, open) female stimulus cage when both were either familiar cagemates (n = 19) or unfamiliar (n = 22) to the subject (B). Boxes show median and interquartile range with 10% and 90% whiskers. A linear mixed model, taking additional random factors into account including age and weight differences, confirmed a highly significant preference for sisters and no difference according to previous familiarity (Table 2). Time in stimulus cages is broken down into the inactive light phase, when females nested together, and the active dark phase for familiar cagemates (C) and unfamiliar females (D). Wilcoxon matched-pair tests within and between light phases confirmed that females preferred to nest with sisters during the light phase, whether previously familiar with the females or not.

sister, whether familiar or unfamiliar (Figures 1C and 1D). In the active dark phase, preference to actively interact with an unfamiliar sister remained strong, though this bias reduced when both females were highly familiar cagemates (Figures 1C and 1D). This clear recognition of sisters as preferred nest partners, even when previously unfamiliar, indicates that kin bias is based on a process of phenotype matching rather than individual recognition of familiar relatives [33]. This could be based on recognition of shared genetic markers and/or other cues gained from similarities in maternal environment.

Kinship Recognition Is Based on MUP, but Not MHC, Loci

To establish whether females use MHC and/or MUP haplotypes to recognize close kin as preferred nest partners, we assessed a female's preference between two unfamiliar age-matched relatives (coefficient of relatedness both r = 0.19 or both r = 0.25). These differed in their match to the subject at one or both of the two putative markers and derived from different parents from the subject. Very tight linkage of genes within the MHC and MUP clusters allowed sharing of complex haplotypes to be tracked very reliably within family pedigrees through recent common descent, with animals from the same family line sharing one haplotype (partial match), both haplotypes (full match), or none (no match) at each putative marker (Figure 2A). Microsatellite markers spread across each region checked for any recombination events, but these were rare (0.2% of MHC and 0.7% of MUP haplotypes inherited; see the Supplemental Experimental Procedures and Figures S2 and S3). We also found tight linkage between MHC and the cluster of 38 *Esp* genes that encode exocrine-gland-secreting peptide (ESP) pheromones involved in mouse olfactory signaling [34] (see the Supplemental Experimental Procedures). Thus, any effects due to MHC sharing could potentially be explained by differences in MHC and/or ESP type. Figures 2B–2E and Table 1 provide illustrative examples of the matching versus non-matching stimulus female genotypes selected for each type of test, according to both a subject's own genotype and a subject's parental genotypes.

Full Self Match at MHC or MUP

First, we tested whether females preferred unfamiliar partners that fully matched themselves (both haplotypes shared) at either MHC or MUP when sharing was controlled across the rest of the genome, including the other marker (Figure 2B; Table 1, test 1). Females strongly preferred to associate with partners that shared their own MUP type over those that shared no MUP haplotype through common descent ($p = 0.001$; Figure 3A). Preference to nest with a MUP-matching partner was evident during the inactive light period, in addition to more time being spent with the matching partner during the active dark period (Figure 3C). By contrast, there was no preference for partners that fully matched the female's own MHC type over those that shared no MHC haplotype (Figure 3B),

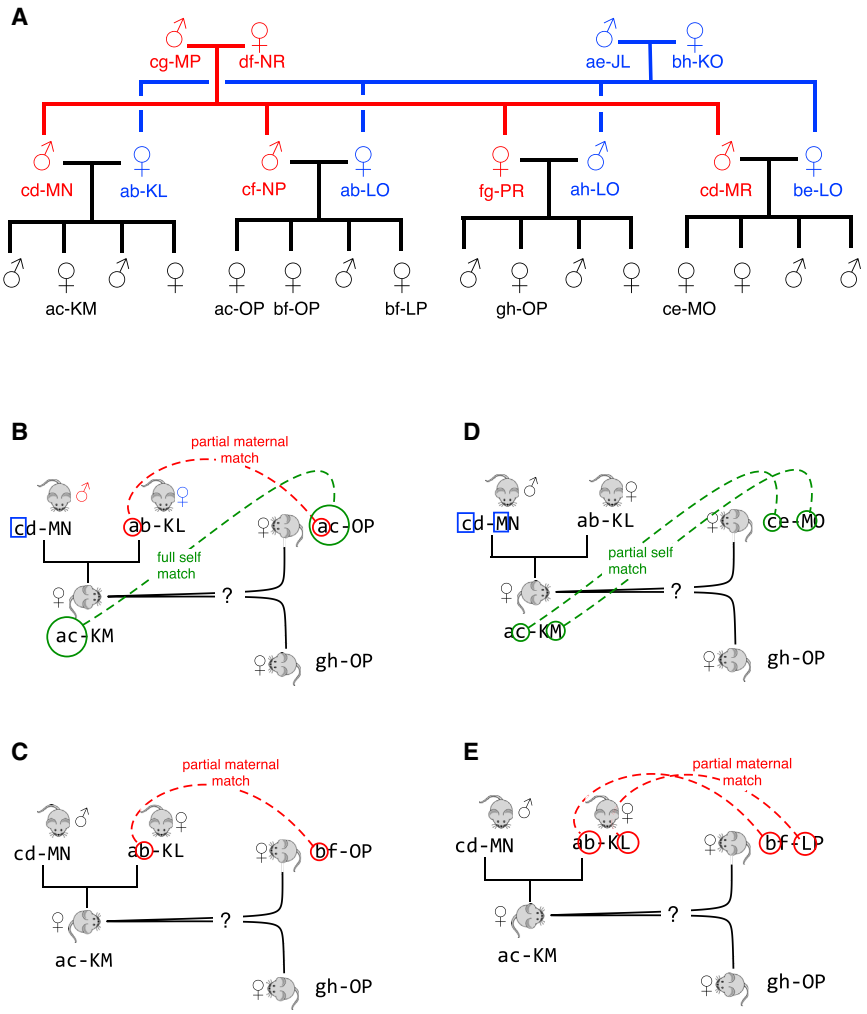

Figure 2. Testing Matches at MHC and/or MUP while Controlling for Genome-wide Sharing

(A) Family lines were created by breeding two unrelated families of outbred sibs (red, blue) that were then crossed to provide multiple litters of unfamiliar double cousins (r = 0.25) as illustrated (black) or a set of double cousins was then crossed with an unrelated set of sibs to provide multiple litters related at r = 0.19 (not shown). Within each line, litters were equally related but had different parents and family experience of MHC (lower case) and MUP (upper case) haplotypes (see also Figures S2 and S3).

(B–E) Examples of matching and non-matching stimulus animals used to assess nest partner preference according to their match to a subject female (highlighted in green) and her familial exposure (red, maternal; blue boxes, paternal, though sires themselves were not present during rearing). Stimulus females were equally related and unfamiliar to the subject. Shown are full self match and partial maternal/paternal match at MHC (B), partial maternal but no self match at MHC (C), partial self/paternal match at MHC and MUP (D), and partial maternal but no self match at MHC and MUP (E). Table 1 provides full list of test types with example genotypes.

a lack of preference that persisted through both active and inactive periods.

Haplotype Imprinting

Recognition of partners that match their own MUP type could be achieved by self-referent matching [35], but could also be achieved by imprinting on cues from relatives learned during development. Offspring can imprint on odors of the animals they are reared with [14, 19, 36], particularly on those from their mother, with which they share one allele at every locus and are exposed to intimately in utero and throughout lactation. As partners that fully match the subject's MUP type carry one MUP haplotype that is also familiar through a partial (single MUP haplotype) match to the subject's mother and to other offspring in the nest (Figure 2B), preference could be due to a match to themselves and/or to a partial match to their mother and other sibs. To distinguish between these mechanisms, we tested whether females preferred unfamiliar partners that shared a haplotype with the subject's mother and littermates, but not with themselves (potentially learned through familial imprinting), over an equally related female that carried two novel haplotypes at the focal marker that they had not experienced during rearing (Figure 2C; Table 1, pooled responses to test 2, 3, or 6 where the haplotypes carried by the non-matching female were both novel).

Females failed to associate preferentially with partners that matched a highly familiar maternal MUP haplotype (Figure 3D). Thus, preference for a full match to their own MUP type was not due to familial imprinting during rearing. There was also no evidence for familial imprinting on maternal MHC haplotypes experienced during rearing (Figure 3E). Indeed, unexpectedly, the effect of MHC on partner preference was opposite to that predicted by the hypothesis that females could recognize kin based on a 50% match (one shared haplotype) to their familiar mother's MHC type [19]. From a theoretical viewpoint, it is unclear why this might be, and further studies will be needed to establish whether this apparent opposite bias has any functional significance. Here, we focused only on identifying the shared genetic markers that females use to preferentially associate with kin.

Preference for a full MUP match to themselves, but not for a single-haplotype match to their mother and littermates, might be because females use only self-referent matching. Alternatively, they may recognize a full match to any MUP phenotypes learned from themselves or imprinted from known relatives. To test this, we asked whether females prefer partners that fully match their mother's MUP type over equivalently related females that do not (Table 1, test 3). Preference for a full match to maternal MUP type was not significant (Figure 3F). This contrasts with the consistent preference when partners matched their own MUP type (Figure 3A), suggesting that imprinting on other familiar MUP types experienced during rearing does not have a strong effect on partner preference. Neither was there any indication of preference for partners that shared the subject's

Table 1. Example Subject and Stimulus Trios Used for Each Test of Kin Recognition

Test	Subject Type[a]	Dam Type	Stimulus	
			Matching	Non-matching
1. Full Self Match (and Partial Maternal Match)				
Marker 1 (focal)	ac	ab	ac	gh
Marker 2	KM	KL	OP	OP
Relatedness			0.19/0.25	0.19/0.25
2. Partial Maternal Match				
Marker 1 (focal)	ac	ab	bf	gh
Marker 2	KM	KL	OP	OP
Relatedness			0.19/0.25	0.19/0.25
3. Full Maternal Match (and Partial Self Match)				
Marker 1 (focal)	ac	ab	ab	gh
Marker 2	KM	KL	OP	OP
Relatedness			0.25	0.25
4. Partial Self/Paternal Match				
Marker 1 (focal)	ac	ab	ce	gh
Marker 2	KM	KL	OP	OP
Relatedness			0.19/0.25	0.19/0.25
5. Partial Self/Paternal Match, Both Markers				
Marker 1 (focal)	ac	ab	ce	gh
Marker 2 (focal)	KM	KL	MO	OP
Relatedness			0.19/0.25	0.19/0.25
6. Partial Maternal Match, Both Markers				
Marker 1 (focal)	ac	ab	bf	gh
Marker 2 (focal)	KM	KL	LP	OP
Relatedness			0.19/0.25	0.19/0.25
7. Genetic Background				
Marker 1	ac	ab	ef	ij
Marker 2	KM	KL	OP	TV
Relatedness (focal)			0.19	0

Arbitrary example of haplotypes carried by one subject female at two genetic markers (upper- or lowercase) inherited from unrelated heterozygous parents. Prior to testing, subjects had experience of all haplotypes carried by themselves, their mothers, and their littermates (sire not present during rearing).

[a]Separate tests (1–7) were based on haplotype matching at the focal genetic marker(s) (MHC, MUP, and background), where matching was to the subject itself and/or to the subject's mother (matching haplotypes are underlined). In tests 2 and 6, the matching stimulus shared a haplotype with the subject's mother and some littermates, but not with the subject. In tests 4 and 5, the matching stimulus shared a haplotype with the subject and some littermates (paternally derived), but not with the subject's mother. Stimulus animals were of equivalent relatedness (either r = 0.19 or r = 0.25), except in test 7. For tests 1–4, MHC or MUP acted as the focal marker in separate tests, with matching at the other marker controlled (either no match, as in the example, or haplotypes were equivalently matched by both stimulus animals). In all tests, there was no sharing between subjects and the nonmatching stimulus at the focal marker. For test 7, MUP and MHC haplotypes of both stimulus animals were unfamiliar to the subject prior to testing (i.e., not shared with the subject, the subject's parents, or the subject's littermates).

full maternal MHC type (Figure 3G). The sample size for this test was small (n = 13) due to the limited availability of appropriate unfamiliar stimulus females, but the direction of response was opposite to that predicted by a kinship marker.

Lack of Preference for Single-Haplotype Matching

As females preferentially associated with those sharing a full MUP match to themselves, we asked whether they also prefer partners that share just one of their two MUP haplotypes. Unrelated animals are very unlikely to share both haplotypes at a highly polymorphic gene cluster, providing reliable exclusion of non-kin. However, this supports recognition of only a proportion of close relatives (approximately one-third of full sibs in an outbred population with eight different haplotypes [37]). Many more close relatives share a single polymorphic haplotype, but this is also considerably more likely between non-kin too (approximately half will share a single haplotype in a population with eight different haplotypes [37]). Thus, use of single-haplotype sharing would allow more relatives to be recognized but at the cost of much less reliable exclusion of non-kin. This high risk of mistaken association with matching non-kin could be halved if a single haplotype had to be matched at both MUP and MHC [37].

To assess the effect of single-haplotype matching to themselves on nest partner preference, we tested matching at the female's paternally inherited haplotype, as the father was not present in the nest during rearing (Table 1, tests 4 and 5; note that recognition of the maternally inherited haplotype was tested in the full maternal match model, test 3). Females displayed no preference at all for partners that matched their paternally inherited MUP haplotype (Figure 3H) or that matched their paternally inherited MHC haplotype (Figure 3I). Instead, they tended to spend more time with an equally related female with no match. Sharing a single haplotype at both markers simultaneously (Figure 2D; Table 1, test 5) did not significantly improve discrimination based on each marker alone (Figure 3J; Table 2). Thus, full sharing with themselves at the MUP marker influences nest partner preference between female house mice, but not partial sharing. Full sharing is a conservative mechanism that reliably excludes non-kin as preferred partners and is likely to reflect very close kinship, even though only a limited proportion of close kin will be recognized using this mechanism.

The high risk that many non-kin will share a single haplotype at a single kinship marker is the same whether self-referent or maternal comparison is used to recognize kin. Thus, we also tested whether females use a single maternal haplotype match at both MHC and MUP to reduce this risk through a maternal imprinting mechanism (Table 1, tests 2 and 6); this template would allow recognition of all maternal sibs because they inherit a maternal haplotype at both putative markers. However, there was no evidence that sharing a single maternal MUP haplotype and a maternal MHC haplotype influenced nest partner preference (Figures 3K–3M; Table 2).

Although it has been suggested that mice might recognize a large proportion of close relatives by recognizing separate maternal MHC haplotypes inherited by other kin [19], currently there is no evidence that mice can perform such single-haplotype matching in other individuals at either MHC or MUP. Such recognition mechanisms may be limited by constraints on the ability to resolve complex polymorphic phenotypes into the

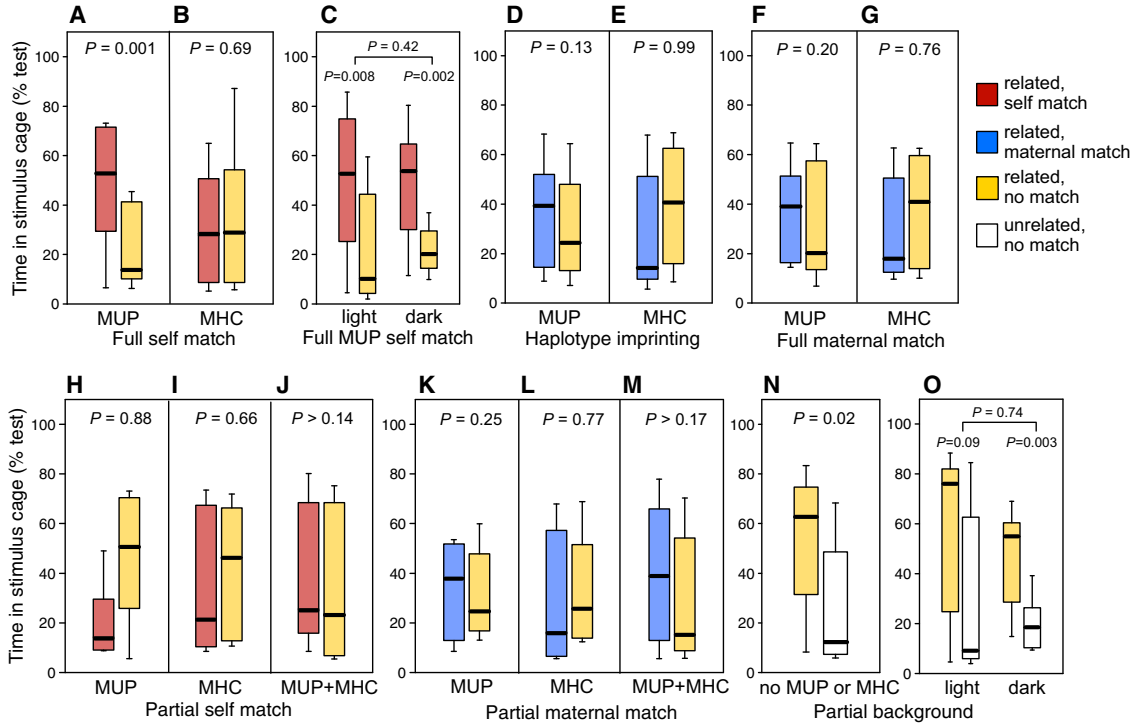

Figure 3. The Influence of Different Genetic Markers on Nest Partner Preference

Nest partner preference (Figure 1A) was assessed over 36 hr between unfamiliar females that were either of the same relatedness (both r = 0.19 or 0.25) but differed in match at MUP and/or MHC (A–M) or differed in relatedness (r = 0.19 versus 0) but shared no MUP or MHC with the subject or subject's mother or littermates (N and O). Full match indicates both haplotypes; partial match indicates one haplotype, shared with the subject itself (red bars in A–C and H–J) or only with its mother and littermates (blue bars in D–G and K–M). The matching stimulus for haplotype imprinting (D and E) carried a haplotype familiar from the subject's mother and littermates at the focal marker but not shared with itself, while neither haplotype of the non-matching stimulus had been experienced during rearing. Boxes show median and interquartile range with 10% and 90% whiskers (n sizes: A, 19; B, 19; C, 19; D, 35; E, 33; F, 19; G, 13; H, 14; I, 12; J, 16; K, 10; L, 11; M, 16; N, 16; and O, 16). p values from linear mixed models assess preference for the female that matches the relevant genetic marker (A, B, and D–N; Table 2). For genetic markers in which there was significant preference, time in stimulus cages is broken down into the inactive light phase, when females nested together, and the active dark phase (C, full MUP match to itself; O, background relatedness but no MUP or MHC haplotypes shared or familiar). Wilcoxon matched-pair tests within and between light phases confirmed that bias was similar during light and dark phases, though nesting together was more variable for shared relatedness but no MUP or MHC haplotypes (O). No preference for nesting with a matching female during the light phase was evident in tests of other genetic markers.

contributions of separate haplotypes, particularly when this must also be achieved on varying genetic backgrounds. Further, to provide the same reliable exclusion of non-kin that can be achieved by full sharing with themselves at a single highly polymorphic locus, single-haplotype matching would require assessment across multiple independent polymorphic markers [37] and would not be achieved by partial matching at MUP and MHC markers alone.

Other Genetic Loci Contribute to Kinship Recognition

Attention has focused on odors associated with MHC and MUP types as potential kinship markers because of very high levels of polymorphism at these loci, together with proven influence on individual scent. However, many genes influence individually variable scents in mice [38, 39]. We asked whether females use matching at other genetic loci to recognize unfamiliar relatives when no MUP or MHC haplotypes are shared (either through common inheritance or experienced during rearing). Females were tested with an unfamiliar relative from different parents (r = 0.19) versus an age-matched nonrelative (matched female from an unrelated family line), when neither had any MUP or MHC haplotypes shared with the female, her mother, or littermates (Table 1, test 7). Females preferred the related partner (p = 0.02; Figure 3N). There was no significant difference in this preference during different phases of the light cycle (p = 0.74), though it may be noted that preference was very consistent during the active dark phase (p = 0.003) and a little more variable when females nested together during the inactive light period (p = 0.09; Figure 3O). Thus, females also use other, as-yet-unidentified loci not closely linked to MUP or MHC to select relatives as preferred partners, though a small number preferred to nest with the unrelated female. Nonetheless, the consistency of overall preference for a female related at only r = 0.19 when no specific loci were selected to match to the subject suggests that this recognition involves sharing integrated across multiple additional unlinked alleles. This fits with general observations that overall similarity in complex mammalian individual odors co-varies continuously with the degree of genetic similarity between individuals, albeit with a high degree of variance [40–43]. As yet, it is not known whether

Table 2. Mixed-Effects Modeling of Nesting Partner Preferences

Dataset and Model	F Statistic	Probability
Figure 2B: Sister versus Unrelated (Familiar or Unfamiliar, n = 41)		
Relatedness	$F_{1,39.9} = 12.62$	*p = 0.001*
Familiarity[a]	$F_{1,30.1} = 0.51$	p = 0.48
Figure 3A: Full Self Match at MUP (n = 19)		
Match at MUP	$F_{1,16.3} = 12.61$	*p = 0.001*
Figure 3B: Full Self Match at MHC (n = 19)		
Match at MHC	$F_{1,17.8} = 0.26$	p = 0.69
Figure 3D: Haplotype Imprinting at MUP (n = 35)		
Maternal and littermate match at MUP	$F_{1,31.1} = 1.35$	p = 0.13
Figure 3E: Haplotype Imprinting at MHC (n = 33)		
Maternal and littermate match at MHC	$F_{1,27.5} = 5.27$	p = 0.99
Figure 3F: Full Maternal Match at MUP (n = 19)		
Match at MUP	$F_{1,16.0} = 0.76$	p = 0.20
Figure 3G: Full Maternal Match at MHC (n = 13)		
Match at MHC	$F_{1,9.9} = 0.55$	p = 0.76
Figure 3H and 3J: Partial Self/Paternal Match at MUP (n = 30)		
Match at MUP versus match at MUP and MHC[b]	$F_{1,27.2} = 1.21$	p = 0.14
Match at MUP	$F_{1,29.0} = 1.36$	p = 0.88
Figure 3I and 3J: Partial Self/Paternal Match at MHC (n = 28)		
Match at MHC versus match at MHC and MUP[b]	$F_{1,20.8} = 0.004$	p = 0.48
Match at MHC	$F_{1,28.0} = 0.16$	p = 0.66
Figure 3K and 3M: Partial Maternal Match at MUP (n = 26)		
Match at MUP versus match at MUP and MHC[b]	$F_{1,26.0} = 0.26$	p = 0.31
Match at MUP	$F_{1,22.9} = 0.36$	p = 0.25
Figure 3L and 3M: Partial Maternal Match at MHC (n = 28)		
Match at MHC versus match at MHC and MUP[b]	$F_{1,25.4} = 0.97$	p = 0.17
Match at MHC	$F_{1,15.2} = 0.57$	p = 0.77
Figure 3N: Partial Background (r = 0.19), no MUP or MHC (n = 16)		
Relatedness	$F_{1,\ 9.6} = 5.76$	*p = 0.02*

Results are presented for the fixed effect of greater time spent with the related or matching partner, with significant results shown in italics ($p < 0.05$). Other variables were included as random effects (subject ID, subject line, enclosure ID, matching at the non-focal marker, stimulus animal age, and weight difference) as relevant to specific models (see "Data Analysis" in the Supplemental Experimental Procedures).

[a]In the sister versus nonrelative model, the effect of familiarity on the bias in time spent with a sister versus nonrelative was tested by fitting an interaction term between relatedness and familiarity.

[b]In partial (single-haplotype) matching models, the effect of sharing at both markers was assessed first before pooling data to examine matching at the focal marker.

animals use an integrated similarity across all scent components to estimate relatedness or selectively assess scent components that correlate most strongly with sharing across the genome to provide the most reliable estimate of their degree of relatedness [41].

Investigation of Scent from Animals with Matching Kinship Markers

To examine whether females perceive a difference in urine scents of relatives due to similarity to own and known relative scents and whether this corresponds to matching at specific genetic markers, we compared initial investigation of urine from pairs of stimulus females. Tests were carried out before females met the scent donors themselves in our functional assay of nest partner preference. In agreement with studies in other species [42, 43], females spent less time investigating urine from an unfamiliar sister (r = 0.5) than that from an unrelated female during brief 10 min tests (p = 0.02; Figure 4B). The same discrimination was shown when the sister and unrelated female urine donors had been their familiar cagemates for at least 4 months prior to testing (p = 0.002; Figure 4A). Thus, investigation bias is not simply due to reduced "novelty" of scent from an unfamiliar sister, but reflects a persistent perception that scent from a close genetic relative requires less investigation due to its similarity to their own and/or familial odors imprinted during rearing. However, when relatedness was only r = 0.19 and the relative carried no MUP or MHC haplotypes that were familiar to the female, scent investigation was just as prolonged as that toward urine from an unrelated female (Figure 4C). Despite this, females still associated preferentially with the related female in nest partner tests (Figures 3N and 3O). We cannot distinguish whether this extended urine investigation provided information on kinship (which led to the association preference) or whether the cues used to recognize background relatedness were not detectable in urine.

When two donors were equally related to the female (r = 0.19 or 0.25), a match to the female's own MHC type (Figure 4E) or to one highly familiar maternal MUP or MHC haplotype (Figures 4F and 4G) failed to influence investigation of unfamiliar scent. By contrast, a match to the female's own MUP type encountered on a different genetic background increased (rather than reduced) the duration of investigation (p = 0.03; Figure 4D). Thus, recognition of MUP sharing was not simply due to greater familiarity of scent, distinct from the response to a sister's urine. The phenotype of involatile MUPs in urine was very similar between females sharing the same MUP genotype, even when their overall relatedness was only r = 0.19 (Figure S1). However, differences in other urinary volatile and peptide components at this level of relatedness were sufficient to stimulate as much investigation as an unrelated stimulus (see Figure 4C). Significant bias for more prolonged investigation of urine that matched the female's own MUP type on this different genetic background most likely reflects the processing time required to assess the similarity of an involatile MUP phenotype alongside other differences in a female's scent. It also confirms that females could detect sharing of MUP type through urine scent, providing a mechanism to select preferred nest partners based on shared MUP type.

DISCUSSION

We have shown that female house mice use genetic kinship markers to preferentially establish pre-reproductive nesting alliances with close kin, regardless of any prior familiarity. We have also shown that both MUP genotype and sharing at multiple

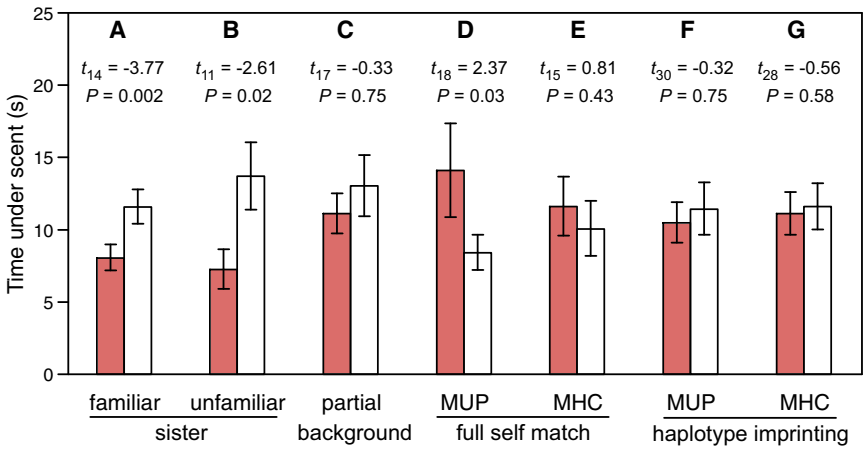

Figure 4. Discrimination of Urine Samples with Different Genetic Markers of Relatedness

Females were given a 10 min choice between 10 μl urine samples from a female with a specific marker of genetic relatedness (red filled bars) versus a control without (open bars), streaked on the ceiling on opposite sides of a divided arena. Time spent immediately under the urine sample (5.5 cm diameter circle) was recorded blind to urine identity (data are means ± SE). Urine choices shown were from a sister (r = 0.5) versus unrelated female when both donors were highly familiar (A) or both unfamiliar (B); a related (r = 0.19) versus unrelated donor where both carried novel MUP or MHC haplotypes that were not experienced by the subject during rearing (C); equally related females (both r = 0.19 or 0.25) that shared the same MUP (D) or MHC (E) type as the subject versus no MUP or MHC haplotype shared; or equally related females (both r = 0.19 or 0.25) that shared at least one MUP haplotype (F) or MHC haplotype (G) with the subject's mother versus a control that carried novel haplotypes not experienced by the subject during rearing. Matched-pair t tests compared time spent under the two stimuli in each test. Familiarity in sister tests had no effect on bias ($F_{1,25}$ = 0.26, p = 0.62). Urine donors in all other tests were unfamiliar. Example urinary MUP phenotypes for females sharing both, one or no MUP haplotypes are illustrated in Figure S1.

unidentified loci across the genome act as genetic kinship markers to establish these nesting partnerships. MUP genotype provides sufficient polymorphism to act as a kinship marker because of recent rapid expansion in the central region of the *Mup* gene cluster in commensal house mice, coincident with their separation from other *Mus* species [21]. In most other species examined to date, *Mup*-like genes show little or no polymorphism (in humans, there is only a single *Mup* pseudogene), although there has been completely independent expansion of these genes also in the Norway rat (*Rattus norvegicus*) [22]. Thus, MUP polymorphism is a species-specific signal comprising a set of specialized communication proteins that, in mice, are excreted in the urine of both sexes [27]. The individual scent signatures that MUPs encode also reflect close kinship through shared inheritance of tightly linked haplotypes. Importantly, these shared signatures are readily recognized against the heterogeneous genetic background of individual outbred animals (see also [23, 24]), a feature essential for genetic kinship markers. Although polymorphic MUP isoforms differ from each other by only a few amino acid changes [44], they are discriminated through vomeronasal sensory neurons using a combinatorial-coding strategy [25]. In addition, MUPs influence individual volatile odor signatures through binding and release of a wide range of urinary volatiles, with isoforms differing in specific binding affinities [45–48]. Further work will be needed to establish whether one or both of these mechanisms are involved in discriminating relatives that share the same MUP phenotype.

The rapid evolution of polymorphic MUP types in house mice most likely reflects strong selection pressure for reliable communication of both individual identity and close kinship in this social species. This will be particularly important in the context of cooperative breeding and communal nursing, when adult females make considerable investment in the offspring of others. That polymorphism in genetic markers could evolve specifically to promote nepotistic behavior (favoring of relatives) is controversial. The fitness advantage that is expected to accrue for common haplotypes could result in erosion of the variability required for recognition [49, 50]. Thus, it has been proposed that extrinsic processes must be necessary to maintain diversity in markers used for genetic kin recognition. For example, the primary role of MHC in immune function provides strong balancing selection to maintain its diversity, providing a polymorphic genetic marker that might then be used for kin recognition [50, 51]. However, mice did not use MHC sharing to select closely related nest partners, regardless of indisputable diversity at MHC. Instead, they used MUP sharing. Polymorphic MUP patterns in mice function only as a specialized communication signal. The use of MUP sharing to identify very closely related nesting partners, though, may be paralleled by a role for the same marker in inbreeding avoidance [3], although properly controlled tests like those presented here are still needed for confirmation. MUP polymorphism also provides an individual genetic signature that allows male mice to advertise their individual competitive ability through scent marks [23–25]. Frequency-dependent selection on MUP through roles in both inbreeding avoidance and individual recognition [52] could help to maintain variability among haplotypes necessary for the reliable recognition of closely related cooperative partners [53].

House mice use sharing at MUP in addition to shared background genes to discriminate preferred partners. In the absence of MUP sharing, those related across the genome are preferred to non-kin, but there is a strong preference for partners of equivalent relatedness that also share the female's MUP type. Inclusive fitness benefits gained from cooperating with relatives will depend on how closely related animals are, and thus the proportion of genes they share. A highly polymorphic locus like MUP is only likely to be fully shared between very close relatives, with increased likelihood of sharing if animals become more inbred and share a greater proportion of their genes. Thus, it is a reliable signal that relatedness across the genome is very high (most likely at least full sibs), even though close relatives will not all share the same type in outbred populations. Familial imprinting on MUP types during rearing could allow a greater range of relatives to be recognized than achieved just by self-reference.

However, animals are likely to encounter a wide range of relatedness in the nest due to frequent multiple paternity of litters in house mice [54, 55] and communal nesting even when closely related partners are not available [28, 29]. Imprinting on such cues would not provide the same reliable indicator of very close relatedness as a full match to themselves. When a full MUP match to themselves was not available, females preferred partners sharing at other loci not closely linked to MUP or MHC. Integration of sharing over multiple loci may allow animals to estimate their degree of genetic similarity [42]. However, the correlation between odor similarity and genetic similarity can be quite crude [40, 41] and could limit the sensitivity of this estimate. By contrast, full sharing at a single highly polymorphic gene cluster like MUP (or MHC) provides a simple reliable indicator that many genes are likely to be shared but cannot indicate different degrees of relatedness, as close relatives share the full range of none, one, or both haplotypes.

The absence of preference based on MHC sharing, whether through common inheritance or familial imprinting, will be surprising in view of the substantial literature showing that MHC type influences individual odors and social responses among laboratory mice [8, 14]. Indeed, the hypothesis that MHC odors provide a kinship marker stems largely from mouse studies [7]. An early influential study found that females rearing offspring communally in semi-natural enclosures had greater MHC sharing than a random model of partner choice among mice with a 50% wild-derived genetic background [18]. However, this was confounded with prior familiarity and genetic background that might also explain biases. Sisters previously reared together in cages could be removed from analyses, but there was no control of parentage and experience of those born in enclosures, background relatedness, or MUP sharing. By contrast, all of these factors were completely controlled with our approach. We could test directly (1) the separate effects of sharing MHC, MUP, and genetic background, (2) the effect of full-genotype or single-haplotype matching, and (3) reference to own genotype or familial imprinting. We found no evidence for any preference based on MHC matching, even in the most extreme choice of a full MHC match to themselves (which simultaneously includes maternal, paternal, and littermate matches, too) compared to no MHC haplotypes matched. Given that MHC and ESP regions exhibited strong linkage in our mice, this also implies that mice did not use *Esp* genes as a marker for kin recognition either. To date, there is no convincing evidence from mouse studies that MHC is used as a genetic kinship marker among genetically heterogeneous animals, or that MHC can provide a consistent kinship signature that is recognizable across different genetic backgrounds [24, 39, 56–59], in strong contrast to recognition of MUP type.

Evidence that other species use MHC as a genetic kinship marker is also surprisingly weak when correlations with genome-wide sharing have been controlled. Although MHC-homozygous (but not MHC-heterozygous) tadpoles of African clawed frogs associate preferentially with those of the same MHC type among familiar sibs [5, 10], they show the opposite preference for different MHC types among unfamiliar non-sibs [11]. As tadpoles from wild-caught parents show only very weak preference to associate with unfamiliar sibs over non-sibs [11], MHC preferences are unlikely to reflect genetic kin recognition. Similarly, there is some evidence that juvenile arctic char prefer the same homozygous MHC class IIb genotype among unfamiliar sibs, but no such discrimination was evident among non-sibs. Further, other unlinked genes were used to discriminate sibs from non-sibs when both shared the subject's MHC class IIb genotype [4, 9]. As sample sizes were extremely small (n = 5), further work is urgently needed to understand the influence of MHC sharing on social associations in arctic char and other species. The approach that we have demonstrated here could be applied to a wide range of vertebrates to test the use of MHC and other candidates as genetic kinship markers. While the idea that MHC could provide a vertebrate-wide genetic kinship marker is very attractive because of its potential generality, appropriately controlled evidence in support is sorely lacking. Instead, our study suggests that species-specific kinship markers evolve when there is strong advantage for reliable recognition of close kinship.

In conclusion, we have demonstrated that a species-specific polymorphic signal (MUP), but not MHC, is an important signal for discrimination of close kinship in the house mouse, on top of information provided by sharing at multiple loci across the genome. This calls for further investigation to establish the genetic markers that underlie kin recognition in other vertebrates. It remains to be discovered whether other species that breed cooperatively, in situations where related and unrelated animals mix, also evolve specific genetic kinship markers that allow reliable discrimination of those that are very closely related. In addition to identifying genetic markers and templates used for kin recognition in a cooperative context in the mouse, our study provides no support for the general assumption that MHC-associated scents provide a vertebrate-wide mechanism for kin recognition.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and three figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.08.045.

AUTHOR CONTRIBUTIONS

J.L.H., R.J.B., P.S., and S.P. designed the study and gained funding. J.L.H., A.M.H., and J.P.G. designed the behavioral experiments, which were performed by J.P.G., A.M.H., J.L.H., and A.J.D. S.P. and R.J.B. advised on genotyping and MUP phenotyping, respectively, which were carried out by A.J.D., J.P.G., A.M.H., and J.L.H. J.P.G. and J.L.H. carried out all statistical analyses. J.L.H. and J.P.G. drafted the manuscript, to which all authors contributed.

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REFERENCES

1. Hamilton, W.D. (1964). The genetical evolution of social behaviour. I. J. Theor. Biol. *7*, 1–16.
2. Grafen, A. (1990). Do animals really recognize kin? Anim. Behav. *39*, 42–54.
3. Sherborne, A.L., Thom, M.D., Paterson, S., Jury, F., Ollier, W.E.R., Stockley, P., Beynon, R.J., and Hurst, J.L. (2007). The genetic basis of inbreeding avoidance in house mice. Curr. Biol. *17*, 2061–2066.
4. Olsén, K.H., Grahn, M., Lohm, J., and Langefors, Å. (1998). MHC and kin discrimination in juvenile Arctic charr, Salvelinus alpinus (L.). Anim. Behav. *56*, 319–327.
5. Villinger, J., and Waldman, B. (2008). Self-referent MHC type matching in frog tadpoles. Proc. Biol. Sci. *275*, 1225–1230.
6. Thom, M.D., Stockley, P., Beynon, R.J., and Hurst, J.L. (2008). Scent, mate choice and genetic heterozygosity. In Chemical Signals in Vertebrates, *Volume 11*, J.L. Hurst, R.J. Beynon, S.C. Roberts, and T. Wyatt, eds. (Springer), pp. 291–301.
7. Brown, J.L., and Eklund, A. (1994). Kin recognition and the major histocompatibility complex - an integrative review. Am. Nat. *143*, 435–461.
8. Penn, D.J. (2002). The scent of genetic compatibility: sexual selection and the major histocompatibility complex. Ethology *108*, 1–21.
9. Olsén, K.H., Grahn, M., and Lohm, J. (2002). Influence of MHC on sibling discrimination in Arctic char, Salvelinus alpinus (L.). J. Chem. Ecol. *28*, 783–795.
10. Villinger, J., and Waldman, B. (2012). Social discrimination by quantitative assessment of immunogenetic similarity. Proc. Biol. Sci. *279*, 4368–4374.
11. Villinger, J. (2007). Kin recognition and MHC discrimination in African clawed frog (*Xenopus laevis*) tadpoles. PhD thesis (University of Canterbury).
12. Yamazaki, K., Boyse, E.A., Miké, V., Thaler, H.T., Mathieson, B.J., Abbott, J., Boyse, J., Zayas, Z.A., and Thomas, L. (1976). Control of mating preferences in mice by genes in the major histocompatibility complex. J. Exp. Med. *144*, 1324–1335.
13. Jordan, W.C., and Bruford, M.W. (1998). New perspectives on mate choice and the MHC. Heredity (Edinb) *81*, 239–245.
14. Yamazaki, K., and Beauchamp, G.K. (2007). Genetic basis for MHC-dependent mate choice. Adv. Genet. *59*, 129–145.
15. Yamazaki, K., Beauchamp, G.K., Kupniewski, D., Bard, J., Thomas, L., and Boyse, E.A. (1988). Familial imprinting determines H-2 selective mating preferences. Science *240*, 1331–1332.
16. Beauchamp, G.K., Yamazaki, K., Bard, J., and Boyse, E.A. (1988). Preweaning experience in the control of mating preferences by genes in the major histocompatibility complex of the mouse. Behav. Genet. *18*, 537–547.
17. Potts, W.K., Manning, C.J., and Wakeland, E.K. (1991). Mating patterns in seminatural populations of mice influenced by MHC genotype. Nature *352*, 619–621.
18. Manning, C.J., Wakeland, E.K., and Potts, W.K. (1992). Communal nesting patterns in mice implicate MHC genes in kin recognition. Nature *360*, 581–583.
19. Penn, D., and Potts, W. (1998). MHC-disassortative mating preferences reversed by cross-fostering. Proc. Biol. Sci. *265*, 1299–1306.
20. Piertney, S.B., and Oliver, M.K. (2006). The evolutionary ecology of the major histocompatibility complex. Heredity (Edinb) *96*, 7–21.
21. Mudge, J.M., Armstrong, S.D., McLaren, K., Beynon, R.J., Hurst, J.L., Nicholson, C., Robertson, D.H., Wilming, L.G., and Harrow, J.L. (2008). Dynamic instability of the major urinary protein gene family revealed by genomic and phenotypic comparisons between C57 and 129 strain mice. Genome Biol. *9*, R91.
22. Logan, D.W., Marton, T.F., and Stowers, L. (2008). Species specificity in major urinary proteins by parallel evolution. PLoS ONE *3*, e3280.
23. Hurst, J.L., Payne, C.E., Nevison, C.M., Marie, A.D., Humphries, R.E., Robertson, D.H., Cavaggioni, A., and Beynon, R.J. (2001). Individual recognition in mice mediated by major urinary proteins. Nature *414*, 631–634.
24. Cheetham, S.A., Thom, M.D., Jury, F., Ollier, W.E.R., Beynon, R.J., and Hurst, J.L. (2007). The genetic basis of individual-recognition signals in the mouse. Curr. Biol. *17*, 1771–1777.
25. Kaur, A.W., Ackels, T., Kuo, T.H., Cichy, A., Dey, S., Hays, C., Kateri, M., Logan, D.W., Marton, T.F., Spehr, M., and Stowers, L. (2014). Murine pheromone proteins constitute a context-dependent combinatorial code governing multiple social behaviors. Cell *157*, 676–688.
26. Thom, M.D., Stockley, P., Jury, F., Ollier, W.E.R., Beynon, R.J., and Hurst, J.L. (2008). The direct assessment of genetic heterozygosity through scent in the mouse. Curr. Biol. *18*, 619–623.
27. Hurst, J.L., and Beynon, R.J. (2013). Rodent urinary proteins: genetic identity signals and pheromones. In Chemical Signals in Vertebrates, *Volume 12*, M.L. East, and M. Dehnhard, eds. (Springer), pp. 117–133.
28. Konig, B., and Lindholm, A.K. (2012). The complex social environment of female house mice (*Mus domesticus*). In Evolution of the House Mouse, M. Macholan, S.J.E. Baird, P. Munclinger, and J. Pialek, eds. (Cambridge University Press), pp. 114–134.
29. Rusu, A.S., and Krakow, S. (2004). Kin-preferential cooperation, dominance-dependent reproductive skew, and competition for mates in communally nesting female house mice. Behav. Ecol. Sociobiol. *56*, 298–305.
30. Weidt, A., Lindholm, A.K., and König, B. (2014). Communal nursing in wild house mice is not a by-product of group living: females choose. Naturwissenschaften *101*, 73–76.
31. Wilkinson, G.S., and Baker, A.E.M. (1988). Communal nesting among genetically similar house mice. Ethology *77*, 103–114.
32. Dobson, F.S., Jacquot, C., and Baudoin, C. (2000). An experimental test of kin association in the house mouse. Can. J. Zool. *78*, 1806–1812.
33. Lacy, R.C., and Sherman, P.W. (1983). Kin recognition by phenotype matching. Am. Nat. *121*, 489–512.
34. Abe, T., and Touhara, K. (2014). Structure and function of a peptide pheromone family that stimulate the vomeronasal sensory system in mice. Biochem. Soc. Trans. *42*, 873–877.
35. Hauber, M.E., and Sherman, P.W. (2001). Self-referent phenotype matching: theoretical considerations and empirical evidence. Trends Neurosci. *24*, 609–616.
36. D'Udine, B., and Alleva, E. (1983). Early experience and sexual preferences in rodents. In Mate Choice, P. Bateson, ed. (Cambridge University Press), pp. 311–330.
37. Paterson, S., and Hurst, J.L. (2009). How effective is recognition of siblings on the basis of genotype? J. Evol. Biol. *22*, 1875–1881.
38. Boyse, E.A., Beauchamp, G.K., and Yamazaki, K. (1987). The genetics of body scent. Trends Genet. *3*, 97–102.
39. Overath, P., Sturm, T., and Rammensee, H.G. (2014). Of volatiles and peptides: in search for MHC-dependent olfactory signals in social communication. Cell. Mol. Life Sci. *71*, 2429–2442.
40. Boulet, M., Charpentier, M.J., and Drea, C.M. (2009). Decoding an olfactory mechanism of kin recognition and inbreeding avoidance in a primate. BMC Evol. Biol. *9*, 281.
41. Hurst, J.L., and Beynon, R.J. (2010). Making progress in genetic kin recognition among vertebrates. J. Biol. *9*, 13.
42. Todrank, J., and Heth, G. (2003). Odor-genes covariance and genetic relatedness assessments: rethinking odor-based "recognition" mechanisms in rodents. Adv. Stud. Behav. *332*, 77–130.

43. Holmes, W.G., and Mateo, J.M. (2007). Kin recognition in rodents: issues and evidence. In Rodent Societies: An Ecological and Evolutionary Perspective, J.O. Wolff, and P.W. Sherman, eds. (University of Chicago Press), pp. 216–228.
44. Beynon, R.J., Armstrong, S.D., Gómez-Baena, G., Lee, V., Simpson, D., Unsworth, J., and Hurst, J.L. (2014). The complexity of protein semiochemistry in mammals. Biochem. Soc. Trans. *42*, 837–845.
45. Darwish Marie, A., Veggerby, C., Robertson, D.H.L., Gaskell, S.J., Hubbard, S.J., Martinsen, L., Hurst, J.L., and Beynon, R.J. (2001). Effect of polymorphisms on ligand binding by mouse major urinary proteins. Protein Sci. *10*, 411–417.
46. Armstrong, S.D., Robertson, D.H.L., Cheetham, S.A., Hurst, J.L., and Beynon, R.J. (2005). Structural and functional differences in isoforms of mouse major urinary proteins: a male-specific protein that preferentially binds a male pheromone. Biochem. J. *391*, 343–350.
47. Kwak, J., Grigsby, C.C., Rizki, M.M., Preti, G., Köksal, M., Josue, J., Yamazaki, K., and Beauchamp, G.K. (2012). Differential binding between volatile ligands and major urinary proteins due to genetic variation in mice. Physiol. Behav. *107*, 112–120.
48. Phelan, M.M., McLean, L., Armstrong, S.D., Hurst, J.L., Beynon, R.J., and Lian, L.Y. (2014). The structure, stability and pheromone binding of the male mouse protein sex pheromone darcin. PLoS ONE *9*, e108415.
49. Crozier, R.H. (1986). Genetic clonal recognition abilities in marine-invertebrates must be maintained by selection for something else. Evolution *40*, 1100–1101.
50. Rousset, F., and Roze, D. (2007). Constraints on the origin and maintenance of genetic kin recognition. Evolution *61*, 2320–2330.
51. Gardner, A., and West, S.A. (2007). Social evolution: the decline and fall of genetic kin recognition. Curr. Biol. *17*, R810–R812.
52. Thom, M.D.F., and Dytham, C. (2012). Female choosiness leads to the evolution of individually distinctive males. Evolution *66*, 3736–3742.
53. Holman, L., van Zweden, J.S., Linksvayer, T.A., and d'Ettorre, P. (2013). Crozier's paradox revisited: maintenance of genetic recognition systems by disassortative mating. BMC Evol. Biol. *13*, 211.
54. Stockley, P., Bottell, L., and Hurst, J.L. (2013). Wake up and smell the conflict: odour signals in female competition. Philos. Trans. R. Soc. Lond. B Biol. Sci. *368*, 20130082.
55. Auclair, Y., König, B., and Lindholm, A.K. (2014). Socially mediated polyandry: a new benefit of communal nesting in mammals. Behav. Ecol. *25*, 1467–1473.
56. Willse, A., Kwak, J., Yamazaki, K., Preti, G., Wahl, J.H., and Beauchamp, G.K. (2006). Individual odortypes: interaction of MHC and background genes. Immunogenetics *58*, 967–982.
57. Novotny, M.V., Soini, H.A., Koyama, S., Wiesler, D., Bruce, K.E., and Penn, D.J. (2007). Chemical identification of MHC-influenced volatile compounds in mouse urine. I: quantitative proportions of major chemosignals. J. Chem. Ecol. *33*, 417–434.
58. Röck, F., Hadeler, K.P., Rammensee, H.G., and Overath, P. (2007). Quantitative analysis of mouse urine volatiles: in search of MHC-dependent differences. PLoS ONE *2*, e429.
59. Kwak, J., Willse, A., Preti, G., Yamazaki, K., and Beauchamp, G.K. (2010). In search of the chemical basis for MHC odourtypes. Proc. Biol. Sci. *277*, 2417–2425.