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The scent of age

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In many species, older males are often preferred mates because they carry 'good' genes that account for their viability. How females discern a male's age is a matter of question. However, for animals that rely heavily on chemical communication there is some indication that an animal's age can be determined by its scent. To investigate whether there are changes in body odours with age, and if so their composition, mice were trained in a Y-maze to discriminate urine odours of donor mice of different ages: Adult (3–10 months old) and Aged (more than 17 months old). Trained mice could discriminate between these two age groups by odour alone. To determine the chemical basis for these discriminations, studies were performed using gas chromatography and mass spectrometry. These analyses demonstrated differences in the ratio of urinary volatiles with age. The most prominent differences involved significantly greater amounts of 2-phenylacetamide and significantly lower amounts of methylbutyric acids in Aged animals relative to Adult animals. Fractionating and manipulating the levels of these compounds in the urine demonstrated that the mice can distinguish age based on variation in amounts of these specific compounds in the combined urine.

Keywords: mice; age discrimination; body odour; urinary volatiles; mating; pheromones

1. INTRODUCTION

An older male, whose survival attests to his viability, may often be a preferred mate by females (Trivers 1972; Halliday 1978, 1983; Manning 1985; Kokko 1998; Brooks & Kemp 2001). This is a specific example of the more general 'good genes' hypothesis, or indicator mechanism for choosy behaviour in females (Andersson 1994). For such a process to occur, some male phenotypic trait(s) must both reliably indicate male genetic quality and influence the pattern of mate choice by females.

For animals that rely on chemical communication for the regulation of social and sexual interactions, there is some indication that an animal's age can be determined by its scent. For example, Ferkin (1999) showed that both male and female meadow voles are attracted to the odours of older conspecifics. That odour changes with age is suggested by the research of Robinson and colleagues who showed that the composition of mouse urine varies with age (Robinson *et al.* 1976; Miyashita & Robinson 1980). Similarly, Müller-Schwarze (1971) demonstrated that the volatile composition of black-tailed deer (*Odocoileus hemionus columbianus*) tarsal glands varies between juvenile and adult males, and Goodrich & Mykytowycz (1972) showed that the chromatographic profile and odour (as perceived by humans) of rabbit anal gland secretions varies with age.

Some changes in body odour with age have obvious causes. Dietary and hormonal changes that occur at specific ages and physiological changes that occur in very old individuals are known to be accompanied by body odour changes (Mennella & Beauchamp 1991; Siegel *et al.* 1992; Haze *et al.* 2001). But within normal reproductive ages, are there still age-related changes in body odour? It is the purpose of the experiments described here to address this question. Using a standard odour training paradigm (Yamaguchi *et al.* 1981), we show that as mice age, their odours change, allowing older male mice to be discriminated from otherwise identical adult, sexually mature individuals. Moreover, we provide evidence for the identity of some of the volatile compounds underlying these changes and suggest that their presence may reflect age-related changes in the animal's immune system.

2. MATERIAL AND METHODS

(a) *Mice*

Odour-donor and trained odour-sensor mice were of the inbred strain C57BL6J-H-2^k (referred to here as K mice). Mice were bred at the Monell Chemical Senses Center. Animals were maintained at 25 °C and photoperiod 12 : 12 L : D and were provided with continuous food and water. Twenty-four hours prior to behavioural tests sensor mice were deprived of water. All tests were conducted during the light phase of the photoperiod.

(b) *Collection of urine samples*

Urine samples were collected from Adult (3–10-month-old) and Aged (17–21-month-old) male K mice by gentle abdominal pressure. Samples were stored in sterile tubes at −20 °C. Owing to the small amount of urine typically produced, the test samples consisted of pooled urine collected from two to six individual animals from the same age group. A panel of 15–30 donors provided the urine samples used for the training trails.

(c) *Y-maze training and testing*

Five adult K mice (three males) were trained in a Y-maze, using water reward, to detect differences in urine samples based

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on the age of the donors (Yamaguchi *et al.* 1981). After successful training (more than 80% concordance for each trained mouse), unrewarded trials were interspersed, at an average frequency of one in four, with rewarded trials to accustom the mice to occasional absence of reward after a correct response. The mice performed with comparable accuracy during these trials. Generalization trials were then instituted so as to test novel urine samples without reward and thus ensure that the trained mice learned to distinguish the class distinction (in this case Aged versus Adult) rather than learned to distinguish the individual samples used during training. The generalization procedure lends itself to blind testing of coded samples because the operator of the maze is not required to supply a reward for concordant choices.

(d) *Sample preparation for Y-maze and gas chromatography analyses*

The mouse urine was pretreated by centrifugal ultrafiltration in Centricon-10 tubes (Amicon, 10 000 MW cutoff) at 5000*g* (6500 r.p.m.) at 5 °C. The filtrate was then acidified to a pH of 4.4–4.6 by adding 200 mg of KH_2PO_4-H_2O and extracted with 15 ml of HPLC-grade diethyl ether (Sigma-Aldrich Corp., St Louis, MO, USA) for 2 h by constant agitation on a Tekmar VXR flatbed shaker (Janke & Kunkel, Staufen, Germany). Following extraction the sample was divided into two equal aliquots for chemical analysis (gas chromatography (GC)) and bioassay. These samples were concentrated to *ca.* 0.5 ml in a SC210A SpeedVac concentrator (ThermoSavant, Holbrook, NY, USA), and then lyophilized to remove any remaining water. Samples for GC were re-dissolved in 100 µl of methyl acetate, while samples for bioassay were re-dissolved in 1 ml of sodium succinate buffer (0.05 M). A total of 30 separate samples, 15 each of the Aged and Adult groups, were prepared.

The chemical analysis was done on a Varian 3300C gas chromatograph. The gas chromatograph was fitted with a Restek Stabilwax column (30 m × 0.32 mm × 0.5 µm) (Restek, Bellefonte, PA, USA). The carrier gas was helium at 90 kPa. Oven temperature was maintained at 80 °C for 2 min and was then programmed at 5 °C min^{-1} to 240 °C. The injector temperature was held constant at 220 °C. Detection was by flame ionization. Analysis of peak heights (as percentage of the total) was undertaken for 38 representative compounds present in both Adult and Aged urine. The variation in peak heights between the Adult and Aged urine was assessed using the Mann–Whitney test. Samples were prepared and analysed as pairs of Aged and Adult urine to rule out effects owing to temporal differences in the extraction and analytical procedures.

HPLC fractionation was undertaken on a modular system comprising a Shimazu LC-9A pump, SLC-6B system controller, a SPD-6AV variable wavelength u.v.–visible spectrophotometric absorbance detector (Shimadzu America, Piscataway, NJ, USA) and a Rheodyne 7125 injector with a 100 µl sample loop (Rheodyne L.P., Rohnert Park, CA, USA). A microsorb-MV 86-200-C5 (Rainin Instrument Co., Inc.) reverse-phase column was used for all analytical separations. The mobile phase consisted of 5 mM phosphoric acid (A) and acetonitrile (B). Gradient elution from 99.9% A and 0.1% B to 71% A and 29% B in 30 min to 100% B in 5 min was employed. Samples of urine (1 ml) were extracted as previously described and evaporated to dryness. Samples were then re-dissolved in 50 µl of acetonitrile before injection into the HPLC. The eluted compounds were divided into three fractions: (i) fraction 1 (2.0–10.9 min); (ii) fraction 2 (10.9–16.7 min); and (iii) fraction 3 (16.7–23.0 min). Following collection, the fractions were divided into two equal aliquots for GC analysis and bioassay.

The identification of odour compounds was undertaken by GC quadrupole mass spectrometry (Thermoquest GC 8000 Top/Finnigan Voyager MS; ThermoFinnigan, Bremen, Germany). Identical column and oven conditions to those described for the chemical analyses were employed. Compounds were identified by comparing retention times with the retention times of standard chemicals (Sigma–Aldrich) under the same chromatographic conditions. Mass spectra from NIST92 and in-house libraries were also used.

3. RESULTS

(a) *Behavioural studies*

Mice were successfully trained to distinguish between the urine of Aged (17–21-month-old) and Adult (3–10-month-old) individuals. Concordance scores of greater than 80% were obtained for each of four trained mice tested and were confirmed by generalization (unrewarded) trials (table 1, test 1). To initially determine whether the distinction observed was attributable to quantitative rather than qualitative changes in odorant composition as a function of ageing, Y-maze generalization assays with urine samples at various concentrations were performed. Test animals discriminated between urine samples of five times diluted Aged versus undiluted Adult, and vice versa, confirming that the distinctions were not due to simple concentration effects (table 1, test 2).

(b) *Chemical studies*

Previous work has shown that the acidic components of urine play an important role in influencing individual differences in urine odour according to MHC type (Singer *et al.* 1997). Consequently, we investigated the acidified extract of both Aged and Adult urine for age-related changes in composition. Five odour-sensor mice each exhibited discrimination in generalization trials (table 1, test 3). GC analysis showed no absolute differences in the composition of both extracts, although differences were observed in the ratio of acidic components. From 38 representative peaks, chosen for comparison based on our ability to reliably measure and identify them, the peak heights of eight differed significantly between Adult and Aged urine (table 2). These eight components were identified by GC-MS and by retention time matching against authentic samples. Out of these eight compounds, 2-phenylacetamide, indole, phenol and α-cedrene were greater in the Aged relative to the Adult samples, and methylbutyric acids (isovaleric acid and 2-methylbutyric acid) were proportionally less.

(c) *Fractionation of extracts and Y-maze bioassays*

Urine extracts were fractionated into three portions by reverse-phase HPLC. These fractions were presented to bioassay mice trained to distinguish between five times diluted Aged mouse urine and five times diluted Adult mouse urine. Training and generalization trials showed that the mice distinguished between Aged and Adult fraction 1 and fraction 3 (table 1, tests 4 and 6, respectively), but not fraction 2 (table 1, test 5). Thus, the odour-sensor mice could distinguish age based on at least two different

Table 1. Percentage concordance of trained mice to whole, diluted and fractionated urine samples.
(Statistical tests (Fisher Exact) were computed after combining data from all test mice (four in tests 1 and 2; five in all others) into a single value (see Yamaguchi *et al.* (1981) for a discussion). This procedure reflects the behaviour of all test mice: for all training trials each test mouse performed at greater than chance (50%). For generalization trials this was also the case except for test 5 where no generalization trials were conducted, test 7 where one animal responded equally to the two alternatives, and test 8 where overall responding was random. n.t., not tested; if training on a fraction was not significant, generalization trials were not conducted. n.s., not significant; Ph, 2-phenylacetamide; I, indole; B, benzoic acid.)

test	training trials		generalization trials	
1	whole urine	82% ($n = 96$; $p < 0.001$)	whole urine	80% ($n = 19$; $p < 0.01$)
2	whole urine	81% ($n = 112$; $p < 0.001$)	diluted urine[a]	93% ($n = 15$; $p < 0.01$)
3	ether extracts	91% ($n = 73$; $p < 0.001$)	ether extracts	84% ($n = 25$; $p < 0.001$)
4	fraction I	91% ($n = 173$; $p < 0.001$)	fraction I	82% ($n = 33$; $p < 0.001$)
5	fraction II	46% ($n = 106$; n.s.)	n.t.	
6	fraction III	74% ($n = 241$; $p < 0.001$)	fraction III	88% ($n = 32$; $p < 0.001$)
7	fraction III	78% ($n = 180$; $p < 0.001$)	fraction III + Ph & I	88% ($n = 24$; $p < 0.01$)
8	fraction III	80% ($n = 135$; $p < 0.001$)	fraction III +B	50% ($n = 18$; n.s.)

[a] Combined data: five times diluted Aged versus undiluted Adult (88%, eight trials) and undiluted Aged versus five times diluted Adult (100%, seven trials).

mixtures as well as the combined urine. Such redundancy in the odour cues may be advantageous in preventing misinterpretation of the 'ageing odour'.

Of the four identified chemicals that increased with age in mouse urine (table 2), two, 2-phenylacetamide and indole, were found in the active fraction 3. We could not detect any of the chemicals that increased with age in the active fraction 1. A decrease in isovaleric and 2-methylbutyric acid, however, was observed in Aged relative to Adult urine in fraction 1. To determine whether changes in the concentrations of these compounds might be responsible for the discrimination of Adult versus Aged urine, we tested whether the odour-sensor mice, initially trained to discriminate Adult versus Aged fraction 3 urine, discriminated between samples of normal Adult urine fraction 3 and spiked samples in which the concentration of 2-phenylacetamide and indole was altered to the concentration determined by our procedure to be present in Aged urine (table 1, test 7). In fraction 3, the final concentrations of these chemicals were: 9.11 ± 3.11 and 18.4 ± 3.00 μg ml^{-1} Adult and Aged (2-phenylacetamide); 2.86 ± 0.62 and 4.59 ± 1.92 μg ml^{-1} Adult and Aged (indole). As a control (table 1, test 8) benzoic acid (10 μg per 1 ml urine) was added. This compound did not change as a function of age (table 2).

The results of the generalization tests supported the hypothesis that 2-phenylacetamide and indole have a behaviourally significant role in mediating the discrimination made by trained mice between Aged versus Adult urine samples. When mice trained to choose the arm of the maze scented with Aged odour over Adult odour were confronted with the choice Adult plus phenylacetamide and indole versus Adult samples spiked with equal amounts of water, they significantly chose the former (table 1, test 7). By contrast, they behaved randomly during control trials (table 1, test 8: Adult plus benzoic acid versus Adult plus water).

4. DISCUSSION

These studies demonstrate that the age-related changes in urine odour allow mice to discriminate age and that this discrimination is, in part, due to changes in the relative amounts of specific volatile compounds in urine. What mechanism(s) might underlie age-related changes in these compounds are unknown. For several reasons we do not believe exogenous sources can account for their differential presence. It is known that some insect repellants (Qiu & Jun 1996), muscarinic M3 receptor antagonists (Mitsuya *et al.* 1999) and anticoagulants (Su *et al.* 2001) are derived from 2-phenylacetamide. In addition, this compound has been observed in urine of mice treated with the cutaneous insect repellant *N*,*N*-diethylphenylacetamide (DEPA) (Rao *et al.* 1989). In our study, however, we used pathogen-free mice that were bred and housed in our sterile animal facility. There was no evidence in the histories of these mice to suggest exposure to DEPA and certainly no reason to believe that hypothetical exposure would be age related. Moreover, no 2-phenylacetamide derivatives were detected in GC chromatograms (data not shown). It has been reported that 2-phenylacetamide is synthesized by L-phenylalanine oxidase from *Pseudomonas* sp. (Koyama 1982). However, there is no evidence for *Pseudomonas* in our mouse colony and, even if there were, there is no evidence that *Pseudomonas* infection increases with age in mouse skin or in intestinal microflora (Pesti & Gordon 1973; Mitsuoka 1984).

An intriguing explanation for the increase of 2-phenylacetamide with age is related to age-related changes of the immune system. It is known that 2-phenylacetamide is metabolized from phenylalanine by means of oxygenation and decarboxylation by peroxidase (Kanehisa & Goto 2000). Although we do not know the true source of 2-phenylacetamide in mouse urine, myeloperoxidase is primarily found in granulocytes (Olsen & Little 1983; Putter & Becher 1983) that proliferate during the ageing process in humans (Mohacsi *et al.* 1996) and mice (Matsumoto *et al.* 1998; O'Keefe *et al.* 1999). Perhaps age-related changes of the immune system including changes in granulocyte function (Miyaji *et al.* 2000; Lord *et al.* 2001) underlie the increase in the relative amount of 2-phenylacetamide in older mouse urine.

Indole, however, can be synthesized from tryptophan by means of intestinal microbial flora and is absorbed into

Table 2. Analysis of peak heights (as per cent of the total) for 38 representative compounds present in both Adult and Aged urine.

		Adult		Aged		
peak number	peak identity	median[a]	interquartile range	median[a]	interquartile range	Mann–Whitney *p* value Adult versus Aged[b]
1		0.43	0.17–1.38	0.42	0.27–0.69	
2		0.75	0.37–1.37	0.47	0.21–0.77	$p = 0.002$
3	α-cedrene	0.23	0.16–0.42	0.48	0.40–0.81	$\mathbf{p = 0.0004}$
4		0.43	0.10–1.32	0.40	0.11–1.71	
5		0.68	0.40–2.61	0.74	0.54–1.47	
6	methylbutyric acids	8.23	3.09–10.8	3.44	1.79–8.38	$p = 0.006$
7		1.21	0.62–2.13	1.52	0.95–2.38	
8		1.36	0.73–2.05	1.16	0.33–1.60	
9		2.31	1.57–3.99	2.33	1.46–4.62	
10	dimethyl sulphone	2.74	1.34–5.80	3.32	2.04–7.09	
11		1.00	0.38–2.05	0.71	0.02–1.29	
12		0.29	0.02–0.86	0.33	0.02–0.92	
13	phenol	0.63	0.54–0.86	0.86	0.64–1.19	$\mathbf{p = 0.0005}$
14		1.00	0.62–1.27	0.98	0.70–1.43	
15	*p*-cresol	1.78	0.60–2.94	1.93	0.97–5.06	
16		3.29	2.66–4.62	3.14	2.41–4.31	
17		0.34	0.01–0.66	0.40	0.02–0.76	
18		0.40	0.15–3.38	0.42	0.20–1.10	
19	4-ethylphenol	0.85	0.73–1.71	0.73	0.64–1.29	
20		0.96	0.18–1.81	1.16	0.10–1.93	
21		0.99	0.73–1.62	1.18	0.80–2.14	
22		1.60	0.61–14.2	1.16	0.64–17.6	
23		2.86	1.90–3.85	2.68	2.10–4.82	
24	benzoic acid	13.4	6.42–16.4	13.1	7.99–15.4	
25	indole	0.66	0.50–0.86	0.86	0.63–1.50	$\mathbf{p = 0.0005}$
26		1.79	0.99–2.43	2.77	1.55–4.89	$\mathbf{p = 0.0004}$
27		2.91	2.01–4.97	2.68	0.92–3.73	
28		0.80	0.43–1.34	0.58	0.32–1.07	
29	phenylacetic acid	24.7	15.9–41.5	29.0	17.4–41.0	
30		1.65	0.68–1.91	1.49	0.80–1.96	
31		4.32	1.71–7.89	1.79	0.93–3.73	$p = 0.000\ 04$
32		2.53	1.20–3.12	1.93	1.55–3.10	
33		1.60	0.76–2.12	1.93	1.62–2.50	
34	2-phenylacetamide	1.70	0.77–2.29	2.79	1.83–3.40	$\mathbf{p = 0.000\ 02}$
35		0.66	0.17–1.00	0.67	0.30–1.18	
36		0.18	0.01–0.33	0.20	0.09–0.29	
37		0.82	0.21–1.25	0.82	0.40–1.28	
38		3.11	1.71–4.35	3.24	1.47–5.37	

[a] Median height from 15 urine samples.
[b] *p* value in bold signifies an increase in peak height with age. No line means the peak height decreased with age.

the body in substantial amounts (Marklova 1999). The compound is known to be oxidized to indoxyl, oxindole, 6-hydroxyindole and other related compounds by the cytochrome-P450/NADPH-P450 reductase system (Gillam *et al.* 2000). Increases of urinary indole seen here could be the result of age-related changes of intestinal microbial flora and/or the metabolic activity of amino acid intermediates.

Among the other compounds that vary significantly with age, α-cedrene, a sesquiterpene, was not observed in active fractions 1 or 3. This suggests that it is not involved in the behavioural discrimination observed. However, the possible source of this compound in the cedar animal bedding and the significant age-related difference in its relative concentration (table 2) raises the intriguing possibility that either uptake (e.g. via consumption) or retention is altered somehow by age. Additionally, both phenol and the unidentified compound No. 26 could not be detected in fractions 1 or 3, suggesting that they do not contribute to the behaviourally active odour change.

It has been reported that voles are attracted to the odours of older voles (Ferkin 1999) as might be expected from theoretical predictions based on a 'good genes' model (Brooks & Kemp 2001). If, as we suggest here, one cue for ageing is age-related changes in immune function and older animals are favoured by females, a potential conflict is evident. Males might be tempted to exhibit premature signs of age to increase their likelihood of passing on their genes. Countering this would be a loss of immune function (presuming that the changes in body odour are necessary consequences of functional loss) that would be detrimental to the male's health. Indeed, this

conflict might be a way to ensure that signals of age remain honest. Clearly, further experimental work is needed to determine, in mice, whether aged males are preferred mates by females. The current studies provided a strong reason to conduct such studies not only in mice but also in other mammals including humans that rely strongly on chemicals for communicating social and sexual messages.

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REFERENCES

Andersson, M. 1994 *Sexual selection*. Princeton University Press.

Brooks, R. & Kemp, D. J. 2001 Can older males deliver the good genes? *Trends Ecol. Evol.* **6**, 308–313.

Ferkin, M. H. 1999 Attractiveness of opposite-sex odor and responses to it vary with age and sex in meadow voles (*Microtus pennsylvanicus*). *J. Chem. Ecol.* **25**, 757–769.

Gillam, E. M., Notley, L. M., Cai, H., De Voss, J. J. & Guengerich, F. P. 2000 Oxidation of indole by cytochrome P450 enzymes. *Biochemistry* **39**, 13 817–13 824.

Goodrich, B. S. & Mykytowycz, R. 1972 Individual and sex differences in the chemical composition of pheromone-like substances from the skin glands of the rabbit, *Oryctolagus cuniculus*. *J. Mammal.* **53**, 540–548.

Halliday, T. R. 1978 Sexual selection and mate choice. In *Behavioural ecology: an evolutionary approach* (ed. J. R. Krebs & N. B. Davies), pp. 180–213. Oxford: Blackwell Scientific.

Halliday, T. R. 1983 Sexual selection and mate choice. In *Mate choice* (ed. P. Bateson), pp. 3–32. Cambridge University Press.

Haze, S., Gozu, Y., Nakamura, S., Kohno, Y., Sawano, K., Ohta, H. & Yamazaki, K. 2001 2-Nonenal newly found in human body odor tends to increase with aging. *J. Invest. Dermatol.* **116**, 520–524.

Kanehisa, M. & Goto, S. 2000 KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28**, 27–30.

Kokko, H. 1998 Good genes, old age and life history tradeoffs. *Evol. Ecol.* **12**, 739–750.

Koyama, H. 1982 Purification and characterization of a novel L-phenylalanine oxidase (deaminating and decarboxylating) from *Pseudomonas* sp. P-501. *J. Biochem., Tokyo* **92**, 1235–1240.

Lord, J. M., Butcher, S., Killampali, V., Lascelles, D. & Salmon, M. 2001 Neutrophil ageing and immunesenescence. *Mech. Ageing Dev.* **122**, 1521–1535.

Manning, J. T. 1985 Choosy females and correlates of male age. *J. Theor. Biol.* **116**, 349–354.

Marklova, E. 1999 Where does indolylacrylic acid come from? *Amino Acids* **17**, 401–413.

Matsumoto, S., Okabe, Y., Setoyama, H., Takayama, K., Ohtsuka, J., Funahashi, H., Imaoka, A., Okada, Y. & Umesaki, Y. 1998 Inflammatory bowel disease-like enteritis and caecitis in a senescence accelerated mouse P1/Yit strain. *Gut* **43**, 71–78.

Mennella, J. A. & Beauchamp, G. K. 1991 Maternal diet alters the sensory qualities of human milk and the nursling's behavior. *Pediatrics* **88**, 737–744.

Mitsuoka, T. 1984 The effect of nutrition on intestinal flora. *Nahrung* **28**, 619–625.

Mitsuya, M. (and 12 others) 1999 J-104129, a novel muscarinic M3 receptor antagonist with high selectivity for M3 over M2 receptors. *Bioorg. Med. Chem.* **7**, 2555–2567.

Miyaji, C., Watanabe, H., Toma, H., Akisaka, M., Tomiyama, K., Sato, Y. & Abo, T. 2000 Functional alteration of granulocytes, NK cells, and natural killer T cells in centenarians. *Hum. Immunol.* **61**, 908–916.

Miyashita, K. & Robinson, A. B. 1980 Identification of compounds in mouse urine vapor by gas chromatography and mass spectrometry. *Mech. Ageing Dev.* **13**, 177–184.

Mohacsi, A., Kozlovszky, B., Kiss, I., Seres, I. & Fulop Jr, T. 1996 Neutrophils obtained from obliterative atherosclerotic patients exhibit enhanced resting respiratory burst and increased degranulation in response to various stimuli. *Biochim. Biophys. Acta* **1316**, 210–216.

Müller-Schwarze, D. 1971 Pheromones in black-tailed deer (*Odocoileus hemionus columbianus*). *Anim. Behav.* **19**, 141–152.

O'Keefe, T. L., Williams, G. T., Batista, F. D. & Neuberger, M. S. 1999 Deficiency in CD22, a B cell-specific inhibitory receptor, is sufficient to predispose to development of high affinity autoantibodies. *J. Exp. Med.* **189**, 1307–1313.

Olsen, R. L. & Little, C. 1983 Purification and some properties of myeloperoxidase and eosinophil peroxidase from human blood. *Biochem. J.* **209**, 781–787.

Pesti, L. & Gordon, H. A. 1973 Effects of age and isolation on the intestinal flora of mice. *Gerontologia* **19**, 153–161.

Putter, J. & Becher, R. 1983 Peroxidases. *Meth. Enzyme Anal.* **3**, 286–293.

Qiu, H. & Jun, H. W. 1996 Solid-phase extraction and liquid chromatographic quantitation of insect repellent N,N-diethyl-m-toluamide in plasma. *J. Pharmacol. Biomed. Anal.* **15**, 241–250.

Rao, S. S., Swamy, R. V. & Ramachandran, P. K. 1989 Toxicity and metabolism of a new insect repellent N,N-diethylphenylacetamide in mice, rats and guinea pigs on cutaneous application. *Toxicology* **58**, 81–89.

Robinson, A. B., Dirren, H., Sheets, A., Miquel, J. & Lundgren, P. R. 1976 Quantitative aging pattern in mouse urine vapor as measured by gas-liquid chromatography. *Exp. Gerontol.* **11**, 11–16.

Siegel, S. F., Finegold, D. N., Urban, M. D., McVie, R. & Lee, P. A. 1992 Premature pubarche: etiological heterogeneity. *J. Clin. Endocrinol. Metab.* **74**, 239–247.

Singer, A. G., Beauchamp, G. K. & Yamazaki, K. 1997 Volatile signals of the major histocompatibility complex in male mouse urine. *Proc. Natl Acad. Sci. USA* **94**, 2210–2214.

Su, T. (and 14 others) 2001 Design and synthesis of glycolic and mandelic acid derivatives as factor Xa inhibitors. *Bioorg. Med. Chem. Lett.* **11**, 2279–2282.

Trivers, R. L. 1972 Parental investment and sexual selection. In *Sexual selection and the descent of man 1871–1971* (ed. B. Campbell), pp. 136–179. Portsmouth: Heinemann.

Yamaguchi, M., Yamazaki, K., Beauchamp, G. K., Bard, J., Thomas, L. & Boyse, E. A. 1981 Distinctive urinary odors governed by the major histocompatibility locus of the mouse. *Proc. Natl Acad. Sci. USA* **78**, 5817–5820.