mouse at day 6 to below 10,000 per mouse from day 11 onwards (data not shown). Therefore, just two weeks after secondary immunization, the majority of specific plasma cells reside in the bone marrow, as expected[7].

We determined the life-span of ovalbumin-specific plasma cells residing in the bone marrow using bromodeoxyuridine (BrdU) incorporation as a measure for DNA synthesis, a method that had already been used successfully to measure the life-span of memory B cells[8]. We immunized and boosted two groups of mice with ovalbumin. Group A was given BrdU in drinking water for the first 19 days after secondary immunization. For group B, feeding with BrdU started at day 19 after the secondary immunization and was maintained for more than 90 days. We measured BrdU incorporation by ovalbumin-specific plasma cells from each group at various times (Fig. 1b, c). Nearly all of the specific plasma cells developed within two months of secondary immunization. Later, only very few additional plasma cells were found to be labelled in group B, or unlabelled in group A. Group A consistently shows 5–15% more 'new' plasma cells, which probably represent the unlabelled plasma cells from the primary immunization, so they are really very 'old' plasma cells. Apparently, these plasma cells had not proliferated on secondary immunization with ovalbumin. Between primary and secondary immunization, the specific serum titre increased by 10–40 times, corresponding to the 20-fold increase in bone-marrow plasma cells.

To conclude, more than 60% of the plasma cells at day 110 after the booster injection lived for more than 90 days without DNA synthesis. They may live longer, as suggested by the asymptotic slope of the incorporation curve. Our data provide clear evidence that most plasma cells, present in constant numbers in the bone marrow, are long-lived and not derived from the differentiation of proliferating, activated B cells. These plasma cells may be as long-lived as memory B cells. Our result has implications for the design of vaccination strategies and may aid the understanding of allergy and humoral autoimmunity.

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Importance of ancestral DNA ages

Recent publications consider the age of a common ancestor of samples of human DNA sequences[1–9]. In particular, variation in Y chromosomes has been interpreted by different authors to give very different estimates of the time to the most recent common ancestor of the sample[1–8]. The cause of the differences is that the data are being interpreted by various authors in terms of their preferred models of human demography. The data are so lacking in power that these estimates depend hardly at all on the data, and almost entirely on the demographic model assumed. However, if we knew the demography of early humans, we would have no real interest in the time to the most recent common ancestor of an individual gene, as the time is important only because it tells us about the demography.

Generally, the data are a sample of sequences, D. Assumptions about the neutral mutation rate, μ, and a demographic model, N (often including neutrality and a constant effective population size) are used to calculate $P(T|D,N,\mu)$, the probability that the sequences had a time to most recent common ancestry of T, conditional upon D, N and μ. From these, one can calculate an expected value of T, $E(T)$.

Different values of $E(T)$ originate from differing choices of N. A clear example concerns analyses of the data of Dorit *et al.*[1], who found no variation in a sample of 38 Y chromosomes for a 729-base-pair intron from the *ZFY* gene. They estimated $E(T)$ using two models. One incorporated a constant population size, with all values of T equally likely prior to the data. Another model assumed a population expansion again with a constant prior distribution of T. $E(T)$ values were 270,000 and 27,000 years, respectively. Others sought explicitly to condition on the basis of a demographic model. Fu and Li[2] considered a constant population size model, and, depending upon the effective population size (from 2,500 to 30,000) obtained values for $E(T)$ ranging from 92,000 years to 703,000 years. Donnelly *et al.*[3] assumed a lognormal prior distribution of population size with a mean of 36,000. $E(T)$ values ranged from 254,000 to 460,000 years, depending on μ. Weiss and von Haeseler[4] imagined populations exponentially increasing, with various growth rates, creating a range of $E(T)$ values from 17,000 to 286,000 years.

The explanation of this 40-fold range of expected times is that, when data sets lack power, $E(T)$ depends more on N than on D. An extreme example is the analysis by Fu and Li[5] of the data of Knight *et al.*[9]. The sequence diversity of an Alu insertion at the α-globin-2 locus suggested an $E(T)$ of under 100,000 years. In their re-analysis, Fu and Li calculate $E(T)$ values over 450,000 years. They use a neutral model with a constant 10,000 individuals, such that $E(T|N) \approx 800{,}000$ years, prior to the data, the lack of power of which leaves $E(T|D,N,\mu)$ high.

The demographic models are assumed to be known without error, and are usually not tested for consistency with the data. Yet T is interesting only for the information that it supplies about N, the demography of the human species. It is N, not T, that can be related to the fossil record, for example. We wish to know human population sizes in the past, and to investigate the possibility of demographic expansions during the recent spread from Africa. Values for times to common ancestry calculated on the basis of an assumption that there has been no expansion are merely confusing.

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correction

In "Structures of mollusc shell framework proteins", by S. Sudo *et al.* (*Nature* **387,** 563–564; 1997) the deduced amino-acid sequence of pMSI2 (Fig. 1b) contained an error at position 69. The corrected sequence is reproduced below.

b

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MKPFVTLASLIVLIASASADGYDDYKKYGSVGYGPGISLGGGGLGGGGGIISVGGGGGLGGGLGGGLGCG   70
LVGVGGGGLIGGGFGPGRVSGTINAGGGVFASGSGLGGGLSPAGRGAAQGAATLSALQIASGRPGRVSGVS  140
VGTGGGRAVVSGSATPVGGFGVPYGGYGYNYGVPSYGVGLPSYGVSLPSYGVGLGGGYGGYGYGLDLASF  210
QGSTYGNLATGQINTAVVAFHMAVLLESMEAESDTEVDTEDMDSEEDMESEEDTESEEDTESEEDTESEE  280
DMESEEDMDSESSVVDQVSMVYPNHFTGDVLFCQVRLQELEFPALEALVSVVLE                   334
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have wild progenitors or relatives with leaf domatia[1,2,11,12]. It may be possible to breed or engineer plants for better expression of leaf traits such as domatia that increase predator populations and efficacy. For plants that already have leaf domatia, these traits may mediate an important, yet poorly documented, mutualism.

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Salt enhances flavour by suppressing bitterness

Salts are used as flavouring agents in the cuisines of many cultures[1], the most commonly used being NaCl. They impart their own salty taste and enhance other flavours. The apparent ability to increase the intensity of other desirable flavours[2,3] is puzzling as virtually all published psychophysical studies show that NaCl either suppresses or has no effect on other flavours[3,4]. To reconcile this contradiction we have proposed[5] that salts selectively filter flavours, such that unpleasant tastes (such as bitterness) are more suppressed than palatable ones (such as sweetness) thereby increasing the salience and/or intensity of the latter. We now present evidence to support this idea.

We used mixtures of aqueous solutions of a bitter substance, urea, which is strongly suppressed by sodium-containing compounds[6]; a sweetener, sucrose; and a salt, sodium acetate, which has a fairly mild taste[6] and so is suitable for studying the flavour-modifying effects of sodium ions. Subjects (21 volunteers) were required to judge the extent of bitterness, sweetness and 'otherness' of all possible combinations of three concentrations of urea (0.0, 0.5, 1.0 M), four of sucrose (0.0, 0.1, 0.3, 0.5 M) and three of salt (0.0, 0.1, 0.3 M) using the method of magnitude estimation[6]. We evaluated the solutions, 12 per day (twice) over three consecutive days, in a counterbalanced order. Data were standardized and normalized[6].

As predicted, there was a selective suppression of the taste components by sodium acetate (Fig. 1). The bitterness of urea was suppressed much more by the salt than was the sweetness of sucrose. Consequently, the sucrose–urea mixtures with added salt were relatively less bitter and more sweet than when sodium acetate was not added. Moreover, at the higher concentrations of sucrose (0.3, 0.5 M) and both concentrations of urea (0.5, 1.0 M), the absolute sweetness intensity was increased by adding either 0.1 or 0.3 M sodium acetate compared with when no sodium acetate was added (one example is shown in Fig. 1). This presumably occurred by releasing sweetness from suppression by the bitterness of urea[7]. As expected[8], the addition of sodium acetate to sucrose in the absence of urea never had an enhancing effect on sweetness (data not shown).

Although this simple three-component aqueous system does not fully mimic the complex food systems in which salts are used, it illustrates at least one mechanism by which a salt increases both the relative and absolute intensity of palatable components of foods. This mechanism has not commonly been considered in taste mixture studies, which have tended to concentrate either on two-component mixtures, or on complex foods where interpretations are difficult.

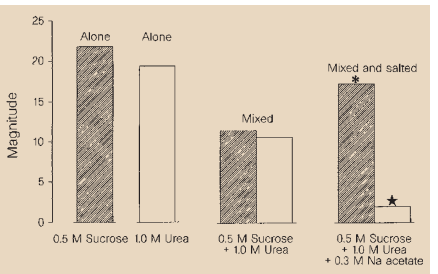

Figure 1 The normalized reported magnitude of the taste of various solution mixtures is shown. The intensity of urea and sucrose at the highest concentrations were roughly the same (left). Statistical analysis revealed that in mixtures, the highest concentrations of sucrose and urea (without sodium acetate), mutually and roughly equally suppressed their intensities (centre). When sodium acetate was added, also at the highest concentration, intensity of the bitterness greatly decreased, being suppressed by sodium ions[6], whereas the sweetness intensity increased to levels that approximated the sweetness in pure deionized water (right). Relative to binary mixture levels, asterisk denotes increase ($P<0.0001$) and star denotes decrease ($P<0.0001$). These trends were evident for other concentrations tested. Detailed analyses available from the authors.

Our data show that, in addition to adding desired saltiness to food, salts potentiate flavour[9] through the selective suppression of bitterness (and perhaps other undesirable flavours), and the release from suppression of palatable flavours such as sweetness. The desire for NaCl and other salts in foods as diverse as (often bitter) vegetables, oily foods and meats may be due in part to their ability to suppress unpleasant flavours[10]. This may explain why it is difficult to make low-sodium foods acceptable.

Biophysical evidence[11] implies that it will be extremely difficult to develop a salty-tasting sodium-free substitute for NaCl. However, the multiple sensory functions of salts in foods should be considered, as the differential flavour-suppressing effect shown here might be duplicated by non-sodium substances, such as bitterness blockers.

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Structures of mollusc shell framework proteins

Mollusc shells consist of the nacreous mother-of-pearl layer and the prismatic layer. Both layers are microlaminate composites of $CaCO_3$ crystals (aragonite in the nacre and calcite in the prismatic layer) and biopolymers. The main biopolymers are structural proteins, insoluble in water and methanoic acid, which determine the framework of each shell layer[1–4] and bind soluble polyanionic proteins[2–4] which determine the type of $CaCO_3$ crystal that grows[5,6]. Here we report the sequences and structures of the framework proteins for the nacreous and prismatic layers of the pearl oyster, *Pinctada fucata*.

We ground and decalcified the nacreous layer of the mollusc shell using 50 per cent methanoic acid, and cleaved the protein by treatment with cyanogen bromide (CNBr),

before separation by Tricine-SDS–PAGE. We then western blotted the cleaved peptide onto a polyvinyl pyrrolidone difluoride filter, and obtained the amino-acid sequence, GGGFGVGLGGG (single-letter code).

We isolated two complementary DNA clones, pMSI1 and pMSI2, from a λgt10 cDNA library constructed with poly(A)$^+$ RNA from the mantle of the oyster, using a degenerate oligonucleotide probe corresponding to the amino-acid sequence FGVGLG. The pMSI1 clone encodes a glycine- and alanine-rich protein (738 amino acids) which contains the sequence GGGFGVGLGGG, matching the isolated peptide (Fig. la). The pMSI2 clone encodes a glycine-rich protein (334 amino acids) containing the sequences GVGLG and GVGL (Fig. 1b).

Both proteins appear to possess signal peptides, as predicted by hydropathy analysis, and have relative molecular masses of 60,000 and 31,000, respectively. We have designated these mature proteins MSI60 and MSI31. The amino-acid compositions of MSI60 and MSI31 corresponded well with those previously reported[3] for the insoluble proteins from the nacreous and prismatic layers of *Pinctada fucata*, respectively.

MSI60 contains 11 poly(alanine) blocks and two alanine-rich domains, between poly(glycine)-rich regions (Fig. 1a). An X-ray diffraction study[7] of mollusc shell proteins has shown that the insoluble matrix protein in the nacreous layer adopts an antiparallel β-sheet conformation. β-sheet domains in *Bombyx mori* cocoon silks are formed from GA/GS repeats and poly(alanine) repeats are seen in spider dragline silks[8]. The poly(alanine) blocks in spider silks are 4–8 residues long[8,9], whereas those in MSI60 are of 9–13 residues (Fig. 1a). Longer poly(alanine) blocks in MSI60 may form longer, densely packed crystals of β-sheets. The MSI60 protein also has 39 poly(glycine) blocks of 3–15 residues, distributed throughout the molecule (Fig. 1a). We suggest that these poly(glycine) blocks participate in the formation of crystalline β-sheets whereas the poly(aspartate) blocks in the amino- and carboxy-terminal regions may bind calcium ions. Cysteine residues located in N- and C-terminal regions could form intermolecular (and intramolecular) disulphide bonds in the nacreous sheets.

MSI31, the framework protein of the prismatic layer, has no poly(alanine) blocks, but has 10 poly(glycine) blocks of 3–5 residues in the N-terminal half of the molecule and a large acidic region including six consecutive ESEEDX motifs in the C-terminal half (Fig. 1b). X-ray diffraction[7] showed that the insoluble matrix protein in the prismatic layer is also composed of β-pleated-sheets, randomly oriented in the plane of the shell with the side chains perpendicular. The poly(glycine)-rich region in MSI31 may participate in the formation of β-pleated-sheet structures and the C-terminal acidic domain in the binding of calcium ions or proteins. Two cysteine residues in the N and C termini of MSI31 would also participate in the formation of intermolecular disulphide bonds in the prismatic walls.

a

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MKLLVVLTTLVGFSSALSFGCNYRPVLGFNSQYMLGGLRLFCMPAMVYDPWACGCVSAWSSAGLYGVGGG   70
GGAWGAGGAGGADGGRGGGGGDWEYDYDDDSDDDDEWDWDDDGGMGAGAGGGAGGGAGGAGAGAGAG   140
AGAGAGLGLGLGGGLGGGLGGLGGLGGLGGGDDLFDLDFDDLGAALALGGAGGAGGAAAAAAAAAAGG   210
GVGGAAAAAAAAAAGGGAGRLGGAAAAAAAAAAAGGAGGLGGLGGGLGGLGGGLGGLGGLGGLGGYG   280
GSAAAAAAAAAAAGGGGLGGVGFYGGRGGRRGRGRGGRRRAAAAAAAAAAAAAGGGGGGGGGGGGGGA   350
GAAAAAAAAASASASRQMSGIRDALGDIKDLLRSNGASAKASAKASAVASTKSQIDDLKDVLKDLAGLL   420
KSSASASASASASASAGGGGGGGNGGGNGGGGGGAGALAAALAAAGAGGGLGGGGGGGALAAALAAAGA   490
GGGGFGGLGGLGGLGGGSAAAAAAAAAASGGGGRALRRALRRQMRGGGSAAAAAAAAAAAGGGWGGGM   560
GGGFGVGLGGGFGGGFGGGSSAAAAAAAAAAGFGGGGRRGRGRGRGGDGDGNGASAVAAAAAAAAAGG   630
SAADVAAAAAAAAMYGDGADGPDFDNGFGGGNGNGGGGSGGGGSGGGGSGGGGSGGGGGGSGGSGGGGGSG   700
GSGGGGSGGGGNNGWGNNGNNKYDDDDCDEYGNPIRRG                                  738
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b

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MKPFVTLASLIVLIASASADGYDDYKKYGSVGYGPGISLGGGGLGGGGGIISVGGGGGLGGGLGGGLGGG   70
LVGVGGGGLIGGGFGPGRVSGTINAGGGVFASGSGLGGLSPAGRGAAQGAATLSALQIASGRPGRVSGVS   140
VGTGGGRAVVSGSATPVGGFGVPYGGYGYNYGVPSYGVGLPSYGVSLPSYGVGLGGGYGGYGYGLDLASF   210
QGSTYGNLATGQINTAVVAFHMAVLLESMEAESDTEVDTEDMDSEEDMESEEDTESEEDTESEEDTESEE   280
DMESEEDMDSESSVVDQVSMVYPNHFTGDVLFCQVRLQELEFPALEALVSVVLE                  334
```

Figure 1a, Deduced amino-acid sequence (single-letter code) of pMSI1 (DDBL/EMBL/Genbank accession number D86074). The putative signal peptide is indicated by a dotted line, and the sequence detected by Edman degradation of CNBr-cleaved peptides is underlined. Poly(alanine) blocks are highlighted. **b,** Deduced amino-acid sequence of pMSI2 (accession number D86073). The putative signal peptide is again shown by a dotted line, and sequences whose nucleotide sequences correspond to the degenerate oligonucleotide probe are underlined.

In situ hybridization shows that the outer epithelia of the pallial of the mantle expresses MSI60 messenger RNA (Fig. 2a), and that the outer epithelia of the mantle edge expresses MSI31 mRNA (Fig. 2b). The pallial of the oyster mantle also expresses mRNA for a soluble protein found in the nacreous layer that has carbonic anhydrase activity[10]. These findings suggest that the proteins in the pallial construct the nacreous layer and the proteins from the edge of the mantle construct the prismatic layer. As MSI60 and MSI31 have no carbonic anhydrase active-site sequence and no *N*-glycosylation site, these proteins may selectively bind soluble aspartate-rich matrix glycoproteins[5,6] and carbonic anhydrase[10] responsible for the formation of $CaCO_3$ crystals, after they construct the frameworks of the nacreous and prismatic layers[6].

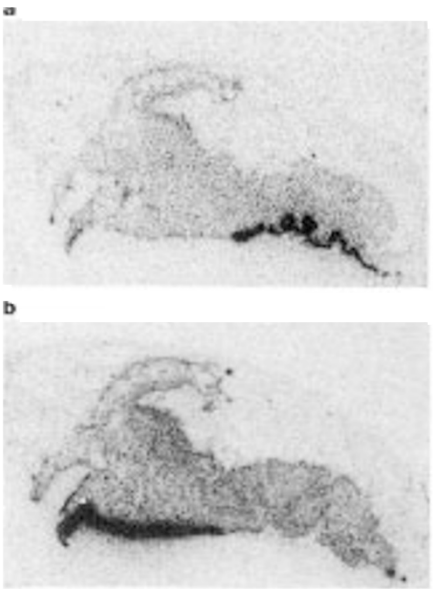

Figure 2 *In situ* hybridization of **a,** MSI60 and **b,** MSI31 mRNA in the mantle of *P. fucata*. The edge of the mantle is to the left and the outer epithelial region is at the bottom.

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