(premolar attributed to *Paranthropus aethiopicus*); KNM-ER 801 (molar attributed to *P. boisei*[27]); KNM-ER 1805E (premolar attributed to *H. habilis*[27]); KNM-ER 1482B (molar attributed to *H. rudolfensis*[27]); KNM-ER 809B (molar attributed to *H. ergaster*[27]); KNM-ER 3733 (lower right P4 attributed to *H. ergaster*[27]); Sangiran S7-37 (upper right M1 and P4 attributed to *H. erectus*); and Tabun C1 (fragment of lower left first molar metaconid; attributed to Neanderthal).

Dental development in Sangiran S7-37

To estimate the timing of dental development in Sangiran S7-37 (Fig. 2), we counted the number of daily increments in the protocone and paracone of M1, which were equal. However, as the paracone initiates before the protocone, a month or so before birth, we added 30 days of prenatal and 30 days of postnatal enamel formation time, presumed lost through wear and/or plane of section. Total crown formation time in M1 was then 2.5 years to the mesiobuccal cervix. A strong accentuated line in both M1 and P4 (short vertical line in Fig. 2b) occurred 3.3 years into postnatal development and allowed the development of these teeth to be cross-matched. P4 mineralization initiated about 18 days after M1 crown completion. P4 crown formation time took 2.7 years. Root extension rates were calculated using counts and measurements of daily incremental markings in root dentine and averaged 10.7 μm^{-1} in M1 and 11 μm^{-1} in P4 (7–8 mm of root growth over 3 years in a modern human M1 would extend at 6.4–7.3 μm^{-1}). We observed about 8 mm of root formed below the buccal cervix of M1 and about 10 mm in M2 in hominin fossils[7,14] where these teeth were just in functional occlusion with wear (see also other fossil hominins, for example LH2 from Laitoli, Tanzania and and Taung from South Africa). Thus, at gingival emergence we expect there would have been about 1 mm less root formed. On this basis we estimate that gingival emergence for M1 occurred at about 4.4 years of age and for P4 (M2) at about 7.6 years of age in Sangiran S7-37.

Received 13 July; accepted 1 October 2001.

1. Bermudez De Castro, J.-M. *et al.* A modern human pattern of dental development in Lower Pleistocene hominids from Atapuerca-TD6 (Spain). *Proc. Natl Acad. Sci. USA* **96,** 4210–4213 (1999).
2. Wood, B. & Collard, M. The human genus. *Science* **284,** 65–71 (1999).
3. Moggi-Cecchi, J. in *The Origin of Humankind* (eds Aloisi, M., Battaglia, B., Carafoli, E. & Danieli, G. A.) 35–50 (IOS, Amsterdam, 2000).
4. Smith, B. H. Dental development as a measure of life history in primates. *Evolution* **43,** 683–688 (1989).
5. Allman, J. & Hasenstaub, A. Brains, maturation times, and parenting. *Neurobiol. Aging* **20,** 447–454 (1999).
6. Kelley, J. in *Human Evolution through Developmental Change* (eds McNamara, K. J. & Minugh-Purvis, N.). (Johns Hopkins Univ. Press, Baltimore, in the press).
7. Walker, A. & Leakey, R. *The Nariokotome* Homo erectus *Skeleton* (Harvard Univ. Press, Cambridge, Massachusetts, 1993).
8. Grine, F. E. & Franzen, J. L. Fossil hominid teeth from the Sangiran Dome (Java, Indonesia). *Courier Forschungsinstitut Senkenberg* **171,** 75–103 (1994).
9. Bromage, T. G. Enamel incremental periodicity in the pig-tailed macaque: A polychrome fluorescent labelling study of dental hard tissues. *Am. J. Phys. Anthropol.* **86,** 205–214 (1991).
10. FitzGerald, C. M. Do enamel microstructures have regular time dependency? Conclusions from the literature and a large scale study. *J. Hum. Evol.* **35,** 371–386 (1998).
11. Antoine, D., Dean, C. & Hillson, S. in *Dental Morphology 1998* (eds Mayhall, J. T. & Heikkinen, T.) 48–55 (Oulu Univ. Press, Finland, 1999).
12. Beynon, A. D. & Dean, M. C. Crown formation time of a fossil hominid premolar tooth. *Arch. Oral Biol.* **32,** 773–780 (1987).
13. Beynon, A. D., Dean, M. C., Leakey, M. G., Reid, D. J. & Walker, A. Comparative dental development and microstructure of *Proconsul* teeth from Rusinga Island, Kenya. *J. Hum. Evol.* **35,** 163–209 (1998).
14. Dean, M. C., Beynon, A. D., Thackeray, J. F. & Macho, G. A. Histological reconstruction of dental development and age at death of a juvenile *Paranthropus robustus* specimen, SK 63, from Swartkrans, South Africa. *Am. J. Phys. Anthropol.* **91,** 401–419 (1993).
15. Boyde, A. in *Primate Life History and Evolution* (ed. DeRousseau, J.) 229–267 (Wiley-Liss, New York, 1990).
16. Risnes, S. Growth tracks in enamel. *J. Hum. Evol.* **35,** 331–350 (1998).
17. Schwartz, G. T. Taxonomic and functional aspects of the patterning of enamel thickness distribution in extant large-bodied hominoids. *Am. J. Phys. Anthropol.* **111,** 221–240 (2000).
18. Beynon, A. D. & Wood, B. A. Variations in enamel thickness and structure in East African hominids. *Am. J. Phys. Anthropol.* **70,** 177–193 (1986).
19. Grine, F. E. & Martin, L. B. in *Evolutionary History of the Robust Australopithecines* (ed. Grine, F. E.) 3–42 (Aldine de Gruyter, New York, 1988).
20. Smith, B. H. Patterns of dental development in *Homo, Australopithecus, Pan* and *Gorilla*. *Am. J. Phys. Anthropol.* **94,** 307–325 (1994).
21. Beynon, A. D. & Dean, M. C. Distinct dental development patterns in early fossil hominids. *Nature* **335,** 509–514 (1988).
22. Dean, M. C. Progress in understanding hominoid dental development. *J. Anat.* **197,** 77–101 (2000).
23. Bromage, T. G. & Dean, M. C. Re-evaluation of the age at death of immature fossil hominids. *Nature* **317,** 525–527 (1985).
24. Schwartz, G. T., Reid, D. J. & Dean, M. C. Developmental aspects of sexual dimorphism in hominoid canines. *Int. J. Primatol.* **22,** 837–860 (2001).
25. Lovejoy, C. O., Cohn, M. J. & White, T. D. Morphological analysis of the mammalian postcranium: A developmental perspective. *Proc. Natl Acad. Sci. USA* **96,** 13247–13252 (1999).
26. Jernvall, J. & Han-Sung, J. Genotype, phenotype and developmental biology of molar tooth characters. *Yearb. Phys. Anthropol.* **43,** 171–190 (2000).
27. Wood, B. A. *Hominid Cranial Remains* Koobi Fora Research Project Vol. 4. (Clarendon, Oxford, 1991).
28. Ramirez Rozzi, F. V. Can enamel microstructure be used to establish the presence of different species of Plio-Pleistocene hominids from Omo, Ethiopia? *J. Hum. Evol.* **35,** 543–576 (1998).
29. Smith, B. H. & Tompkins, R. L. Towards a life history of the Hominidae. *Ann. Rev. Anthropol.* **24,** 257–279 (1995).
30. Reid, D. J. & Dean, M. C. The timing of linear hypoplasias on human anterior teeth. *Am. J. Phys. Anthropol.* **113,** 135–139 (2000).

Acknowledgements

We thank The Government of Kenya; The National Museums of Kenya; Forschungsinstitut Senckenberg, Frankfurt am Main, Germany; the Natural History Museum, London; and F. Thackeray of the Transvaal Museum, South Africa for access to fossil material. We thank D. Antoine, B. Berkovitz, D. Beynon, D. Clements, C. FitzGerald, L. Humphrey, J. Jernvall, J. Kelley, C. Kiarie, R. Krusynski, D. Lieberman, G. Macho, P. O'Higgins, J. Pendjiky, F. Ramirez Rozzi, H. Smith, P. Smith, F. Spoor, P. Walton and B. Wood for their help. This research was enabled by research grants to C.D. from the Royal Society and the Leverhulme Trust.

Correspondence and requests for materials should be addressed to C.D. (e-mail: ucgacrd@ucl.ac.uk).

Individual recognition in mice mediated by major urinary proteins

Jane L. Hurst*, Caroline E. Payne*, Charlotte M. Nevison*, Amr D. Marie†, Richard E. Humphries*, Duncan H. L. Robertson†, Andrea Cavaggioni‡ & Robert J. Beynon†

* *Department of Veterinary Clinical Science and Animal Husbandry, University of Liverpool, Leahurst, Neston CH64 7TE, UK*
† *Department of Veterinary Preclinical Sciences, University of Liverpool, Liverpool L69 3BX, UK*
‡ *Dipartimento di Anatomia e Fisiologia Umana, Università di Padova, 35100 Padova, Italy*

The ability to recognize individuals is essential to many aspects of social behaviour, such as the maintenance of stable social groups, parent–offspring or mate recognition, inbreeding avoidance and the modulation of competitive relationships. Odours are a primary mediator of individuality signals among many mammals[1]. One source of odour complexity in rodents, and possibly in humans, resides in the highly polymorphic major histocompatibility complex (MHC)[2]. The olfactory acuity of mice[3] and rats[4] allows them to distinguish between the urinary odours of congenic strains differing only in single genes within the MHC, although the chemical mediators or odorants are unknown. However, rodent urine also contains a class of proteins, termed major urinary proteins (MUPs)[5], that bind and release small volatile pheromones[6,7]. We have shown that the combinatorial diversity of expression of MUPs among wild mice might be as great as for MHC, and at protein concentrations a million times higher[8]. Here we show in wild house mice (*Mus domesticus*) that urinary MUPs play an important role in the individual recognition mechanism.

The only known function of MUPs is in chemical signalling. MUPs of male mice bind volatile signalling pheromones and release them slowly from urinary scent marks[9]. These volatiles are attractive to male[10,11] and female[12] mice, stimulate oestrus in prepubertal[13] and adult[14,15] females, and stimulate aggression between males[16]. In addition, the urinary proteins themselves stimulate increased competitive scent marking[10] and, if derived from a male of an unfamiliar strain, block pregnancy in females[17]. MUPs are expressed by both dominant and subordinate male mice[18] and both urine types stimulate increased scent marking by competitive males but not by subordinate males[18,19]. MUPs are encoded by a multigene family on chromosome 4 (ref. 20), and there are multiple alleles at each locus. The urinary MUPs are readily analysed[21], and it has become clear that MUPs in the urine of wild house mice exhibit a very high level of polymorphism. Individual mice each express a combination of about 7–12 MUPs and we have found many different MUP patterns, even among mice captured from the same population[22]. It is difficult to reconcile such molecular diversity with a simple role of

© 2001 Macmillan Magazines Ltd

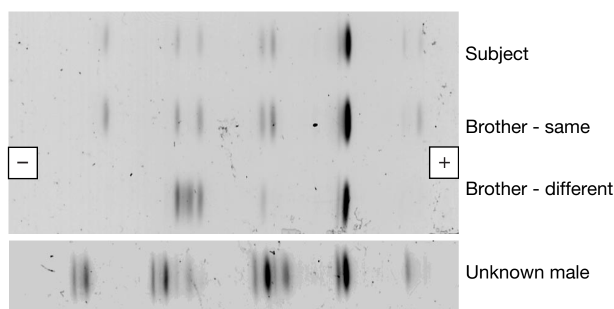

Figure 1 Urinary MUP types. MUPs are readily resolved by isoelectric focusing in narrow range (pH 4.2–4.9) immobilized gradient gels (AP Biotech, UK). Urine (5 μl of a 1 in 10 dilution) was focused for 15 kV h at 10 °C and stained with Coomassie blue. The overall pattern of bands is the MUP type, and the figure shows representative MUP types of a test subject, of a brother with the same MUP type, of a brother with a different MUP type and the MUP type of an unfamiliar male.

ligand binding and release, and we have previously suggested that the polymorphism in this class of proteins contribute to the individuality signals in urine deposits[8,10,19].

We therefore conducted two sets of tests to establish whether differences in MUP profile allow mice to distinguish their own scent from that of other males. The wild house mice were derived from outbred crosses between animals captured from geographically separated populations, to maintain the normal genetically heterogeneous background of wild mice. We also examined natural responses rather than a trained ability to discriminate, making use of the fact that when male mice encounter scent marks in their territory from another male, they spend longer investigating another male's scent than their own and increase their own rates of urine mark deposition to counter-mark the competitor's scent[10,18,23]. We used matched-pair *t*-tests and Wilcoxon matched-pair exact tests respectively to assess the specific hypothesis of increased investigation and scent marking towards urine stimuli from other males.

In the first set of tests, we compared the responses of the mice to their own urine, to urine from an unrelated male of different MUP type, and to urine from sibling males of the same or different MUP type as the subject (Fig. 1). In each test, stimulus urine was introduced into a male's home enclosure on one of two squares of absorbent paper for comparison with a control test in which both squares were marked only with water. The second square, marked only with water, also allowed us to examine whether increased scent marking was apparent only in the immediate vicinity of a stimulus or was distributed more widely around the home area. First we confirmed that males responded to the urine marks of an unrelated male but not to their own scent marks (Fig. 2). As expected, males spent more time investigating urine from an unrelated male than water-marked squares in the control test ($t_7 = -2.72$, $P < 0.025$) and deposited more scent marks on the unrelated urine square (Wilcoxon matched-pair exact test, $z = -2.38$, $P < 0.005$). In contrast, a square marked with their own urine stimulated no more investigation ($t_6 = -0.61$, not significant, NS) or scent marks ($z = -0.28$, NS) than water-marked squares in the control test. The scent-marking response induced by unrelated male urine also increased on the water-marked square presented simultaneously (number of scent marks on water-marked tile in unrelated male urine test compared with control test, $z = -2.52$, $P < 0.005$). Thus, scent marking was measured as the total number of marks deposited on both introduced squares in each test for all further analyses. Wilcoxon matched-pair exact tests confirmed that males deposited significantly more total marks in response to unrelated male urine than in either the control ($z = -2.55$, $P < 0.005$) or own urine test ($z = -2.19$, $P < 0.025$) whereas their own urine did not stimulate an increase in total scent marking ($z = 0.65$, NS; Fig. 2c). Investigation was directed towards the urine stimulus only (Fig. 2a), and thus only investigations of the urine-marked squares were compared.

To examine whether MUP type was important in recognizing scent marks as different from own, we compared the responses of the mice to their own urine and to urine from brothers of the same or different MUP type (Fig. 1). Urine from a brother of different MUP type stimulated significantly more investigation ($t_6 = 2.99$, $P < 0.025$) and scent marks ($z = -2.67$, $P < 0.005$) than own urine (Fig. 2), showing clear recognition of the brother's scent

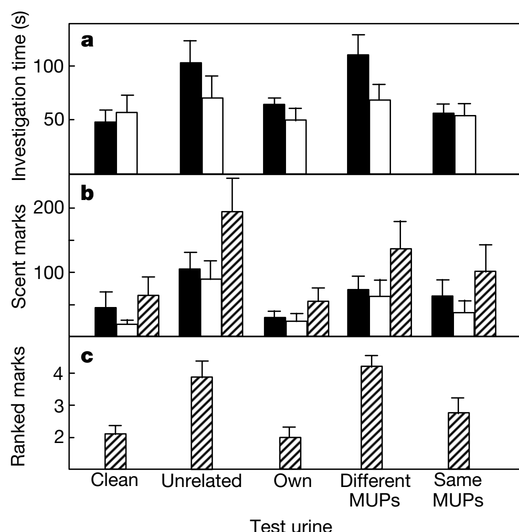

Figure 2 The effect of MUP type on investigation and countermarking of urine marks. **a**, **b**, Investigation time (**a**) and scent marking (**b**) by adult male wild house mice on paired stimulus squares streaked with urine (filled bars) or water (open bars) in five separate tests. Cross-hatched bars show scent marks totalled for each test. **c**, Rank of total scent marks deposited on both stimulus squares across the five tests. Data are means ± standard error of the mean, s.e.m.

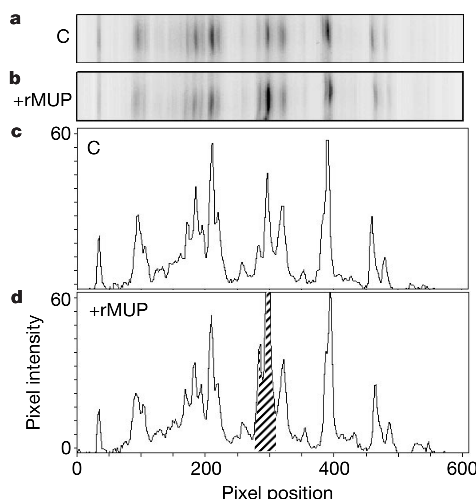

Figure 3 Modification of MUP type by addition of recombinant MUP. A male's own urine sample (C) was modified by addition of recombinant MUP (+rMUP) to a level of 20% of the total protein. Isoelectric focusing (**a**, **b**), followed by densitometry (**c**, **d**) was used to analyse the modification of the urine MUP type by the added protein (a representative example is shown). The cross-hatched area of the +rMUP densitometric trace highlights the change in protein profile elicited by the added rMUP.

© 2001 Macmillan Magazines Ltd

marks. Brother's urine of the same MUP type failed to stimulate any more investigation ($t_6 = 0.34$, NS) or scent marking ($z = -1.6$, NS) than own urine, suggesting that urine of the same MUP type was not distinguished from own urine, despite many other genetic differences between brothers. Brother's urine of different MUP type stimulated significantly more investigation ($t_7 = -3.25$, $P < 0.01$) and scent marking ($z = 1.84$, $P < 0.05$) than that from a brother of the same MUP type as the subject, suggesting that urinary MUP type is an important factor in recognition of individual scent donors.

Brothers were previously familiar to the test animal (although animals were separated at least four weeks before testing) and unrelated stimulus donors were unfamiliar. However, there was no difference in investigation ($t_7 = 0.28$, NS) or scent marking ($z = -0.53$, NS) in response to urine of different MUP type, whether from an unrelated male or a brother (Fig. 2). Males respond to any competing scents from other males within their territories, whether these come from a familiar relative or an unfamiliar unrelated male[23]. Thus, responses were not due to differences in familiarity. The MUP types of brothers often differed by only a few MUP bands (Fig. 1), but this still stimulated a strong response. The entire pattern of MUPs expressed thus appears to be used to discriminate own urine from that of another male.

To show that MUPs themselves were responsible for the response, we modified natural urine samples with recombinant MUP (rMUP), expressed in the yeast *Pichia pastoris*, the structure of which has been shown to resemble closely that of the protein from urine[24]. Mice were presented with own urine or with own urine to which rMUP had been added (Fig. 3). Although own urine mixed with rMUP did not induce any more investigation than own urine alone ($z = -0.49$, NS), the addition of rMUP stimulated significantly more scent-marking activity ($z = -2.045$, $P < 0.025$, Fig. 4). As in previous tests, marking increased on both the stimulus and water-marked squares presented simultaneously.

The response to urine supplemented with rMUP confirms that mice can perceive differences in urinary MUP type. The characteristics of MUPs—genome derived, a very high level of individual heterogeneity, stable expression patterns, involatility, expression in large quantities and resistance to degradation—are all particularly suited to a role in communication of individual identity in scent marks. This might be true for both sexes, since wild-caught female mice also excrete substantial quantities of MUPs with similar heterogeneity[22]. Indeed, to our knowledge, no other role for MUPs expressed by female mice has been postulated. Further, MUPs are expressed by all adult males, regardless of status. Social status among male mice is signalled through levels of sesquiterpenes excreted into the urine from preputial glands[25]. However, scent signals deposited in the environment also need to provide information on the identity of the signaller. Females discriminate between competing males according to which male's marks were more freshly deposited (that is, the counter-marking male and winner of a competitive encounter), so unambiguous information on the identity of a scent mark owner is also crucial for mate selection[19]. It is not clear whether mice detect urinary MUP type through differences in volatile ligands or in the MUPs themselves. Volatile ligands released from MUPs attract mice to investigate urine scent marks, leading to direct contact[10] and therefore bringing the involatile proteins into direct contact with receptors in the vomeronasal organ similar to those of rats that bind α2u-globulins[26], the equivalent lipocalins in rat urine. MUPs stripped of their volatile ligands elicit immediate early gene *egr-1* expression in mitral cells of both the anterior and posterior accessory olfactory bulb in mice and appear to convey the strain recognition signals of the male pheromone responsible for pregnancy block[17]. Although most polymorphism occurs on the surface of the protein, some variants occur in the cavity-binding site and we have shown that these variants are able to modulate binding of ligands[27]. Thus, MUP type may also affect volatile urinary odours through differential binding and release of volatile odorants. In this context, it is particularly interesting that noticeable differences in MHC-based odours among MHC-congenic inbred strains involve volatiles bound and released by urinary proteins[28,29]. It has been assumed that these odours derive from volatiles bound to MHC proteins or their degradation products[30], but a role for MUPs, which have evolved to bind lipophilic molecules, has not been examined. MUP type and MHC haplotype, derived from gene clusters on different chromosomes and inherited independently, may combine to provide a highly polymorphic individual identity signal that is unlikely to be shared even between relatives. However, in this study of wild mice, using a behavioural response that reflects natural behaviour, there was no significant response to brothers of the same MUP type, even though these males were likely to be of different MHC type to the subjects. Thus, MUPs may constitute a significant part of the individuality signal, although the interplay between the two systems, and the precise mechanisms of chemical mediation, are yet to be resolved. □

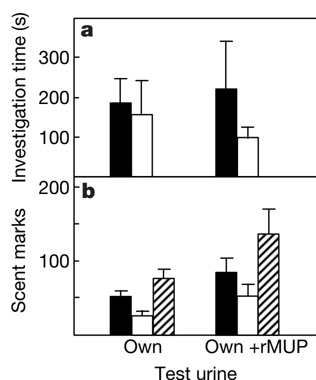

Figure 4 The effect of recombinant MUP on investigation and counter-marking of urine marks. Investigation (**a**) and scent marking (**b**) by adult male wild house mice presented with their own urine or their own urine mixed with recombinant MUP (means ± s.e.m.) was assessed in two separate tests. In each test, mice were presented with both a urine (filled bars) and water (open bars) stimulus, as described in the Methods. Cross-hatched bars show scent marks totalled for each test.

Methods

In the first set of tests, adult male house mice (F_1 or F_2 outbred crosses of mice captured from geographically separate locations) were housed in separate laboratory enclosures (1.2×1.2 m)[10] and presented with different urine stimuli (own, unrelated unfamiliar male, male sibling of different MUP type to own and male sibling of same MUP type as own) and a control test (two water stimuli) in a balanced order at weekly intervals. In each test, 2×5 μl urine and 2×5 μl water were streaked in the centres of separate 15×15 cm Perspex tiles covered in absorbent paper (Benchkote) and placed against opposite walls of a male's home enclosure. Investigatory behaviour (time in contact with each tile) was video recorded for the first 30 min of each test. Tiles were removed after 21 h and the number of marks counted under an ultraviolet lamp (marks smaller than 1×1 mm were not counted to exclude footprints). To stimulate competitive scent-marking behaviour[10], a mesh grille (5 cm diameter) provided olfactory contact between pairs of neighbour unrelated males before and between tests. In addition, nest material from caged females was introduced for 20 h immediately before each test. During tests, grilles were covered and female odours removed. Because subordinate or non-competitive males suppress scent marking and do not respond to the urine of other males by increasing their scent-marking rates[18], we first screened males for their willingness to scent-mark a tile streaked with unrelated male urine. Nine out of 18 males tested deposited very few scent marks (<20) and were excluded from further analyses[10].

In the second set of tests, adult male subjects were tested as above with their own urine and independently with a sample of their own urine modified by addition of MUP to a level of 20% of the total protein. Recombinant MUP was prepared by heterologous expression in *Pichia pastoris*[24] and was purified by a four-step process, including two stages of high-resolution anion exchange chromatography. In some individuals, the rMUP co-migrated with existing MUPs, although this cannot be taken as proof that they were the same protein[21]. In others, new bands were added to the MUP profile. The urine (own or mixed with rMUP, Fig. 3) was allowed to stand for 30 min (to allow rMUP to equilibrate with the semiochemical pool in the urine) before an amount of protein equivalent to that in 10 μl of own urine (range 40–130 μg protein) was deposited on the test substrate. In addition, 2×5 μl water was deposited on a second piece of Benchkote and placed against

© 2001 Macmillan Magazines Ltd

the opposite wall of the male's home enclosure. Investigation and scent marking were measured as described above. Tests were conducted four days apart in balanced order. Before tests, a female was introduced into each male's enclosure for 24 h, the female was then removed and males in neighbour enclosures were allowed a single interaction to stimulate more males to show competitive scent marking. Eleven out of 15 subjects deposited at least 20 marks on each tile and were included in analysis.

Received 16 August; accepted 17 September 2001.

1. Brown, R. E. & McDonald, D. W. *Social Odours in Mammals* Vols 1 & 2 (Clarendon, Oxford, 1985).
2. Yamazaki, K., Singer, A. & Beauchamp, G. K. Origin, functions and chemistry of H-2 regulated odorants. *Genetica* **104,** 235–240 (1999).
3. Yamazaki, K. *et al.* Sensory distinction between H-2b and H-2bm1 mutant mice. *Proc. Natl Acad. Sci. USA* **80,** 5685–5688 (1983).
4. Singh, P. B., Brown, R. E. & Roser, B. MHC antigens in urine as olfactory recognition cues. *Nature* **327,** 161–146 (1987).
5. Beynon, R. J., Robertson, D. H. L., Hubbard, S. J., Gaskell, S. J. & Hurst, J. L. in *Advances in Chemical Communication in Vertebrates* (eds Johnston, R. E., Muller-Schwarze, D. & Sorensen, P.) 137–147 (Plenum, New York, 1999).
6. Bacchini, A., Gaetani, E. & Cavaggioni, A. Pheromone binding proteins of the mouse, *Mus musculus*. *Experientia* **48,** 419–421 (1992).
7. Robertson, D. H. L., Beynon, R. J. & Evershed, R. P. 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 (1993).
8. Beynon, R. J. *et al.* in *Chemical Signals in Vertebrates* (eds Marchelewska-Koj, A., Muller-Schwarze, D. & Lepri, J.) 149–156 (Plenum, New York, 2001).
9. Hurst, J. L., Robertson, D. H. L., Tolladay, U. & Beynon, R. J. Proteins in urine scent marks of male house mice extend the longevity of olfactory signals. *Anim. Behav.* **55,** 1289–1297 (1998).
10. Humphries, R. E., Robertson, D. H. L., Beynon, R. J. & Hurst, J. L. Unravelling the chemical basis of competitive scent marking in house mice. *Anim. Behav.* **58,** 1177–1190 (1999).
11. Mucignat-Caretta, C. & Caretta, A. Urinary chemical cues affect light avoidance behaviour in male laboratory mice, *Mus musculus*. *Anim. Behav.* **57,** 765–769 (1999).
12. Jemiolo, D., Alberts, J., Sochinski-Wiggins, S., Harvey, S. & Novotny, M. Behavioural and endocrine responses of female mice to synthetic analogs of volatile compounds in male urine. *Anim. Behav.* **33,** 1114–1118 (1985).
13. Novotny, M. V., Ma, W., Wiesler, D. & Zidek, L. 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* **266,** 2017–2022 (1999).
14. Jemiolo, B., Harvey, S. & Novotny, M. Promotion of the Whitten effect in female mice by synthetic analogs of male urinary constituents. *Proc. Natl Acad. Sci. USA* **83,** 4576–4579 (1986).
15. Marchlewska-Koj, A., Cavaggioni, A., Mucignat-Caretta, C. & Olejniczak, P. Stimulation of estrus in female mice by male urinary proteins. *J. Chem. Ecol.* **26,** 2355–2366 (2000).
16. Novotny, M., Harvey, S., Jemiolo, B. & Alberts, J. Synthetic pheromones that promote inter-male aggression in mice. *Proc. Natl Acad. Sci. USA* **82,** 2059–2061 (1985).
17. Brennan, P. A., Schellinck, H. M. & Keverne, E. B. 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 (1999).
18. Nevison, C. M., Barnard, C. J., Beynon, R. J. & Hurst, J. L. The consequences of inbreeding for recognising competitors. *Proc. R. Soc. Lond. B* **267,** 687–694 (2000).
19. Hurst, J. L. *et al.* in *Chemical Signals in Vertebrates* (eds Marchelewska-Koj, A., Muller-Schwarze, D. & Lepri, J.) 43–50 (Plenum, New York, 2001).
20. Bishop, J. O., Clark, A. J., Clissold, P. M., Hainey, S. & Francke, U. Two main groups of mouse major urinary protein genes, both largely located on chromosome 4. *EMBO J.* **1,** 615–620 (1982).
21. Robertson, D. H., Cox, K. A., Gaskell, S. J., Evershed, R. P. & Beynon, R. J. Molecular heterogeneity in the major urinary proteins of the house mouse *Mus musculus*. *Biochem. J.* **316,** 265–672 (1996).
22. Payne, C. E. *et al.* in *Chemical Signals in Vertebrates* (eds Marchelewska-Koj, A., Muller-Schwarze, D. & Lepri, J.) 233–240 (Plenum, New York, 2001).
23. Hurst, J. L. Urine marking in populations of wild house mice *Mus domesticus* Rutty. 1. Communication between males. *Anim. Behav.* **40,** 209–222 (1990).
24. Lücke, C. *et al.* Solution structure of a recombinant mouse major urinary protein. *Eur. J. Biochem.* **266,** 1210–1218 (1999).
25. Novotny, M., Harvey, S. & Jemiolo, B. Chemistry of male dominance in the house mouse, *Mus domesticus*. *Experientia* **46,** 109–113 (1990).
26. Krieger, J. *et al.* Selective activation of G protein subtypes in the vomeronasal organ upon stimulation with urine-derived compounds. *J. Biol. Chem.* **274,** 4655–4662 (1999).
27. Marie, A. D. *et al.* Effect of polymorphisms on ligand binding by mouse major urinary proteins. *Protein Sci.* **10,** 411–417 (2001).
28. Singer, A. G., Tsuchiya, H., Wellington, J. L., Beauchamp, G. K. & Yamazaki, K. Chemistry of odortypes in mice—fractionation and bioassay. *J. Chem. Ecol.* **19,** 569–579 (1993).
29. Singer, A. G., Beauchamp, G. K. & Yamazaki, K. Volatile signals of the major histocompatibility complex in male mouse urine. *Proc. Natl Acad. Sci. USA* **94,** 2210–2214 (1997).
30. Pearse-Pratt, R., Schellinck, H., Brown, R., Singh, P. B. & Roser, B. Soluble MHC antigens and olfactory recognition of genetic individuality: the mechanism. *Genetica* **104,** 223–230 (1999).

Acknowledgements

This work was supported by Biotechnology and Biological Sciences Research Council (BBSRC) grants to J.L.H. and R.J.B., a BBSRC studentship to C.E.P. and an Egyptian government scholarship to A.D.M.

Competing interests statement

The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to J.L.H. (e-mail: jane.hurst@liverpool.ac.uk).

Drosophila Stardust interacts with Crumbs to control polarity of epithelia but not neuroblasts

Yang Hong*, Beth Stronach†, Norbert Perrimon†, Lily Yeh Jan* & Yuh Nung Jan*

* *Howard Hughes Medical Institute, Department of Physiology and Biochemistry, University of California San Francisco, San Francisco, California 94143-0725, USA*

† *Department of Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, Massachusetts 02115, USA*

Establishing cellular polarity is critical for tissue organization and function. Initially discovered in the landmark genetic screen for *Drosophila* developmental mutants[1–4], *bazooka*, *crumbs*, *shotgun* and *stardust* mutants exhibit severe disruption in apicobasal polarity in embryonic epithelia, resulting in multilayered epithelia, tissue disintegration, and defects in cuticle formation[5]. Here we report that *stardust* encodes single PDZ domain MAGUK (membrane-associated guanylate kinase) proteins that are expressed in all primary embryonic epithelia from the onset of gastrulation. Stardust colocalizes with Crumbs[6] at the apicolateral boundary, although their expression patterns in sensory organs differ. Stardust binds to the carboxy terminus of Crumbs *in vitro*, and Stardust and Crumbs are mutually dependent in their stability, localization and function in controlling the apicobasal polarity of epithelial cells. However, for the subset of ectodermal cells that delaminate and form neuroblasts, their polarity requires the function of Bazooka[7,8], but not of Stardust or Crumbs.

The *stardust* (*sdt*) mutation is not complemented by *Df(1)HA11*, a deletion of regions 7D14–7D22 (ref. 9). *HA11* was mapped to a region of about 85 kilobases (kb) (B.S., unpublished data), predicted to contain six open reading frames of more than 300 amino acids each by the genome annotation database of *Drosophila* (GadFly, http://www.bdgp.org). One of these open reading frames, CG1617, encodes a previously unknown MAGUK protein containing a single PDZ (PSD-95, Discs Large, ZO-1) domain, a SH3 (Src homology region 3) domain and a GUK (guanylate kinase) domain. We pursued the possibility that this MAGUK protein corresponds to Sdt, because other proteins with similar motifs are important for cell–cell junctions and cellular polarity[10–14].

To obtain full-length complementary DNAs, we screened an embryonic cDNA library and identified a large transcription unit that includes CG1617 and CG15341. Three cDNAs for this *sdt* candidate gene, *sdt1*, *sdt2* and *sdt3* (Fig. 1a), differ at their 5′ ends owing to alternative splicing, and code for two isoforms of potential Sdt protein: SdtA, with 1,292 amino acids; and SdtB, with 860 amino acids and lacking the 432 amino acids encoded by alternatively spliced exon 3 (hatched bar in Fig. 1d). *In vitro* translation of *sdt1* and *sdt3* yielded products of the predicted size (Fig. 1b). Blast analyses (http://www.ncbi.nlm.nih.gov/BLAST) identified homologues of SdtB in mouse, recently identified as Pals1 (proteins associated with mammalian Lin-7 (ref. 15), and in *Caenorhabditis elegans* a predicted protein of unknown function (see Supplementary Information). No homology to the amino-acid sequence of exon 3 in SdtA was found.

The gene that gives rise to these three cDNAs is *sdt*, because three independent strong hypomorphic or null alleles of *sdt*—*XN05*, *XP96* and *EH*—induced by ethylmethane sulphonate (EMS)[9,16] each carry a single nucleotide alteration in the coding sequences for *sdt1-3*. *XN05* contains a nonsense mutation in exon 6 (Fig. 1d). *XP96* contains a mutation at the 3′ splice junction of exon 6; a failed splicing would incorporate a stop codon that immediately follows

© 2001 Macmillan Magazines Ltd