400–550 cm^{-1} for films produced with an ion energy of 30 eV (the energy that produced the maximum endohedral signal in the laser-desorption mass-spectrometry results). The double maximum that we observe shows close similarities with the predicted P and R branches but is shifted by about 50–75 cm^{-1} to the blue, indicating that the endohedral potential is slightly deeper and narrower than predicted theoretically.

As our method produces films that contain only the particular fullerene deposited and its endohedral version in a ratio of around 3:1 (for Li), we can investigate the properties of the endohedral material without further purification. Similar results using Na^+, K^+ and Rb^+ ion beams show that the method can be extended to larger ions and might also be applicable to non-alkali-metal systems.

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Genome analysis

SIR — The recent commentary from Venter, Smith and Hood[1] proposed a simplified approach to sequencing the human genome. Many parts of this proposal are very attractive and make good use of the dispersed facilities that exist internationally for small, large and massive scale DNA sequence determination.

The ultimate use of the "sequence-tagged connector" (STC) approach depends very much on the quality of the library that is to act as a shared public resource; the authors do not discuss this problem in detail. They cite two studies[2,3] to argue that bacterial artificial chromosome (BAC) clones "seem to represent human DNA far more faithfully than their YAC (yeast artificial chromosome) or cosmid counterparts". Both refs 2 and 3 show that DNA is stable in BAC clones that are subject to serial propagation. The basis of instability of cosmids under these circumstances has been discussed by several workers[4] but this misses a key point: the technical problem has been that regions of DNA simply do not appear in properly grown cosmid (and YAC) libraries, not that a high proportion of deleted cosmids are found in such cases (although this is sometimes found in badly handled libraries). This suggests that the difficulty is not simply in the intrinsic instability of high-copy cosmid-replication systems. It is still unclear that BACs are any more successful with the problematic regions because, by definition, these regions have not been isolated from human DNA.

A second issue that also needs to be addressed is the actual distribution of ends of DNA molecules generated by random shear (or partial digestion) of total genomic DNA. Very little is known about the detailed distribution of end-sequences under these experimental conditions, particularly after the necessarily gentle procedures for isolation of large DNAs have been used. In most library constructions this is not normally a problem but in the STC proposal it becomes a significant issue: clustering of end points or under-representation of regions would generate gaps in STC coverage. Of course, these can be identified by the developing physical mapping reagents, which is precisely the role that these maps have always occupied in 'traditional' descriptions of the genome project.

When they first appeared, YAC libraries were widely held to overcome many of the difficulties associated with cosmids: it is only now that we have a clear understanding of their deficiencies. Indeed, Venter *et al.* point out that in the T-cell receptor region, 1 BAC clone in 17 has a deletion, suggesting 5–6% of BAC clones could be rearranged.

The reliance of the STC proposal on a single library has obvious advantages, but does require that the properties of the target library be very well understood. It is not clear that any single cloning vector is yet understood in these terms. As a consequence, the undeniable cost effectiveness, ready availability and ease of resource sharing of the library underpinning the STC proposal, should not be allowed to obscure the heavy reliance of this approach on an untested quantity, that of clone distribution and stability in a rigorous sense. It would be a mistake to assume, once again, that technical advances can be frozen by selection of exclusive approaches. In genome analysis, flexibility of resources as well as approach must remain a key strategy.

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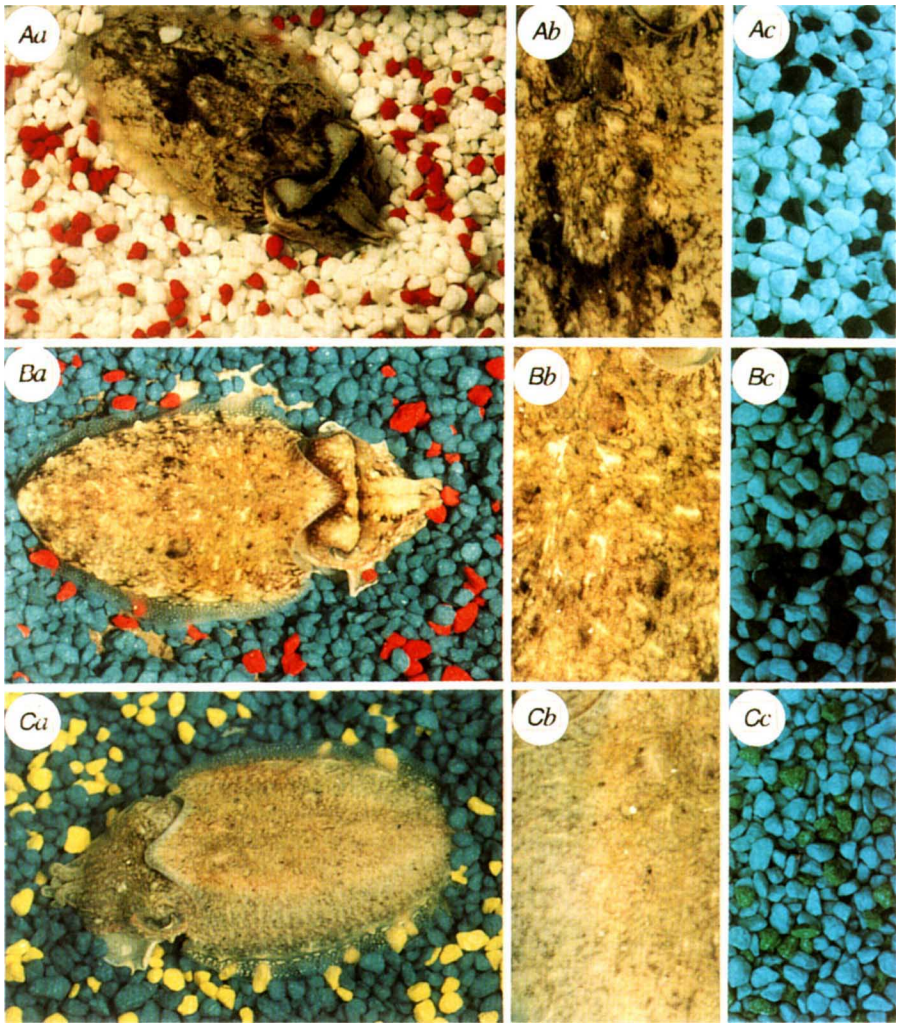
Colour-blind camouflage

SIR — Cuttlefish are remarkable for their powers of camouflage, which is in part due to their ability to generate disruptive patterns in the skin, using neurally controlled chromatophores. We show here, however, that cuttlefish produce patterns on variegated backgrounds only if these contain appreciable differences in intensity: differences in wavelength are not responded to. This result may seem counterintuitive, but it supports other evidence that cuttlefish (like octopuses) are colour blind.

Although it is well known that cephalopods that live on the bottom of the sea use chromatophores to camouflage themselves on various backgrounds, it is not generally realized that like most cephalopods (with the notable exception of the firefly squid, *Watasenia*[1,2]) they are almost certainly colour-blind[3–5]. How do such animals match their background so well? One theory[6,7] has emphasized that they achieve a degree of "general colour resemblance"[8] with iridophores and leucophores, which can reflect the predominant wavelengths in the immediate environment. Yet effective camouflage does not depend on general colour resemblance alone. One powerful and widespread technique used by animals for crypsis is patterning, especially the various forms of "disruptive" patterns[8] that break up the overall form of the body. Patterning in cephalopods is effected by the chromatophores, and because these are neurally controlled the animal can use them to generate, as appropriate, a range of finely graded patterns in its skin, from uniform, through stipples and mottles to bolds and disruptives[9].

Cuttlefish (*Sepia officinalis*) have numerous, small chromatophores containing either yellow, orange-red or dark brown pigment granules[9], and they have large eyes with a single visual pigment (λ_{max} 492 nm; ref. 10). We tested their patterning responses to a series of specially designed backgrounds. We placed individuals in small tanks containing a gravel substrate, and once they had settled we



◀ Fig. 1 *Aa*, Whole animal (mantle length about 100 mm) photographed in daylight on a prepared background — red gravel on white; *Ab*, detail of mantle skin of the same animal (magnification X 2); *Ac*, the same background photographed through a green interference filter (λ_{max} 490). Kodachrome 64. *B*, As *A*, but with red gravel on blue. *C*, As *A*, but with yellow gravel on blue.

photographed them in daylight, with and without a 'green' interference filter (λ_{max}490). We used white, blue, green, yellow and red gravels, singly and in various paired combinations, with a few stones of one colour evenly scattered on a background of the other.

Examples of the responses of cuttlefish to three combinations of coloured gravel are shown in Fig. 1, which shows that the animals are not matching their body colour to that of the gravel background. This is even more obvious when the mantle skin is examined in close-up. Yet the photographs also show that the patterning response to the three backgrounds differs considerably. A cuttlefish tends to become uniformly dark on red gravel, and uniformly pale on white, but when confronted with red gravel on white (Fig. 1*Aa*) it produces a bold, coarse patterning, with 'dark mottle'[9] on the mantle and 'disruptive'[9] on the head and arms. On a background of red gravel on blue, however, it produces a much less bold pattern: 'light mottle'[9] (Fig. 1 *Ba*); and on a background of yellow gravel on blue it shows an extremely fine, almost uniform pattern: 'stipple'[9] (Fig. 1 *Ca*). The lack of patterning with yellow on blue gravels, which make such a striking background for humans, may seem strange but is understandable if we consider the contrast of the gravels as they would appear to an animal with a single visual pigment peaking in the green (Fig. 2). It can be seen now that the yellow and blue gravels must appear very similar (Fig. 1 *Cc*); the red gravel is substantially darker than the blue, however (Fig.1 *Bc*), and the red contrasts markedly with the white (Fig. 1 *Ac*).

Our photographs provide evidence that cuttlefish do not respond to different wavelengths in the substrate as they regulate their chromatophores. However, where they perceive different intensities in the background they generate patterns in the skin: the higher the contrast in the background the bolder the pattern. Clearly, an animal with only a single visual pigment can produce patterns for crypsis where there are intensity differences in the background. This demonstration should finally lay to rest doubts about how cephalopods can camouflage themselves despite being colour blind.

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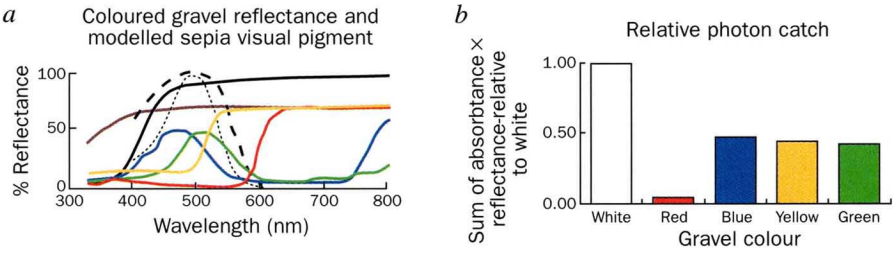

Fig. 2*a*, Spectral characteristics of gravels used (light grey line, white) and *Sepia* lens transmittance. Also plotted are a visual pigment nomogram curve (kindly supplied by Gary Bernard) for *Sepia* visual pigment (at λ_{max}492 nm), and calculated absorptance of a photoreceptor based on 0.008 μm^{-1} unit absorbance and a photoreceptor length of 200 μm. Measurements made with "Sub-Spec", a custom-made spot spectrophotometer (Oriel Instruments/Andor Technology), an underwater multi-purpose CCD spectrograph, a xenon arc lamp and a "Spectralon" white standard as reference. *b*, Calculated photon catch of a *Sepia* photoreceptor looking at each gravel type relative to white gravel. The area of each histogram block is the sum of the products of each gravel reflectance curve and absorptance curve at 1-nm intervals. The lens is assumed to act as a perfect 400-nm cut-off filter so data were summed over the range 400–600 nm only. Blue, yellow and green gravels have very similar relative brightnesses to the presumed monochromatic *Sepia* visual system. Experiments were carried out at the Marine Biological Association Laboratory, Plymouth PL1 2PB, UK.

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