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Research report

Female recognition and assessment of males through scent

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ABSTRACT

Scents play key roles in mediating sexual behaviour in many vertebrates, both in the recognition of opposite sex conspecifics and in assessing the suitability of different individuals as potential mates. The recognition and assessment that underlies female attraction to male scents involves an important interaction between the main and accessory (vomeronasal) olfactory systems. Female mice gain information through the vomeronasal system on nasal contact with a scent source that is essential to stimulate attraction to an individual male's scent. Three highly polymorphic multigene families contribute involatile proteins and peptides to mouse scents that are detected through specific vomeronasal receptors during contact with scent. Major urinary proteins (MUPs) provide an individual genetic identity signature that underlies individual recognition and assessment of male competitive ability, kin recognition to avoid inbreeding, and genetic heterozygosity assessment. Familiar mates are recognised in the context of pregnancy block using MHC peptides, while exocrine-gland secreting peptides (ESPs) are likely to play additional roles in sexual assessment. By associating this involatile information in individual male scents, gained on initial scent contact, with the individual male's airborne volatile signature detected simultaneously through the main olfactory system, females subsequently recognise and are attracted by the individual male's airborne volatile signature alone. This allows much more rapid recognition of scents from familiar animals without requiring physical contact or processing through the vomeronasal system. Nonetheless, key information that induces attraction to a male's scent is held in involatile components detected through the vomeronasal system, allowing assessment of the genetic identity and attractiveness of each individual male.

1. Introduction

Scents play an integral role in mediating reproductive interactions in many vertebrates including mammals. Initial attention focused largely on the scents that prime reproductive physiology according to the social environment, particularly among laboratory mice. These can have a wide variety of affects, including the acceleration or delay of reproductive development among young animals, the synchrony or inhibition of oestrus among females, interruption of pregnancy establishment and, among males, modulation of luteinizing hormone levels, sperm density, sperm motility and spermatogenesis [25,77]. Scents also play key roles in mediating sexual behaviour itself. Research into the olfactory and neural pathways underlying sex-biased responses to conspecific scent signals has focused on the pathways underlying sex recognition and the control of sexual behaviour. However, there has been relatively little consideration of the complexity of the scent signals used in sexual signalling, particularly on the need to assess the suitability of potential mates beyond simple sex recognition. Here I will argue that to understand the pathways underlying reproductive behaviour, we must consider the functional significance of the information that is being processed and its importance for individual reproductive success, both to guide and to interpret investigations of the signals and the neural pathways that are involved. Laboratory rodents play a key role in these investigations because they are easily manipulated and bring advantages of genetic homogeneity and targeted manipulation. However, this homogeneity introduces its own complications which need to be taken into account when interpreting the responses of laboratory animals, as explained below.

2. Interaction between the main and accessory olfactory systems

Much research to date has addressed the separate roles of the main and accessory olfactory system in sex recognition and the control of sexual behaviour (recent reviews by [19,72,113]). The main olfactory system detects airborne scents (volatile chemical components and small airborne peptides) via receptors in the main olfactory epithelium (MOE) and can thus detect scents at some distance from their source. By contrast, the accessory olfactory system detects volatile and involatile molecules that are pumped to the vomeronasal organ (VNO) when animals make nasal contact with a

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scent source [16,47,89]. Although these two systems detect partially overlapping sets of chemosignals, each system appears to mediate different social and sexual responses [113,134]. Because the main olfactory system can detect scents in the air, this system is key in allowing animals to detect the presence of scents in the environment. This may stimulate animals to approach the source to gain further information, particularly if the volatile scent detected is unfamiliar or has not been encountered recently. Once animals are in close nasal contact with the scent source, the VNO pump is activated to gain additional information through the accessory olfactory system.

Attempts to understand the different roles of these two systems generally involve debilitation of one system to see what responses are controlled by the system remaining intact, or are disrupted presumably because responses depend on processing through the debilitated system. This approach provides very useful information, but there are significant limitations that need to be considered when interpreting responses. Detection of airborne scents through the main olfactory system may be necessary to activate delivery of scent to the VNO for example [61,71]. Thus, deficits caused by removal of MOE input may be due to the absence of information detected through the main olfactory system, the accessory olfactory system, or both. Another major limitation is that disruption of one system fails to take into account any important interaction between the systems. The functional importance of this interaction is illustrated by the effect of sexual experience on sexual responses when the VNO is removed. When male mice or hamsters have had no prior sexual experience with females, ablation of the VNO eliminates normal sexual behaviour towards females even if the main olfactory system remains intact. However, if males have sexual experience prior to VNO ablation, they learn to associate airborne volatiles detected through the MOE with scents detected through the VNO. Airborne odours processed through the main olfactory system then are sufficient to stimulate normal sexual responses towards females even if the VNO is ablated [88,99,145]. Females similarly learn to recognise airborne volatiles from males detected through the MOE by association with involatile scent information gained through the VNO during nasal contact with scents. When naïve to adult male scents, females show an inherent attraction to adult male compared to either female scents [92,93] or to castrated male scents [84], but only if they are able to contact the scent source and gain information through the VNO [85]. Once females have had repeated nasal contact with male scents, they become attracted to the airborne volatiles emanating from male scents that were not previously attractive [85,92,93].

Based on studies of laboratory mice, these findings initially suggest that animals have to learn to recognise airborne scents representing the opposite sex through the main olfactory system by association with opposite sex scents that can be inherently recognised through vomeronasal inputs without any prior experience. Such associations could be learnt through contact with scents from adult conspecifics during development in juvenile and prepubertal mice, but a need to learn such sex-specific airborne odours would be somewhat surprising. There are substantial differences in the airborne odours of male and female mice that are readily detected even by the human nose. Why then should animals have to learn to recognise the difference between male and female airborne volatile profiles through association with scents detected on contact? Our recent studies of genetically heterogeneous wild mice reveal that animals do not learn to discriminate between sex-specific airborne scents, but instead they learn the airborne scent profiles of individual mice whose scent they have previously contacted. Thus, the association learnt between contact and airborne scents is for individual recognition rather than for sex recognition [112]. When female mice are able to contact scents from individual wild-derived adult males or females, they show a consistent attraction to spend more time near to male scents regardless of whether or not they have met scent from particular individual donors before (Fig. 1a and b). When unable to contact the scent source, females are only attracted to airborne scents from individual males whose scent they have previously contacted (Fig. 1c–f). This is the case regardless of the prior sexual and social experience of females—even those that have natural social experience of many different males and females in semi-natural populations fail to learn a generalised attraction to airborne volatiles from unfamiliar males whose scent they have not contacted [112]. When experiments are carried out with inbred laboratory mice, by contrast, males or females within the same strain are genetically identical. Thus, it is not surprising that responses to one individual often generalise to other individuals of the same strain and individual-specific responses are not apparent. Even mice from so-called 'outbred' laboratory strains are still likely to share key individual genetic identity signatures (see below) because all classical laboratory strains derive from the same very small gene pool and originate from a single female ancestor [6,33,41,44]. This is clearly not a natural situation and wild mice have much more distinct scent signatures of individual genetic identity. Instead of learning an attraction to 'male' volatile scents, females learn an attraction to the volatile profile of an individual male after direct contact with that male's scent. Familiarity with an individual male's airborne volatile profile *per se* does not make that individual's scent attractive if the female has been unable to contact the scent directly (Fig. 1e). The implication is that females gain essential information about the suitability, and thus attractiveness, of a particular individual male as a potential mate through direct contact investigation of his scent, which allows detection of information through the VNO. Without this information, females do not find a male's scent attractive.

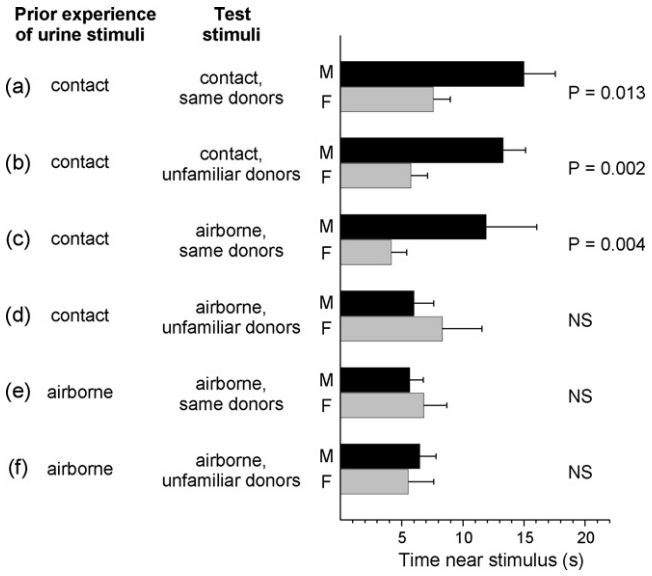

Fig. 1. Preference for male (M, black bars) over female (F, grey bars) urine according to prior experience of urine from the same or different individual donors and whether female mice can contact the stimuli before or during the test. Females spend more time near male urine if they can contact stimuli during the test (a and b). They only spend more time near airborne odours from male urine if they have had prior contact with urine stimuli from the same individual donors (c–f). *p*-values indicate Wilcoxon signed ranks tests. Total time near a stimulus (mean ± S.E.M.) involves two components. First, females spend more time sniffing closely at male than at female scents, a difference that might simply reflect greater processing time required to interpret information in male scents which are more dissimilar from their own. However, females also spend more time in the vicinity of male scents even when not sniffing [112], reflecting attraction that is not likely to be due to scent processing. Under free-ranging conditions, attraction to spend more time near male scents will increase the chances of encountering a male, as male mice scent mark their territories extensively [61]. Adapted from [112].

3. Involatile information in mouse scents

Why should information gained through the vomeronasal system on direct nasal contact with scents be essential for determining whether or not that individual is attractive? Most characterisation of sex-specific scents and associated receptors has focused on low molecular weight volatile components. Adult male mice produce several male-specific volatiles in their urine under androgen control [80,96,124] that can be detected through the MOE as well as through the VNO. However, recent research has highlighted the importance of non-volatile protein and peptide components of scents that have the capacity to provide information about individual genetic identity and competitive quality when detected through the VNO. First, there are specific V2R vomeronasal receptors in the mouse that respond differentially to synthetic peptides that emulate the nine-amino-acid peptide ligands that bind to major histocompatibility complex (MHC) proteins according to their specific anchor residues [79]. As MHC is highly polymorphic and individuals in natural populations have different MHC types that bind different sets of peptide ligands, assessment of these peptides may allow animals to detect the MHC type of a scent owner [14]. The ability to detect these peptides is not exclusive to the VNO, as peptide receptors are also found in the MOE [133], but as peptides are not 'volatile', close contact investigation may still be necessary to draw sufficient peptides into the nasal cavity for detection through the main olfactory system. It is currently supposed that MHC peptide ligands are released when MHC proteins are broken down, and that they are filtered (as waste products) into urine [79]. However, although receptors for these peptides have been clearly demonstrated, the presence of these peptides in urine or other scents has not yet been shown; neither is it clear how long such small peptides, which will be highly susceptible to both endoproteolytic and exoproteolytic attack [129]. A recent study found no evidence for the presence of these peptides in mouse urine, as there was no overlap between the VNO neurons activated by a synthetic MHC class I peptide and those activated by urine from a strain with matching MHC type [50]. By contrast, a previous study reported that there is overlapping activation of the same VSN neurons by synthetic peptides and urine from mice of the relevant MHC type [79]. Other scent sources that might contain these peptides have yet to be examined.

Mouse urine contains a very high concentration of specialised communication proteins called the major urinary proteins or MUPs [12,43]. These are encoded by a cluster of at least 19 functional *Mup* genes on mouse chromosome 4 [95]. Most MUPs are expressed in the liver and efficiently filtered into the urine, while some MUPs are expressed in salivary glands (submaxillary, parotid), lachrymal glands, nasal tissues or in the mammary glands [95,126,127,141]. Urinary MUPs are highly polymorphic and show both individual- and sex-specific expression. Once adult, each individual mouse expresses a fixed pattern of approximately 8–14 different MUP isoforms in their urine [10,11,101] determined by their *Mup* genotype and, to a lesser extent, by their sex as some MUP isoforms are expressed predominantly by males [3,95]. Males also excrete three to four times more MUP in their urine on average than females [12]. However, although expression levels can be quite low among some laboratory females, urinary MUP concentrations among wild females can overlap substantially with those of males. Individual difference in MUP patterns appear to be due largely to genes within the central region of the *Mup* cluster, which encode urinary MUPs that each differ by only a few amino acids. Genes in the peripheral region of the cluster show much greater divergence, encoding either non-urinary MUPs or urinary MUPs that are expressed predominantly by males [95]. The polymorphism among wild mice is sufficiently great that unrelated animals express different individual MUP patterns, and individual variability is apparent even in a small geographically isolated population [11]. By notable contrast, only two basic patterns of MUPs have been observed so far among laboratory strains [33,95,119].

MUPs are small (18–19 kDa) barrel-shaped lipocalin proteins with a central cavity that binds small lipophilic molecules including a number of volatile male pheromones [4,97,120]. This binding to MUPs greatly extends the longevity of urinary volatile signals in the environment: free volatiles not bound to MUPs are lost within a few minutes [121] while those bound to MUPs continue to be released over at least 24 h [54,58]. By binding volatile pheromones, MUPs play a role in concentrating, transporting and delivering volatile signals to the VNO [102]. However, MUPs are signalling molecules in their own right, activating specific V2R vomeronasal receptors that stimulate functional aggressive responses between males [31] and trigger oestrus in females [94]. Notably, most of the variation in MUP isoforms occurs on the surface of the protein rather than at the ligand-binding site [11], suggesting that animals may detect MUP variation directly rather than through differential binding of lipophilic volatiles. However, some MUP isoforms exhibit variation within the central cavity and have different binding affinities for volatile ligands [3,83,128].

Exocrine-gland secreting peptides (ESPs) are encoded by another multigene family on mouse chromosome 17, separated from the class I MHC region by a large cluster of olfactory receptor genes [76]. At least 24 functional ESP genes encode proteins predicted to range in size from 5 to 15 kDa, many of which are expressed in the extraorbital lachrymal gland, Harderian gland and/or submaxillary gland of laboratory mice [76]. Some ESPs show sex-specific expression within strains, although this appears to be strain-dependent. Expression levels of particular ESPs also vary between strains, suggesting that ESP patterns may show individual variation, although the extent of individual- and sex-specific variation in genetically heterogeneous wild mice is not yet known. A small 7-kDa protein (ESP1) that exhibits male-specific expression in the lachrymal glands of the strains so far examined stimulates V2R-expressing vomeronasal sensory neurons when female (but not male) mice make close nasal contact with either the facial area of adult males or with male-soiled bedding [75]. Males probably habituate to their own expression of this protein. Vomeronasal sensory neurons sensitive to ESP1 differ from those that respond to MUPs or to other components of mouse urine [76], showing that ESPs provide distinct scent signals. Differential activation of accessory olfactory bulb neurons when mice sniff closely at the facial region of conspecifics according to strain and sex [81] further suggests that these proteins play a role in identity signalling.

There thus appear to be at least three separate multigene families (MHC, MUP, ESP) that contribute involatile proteins or peptides to mouse scent signals and for which mice have specific receptors in the VNO. Two of these (MUPs and ESPs) show individual- and sex-specific expression consistent with a role in sexual signalling, while all three may have the capacity to provide information on individual genetic identity that could be used in mate selection. In the following sections, I will review evidence for the roles of MHC and MUPs in influencing male attractiveness to females through urine scents. The functional significance of ESP family proteins in sociosexual communication has not yet been addressed, but has the potential also to be involved in female assessment of males through facial scents.

4. Status assessment and recognition of individual males

Females can gain some information about the social status and current health of individual males from the quality of their scents. Female mice are particularly sensitive to the social status of individual males and, under semi-natural conditions, mate

only with dominant males that are able to successfully defend territories [55,108,144]. In part, male status is assessed through changes in androgen-dependent volatiles in urine that are attractive to females [21,64,65]. Subordinate males that are unable to defend their own territories in high-density populations reduce the production of farnesenes from their preputial glands [48] in order to be tolerated within the territory of a dominant male, such that their preputial glands regress and become smaller than those of dominant territory owners [22,35,49]. This makes subordinate male scents unattractive to females regardless of prior familiarity [67], and subordinates are forced to advertise their low status through an altered pattern of scent marking in order to be tolerated within the territory [38,57,59]. A wide range of infections also reduces the attractiveness of scents from infected males [40,105,150] or even stimulates aversion [69]. Although the chemical changes in scents associated with infection are not yet known, mice appear able to detect even subclinical infections or the activation of an immune response [149,150].

Qualitative changes in urine associated with social and health status appear to be detectable through airborne odours alone, and should be apparent directly on encountering a male, regardless of any prior familiarity. However, females can gain more information about longer term status from repeated encounters with males or their scent marks. There are several advantages to assessing male competitive status through scent marks. Scent marks provide physical proof of the ability to defend a territory, as only a successful male can ensure that its marks predominate throughout the defended area, and can be identified by the match between scents on the local substrate and the male himself [45,56]. These broadcast signals are available for challenge by any other male in the vicinity and provide a long-term record of success in competitive challenges. The presence of fresh scents from competitor males indicates failure to prevent such challenges, allowing a female to choose between different males that all attempt to signal dominant social status [63]. This leads to a 'scent war' between males, which not only attempt to exclude any competing males from their scent marked territory but will also rapidly countermark any scent mark challenges from competitors [61]. Female house mice discriminate countermarking based on the relative ages of the scent marks, and thus which was most recently deposited [32,116,117]. Other species such as Syrian hamsters discriminate on the basis of direct overmarking, favouring the individual whose scent has been deposited on top of the other [66].

In order to use any information gained about a male through his scent marks or from previous encounters, females have to be able to recognise individual males and their scents reliably. The distinct polymorphic patterns of involatile MUPs in urine provide a persistent and fixed individual genetic identity signature in mouse urine scent marks [60,61]. Females use this MUP signature to recognise individual scent owners [32] regardless of many other genetic and non-genetic differences that influence individual scents [15,26]. After encountering the scents of two males where one male's fresh scent apparently countermarks the other male's scent, female mice are more attracted to the owner of the countermark [32,117]. They are able to correctly identify the owner of the countermarks as long as (1) females are able to make nasal contact with the males' scent marks during scent mark investigation, and (2) the males express two different MUP patterns [32]. Although many other genetic and non-genetic factors contribute to an individual's scent profile and are important for the recognition of familiar scents previously encountered, the response on encountering unfamiliar or altered scent is to gain further information through contact and sniffing closely at the scent. If, on close investigation, two males share the same MUP pattern, females fail to discriminate between the individual identities of the two males, regardless of other differences in their scents [32]. MUP signatures are not only a genetically fixed characteristic but involatile MUPs are also highly resistant to degradation in scent marks over many weeks, providing a persistent individual identity signature that will be unaffected by environment, metabolic fluctuation or scent age. In normal wild mouse populations, MUP patterns are sufficiently polymorphic that competing adult males are unlikely to share the same pattern—this is only likely to occur between some very close relatives (see Section 6) but male dispersal from natal territories ensures that few are likely to remain in the same area [107].

MHC also exhibits a high degree of polymorphism between individuals that influences mouse urinary scents [15,20,29,98,142]. Differences in MHC-associated scents contribute to recognition of familiar and unfamiliar scents such that detection of a difference in MHC-based scents on a constant genetic background stimulates further close contact investigation of the scent source [29,104,130]. This discrimination has led to widespread assumptions that MHC-associated scents must make a major contribution to individual recognition [5,15,18,20,26,131]. However, although MHC-associated odours contribute to stimulating further investigation of unfamiliar urinary scents, females do not use MHC differences to recognise the urine scent marks of individual males [32]. Similarly, MHC type is not sufficient for males to recognise the individual owners of urine scent marks either [62]. The reliance on MUP signatures to recognise individual scent owners rather than MHC or other polymorphic genes that differ between individuals may be because a MUP signature is easily discriminated from other non-genetic factors that influence scents and will not degrade as scent marks age. By contrast, MHC type has widespread effects on volatile metabolites and testosterone-mediated pheromones in urine [98,142] that are likely to vary with current status. Differential loss of volatile scent components through time also means that volatile profiles change as scent marks age [30]. MHC peptide ligands may provide a more distinctive reflection of MHC identity in scents than volatile metabolites, but their susceptibility to endoproteolytic and exoproteolytic attack is likely to reduce their utility as persistent individual identity cues in scents [129].

5. Recognition of familiar mates

When newly mated female mice are exposed to the urine scents of unfamiliar males of a different laboratory strain from their familiar mate, pregnancy is blocked [27], an effect widely known as the Bruce effect. The effect is mediated by activation of a specific vomeronasal neuroendocrine pathway that inhibits prolactin release [8,111], causing luteolysis and implantation failure. The timing of exposure to unfamiliar male scents is critical as pregnancy block occurs in mice only if females encounter the scents of unfamiliar males during the first 4–5 days after mating (prior to implantation) [100] and scent exposure is coincident with at least two daily surges in prolactin that occur approximately 1 h before the change to light and dark periods [122]. However, the scent of a familiar mate does not have this effect. When females mate, they learn the chemosensory identity of the stud male during a 4–6 h sensitive period immediately after mating [74]. This scent memory prevents the familiar male's scent, or scent from males genetically identical to the mate, from blocking pregnancy through selectively enhanced inhibition in the accessory olfactory bulb [13]. The fitness advantages of this response to females under natural conditions remain unclear and have raised extensive debate (e.g. [7,36,39,78,123,143]), but substantial research under controlled laboratory conditions has provided detail of the signals and mechanisms involved (reviewed by [17–19]).

The urinary scent components used to recognise a mate or an unfamiliar male's scent in the context of pregnancy block are not the same as those underlying individual recognition of scent own-

ers in other contexts (see previous section). Individual (or strain) variation in MUP pattern between males is not effective in blocking pregnancy. When male urine is fractionated into components that are smaller or larger than 12 kDa, the lower molecular weight fraction is more effective in blocking pregnancy than the high molecular weight fraction containing MUPs [102]. However, effectiveness in blocking pregnancy is increased if the lower molecular weight fraction from unfamiliar males is combined with the high molecular weight fraction from either unfamiliar males or the familiar mate. This is probably because MUPs in the high molecular weight fraction bind and concentrate the small volatile molecules that are involved in inducing pregnancy block [102]. This is also consistent with the fact that only fresh urine is effective in blocking pregnancy.

Instead, pregnancy block is highly sensitive to differences in MHC type: congenic mice that differ from the familiar mate only at MHC block pregnancy while those of the same MHC type do not [147]. This is due to the detection of MHC peptide ligands that do not match those of the familiar mate's MHC type. A synthetic "non-matching" peptide added to the familiar mate's urine will block pregnancy while a peptide that matches the familiar male's own MHC type is ineffective [79,138]. The extent to which this response also depends on androgen-dependent cues in male mouse urine is currently unclear. Castration usually eliminates the pregnancy blocking effectiveness of urine from unfamiliar strain males [28,135] while cues from sexually immature males [28] and from unfamiliar females of a different strain [27] are also ineffective. However, urine from congenic females of different MHC type can induce pregnancy block [147] while Thompson and colleagues report that castrated and juvenile male urine induce pregnancy block with or without the addition of MHC peptides [138].

Few studies of the pregnancy block response have utilised wild mice, perhaps because wild females will block pregnancy in response to mild non-social stressors such as handling [34]. Notably, though, the *t*-complex genotype influences the effectiveness of unfamiliar wild males in inducing pregnancy block [36]. The *t*-complex is a large segment of mouse chromosome 17 that causes either embryonic mortality in homozygotes or semilethality and male sterility. However, heterozygotes for a number of recessive *t*-haplotypes are maintained in mouse populations because of segregation distortion in males, such that heterozygote males pass *t*-alleles to 75–97% of their offspring (see [9,36,140] for further explanation). These recessive *t*-haplotypes also suppress genetic recombination over a large region of mouse chromosome 17 including MHC, and *t*-haplotypes are closely linked to MHC such that most *t* chromosomes share the same MHC alleles [42]. This is in strong contrast to individual variation in MHC alleles among non-*t* wild mice, and might perhaps explain why non-*t* wild males (with high variation in MHC types that are more likely to be unfamiliar) are more effective at inducing pregnancy block that those carrying *t*-haplotypes (which share a common MHC haplotype) [36]. Indeed, as *t*-haplotypes either cause death in homozygous embryos or sterility in males that carry two *t*-haplotypes [9], it is interesting to speculate whether the potentially deleterious effects of the *t*-complex might in part have driven the sensitivity of this response to MHC variation. However, further research under more naturalistic conditions is needed to understand the significance of the pregnancy blocking response for female post-copulatory mate choice.

6. Kin recognition and the avoidance of inbreeding

Animals that are likely to meet close kin of the opposite sex once adult need to recognise these animals to avoid inbreeding [110] as this can have substantial deleterious effects [24,136]. A reliable mechanism for recognising close kin in adulthood would be to assess phenotypic similarity to self at highly polymorphic genetic markers that are only likely to be shared by close relatives. This would allow recognition whether or not individuals were previously familiar from a shared period within the same family group during development. To broaden the number of kin recognised, animals might also compare phenotypic similarity at these genetic markers with other familiar animals known to be close relatives (such as the animal's familiar mother) through a process of familial imprinting [103,148]. Scent cues have been widely implicated as the most likely means by which animals might assess relatedness through self-referent matching or familial imprinting because many genetic differences can be discriminated through scent [15].

Attention has focused almost exclusively on the MHC, one of the most polymorphic regions of the vertebrate genome, as a likely marker of genetic relatedness that may facilitate inbreeding avoidance across vertebrates [14,23,68,106,109,118,132,140,151]. Animals that share the same genotype at such a highly polymorphic marker are likely to be very closely related, thus avoidance of those with the same MHC-based scents should reduce the probability of inbreeding [23,109]. Offspring might gain additional fitness benefits since MHC disassortative mating will promote heterozygosity at the MHC itself, conferring increased resistance to pathogens [106]. Despite some initial controversy, the idea that MHC may play a central role as a marker for inbreeding avoidance in a wide range of animals has gained widespread acceptance, although direct evidence for MHC-determined disassortative mating is surprisingly limited. The inherent correlation between MHC and genome-wide similarity (relatedness) in natural populations means that experimental studies need to control for other potential genetic markers that may be used instead. Most direct evidence derives from studies of inbred laboratory mice or hybrid mice, following from a fortuitous observation that males of some MHC congenic strains prefer to mate disassortatively with females of different MHC type [146]. Subsequent studies revealed that preferences are more variable than this: depending on the strain and sex tested, laboratory mice may show disassortative, associative or no apparent preference based on MHC [68]. However, laboratory mice are not an appropriate model for studying inbreeding avoidance for several reasons. They have been selected over many generations for willingness to breed not only with close relatives but with those genetically identical to themselves except for their sex chromosomes [82]. Studies also need to address the levels of genetic similarity found between related and unrelated animals in natural populations, whereas laboratory strains derive from an extremely small gene pool, even when apparently from separate lineages [6,37,41,44]. Mate choice under natural conditions is also determined largely by female behaviour, which is highly constrained in small laboratory cages [82]. Potts and colleagues [108,109] addressed some of these issues by crossing inbred laboratory strains with wild mice to derive mice that had laboratory-derived MHC types but on a genetic background that was 50% from laboratory mice and 50% from wild mice to promote more natural behaviour. When allowed to breed freely in semi-natural enclosures, there was a deficit in MHC homozygous offspring compared to random mating expectations, consistent with MHC disassortative mating. However, other genetic differences that might correlate with MHC could not be assessed and it is not clear whether the deficiency in MHC homozygotes resulted from use of MHC or other correlated genes as markers. Notably, the strains used to create hybrids between laboratory and wild mice carried two different MUP genotypes.

To establish whether wild mice with natural levels of genetic variation at MHC, MUP and across the genome use either or both of these highly polymorphic genetic markers in scent signals to avoid inbreeding, we examined successful matings between each dyad within populations of wild-derived mice in very large semi-natural enclosures. By using known full sib and paternal half sib animals we were able to control for relatedness across the genome while examining whether there was any bias in mating with those shar-

ing neither, one or both haplotypes at MHC or MUP. This revealed that wild house mice use self-referent matching of MUP patterns to avoid inbreeding, but there was no evidence that MHC sharing influences mate selection [129]. Inbreeding avoidance was fully explained by a strong deficit in successful matings between those sharing both MUP haplotypes, which is a good indicator that mice are very closely related. Partial (one haplotype) sharing was not used, but modelling confirmed that a single haplotype shared does not provide a good guide for recognising very close relatives as many non-relatives will also carry the same marker [129]. We found no evidence to support the hypothesis that mice might increase the range of relatives recognised through behavioural imprinting on the separate haplotypes carried by their mother [103]. Although more relatives might be avoided this way, this strategy would also mean avoiding many animals that are not closely related, where inbreeding is not a risk, greatly reducing the choice of mates in the local population. Further, there does not seem to be any evidence that mice are able to recognise partial rather than full sharing of MHC or MUP genotypes in support of this more inclusive mechanism. Instead, it is likely that mice use direct assessment of relatedness through MUP sharing combined with male-biased dispersal from natal areas that contain a high proportion of related females to avoid the risk of inbreeding. This will promote genome-wide heterozygosity including MHC. As sharing of MUP type is clearly detectable through urinary scents, precopulatory mate selection is the most likely mechanism underlying this inbreeding avoidance, but this will require confirmation of response to matching and non-matching urinary scents alone.

7. Assessment of genetic heterozygosity

In addition to the avoidance of inbreeding using genetic markers to promote genetic heterozygosity in offspring, females may also have a direct preference for more heterozygous males as mates [23,51,73]. This preference may be because males that are more heterozygous are more vigorous and successful in intrasexual competition [52,87,125,139], and females may simply choose such males based on signals of male status and competitive success (see Section 4). However, females might also use polymorphic genetic markers to assess male genetic heterozygosity directly even in the absence of direct male competition [24,115]. Female sticklebacks, for example, prefer males with an appropriate number of MHC alleles that, when matched with their own, provide offspring with an optimum level of MHC diversity to maximise their resistance against parasites [1,91,114]. A male's MHC diversity is assessed by self-referent matching of MHC peptide ligands detected in the water in which a male swims, with females being more attracted to males that provide an optimal number of alleles when matched with the female's own MHC diversity [90].

Polymorphic genetic markers could also be used to assess a male's overall level of genetic heterozygosity (or conversely inbreeding) as animals that are inbred are much more likely to be homozygous at a polymorphic locus than outbred animals are. Female house mice show a consistent preference for associating with MUP heterozygous over MUP homozygous males when heterozygosity across the rest of the genome is controlled, but no bias in favour of MHC heterozygous males [137]. This preference is apparent in the time spent co-inhabiting the nest site of a MUP heterozygous male, and is unlikely to be due to any greater quality or vigour of the heterozygous male as the normal correlation between MUP and genome-wide heterozygosity was controlled. Thus, females appear to be able to assess MUP heterozygosity directly from scent cues alone, most likely through the greater number of MUPs expressed by the heterozygous male [137].

8. A new model of recognition and assessment integrating airborne and involatile scents

For many animals, simply recognising a conspecific of the opposite sex is not sufficient to stimulate sexual attraction and a willingness to mate. Typically, animals are selective in their response to different individuals of the opposite sex, needing to assess their suitability as potential mates in terms of both quality and genetic compatibility with the individual assessor's own genome [2,86,140]. As outlined above, the involatile MUP signature in mouse urine provides a stable genetic identity signal that appears to be essential for the recognition and assessment of individual scent owners, for the recognition of kin to avoid inbreeding, and to assess genetic heterozygosity in mice. MHC peptides may also be important for recognising or assessing familiar mates, or for assessing MHC compatibility in some species, while ESPs in tear fluids may play additional roles, as yet unexplored, in sexual and individual identity assessment. The central role that at least some of these protein and peptide components play in the assessment of potential mates, and thus in sexual attractiveness to females, is entirely consistent with the requirement for initial scent contact before female mice show any sexual attraction towards an individual male's scent (i.e., the male's scent becomes more attractive than an equivalent female's scent) [112]. Polymorphic proteins in scents allow unambiguous assessment of direct gene products via specific vomeronasal receptors, providing a fixed identity signal that may also persist over prolonged periods in the environment. However, the involatility of proteins creates considerable limitations for scent communication. Airborne volatiles are essential for the detection of scents at a distance, alerting animals to both the presence and location of a scent source. In order to detect involatile components through the vomeronasal system, animals must then approach and make nasal contact with the scent source, a costly requirement of both time and energy to gain further information. Even after contact, assessment of scents through the vomeronasal system is much slower than the processing of airborne volatile odorants through the main olfactory system. There is a latency of 3–4 s between initial contact and any increased neural activity in the accessory olfactory bulb while water-soluble stimuli are delivered to the vomeronasal organ [81] and vomeronasal receptors are activated [53]. Peak responses to stimuli can take as long as 18 s [81]. Such an inefficient processing system is likely to be extremely costly with respect to time taken to gain information, particularly as animals encounter multiple sources of scent information in many social situations and recognition needs to be rapid. A requirement for contact to recognise and assess competitors may also be dangerous and risk injury. However, by integrating information detected through the vomeronasal system during initial contact investigation of scent with that received simultaneously through the main olfactory system [46], animals can subsequently recognise familiar scents from which they have already gained involatile information by using airborne volatile scents alone [61]. This considerably reduces the need to physically contact the source of familiar airborne scents following recent full contact investigation. Thus, although female mice are not attracted to airborne volatiles from a male if they have not had the opportunity to assess his scent during contact through the vomeronasal system, scent contact results in a learnt association between involatile and airborne scents such that females subsequently find the airborne volatiles alone attractive (see Fig. 1). Nonetheless, the key information that induces attraction to a male is held in the involatile components detected through the vomeronasal system [85,92,93,112], most likely because this provides essential information about the genetic identity of the individual male.

Recognition and assessment of conspecifics through scents thus involves an important interaction between the main and accessory

olfactory systems that controls sexual attraction. This interaction is probably responsible for apparently conflicting findings when studies have debilitated one or other of these two systems, depending on the prior scent experience and learnt associations of the animals used, as well as the measure of response (e.g., scent investigation, sexual attraction or sexual behaviour). The complex airborne volatile profile emitted by animals or their scent marks appears to have key functional significance for the recognition of familiar scents that have previously been fully investigated and are still remembered. Detection of unfamiliar or altered airborne scents through the main olfactory system stimulates approach of the scent source and delivery of scents to the vomeronasal system. Indeed, a very wide range of factors can contribute to detectable differences in airborne volatiles that stimulate such approach and prolonged close contact investigation (evidenced by prolonged investigation or 'dishabituation' in habituation–dishabituation tests). This includes many genetic differences, current physiological status (such as social status, reproductive status and health status), food sources and bacterial flora [15,26,70,131]. Thus, by contrast to the fixed involatile scent signature provided by proteins such as MUPs, an individual's volatile scent profile will vary according to current status and environment but can be used for recognition over the short-term. Animals then gain the substantial advantage of a much faster system that allows rapid recognition of scents from familiar individuals without contact [61]. As animals typically deposit many scent marks around their home area and refresh these regularly, there are many opportunities to refresh and update such scent associations even when individual scent owners are elsewhere.

A challenge for the future will be to understand the neural pathways that underlie the learnt association between scents detected through the main and accessory olfactory systems, together with the neural mechanisms involved in the assessment, collation and memory of information about different individual conspecifics. The scent signals used to recognise and assess potential mates appear to provide an ideal opportunity to significantly progress understanding of the neural control of such complex social behaviour.

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