Scent wars: the chemobiology of competitive signalling in mice

Jane L. Hurst* and Robert J. Beynon

Summary

Many mammals use scent marks to advertise territory ownership, but only recently have we started to understand the complexity of these scent signals and the types of information that they convey. Whilst attention has generally focused on volatile odorants as the main information molecules in scents, studies of the house mouse have now defined a role for a family of proteins termed major urinary proteins (MUPs) which are, of course, involatile. MUPs bind male signalling volatiles and control their release from scent marks. These proteins are also highly polymorphic and the pattern of polymorphic variants provides a stable ownership signal that communicates genome-derived information on the individual identity of the scent owner. Here we review the interaction between the chemical basis of mouse scents and the dynamics of their competitive scent marking behaviour, demonstrating how it is possible to provide reliable signals of the competitive ability and identity of individual males. ***BioEssays*** **26:1288–1298, 2004.**

Introduction

Unlike visual or acoustic signals used in communication between animals, chemical signals can be deposited in the environment as scent marks that persist in the absence of the signaller, often over extended periods. Scent marks can thus be used to provide information to conspecifics even when the scent owner is elsewhere and are widely used among terrestrial vertebrates in the context of territory marking and defence.[1,2] Although it is popularly assumed that the main function of territory scent marks is to keep competitors out of a scent marked area, it turns out that scent marks are not very effective in preventing invasion.[2] Indeed, why should scent marks prevent other animals from gaining access to attractive resources if the owner is not around to defend them? We will argue here that the main role of territorial scent marks is to allow owners to advertise their high competitive ability, by providing a signal that reliably reflects their success in territory defence.

There are three facets to the information content in scent marks. First, they provide information through the chemical components of the scent. Secondly, the spatial and temporal pattern of scent deposition provides further complexity. Finally, since scent signals are often deposited in response to competition with one animal counter-marking the scents of another, there is also information in the pattern of scents deposited by different individuals. Competitive scent marking is thus a battle fought between individuals, a form of ''scent wars'' played out at both molecular and behavioural levels. In this review, we will discuss the theoretical biology underlying competitive scent marking, supported by empirical evidence from the animal in which the process is best understood: the common house mouse, *Mus domesticus*.

Scent marks and territory ownership: the perspective from behavioural ecology

Territory owners need to defend resources from competitors and also attract potential mates. Success in defending a territory is, in itself, proof of the owner's high competitive ability. Advertising this success therefore has competitive and reproductive advantages. Because competitive conflict is costly in terms of energy, time and risk of serious injury, animals recognised to be of high competitive ability are less likely to be challenged.[3,4] Owners that advertise successful territory defence therefore will suppress the number of challenges from other competitors. Males that advertise resource ownership and competitive success also gain a reproductive advantage if females prefer mates of high competitive ability, able to defend desirable resources.[5] However, competitors and potential mates only gain from responding to such advertisement if the owner really is of high competitive ability; there will therefore be strong selection to respond only to reliable signals.[6]

Scent marks are particularly well suited for providing this reliable signal of competitive ability. Indeed, most territorial mammals scent mark the area that they defend.[7] Because only animals that successfully dominate an area can ensure that their scent marks predominate, the spatial pattern and density of the owner's scent marks provide physical proof of

Faculty of Veterinary Science, University of Liverpool.
*Correspondence to: Jane L. Hurst, Faculty of Veterinary Science, University of Liverpool, Leahurst, Neston CH64 7TE.
E-mail: jane.hurst@liv.ac.uk
DOI 10.1002/bies.20147
Published online in Wiley InterScience (www.interscience.wiley.com).

territory ownership.[2,8] Intruders, or subordinates that live within a territory owned by a more dominant animal, cannot be mistaken for the owner if their scent marks do not predominate. Further, only those owners that defend their territory effectively can ensure that no other animals introduce competing signals. Even though a territory might be suffused with an owner's scent, the presence of any fresh signals from competitors would indicate ineffective territory defence.[8,9] By excluding competing males and countermarking any such scent challenges, a successful owner can ensure that its own marks are always the freshest signals within the defended area. Accordingly, territory owners counter-mark intruder scent marks immediately.[1,9] Competitor scents may be over-marked to provide physical evidence of the most recent scent mark,[10] or fresh scents may be placed next to the ageing scents of a competitor providing chemical proof of relative scent age.[11–13] Thus, the spatial and temporal pattern of scent marks deposited by a territory owner and by any other males in the locality indicates the success with which an owner dominates its scent-marked territory (Fig. 1). Scent marking and refreshment rates are particularly high at shared territory borders. This may reflect the need for both neighbours to ensure the relative freshness of their scent in the immediate vicinity of competitors' scent marks.[13]

Scent signals are not directed towards specific recipients but are broadcast to any other animals in the locality. Because scent marks are long lived and persist in the environment, the spatial / temporal pattern of scents from different individuals provides a continuous record of challenges for dominance and crucially, the outcome of those challenges.[9,14] This record is available to visitors and residents. As long as scent marks provide reliable information about the identity of scent owners

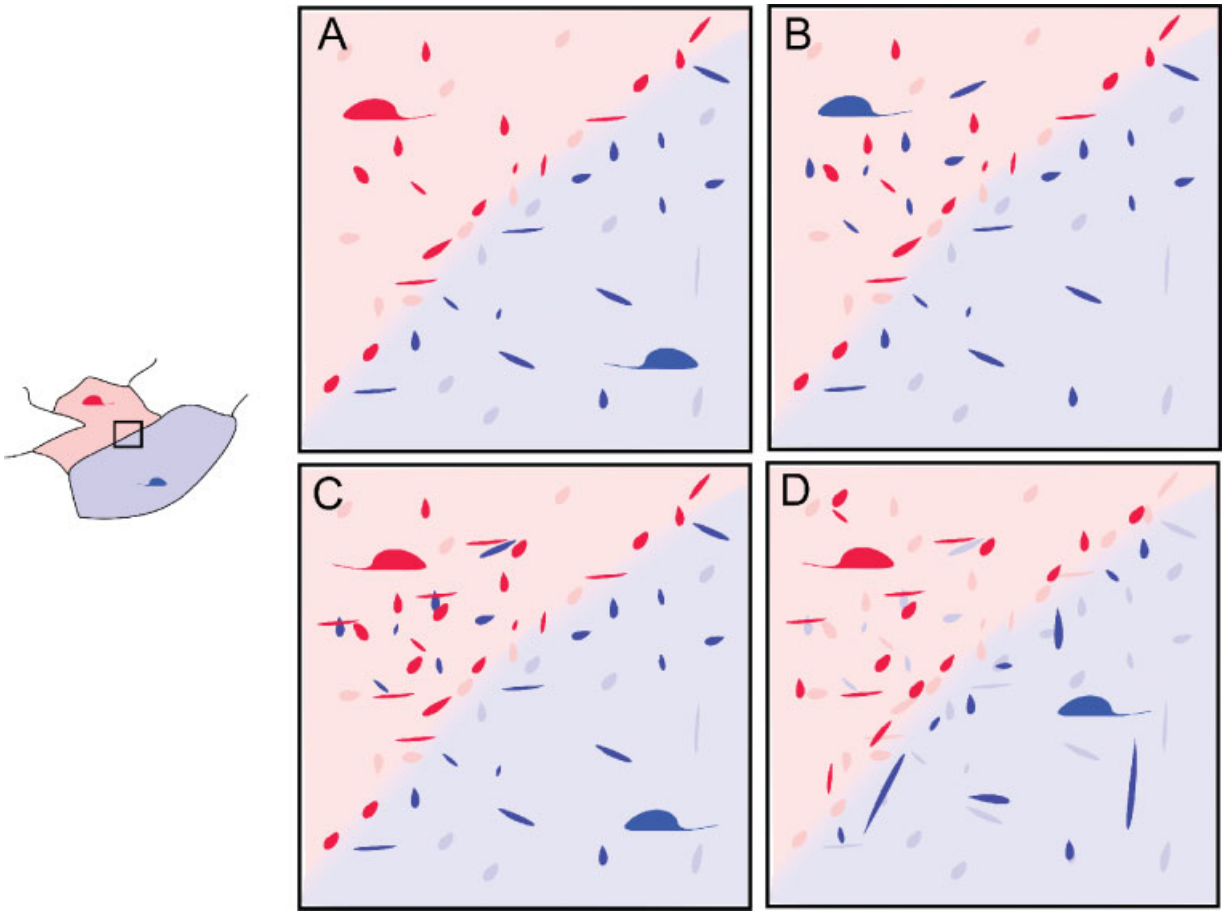

Figure 1. A: Competitive scent marking: Territories are delineated by owner-specific scent marks that are regularly replenished within the territory and particularly at territory boundaries. **B:** If a mouse intrudes into another territory, leaving scent marks, **C:** the territory owner rapidly deposits countermarks near to the marks of the intruder, and **D:** replenishes these marks at a higher rate than usual. In this fashion, the territory owner successfully advertises the ability to defend a territory and, by comparing the relative age of the intruder marks and owner countermarks, potential mates of territory challengers can assess the fitness of the owner. Key: scent marks are colour coded to reflect individual ownership; paler colours reflect scent marks that have aged.

and the relative ages of their signals, conspecifics do not need to witness challenges for dominance. Both challenge and outcome are recorded in scent marks—these thus provide the "Minutes" of meetings between competitors that are made public to all interested third parties, summarising the identities of participants and outcomes of their interactions.[15]

The main function of scent countermarking may therefore be a signal to third parties rather than a signal to the competitor whose scent is countermarked. This would provide a mechanism to assess the proven competitive ability of potential mates. For such complex information to be conveyed, scent marks must contain information about the species, sex and individual identity of a scent owner, social status (dominant territory owner or subordinate) and the freshness of the scent relative to any adjacent scent marks from competitors. From a theoretical viewpoint, on the one hand, territorial scent marks need to be relatively long lasting (i.e. of low volatility) to reduce the need for constant replenishment of signals over a wide area. On the other hand, scents of low volatility will be difficult to detect at a distance and may not provide reliable signals of scent "freshness" to receivers. How do animals satisfy these conflicting requirements? Studies of house mice suggest that this is achieved through a combination of the chemical qualities of the scent and the way that those scents are deposited in the environment.

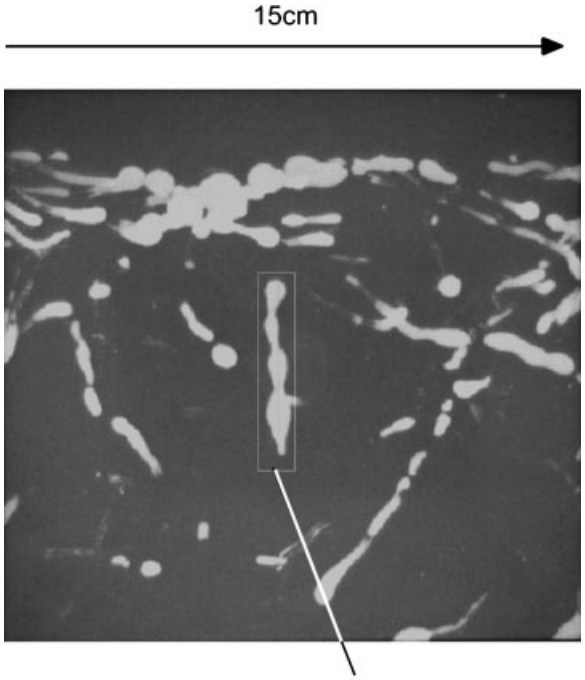

Figure 2. Scent countermarking: Mouse urine scent marks fluoresce under ultraviolet light.[16] When a territory owner encounters a streak of stimulus urine from another male, the owner's rate of scent marking in the vicinity increases over a period of several hours. Countermarks are not deposited deliberately over the top of the intruder's scent but consist of many small streaks and spots of urine deposited each time the owner is in the vicinity of the intruder's scent.

Chemical defence among mice

Male house mice are highly territorial and deposit urine scent marks throughout their defended area.[16–18] They have hairs on the end of the prepuce that aid the deliberate "painting" of tiny quantities of urine in thin streaks and small spots on the substrate.[19] Territory owners increase their rate of scent marking in the vicinity of scent marks from familiar or unfamiliar competitors over several hours, placing their own scents nearby but not deliberately over the top of scents of their competitors (Fig. 2).[12,18] They also attack males that deposit such competing scents.[8] Unfamiliar intruders use the scent marks deposited around a territory to identify a territory owner and are much less likely to challenge a male whose scent signature matches the local scent marks than a male whose scent does not match.[20] Adding a small drop of the territory owner's fresh urine onto one of his scent deposits increases the frequency with which intruders and resident subordinates spontaneously flee when they encounter the owner, without any attack or pursuit. In contrast, introducing urine from a neighbour territory owner reduces evasion and increases challenges against the resident owner, regardless of familiarity with the resident male's aggressive defence of his territory during direct interactions.[9]

Molecular signals of species, sex and social status

Although fulfilling an excretory role, mouse urine also contains "fixed" (genomic) information about the species, sex and individual identity of the owner, as well as "variable" (metabolic) information concerning the owner's current social, reproductive and health status, and its food resources.[21–24] Information concerning the species and sex of the scent owner is inherent in a number of male mouse-specific signalling volatiles in urine expressed under androgen control, including 2-*sec*-butyl-4,5-dihydrothiazole ('thiazole') and 2,3-dehydro-*exo*-brevicomin ('brevicomin') which are present in bladder urine post-puberty and which are considerably reduced by castration.[25] In addition, two sesquiterpenes, E,E-α-farnesene and E-β farnesene ('farnesenes') are produced by preputial glands and added to urine on elimination.[26] These male-specific volatiles are highly attractive to female mice[27,28] and have a number of pheromonal priming effects on female reproductive physiology[29,30] in addition to stimulating aggression between males.[31] While thiazole and brevicomin appear to be produced by all adult males, farnesenes provide additional information concerning male social status since subordinate males partially suppress production of farnesenes

and have smaller preputial glands than dominant male territory owners.[25,26]

Scent signals are only of use if they are detected by others, indicating the need for airborne (volatile) signals that will be detected by animals in the vicinity. However, as they become airborne, volatile scent components are lost from scent marks, reducing the longevity of signals deposited in the environment and increasing the rate at which signals need replenishment. While attention has focused largely on volatile pheromones in scent signals, it has increasingly become apparent that non-volatile proteins and peptides are also important and interact with volatile components in providing scent signals. Mouse urine is characterized by the presence of a high concentration of protein, over 99% of which comprises the Major Urinary Proteins (MUPs, see Fig. 3). These are a group of 18–20 kDa lipocalins, synthesized in the liver, secreted into the plasma and subsequently passed through the glomerular filter into the urine.[36] MUPs have a central cavity that binds lipophilic molecules and their only known functions are in chemical signalling. MUPs are produced by mice of both sexes but adult male urine typically contains 20–40 mg/ml of protein, approximately three to four times as much as female urine.[37] This sex difference occurs at puberty when there is an increase in excretion among males, with staged activation of some MUPs.[34] In males, MUPs bind a number of ligands but principally the two male-specific signalling volatiles thiazole and brevicomin such that almost all thiazole and brevicomin in fresh male urine is bound to these proteins.[38–40] Once urine is deposited as a scent mark, the binding of signalling volatiles to MUPs greatly slows down their evaporation from the scent mark,[41,42] considerably extending the duration over which volatiles are detected from a distance.[12,41] When not bound to MUPs, male signalling volatiles are lost from scent marks within a few minutes, while those bound and released from MUPs continue to be detected over at least 24 hours, drawing attention to scent marks that mice then approach to investigate closely.[12] Once within a few centimetres of a foreign male mark, male signalling volatiles stimulate caution in contacting the scent source (Fig. 4), probably because, in the dark, volatiles released from fresh scent marks are difficult to distinguish from those released by a dangerous male. Thus, the combination of male-specific volatiles bound to and released by non-volatile proteins provides readily detectable signals that are gradually released over many hours.

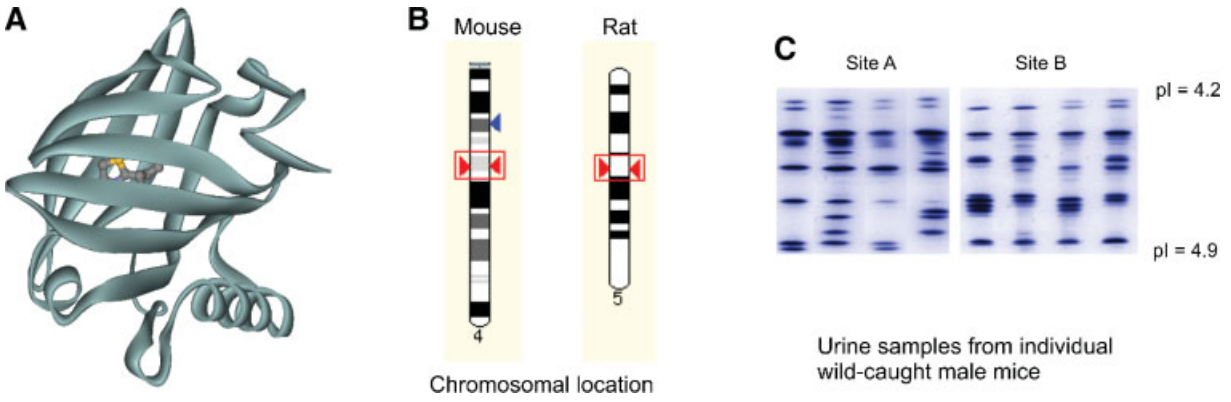

Figure 3. Major urinary proteins (MUPs): **A:** The urine of many species, including man, does not normally contain protein. However, some species, and several rodents in particular, release substantial quantities of protein in their urine. These proteins are synthesised in the liver, and pass through the kidney into the urine, where they can reach concentrations of 40mg/ml. The structure and function of the MUPs from mouse (shown) and rat (often termed α-2U protein) are relatively well understood, and the three-dimensional structures are known (search http://www.rcsb.org/p db/ with the text "major urinary protein"). They are eight-stranded beta barrel structures; the barrel being sheared to form a cross-hatched central cavity. In the mouse, the cavity is known to bind the pheromonally active ligands 2-*sec*-butyl-4,5-dihydrothiazole and 2,3-dehydro-*exo*-brevicomin, although most of the electron density is explained by the thiazole. The mechanism of binding of a range of thiazoles and indeed, other ligands is unusual, inasmuch as it seems to be driven by the enthalpy changes brought about by desolvation of the cavity.[32,33] **B:** In the mouse, most MUPs are encoded between approximately 59.7 and 60.7 MBp of chromosome 4; the equivalent region in the rat genome is between 78 and 79 MBp of chromosome 5 (http://www.ensembl.org). Other MUP-like sequences, located throughout the genomes by virtue of weaker homology matches might encode MUP-related genes that are expressed in other tissues. **C:** A mouse probably expresses at least 5 urinary MUP genes, and in wild populations, this leads to the expression of around 8–14 electrophoretically separable MUP isoforms in urine.[34,35] One of the challenges has been to explain why a simple role of ligand binding and release should require such a complex mixture of virtually identical protein structures. Most of the polymorphic variation between different MUPs is in residues that are on the surface of the structure.[35]

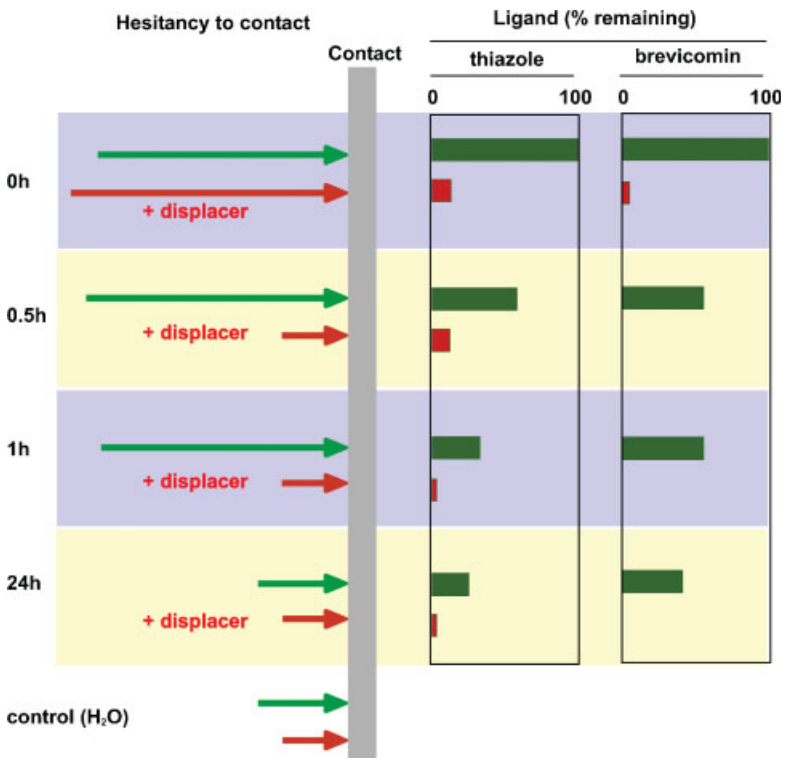

Figure 4. MUPs allow volatile scent marks to persist over time. A male mouse will locate and approach a novel male urine scent mark much more quickly than water,[12] but is hesitant in contacting a fresh scent mark once within 15 cm of the scent. As the scent mark ages, this hesitancy declines, until after 24 hours, the mouse is no more reluctant to approach than to a water control (green arrows). If the scent mark is treated with a competitive displacer (a molecule that occupies the MUP cavity and which displaces the natural ligands) the hesitancy to approach declines to control values within 30 minutes (red arrows). These time scales are consistent with the natural (green histograms) and displaced (red histogram) loss of the natural ligands 2-*sec*-butyl-4,5-dihydrothiazole and 2,3-dehydro-*exo*-brevicomin. Figure adapted from Hurst JL, Robertson DHL, Tolladay U, Beynon RJ. 1998. Anim Behav 55:1289–1297.

Molecular ownership signatures

Scent marks can only provide information on ownership if the scent includes information about identity. Scent ownership signatures need to be sufficiently polymorphic to provide a functionally unique signature to each individual in the local population. They also need to be stable and persistent once deposited, since it is essential that the ownership signal does not change as the scent mark ages. Further, ownership signals need to be a fixed characteristic that can be recognised independently of any metabolic or environmental fluctuations that alter an individual's scent profile.[43,44] Laboratory discrimination tests indicate that many non-genetic factors influence an animal's volatile scent profile, reflecting the owner's current social status, reproductive condition, health status and food sources.[7,23,45] Microbial flora can also have a strong influence on the volatile profile due to the breakdown of metabolites.[23] The complex scent profiles that result from the combination of both genetic and non-genetic influences are often referred to as "individuality odours". The problem that animals must solve, however, is discrimination between fixed information about the owner's identity and variable (metabolic) scent information that might be used to assess the owner's current status.

Although many genetic loci contribute to discriminable differences in the volatile scents of laboratory rodents, many of these are likely to influence scents through indirect affects that alter the metabolic profile. By contrast, the pattern of MUPs expressed in urine is a fixed, genetically determined characteristic that only influences scent signals. MUP profiles remain fixed throughout adult life regardless of status changes or alterations in food resources and, once deposited in scent marks, MUPs can persist without degradation over many weeks or months. Given the diversity of profiles expressed by wild-caught mice, even within geographically isolated populations where genetic heterogeneity is much reduced,[35] MUPs are ideal candidates for providing individual ownership signatures. To test this, we examined competitive countermarking among wild-derived mice according to the MUP profiles expressed in the scent marks of territory owner and intruder.

On encountering urine scent marks from another male in their territory, male mice initially investigate the scent closely and then countermark it by returning repeatedly to deposit fresh scent marks in the vicinity of the intruder's scent over the next few hours. It is the high molecular weight fraction of urine containing MUPs and their bound ligands that stimulates this response.[12] The response is the same whether urine comes from an unrelated or related male, both of which are regarded as competitors among adult male mice. However, urine from a brother that has inherited the same urinary MUP type as the territory owner (a situation likely to occur only between very

close relatives) stimulates initial close investigation but no subsequent functional response that indicates recognition of another male's scent. Territory owners fail to spend more time in the vicinity of intruder urine when this shares the same MUP type as own urine and do not countermark, despite many other genetic differences that outbred wild-derived mice will inherit.[46] This is not because mice fail to detect differences between their own urine and that of the same MUP type. Many genetic loci contribute to volatile urine scents and, on first encounter, mice spend much longer investigating another male's scent marks than their own regardless of MUP type, applying their noses closely to the scent source. However, the lack of a functional countermarking response following close investigation implies that males only recognise that urine marks belong to an intruder when these contain a different MUP ownership signal to their own. To prove that this response is to MUP type rather than other genetic differences linked to MUP genes, we manipulated the animal's own urine scent marks using a highly purified recombinant MUP which, although initially devoid of natural ligands, would be expected to bind urine-derived compounds. When this was added to a territory owner's own urine to change the MUP profile (controlling for total urinary protein concentration), the owner's scent marking increased in the vicinity of the manipulated scent, countermarking the urine as if it came from an intruder male.[46]

Distinguishing between own scent marks and those of other males is one aspect of scent ownership recognition, similar to the self–nonself recognition of the immune system. More general recognition of individual ownership signals requires the ability to distinguish individual scent signatures from different conspecifics. By introducing scent marks from one of two equally familiar neighbours into a male's territory, we have recently shown that territory owners are able to match the scent introduced to the correct neighbour and that this recognition of scent ownership also appears to be due to MUP type, despite many other genetic differences between wild-derived scent owners (unpublished data).

Molecular mechanism underlying MUP ownership signals

If MUP polymorphic variants bind ligands with different affinities, each MUP pattern might also define a characteristic pattern of low molecular weight ligands that provide the ownership signal in scent marks. There is good evidence that the affinity of different MUPs for natural or reporter ligands can vary, particularly when isoforms differ in amino acid substitutions in the central hydrophobic calyx.[47,48] Analysis of the primary sequence of MUPs indicates that much of the heterogeneity resides in an extended patch on the surface of the protein, but with variant residues protruding both into the central cavity and towards solvent.[36] However, comparison of the release kinetics between scent deposits from two mouse strains that express very different MUP patterns indicated no major differences in the rate of loss of the two male signalling volatiles thiazole and brevicomin.[42]

Both behavioural and neurophysiological evidence indicate that scent ownership is signalled either by non-volatile MUP–ligand complexes or by the MUPs themselves rather than by volatile ligands released from MUPs. Direct nasal contact with the scent source is essential to detect the scent ownership signal and stimulate males to countermark another male's scent. When contact is prevented by a porous sheet of nitrocellulose, mice detect volatiles emanating from scent marks and investigate closely but then fail to countermark the scent from another male.[49] While airborne volatiles are detected through the main olfactory system, non-volatile scent stimuli are detected via the vomeronasal system (VNS).[50] The vomeronasal organ (VNO) is a blind-ended, mucus-filled tube linked to the nasal cavity by a narrow duct;[51] stimulus access depends on a vascular pumping mechanism that appears to be activated when animals make nasal contact with a novel stimulus.[52,53] This system plays a key role in the recognition of sex and genetic identity from conspecific scents.[53–55] Removal of the VNO eliminates both male aggression towards an intruder male and the countermarking of intruder scents.[56,57] Further evidence for the role of the VNS in recognition of genetically determined identity scents comes from studies of the olfactory block to pregnancy induced by the scents of unfamiliar males.[58] Shortly after mating, females form an olfactory memory of the stud male's scent in the accessory olfactory bulb, which receives input from the VNO.[59] Before embryo implantation (within 5 days of mating), prolonged contact with urine from a male genetically distinct from the stud male often results in failure of implantation unless the female also maintains contact with scent from the stud male.[60,61] Identity recognition is due largely to low molecular weight components, but is enhanced when these are delivered in the context of urinary proteins.[62] However, the molecular mechanism underlying identity recognition in the context of pregnancy block may differ from that involved in scent mark ownership signaling. Pregnancy block requires exposure to fresh male scents, suggesting the importance of volatile or unstable components that are rapidly lost from ageing scents;[60,63] old scent marks will not influence female reproductive strategies. By contrast, the ownership signal in territorial scent marks needs to be long-lasting. Countermarking of intruder scent is just as strong towards urinary proteins when ligands have been lost through natural ageing or by chemical displacement.[12] This suggests that any MUP ligands involved in recognition are relatively involatile and resistant to displacement, or that animals are able to recognize the MUPs themselves or MUP–ligand complexes. The anterior region of the accessory olfactory bulb, which receives input from the VNO, responds preferentially to MUPs while the main MUP ligands thiazole and brevicomin elicit activity in

the posterior region.[64] VNO receptors for MUPs remain to be identified.

Association between involatile and volatile scent profiles

Animals generally contact scents to investigate closely when they have detected some unfamiliarity, presumably through airborne volatiles. Detection of airborne volatiles through the main olfactory system is much quicker than detection of involatile components through the VNS and does not require physical contact with the scent source. Further, although making contact with a scent mark to detect involatile components is relatively easy, such close contact with a competitor can be considerably more dangerous. In territorial populations, competitors usually sniff towards each other from a distance, and then flee or attack based on information gained only from volatile scents.[8] Volatile scents are thus much more readily detected and avoid the need to approach and contact every scent source. However, as discussed above, volatile scents are also influenced by a wide range of factors and are thus likely to provide much-less-stable identity signals. Close investigation of fresh scent marks provides an opportunity to learn an association between the involatile ownership signal detected through the VNS and volatile scents detected simultaneously through the main olfactory system.[44,65] Recognition of sex-specific volatiles through the main olfactory system requires a learnt association with involatile signals. Naïve female mice show an innate attraction to male scents that they can contact, but are only attracted to volatile male odours alone once they have experienced repeated contact with male scents.[66] Similarly, male mice that have encountered females artificially odorized with perfume subsequently emit ultrasonic courtship vocalizations to the perfume itself, apparently associating the perfume with recognition of a female mouse.[67] Indeed, the detrimental effects of removal or deafferentation of the VNO in laboratory experiments can sometimes be partially overcome by prior social experience,[57] when animals have the opportunity to learn an association with odours detected through the main olfactory system. This ability to associate volatile with involatile signals may also allow animals to recognize familiar individuals from their airborne scents. If a familiar volatile signal changes (e.g. due to changes in status or food source), close contact investigation should update the link between a stable involatile ownership signal and volatile scents (Fig. 5). Fresh scent marks deposited around the territory provide ample opportunity to update this association in advance of encountering the owner.

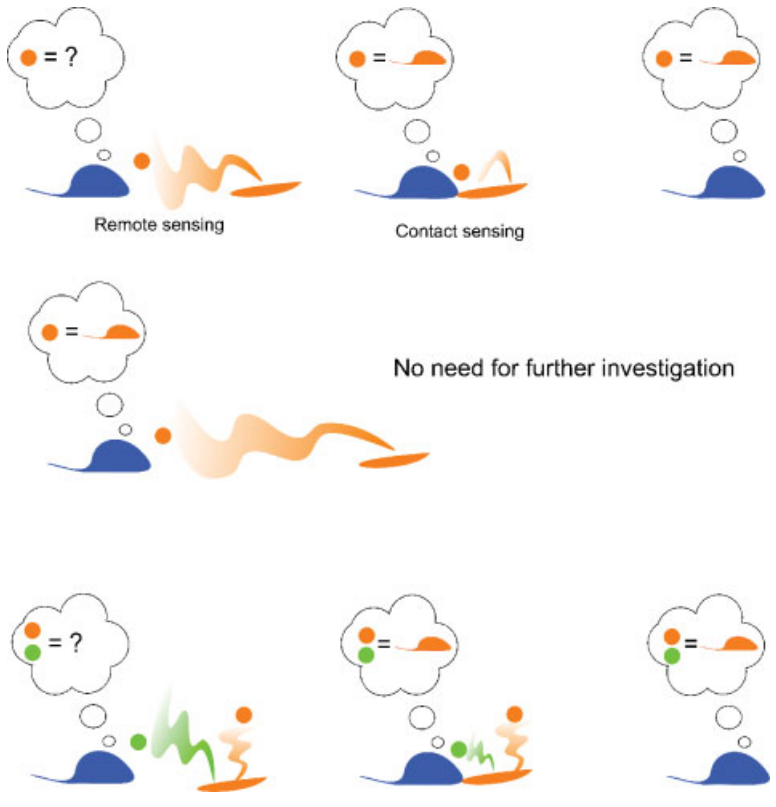

Figure 5. An associative matching of volatile, variable signals to involatile genome-derived signals. When a mouse encounters the volatile signature of a second, novel mouse, it moves up the concentration gradient to the source, and makes contact with the scent mark, at which point constituents of the scent mark may be pumped into the VNS. This allows the receiver animal to build an association between the volatile, variable and involatile (genomically hard-wired) information. Subsequently, if the same pattern of volatiles is encountered, the receiver is aware of the identity of the scent owner. However, if the scent donor undergoes a metabolic shift (e.g. because of a change in diet, social status or infection status) then the volatile odour is perceived as novel, and the drive to associate the odour with the individual signature is activated. In this way, a mouse can retain an image of individuals even in the context of a shifting metabolic profile.

Interaction between MUPs and MHC—an integrative hypothesis

The volatile scent profile is influenced by a second highly polymorphic gene complex, the major histocompatibility complex (MHC) in mice,[68,69] rats[70] and humans.[71,72] Unlike MUPs, which are involved only in scent communication, the primary function of MHC is in self–nonself immune recognition at the cellular level. The molecular mechanism underlying the MHC influence on urinary scents involves a complex mixture of volatile metabolites bound and released by urinary proteins or peptides.[73–75] The urinary proteins might be fragments of MHC molecules themselves, or MUPs.[73,76] The "carrier hypothesis"[75] proposes that soluble fragments of MHC class I and class II molecules in urine differentially bind volatile metabolites in the antigen-binding groove once the peptide that is normally bound tightly in this groove is lost. Further proteolysis of the fragments would then lead to release of the volatiles. The MHC specificity of odours might thus be determined by the highly polymorphic binding characteristics of the antigen-binding groove. How low molecular weight volatiles could be specifically bound to MHC protein fragments that normally bind peptides is unclear.[74]

By contrast, the central calyx of MUPs has evolved to bind small odorant molecules and MUPs are present in considerably greater concentration than MHC fragments in rodent urine.[36] Since many physiological traits are genetically associated with the MHC,[77,78] it seems likely that MHC-type will influence the metabolites present in urine. A plausible alternative hypothesis is that MHC-based developmental and physiological variations modulate volatile metabolites[22] which are then bound and released by MUPs in mouse urine.[76] If this is the case, these two highly polymorphic systems would interact to determine the volatile scents in a scent mark. However, if MHC can influence the owner's metabolic profile, this is likely to be influenced by other genetic and non-genetic factors too, diminishing the stability of the signal of identity or ownership. This may explain why it is difficult for mice or rats to recognize the MHC type of scent owners when metabolism is modified by factors such as food type,[79] infection[80] or genetic background.[81] Animals can discriminate MHC-associated odours when the genetic and environmental background remains reasonably constant. However, we have recently found that MHC-associated odours do not induce a countermarking response, and are neither required nor sufficient for scent ownership recognition.

Discriminating scent marks and countermarks

Although female house mice generally nest and raise their offspring within one male's territory, they often visit or range over several neighbouring male territories[82,83] and extra-territorial matings occur frequently (e.g. 43% of all observed matings in large captive populations occurred when a female travelled to, and mated with, a male owning a nearby territory[84]). Females may thus have a choice between several territory owners as potential mates. Females prefer the owner of a territory that is scent marked exclusively (i.e. containing no scents from competitor males) over an owner whose territory contains countermarks from an intruder male; this preference is maintained even if the territory containing intruder countermarks is itself made much more attractive to females.[85] If both territories contain intruder scent marks, females prefer an owner that has countermarked intruder scent marks over an owner whose scent marks had been countermarked by the intruder.[13] To determine which male's scent was deposited most recently (i.e. as a countermark), female mice assess the age difference between nearby scent marks from competing males, failing to discriminate when both scent marks and countermarks are of similar age.[13] House mice deposit countermarks nearby rather than on top of a competitor's scent marks.[12] They do not attempt to deposit a larger scent mark than that of the competitor, which would contain a greater intensity of volatile signalling molecules. Indeed, under competitive pressure, mice tend to reduce rather than increase the size of individual scent marks[16] and, instead, deposit a large number of small scent marks, returning repeatedly to the same area to deposit more marks over several hours. By gradually depositing urine in small spots and streaks over time rather than one large scent mark, males increase the rate of replenishment, which maximizes the freshness of their scent marks.[14] Thus, each time males deposit a new countermark, they increase the age difference between their own scent and that of the competitor, while volatiles in their own fresh scent marks are likely to attract the attention of others to the aged competitor scents. Notably, males countermark both fresh and aged scents from competitors but deposit most marks near to the aged competitor scent where the contrast will be greatest.[12] While most male signalling volatiles are lost from scents within a few hours,[12,41,42] non-volatile components of scent marks continue to be detected for at least 7 days if males are aware of the presence of scent marks in the area.[12,86]

A receiver animal must be able to pick up the molecular signature that indicates the age of a scent mark. This precludes the use of a single volatile chemical as a molecular timer to establish the age of a scent mark because the receiver would not be able to detect the difference between a small, recent scent mark and a larger deposit that was placed in the environment some time ago. It is most probable that minimally, the ratio of two molecules, or more likely, a complex pattern of more than two molecules, is used as a molecular timer. The most-volatile component(s) decay rapidly whilst the less-volatile components remain in the scent mark and provide a reference against which the loss of the volatile component can be assessed. MUPs are of course, completely involatile, and thus offer a completely stable timebase against which loss of volatiles can be assessed.[14] It follows that this requires the

receiver to make contact with the scent mark, and that the VNO is able to detect shifting patterns of scent constituents. Whether this is due to separate receptors for MUPs and volatiles, or whether the VNO can respond differently to occupied versus unoccupied MUPs remains to be discovered.

Conclusions

Competitive scent signalling has evolved into a complex and dynamic system in which short-lived volatile signals and stable, involatile scent constituents are deployed at rates, and in patterns, that advertise status, define territories and provide a record of challenges for dominance. In the house mouse, proteins, which are not molecules traditionally considered to be present in scents, are key modulators that play multiple roles in this system. The binding and release of male signalling volatiles alerts others to the presence of scent marks from a distance and identifies the owner as a male house mouse. On contact, they communicate individual ownership, either directly or by transporting and presenting bound ligands to VNO receptors. Simultaneous detection of complex involatile and volatile scent profiles provides the opportunity for associative learning, subsequently allowing animals to identify the owners of familiar volatile scents from a distance. The gradual release of volatile ligands from these stable involatile proteins also provides a mechanism that will indicate scent mark age regardless of the amount of scent deposited, allowing others to discriminate which competitor countermarked the other. By providing a record of challenges for dominance and the outcome of those challenges, scent marks thus provide a reliable signal of competitive ability that is freely available to other competitors and to potential mates.

Acknowledgments

Ideas in this review arose from work supported by research grants from the Biotechnology and Biological Research Council to JLH and RJB.

References

1. Johnson RP. 1973. Scent marking in mammals. Anim Behav 21:521–535.
2. Gosling LM. 1982. A reassessment of the function of scent marking in territories. Z Tierpsychol 60:89–118.
3. Maynard Smith J, Price GR. 1973. The logic of animal conflict. Nature 246:15–18.
4. Maynard Smith J, Parker GA. 1976. The logic of asymmetric contests. Anim Behav 24:159–175.
5. Bateson P. 1983. Mate Choice. Cambridge: Cambridge University Press.
6. Johnstone RE. 1994. The evolution of animal signals. In: Krebs JR, Davies NB, editors. Behavioural Ecology An Evolutionary Approach. Oxford: Blackwell Science. p 155–178.
7. Brown RE, McDonald DW. 1985. Social Odours in Mammals, Vol 1 & 2. Oxford: Clarendon Press.
8. Hurst JL. 1993. The priming effects of urine substrate marks on interactions between male house mice, *Mus musculus domesticus* Schwarz and Schwarz. Anim Behav 45:55–81.
9. Hurst JL, Rich TJ. 1999. Scent marks as competitive signals of mate quality. In: Johnson RE, Muller-Schwarze D, Sorensen P, editors. Advances in Chemical Communication in Vertebrates. New York: Plenum Press. p 209–226.
10. Wilcox RM, Johnston RE. 1995. Scent counter-marks: specialized mechanisms of perception and response to individual odors in golden hamsters (*Mesocricetus auratus*). J Comp Psychol 109:349–356.
11. Johnston RE, Schmidt T. 1979. Responses of hamsters to scent marks of different ages. Behav Neural Biol 26:64–75.
12. Humphries RE, Robertson DHL, Beynon RJ, Hurst JL. 1999. Unravelling the chemical basis of competitive scent marking in house mice. Anim Behav 58:1177–1190.
13. Rich TJ, Hurst JL. 1999. The competing countermarks hypothesis: reliable assessment of competitive ability by potential mates. Anim Behav 58:1027–1037.
14. Hurst JL, Beynon RJ, Humphries RE, Malone N, Nevison CM, et al. 2001. Information in scent signals of competitive social status: the interface between behaviour and chemistry. In: Marchelewska-Koj A, Muller-Schwarze D, Lepri J, editors. Chemical Signals in Vertebrates 9. New York: Plenum Press. p 43–52.
15. Hurst JL. 2005. Scent marking and social communication. In: McGregor PK, editor. Communication Networks. Cambridge: Cambridge University Press. In press.
16. Desjardins C, Maruniak JA, Bronson FH. 1973. Social rank in the house mouse: differentiation revealed by ultraviolet visualisation of urinary marking patterns. Science 182:939–941.
17. Hurst JL. 1987. The functions of urine marking in a free-living population of house mice, *Mus domesticus* Rutty. Anim Behav 35: 1433–1442.
18. Hurst JL. 1990. Urine marking in populations of wild house mice *Mus domesticus* Rutty .1. Communication between males. Anim Behav 40: 209–222.
19. Maruniak JA, Desjardins C, Bronson FH. 1975. Adpatations for urinary marking in rodents: prepuce length and morphology. J Reprod Fertil 44: 567–570.
20. Gosling LM, McKay HV. 1990. Competitor assessment by scent matching—an experimental test. Behav Ecol Sociobiol 26:415–420.
21. Brown RE. 1985. The rodents II: suborder Myomorpha. In: Brown RE, Macdonald DW, editors. Social Odours in Mammals. Volume 1. Oxford: Clarendon Press. p 345–457.
22. Boyse EA, Beauchamp GK, Yamazaki K. 1987. The genetics of body scent. Trends Genet 3:97–102.
23. Brown RE. 1995. What is the role of the immune system in determining individually distinct body odours? Int J Immunopharmacol 17:655–661.
24. Malone N, Payne CE, Beynon RJ, Hurst JL. 2001. Social status, odour communication and mate choice in wild house mice. In: Marchlewska-Koj A, Muller-Schwarze D, Lepri J, editors. Chemical Signals in Vertebrates 9. New York: Plenum Press. p 217–224.
25. Harvey S, Jemiolo B, Novotny M. 1989. Pattern of volatile compounds in dominant and subordinate male-mouse urine. J Chem Ecol 15:2061–2072.
26. Novotny M, Harvey S, Jemiolo B. 1990. Chemistry of male dominance in the house mouse, *Mus domesticus*. Experientia 46:109–113.
27. Jemiolo D, Alberts J, Sochinski-Wiggins S, Harvey S, Novotny M. 1985. Behavioural and endocrine responses of female mice to synthetic analogs of volatile compounds in male urine. Anim Behav 33:1114–1118.
28. Jemiolo B, Xie TM, Novotny M. 1991. Socio-sexual olfactory preference in female mice: attractiveness of synthetic chemosignals. Physiol Behav 50:1119–1122.
29. Novotny MV, Ma W, Zidek L, Daev E. 1999. Recent biochemical insights into puberty acceleration, estrus induction and puberty delay in the house mouse. In: Johnston RE, Muller-Schwarze D, Sorensen P, editors. Advances in Chemical Communication in Vertebrates. New York: Plenum Press. p 99–116.
30. Leinders-Zufall T, Lane AP, Puche AC, Ma W, Novotny MV, et al. 2000. Ultrasensitive pheromone detection by mammalian vomeronasal neurons. Nature 405:792–796.
31. Novotny M, Harvey S, Jemiolo B, Alberts J. 1985. Synthetic pheromones that promote inter-male aggression in mice. P Natl Acad Sci USA 82: 2059–2061.

32. Sharrow SD, Novotny MV, Stone MJ. 2003. Thermodynamic analysis of binding between mouse major urinary protein-I and the pheromone 2-sec-butyl-4,5-dihydrothiazole. Biochemistry 42:6302–6309.
33. Bingham RJ, Findley JBC, Hsieh SY, Kalverda AP, Kjeliberg A, et al. 2004. Thermodynamics of binding of 2-methoxy-3-isopropylpyrazine and 2-methoxy-3-isobutylpyrazine to the major urinary protein. J Am Chem Soc 126:1675–1681.
34. Payne CE, Malone N, Humphries RE, Bradbrook C, Veggerby C, et al. 2001. Heterogeneity of major urinary proteins in house mice: population and sex differences. In: Marchelewska-Koj A, Muller-Schwarze D, Lepri J, editors. Chemical Signals in Vertebrates 9. New York: Plenum Press. p 233–240.
35. Beynon RJ, Veggerby C, Payne CE, Robertson DH, Gaskell SJ, et al. 2002. Polymorphism in major urinary proteins: molecular heterogeneity in a wild mouse population. J Chem Ecol 28:1429–1446.
36. Beynon RJ, Hurst JL, Gaskell SJ, Hubbard SJ, Humphries RE, et al. 2001. Mice, MUPs and myths: structure-function relationships of the major urinary proteins. In: Marchelewska-Koj A, Muller-Schwarze D, Lepri J, editors. Chemical Signals in Vertebrates 9. New York: Plenum Press. p 149–156.
37. Beynon RJ, Hurst JL. 2003. Multiple roles of major urinary proteins in the house mouse, *Mus domesticus*. Biochem Soc Trans 31:142–146.
38. Bacchini A, Gaetani E, Cavaggioni A. 1992. Pheromone binding proteins of the mouse, *Mus musculus*. Experientia 48:419–421.
39. Robertson DHL, Beynon RJ, Evershed RP. 1993. Extraction, characterization and binding analysis of two pheromonally active ligands associated with major urinary protein of house mouse (*Mus musculus*). J Chem Ecol 19:1405–1416.
40. Novotny MV, Ma W, Wiesler D, Zidek L. 1999. Positive identification of the puberty-accelerating pheromone of the house mouse: the volatile ligands associating with the major urinary protein. Proc R Soc Lond B Biol Sci 266:2017–2022.
41. Hurst JL, Robertson DHL, Tolladay U, Beynon RJ. 1998. Proteins in urine scent marks of male house mice extend the longevity of olfactory signals. Anim Behav 55:1289–1297.
42. Robertson DHL, Marie AD, Veggerby C, Hurst JL, Beynon RJ. 2001. Characteristics of ligand binding and release by major urinary proteins. In: Marchlewska-Koj A, Muller-Schwarze D, Lepri J, editors. Chemical Signals in Vertebrates 9. New York: Plenum Press. p 169–176.
43. Halpin ZT. 1986. Individual odors among mammals: origins and functions. Adv Stud Behav 16:39–70.
44. Hurst JL, Thom MD, Nevison CM, Humphries RE, Beynon RJ. 2005. The ''scents'' of ownership. In: Mason RT, Muller-Schwarze D, editors. Chemical Signals in Vertebrates 10 (in press).
45. Penn D, Potts WK. 1998. Chemical signals and parasite-mediated sexual selection. Trends Ecol Evol 13:391–396.
46. Hurst JL, Payne CE, Nevison CM, Marie AD, Humphries RE, et al. 2001. Individual recognition in mice mediated by major urinary proteins. Nature 414:631–634.
47. Marie AD, Veggerby C, Robertson DHL, Gaskell SJ, Hubbard SJ, et al. 2001. Effect of polymorphisms on ligand binding by mouse major urinary proteins. Protein Sci 10:411–417.
48. Sharrow SD, Vaughn JL, Zidek L, Novotny MV, Stone MJ. 2002. Pheromone binding by polymorphic mouse major urinary proteins. Protein Sci 11:2247–2256.
49. Nevison CM, Armstrong S, Beynon RJ, Humphries RE, Hurst JL. 2003. The ownership signature in mouse scent marks is involatile. Proc R Soc Lond B Biol Sci 270:1957–1963.
50. Halpern M, Martinez-Marcos A. 2003. Structure and function of the vomeronasal system: an update. Prog Neurobiol 70:245–318.
51. Doving KB, Trotier D. 1998. Structure and function of the vomeronasal organ. J Exp Biol 201:2913–2925.
52. Meredith M. 1994. Chronic recording of vomeronasal pump activation in awake behaving hamsters. Physiol Behav 56:345–354.
53. Luo M, Fee MS, Katz LC. 2003. Encoding pheromonal signals in the accessory olfactory bulb of behaving mice. Science 299:1196–1201.
54. Dulac C, Torello AT. 2003. Molecular detection of pheromone signals in mammals: from genes to behaviour. Nat Rev Neurosci 4:551–562.
55. Brennan PA, Keverne EB. 2004. Something in the air? New insights into mammalian pheromones. Curr Biol 14:R81–R89.
56. Maruniak JA, Wysocki CJ, Taylor JA. 1986. Mediation of male mouse urine marking and aggression by the vomeronasal organ. Physiol Behav 37:655–657.
57. Wysocki CJ, Lepri JJ. 1991. Consequences of removing the vomeronasal organ. J Steroid Biochem Mol Biol 39:661–669.
58. Bruce HM. 1959. An exteroceptive block to pregnancy in the mouse. Nature 184:105.
59. Brennan P, Kaba H, Keverne EB. 1990. Olfactory recognition: a simple memory system. Science 250:1223–1226.
60. Parkes AS, Bruce HM. 1961. Olfactory stimuli in mammalian reproduction. Science 134:1049–1054.
61. Thomas KJ, Dominic CJ. 1987. Evaluation of the role of the stud male in preventing male-induced implantation failure (the Bruce effect) in laboratory mice. Anim Behav 35:1257–1259.
62. Peele P, Salazar I, Mimmack M, Keverne EB, Brennan PA. 2003. Low molecular weight constituents of male mouse urine mediate the pregnancy block effect and convey information about the identity of the mating male. Eur J Neurosci 18:622–628.
63. Rajendren G, Dominic CJ. 1984. Involvement of contact stimuli in the male-induced implantation block (the Bruce effect) in mice. Anim Reprod Sci 7:377–383.
64. Brennan PA, Schellinck HM, Keverne EB. 1999. Patterns of expression of the immediate-early gene egr-1 in the accessory olfactory bulb of female mice exposed to pheromonal constituents of male urine. Neuroscience 90:1463–1470.
65. Guo J, Zhou A, Moss RL. 1997. Urine and urine-derived compounds induce c-fos mRNA expression in accessory olfactory bulb. Neuroreport 8:1679–1683.
66. Moncho-Bogani J, Lanuza E, Hernandez A, Novejarque A, Martinez-Garcia F. 2002. Attractive properties of sexual pheromones in mice: innate or learned? Physiol Behav 77:167–176.
67. Nyby J, Whitney G, Schmitz S, Dizinno G. 1978. Postpubertal experience establishes signal value of mammaliam sex odor. Behav Biol 22:545–552.
68. Yamazaki K, Yamaguchi M, Baranoski L, Bard J, Boyse EA, et al. 1979. Recognition among mice. Evidence from the use of a Y-maze differentially scented by congenic mice of different major histocompatibility types. J Exp Med 150:755–760.
69. Carroll LS, Penn DJ, Potts WK. 2002. Discrimination of MHC-derived odors by untrained mice is consistent with divergence in peptide-binding region residues. Proc Natl Acad Sci USA 99:2187–2192.
70. Singh PB, Brown RE, Roser B. 1987. MHC antigens in urine as olfactory recognition cues. Nature 327:161–164.
71. Wedekind C, Furi S. 1997. Body odour preferences in men and women: do they aim for specific combinations or simply heterozygosity? Proc R Soc Lond B Biol Sci 264:1471–1479.
72. Jacob S, McClintock MK, Zelano B, Ober C. 2002. Paternally inherited HLA alleles are associated with women's choice of male odor. Nat Genet 30:175–179.
73. Singer AG, Tsuchiya H, Wellington JL, Beauchamp GK, Yamazaki K. 1993. Chemistry of odortypes in mice—fractionation and bioassay. J Chem Ecol 19:569–579.
74. Singer AG, Beauchamp GK, Yamazaki K. 1997. Volatile signals of the major histocompatibility complex in male mouse urine. Proc Natl Acad Sci USA 94:2210–2214.
75. Singh PB. 2001. Chemosensation and genetic individuality. Reproduction 121:529–539.
76. Beynon RJ, Hurst JL. 2004. Urinary proteins and the modulation of chemical scents in mice and rats. Peptides 25:1553–1563.
77. Ivanyi P. 1978. Some aspects of the H-2 system, the major histocompatibility system of the mouse. Proc R Soc Lond B Biol Sci 202:117–158.
78. Boyse EA, Beauchamp GK, Yamazaki K. 1983. Critical review: the sensory perception of genotypic polymorphism of the major histocompatibility complex and other genes: some physiological and phylogenetic implications. Hum Immunol 6:177–183.
79. Schellinck HM, Slotnick BM, Brown RE. 1997. Odors of individuality originating from the major histocompatibility complex are masked by diet cues in the urine of rats. Anim Learn Behav 25:193–199.
80. Ehman KD, Scott ME. 2001. Urinary odour preferences of MHC congenic female mice, *Mus domesticus*: implications for kin

recognition and detection of parasitized males. Anim Behav 62:781–789.
81. Eggert F, Holler C, Luszyk D, Muller-Ruchholtz W, Ferstl R. 1996. MHC-associated and MHC-independent urinary chemosignals in mice. Physiol Behav 59:57–62.
82. Wolff RJ. 1985. Mating behaviour and female choice: their relation to social structure in wild caught house mice (*Mus musculus*) housed in a semi-natural environment. J Zool Lond 207:43–51.
83. Hurst JL. 1987. Behavioral variation in wild house mice *Mus domesticus* Rutty—a quantitative assessment of female social organization. Anim Behav 35:1846–1857.
84. Potts WK, Manning CJ, Wakeland EK. 1991. Mating patterns in semi-natural populations of mice influenced by MHC genotype. Nature 352: 619–621.
85. Rich TJ, Hurst JL. 1998. Scent marks as reliable signals of the competitive ability of mates. Anim Behav 56:727–735.
86. Humphries RE, Robertson DHL, Nevison CM, Beynon RJ, Hurst JL. 2001. The role of urinary proteins and volatiles in competitive scent marking among male house mice. In: Marchlewska-Koj A, Lepri J, Muller-Schwarze D, editors. Chemical Signals in Vertebrates 9. New York: Plenum. p 353–360.