

VISUAL PIGMENTS AND THE ACQUISITION OF VISUAL INFORMATION

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Summary

All the information available to the brain for the interpretation of the visual scene comes from the number of photons absorbed by a very limited number of photoreceptor types which are characterized by their spectral sensitivity. In vertebrates there are considerable differences in the spectral absorption of the rods and cones making up the retinal mosaic of different animals and, in some cases, including fish and primates, there are considerable differences between the cone sets of individuals within a species.

Broadly speaking, the spectral sensitivity of the photoreceptors is related to the spectral distribution of the ambient light and this is particularly true of the colour-biased light under water. When an animal migrates from one visual environment to another, its cone complement may change to that suited to the new conditions. However, significant differences between the cone sets of animals living within the same environment and colour vision polymorphism within a species suggest that visual tasks critical to survival or breeding success require particular visual pigment sets. A start has been made in trying to understand what tasks are best served by different pigment sets.

Introduction

Ultimately we would like to understand how the human visual system is adapted to the complex visual scene that surrounds us, but it may be easier to make a beginning by studying visual environments that are simpler. Such environments are to be found underwater where the wavelength-selective absorption of daylight means that animals live in a world where the ambient light is dim and restricted to a narrow region of the spectrum (Dartnall, 1975; Lythgoe, 1979, 1988). Fish living in these dark and homochromatic conditions may have only one receptor class, the rods, but those living in slightly shallower water often have one or two classes of cone in addition to the rods, whereas near the surface they may have three or four classes of cone in addition to the rods (Loew & Lythgoe, 1978; Levine & MacNichol, 1979). At present we do not have enough information about the depths and ambient light levels where particular classes of cone are lost because there is insufficient light for them to be useful. The behaviour and ecology of the

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animal is also important. Diurnal animals are likely to have more classes of cone than those that are nocturnal or live underground or in turbid conditions (Levine & MacNichol, 1979).

Rhodopsins, the visual pigments, are part of a larger class of G-protein-linked molecules which also include muscarinic and adrenergic receptors. All members of the class have seven helical transmembrane segments and, once activated, appear to have similar effector mechanisms which open or close ion gates in the plasma membrane. However, there are significant variations within each type of the class. Adrenergic and muscarinic receptors are divided into subtypes on the basis of their pharmacological action, whereas rhodopsins are classified into subtypes by their absorption spectra and whether the chromophore group is retinal, dehydroretinal, 3-hydroxyretinal (Kirschfeld & Vogt, 1986) or 4-hydroxyretinal (Matsui *et al.* 1988), and perhaps there are others. So far only retinal and dehydroretinal have been found in vertebrates.

Genes have been identified and sequenced for several different opsin molecules, including the human and bovine rod pigment and the blue, green and red cone pigments and four *Drosophila* pigments (Hargrave *et al.* 1983; Nathans *et al.* 1986; Zuker *et al.* 1987). So far all the opsin genes that have been found are also expressed, but it is possible that genes could be present but not expressed, or only expressed at particular stages of an animal's life. The 'deep-sea' opsin of the eel (Carlisle & Denton, 1959; Beatty, 1984) and the blue pigment of the pollack (Shand *et al.* 1988) may be examples of this.

An opsin becomes a light-sensitive visual pigment when a chromophore group in the 11-*cis* configuration is inserted into it. If the chromophore group is retinal, the visual pigment is a rhodopsin, if the chromophore is dehydroretinal, the visual pigment is a porphyropsin. Rhodopsins and porphyropsins differ in both the wavelength of maximum absorption and the breadth of their spectral absorbance curve. The difference is small for blue-sensitive pigments, but increases progressively at longer wavelengths (Dartnall & Lythgoe, 1965; Whitmore, 1988). Thus a rhodopsin of λ_{\max} 565 nm becomes a porphyropsin of λ_{\max} 630 nm with a correspondingly broader spectral absorption curve (Fig. 1). There appear to be no visual pigments that absorb at longer wavelengths than this but, nevertheless, a freshwater fish with a 630 nm porphyropsin can see substantially further into the near infrared than can humans with the rhodopsin analogue which has a λ_{\max} at 565 nm.

Environmental light and visual pigments

Penetration of light into natural water

There are striking differences in the spectral distribution of natural water according to the amount of chlorophyll and the dissolved products of natural decay that it contains (Fig. 2). Pure water is blue partly because of the selective absorption of water molecules, and partly because of Rayleigh scatter, which is greater at short than at long wavelengths. The salts dissolved in sea water have

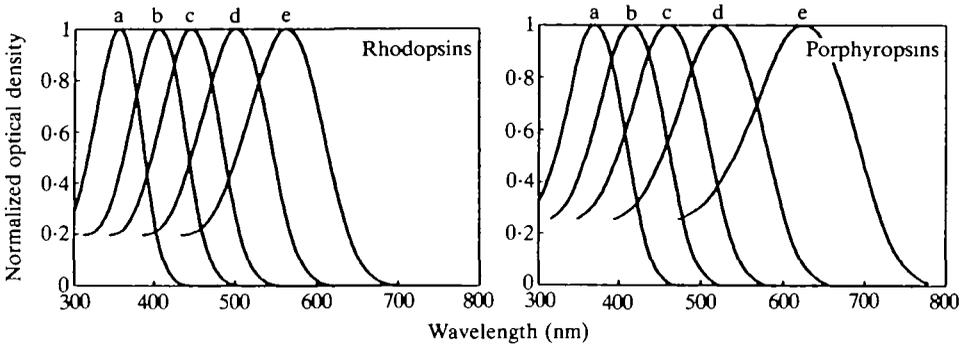


Fig. 1. Absorbance spectra of five rhodopsins compared with their porphyropsin analogues. At longer wavelengths the porphyropsin absorbance spectra become broader and more red-sensitive than their rhodopsin analogues. The templates are based on Knowles & Dartnall (1977) and transformed by Mansfield's method (MacNichol, 1986). The analogue pairs are amongst those listed by Whitmore (1988).

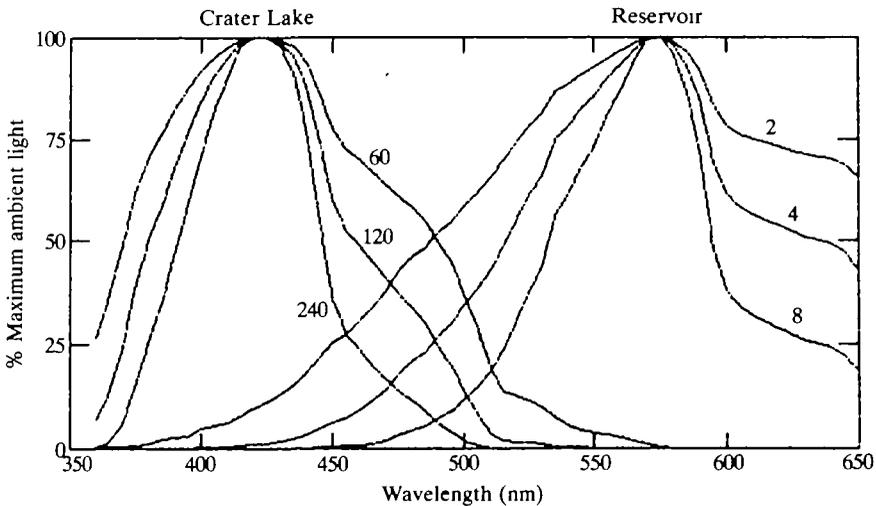


Fig. 2. The spectral distribution of downwelling daylight at optically equivalent depths in very clear water containing little chlorophyll or dissolved organic matter (Crater Lake, Oregon) and fresh water containing significant amounts of chlorophyll and dissolved organic matter (San Vicente reservoir, California). The numbers are depths in metres. Values are calculated from the spectral attenuation coefficient of downwelling light measured by Tyler & Smith (1970) and do not take into account the spectral distribution of daylight, which is variable but will have little effect on the shape of the spectral distribution curves at these depths. Notice the reduction in the bandwidth of available light as the depth increases. At 60 m in Crater Lake and 2 m in the reservoir, surface light intensity is reduced by a minimum of 60%; at 120 m in Crater Lake and 4 m in the reservoir, surface light is reduced by 85%; at 240 m in Crater Lake and 8 m in the reservoir, surface light is reduced by 98%.

almost no effect on its colour and pure fresh water is the same blue as ocean water. In fact, fresh water very rarely looks blue because it contains enough nutrients to support a rich crop of chlorophyll-containing phytoplankton. It is also coloured by the yellow and brown products of vegetable decay that originate from phytoplankton and from run-off from the land (Jerlov, 1976; Baker & Smith, 1982). Compared with most fresh waters and inshore water, the open ocean and the clear blue seas of the Mediterranean are poor in nutrients and remain the blue colour of pure water. Inshore waters such as those that surround the coasts of the North Atlantic vary from blue-green to yellow-green according to the amount of phytoplankton and dissolved organic matter. Fresh water is often green or yellow-green, but in places where it has filtered through forest litter or peat it is stained brown or reddish brown in colour and it is red and near infrared light that penetrates furthest (Spence *et al.* 1971; Muntz & Mouat, 1984).

Deep-sea animals

It is something of a paradox that we should know the most about the visual pigments of those most inaccessible of animals – the deep-sea fishes. At high sun angles visually useful light may penetrate the clearest ocean waters to a depth approaching 1000 m (Dartnall, 1975) but, where there is an overcast sky at night, the maximum depth of vision may only be a few metres below the surface. An alternative source of light is the bioluminescence produced by fishes and invertebrates. Like the ambient daylight at mesopelagic depths, bioluminescence also tends to be blue or blue-green in colour (Widder *et al.* 1983; Herring, 1983). These colours may be used partly because they penetrate furthest through the water, and partly because they are the most useful for camouflage. The visual pigments of 89 species of deep-sea fish have been investigated (Partridge *et al.* 1988, 1989) and there is no doubt that most of them have pure rod retinas containing rhodopsins that absorb most strongly in the 470–490 nm blue region of the spectrum (Fig. 3) (Crescitelli *et al.* 1985; Fernandez, 1978; Denton & Warren, 1957; Munz, 1958; Partridge, 1989; Partridge *et al.* 1988, 1989).

The scales of many pelagic fishes contain guanine crystals that are orientated in such a way that the curved flank of the fish acts as a vertical mirror (Denton & Nicol, 1965). Beneath the immediate surface waters, the light penetrating into the oceans is symmetrical about the vertical axis, and a vertical mirror viewed from most directions will reflect light of the same colour and intensity as the light against which it is seen. This ‘mirror camouflage’ does not work for the vertically upward or downward directions of view. Camouflage against downward-searching predators such as sea birds simply needs dark pigmentation along the dorsal surfaces. Camouflage against upward-searching predators is more difficult since the silhouette is always going to be darker than the bright downwelling daylight. The solution adopted by many vertebrates and invertebrates is to arrange for a bank of downwardly directed photophores giving light that exactly matches the downwelling daylight (Warner *et al.* 1979). Fishes that have well-developed ventral photophores and silvery flanks for spacelight camouflage are likely to live where

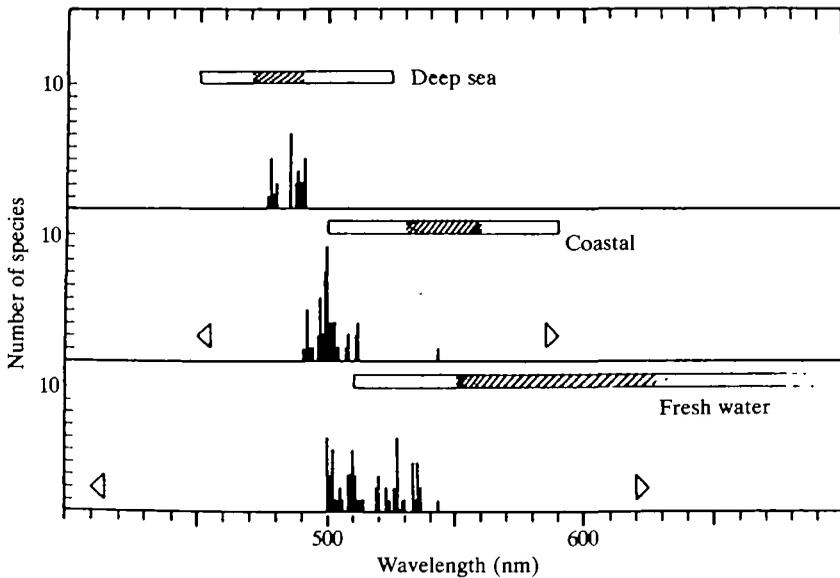


Fig. 3. The relationship between the visual pigments in the rod outer segments of fishes and the spectral waveband available for vision in different aquatic environments. The hatched areas of the horizontal bars represent the absorption maxima of visual pigments which would give greatest sensitivity to fishes living in those waters.

there is residual daylight. Fishes living deeper than 1000 m or those that only venture into shallower water at night tend to have dark bodies and fewer and less-developed photophores. Bioluminescence is present at all depths, but it appears to be more frequent at depths less than about 1000 m (Marshall, 1979).

All the fishes with counterlighting and mirror camouflage that have been examined have one type of retinal rod containing a single rhodopsin. However, a significant percentage of the deep-living darker-coloured species have two classes of rod containing different visual pigments (Partridge *et al.* 1988, 1989). It is as though the presence of homochromatic blue daylight in the shallowest quarter of the ocean demands that photophores should emit blue light and that photoreceptors should also be most sensitive to the blue daylight and the blue light from the photophores. Deeper than this, where there is no daylight, a more versatile visual system using more than one class of photoreceptor is allowed, perhaps tuned to distinguish between different bioluminescent sources by the shape of the spectral radiance curves.

There are two related families of fish whose members fall into the dark-coloured category that are worthy of notice. Members of the Malacosteidae and Melanostomiidae have large red and green photophores situated just below the eye. It is now firmly established that there are two classes of rod in their retinas. In *Pachystomias microdon* the two classes have λ_{\max} values near 513 and 539 nm. In *Malacosteus niger* the λ_{\max} are near 521 and 540 nm (Bowmaker *et al.* 1988;

Partridge *et al.* 1989; Partridge, 1989). In both cases the short-wave rod contains mainly rhodopsin and the long-wave rod mainly porphyropsin. There are slight differences in the λ_{\max} of the two classes obtained by different workers, but this may be largely due to the fact that the rhodopsin rods contain traces of porphyropsin and the porphyropsin rods contain traces of rhodopsin. The difference between a rhodopsin and a porphyropsin is that rhodopsins have 11-*cis* retinal as the chromophore group and porphyropsins have 3-dehydroretinal. It is likely that the opsin in the two classes of rod is the same, and the difference lies solely in the chromophore group. Some other dark-coloured deep-sea fishes, such as *Bathylagus bericoides*, also have two classes of rod but these, judging by the shape of their spectral absorption curves, contain different rhodopsins and there are no porphyropsins (Partridge *et al.* 1988). Differently coloured photophores have not been reported from these species and at present we do not know why they should have two types of rod whereas other species have only one.

Fishes living at intermediate depths

There are few systematic data about the visual pigments in fishes living at depths less than about 200 m in blue oceanic water and we must turn our attention to green coastal water and green or brown fresh water where more data are available (Loew & Lythgoe, 1978; Levine & MacNichol, 1979; Lythgoe, 1988). As a *very rough* guide, visually intermediate depths in coastal water might mean something between 10 m and 50 m in some coastal waters and between 5 and 20 m in fresh water. We owe the most systematic study on the relationship between depth and visual pigments in rods and cones to Levine & MacNichol (1979). They found that fishes that live in tropical fresh water, near and on the bottom, and particularly those that are nocturnal or have a well-developed olfactory apparatus, tend to possess only two classes of cones. One of these cone classes typically contains a porphyropsin of 600–640 nm λ_{\max} which would be most sensitive to the long-wavelength light that penetrates deepest into the type of water where they live. Fishes living at intermediate depths in the green coastal waters of the English Channel also have two cone pigments, but these have rhodopsins and are most sensitive to the green ambient light that prevails in these waters (Fig. 4).

Terrestrial animals and fishes living in shallow water

Fishes that spend at least some of the time in well-lit shallow water are exposed to daylight that is not very different in spectral distribution from that experienced by terrestrial animals. It is interesting that fishes living in these very shallow waters lack the long-wave-sensitive porphyropsins and this includes freshwater fishes which one might expect to have them (Fig. 5). The lack of red-sensitive pigments cannot be explained by any shortage of red light, although the relative amount is less than at greater depths in fresh water. Muntz & Mouat (1984) have noted that the seasonal changes in the ratio of rhodopsin to porphyropsin in rudd and trout follow this trend, since in summer the fish tend to feed near the surface and have high percentages of rhodopsin, whereas in winter they move to deeper water and

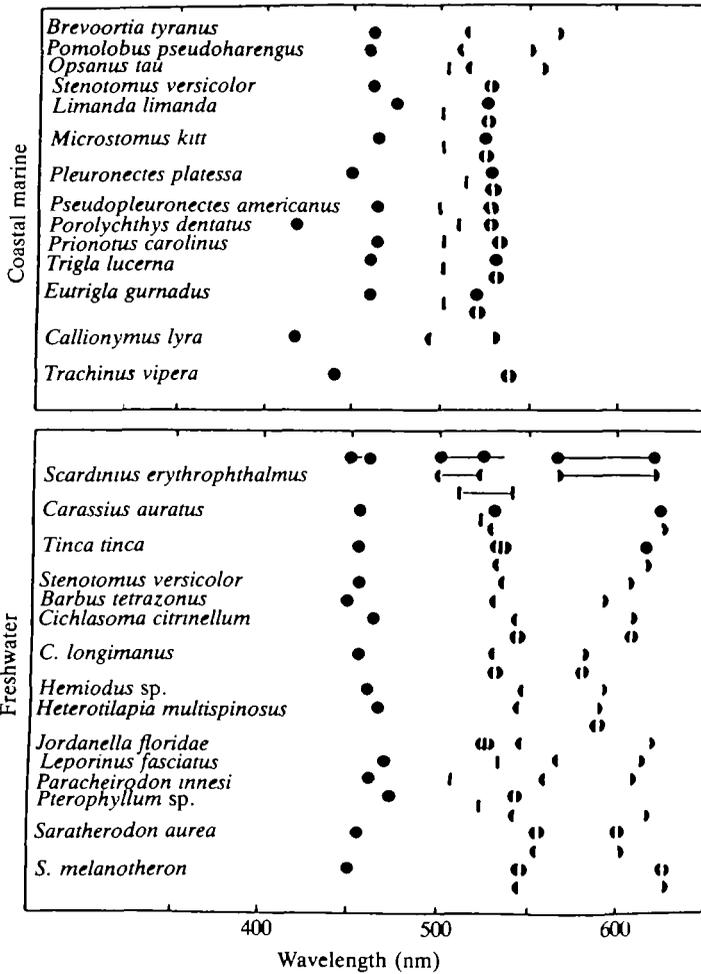


Fig. 4. The visual pigments present in the rods and cones of various species of fish that live at moderate depths in coastal water and freshwater. Long-wave-sensitive visual pigments (mostly porphyropsins) are rare in fish from coastal water, but frequent in fish from fresh water, which is most transparent at longer wavelengths. Filled circles, λ_{\max} of visual pigments in single cones; half circles, λ_{\max} of visual pigments in one member of twin or paired cones; vertical bars, λ_{\max} of the visual pigments in the rods.

their retina contains a higher percentage of porphyropsin. In Fig. 6, the visual pigments of shallow marine and freshwater species are compared with those of terrestrial species. Taking the groups as a whole, there is an overall similarity between them, although there are several differences in detail. Some of these differences may be due to limitation in the gene pool of particular phylogenetic lines. However, we need many more data than we have at present to prove the absence of particular visual pigments and there is little actual evidence to suggest

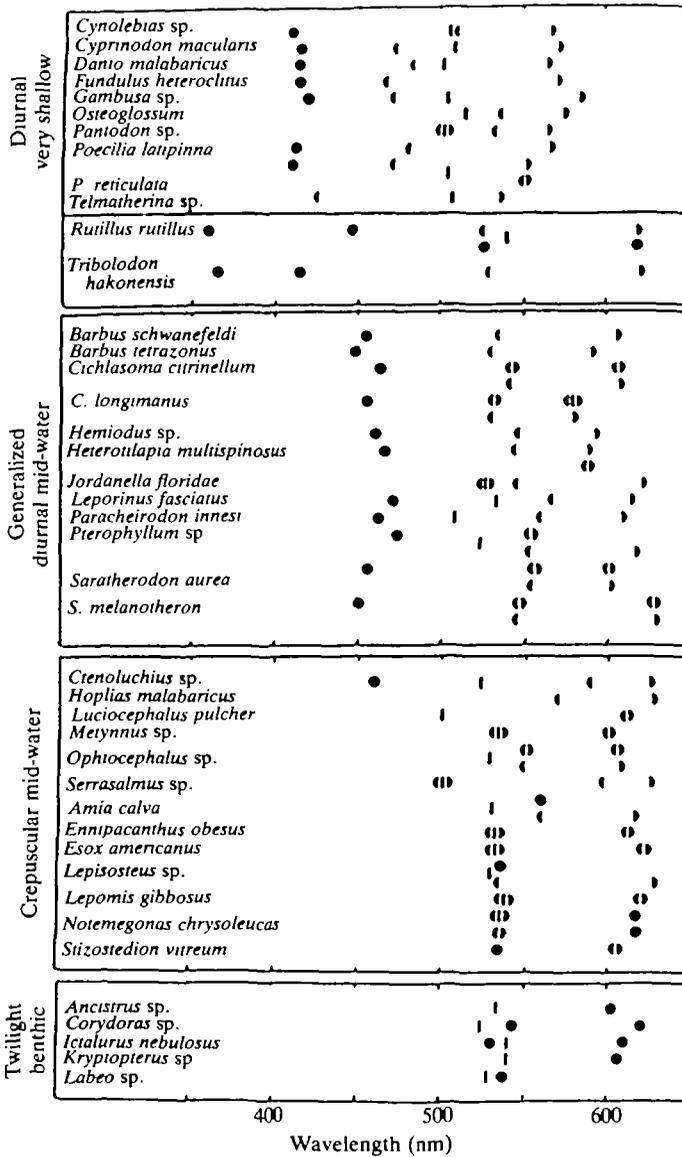


Fig. 5. The relationship between visual pigments and the depth that the fish lives at in fresh waters. Note that the spectral range of visual pigments is reduced as the depth increases and the spectral bandwidth of incident light available for vision becomes narrower. Symbols as for Fig. 4.

that certain groups of animals are genetically incapable of producing particular visual pigments.

Information from rods and cones

So far we have been concerned with the sensitivity of the photoreceptors, but

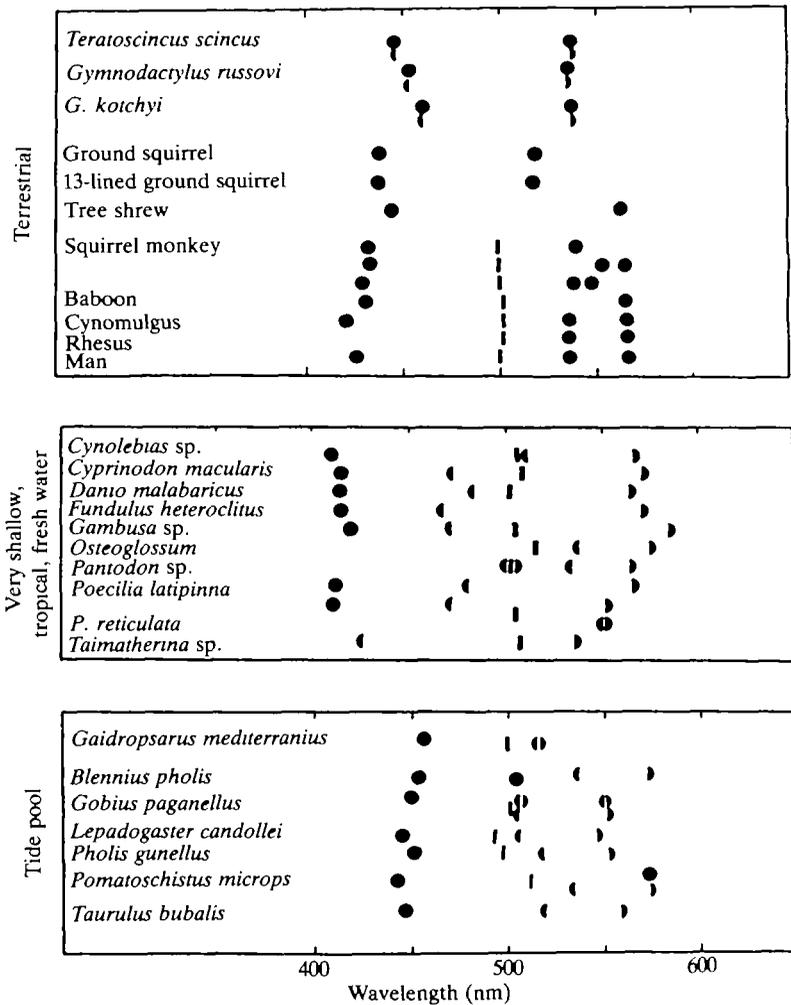


Fig. 6. Comparison between the visual pigments of terrestrial animals and those living in very shallow fresh water and very shallow coastal water. Note that they are all located in approximately the same region of the spectrum. Symbols as for Fig. 4.

what actually matters to an animal is the amount of useful information it can extract from the retinal image. Photoreceptors are photon counters and it is the number of photons that are absorbed that modulates the strength of the neural message originating from the photoreceptors. However, the actual number of photons that arrive at the photoreceptor can only be predicted as a statistical probability and, even at the light levels prevailing at sunset, the number of photons available limits thresholds for the perception of contrast, detail and movement (Barlow, 1964; Land, 1981). A further problem is likely to be spurious signals generated by 'spontaneous' thermal isomerizations of the visual pigment chromophore (Barlow, 1988; Aho *et al.* 1988). There are some 10^9 rhodopsin molecules in

a (frog) rod (Rodieck, 1973) and if the isomerization of one rhodopsin molecule by a photon of light is sufficient to initiate a signal, it follows that almost all the remaining molecules must not isomerize spontaneously if spurious signals are not to mask the light signal with physiological noise. It is possible that the reason why rods tend to contain rhodopsins of λ_{\max} between 470 and 510 nm is because it is those that are least likely to have spontaneous isomerizations. However, there is little or no evidence on the point.

Most visual tasks involve detecting the differences between elements of an image either in space or in time. The larger the change, the more likely it is to be detected at low light levels or when there is a high level of physiological noise. Where two radiances are to be distinguished, it makes sense for the photoreceptors to gather most photons at wavelengths where the two radiances are the most different, always providing that there are enough photons to make statistically reliable judgements. The relationship between the size of a contrast ($\Delta I/I$) to be detected and the level of illumination required to make judgements with varying degrees of confidence has been discussed by Land (1981). In many situations the greatest contrasts occur in regions of the spectrum where radiances are small, and the λ_{\max} of the best visual pigment to detect them is likely to be a compromise between the need to maximize contrast, the need to sample as many photons as possible and the need to employ a noise-free photoreceptor. At high levels of illumination the limits to contrast perception may be set by physiological noise generated by the neural circuitry of retina and brain. However, visual noise, whether of environmental or physiological origin, is likely to be an important factor in setting thresholds and delimiting the spectral band that is useful for vision.

Vision in the ultraviolet and infrared

There is no doubt that many animals can see at shorter wavelengths than we can, others can see into the near infrared and some freshwater fish can see in both the ultraviolet and the infrared. The long-wavelength limit for vision is likely to be set by the absorption of the most long-wavelength-sensitive visual pigment which appears to be a porphyropsin with a λ_{\max} of about 630 nm (Lythgoe, 1988). This will allow useful sensitivity to light longer than 740 nm, where the absorption of a 630 nm porphyropsin falls to about 10 % of its maximum value. Ultraviolet vision is well established by the use of electrophysiological techniques in insects, birds and shallow-living fishes. In fishes, visual pigments that have their λ_{\max} in the 350–400 nm region of the spectrum have been measured directly by microspectrophotometry and they are probably sensitive to light of wavelengths as short as 280 nm, where absorption by aromatic amino acids effectively puts a limit to short-wave vision. There is strong evidence that ultraviolet sensitivity in some insects is promoted by the attachment of a sensitizing pigment to the opsin molecule. In the higher flies such as *Musca*, *Calliphora* and *Drosophila*, the short-wave-sensitive pigment has a 3-hydroxyretinal chromophore and a λ_{\max} of 420 nm. However, it

has attached to it a molecule of 3-hydroxyretinol which sensitizes it to ultraviolet light, the conjugated pigment having a strong peak at 420 nm. In the simuliid flies the pigment is a rhodopsin and the sensitizing pigment is retinol (Kirschfeld & Vogt, 1986; Kirschfeld *et al.* 1988). We humans are only prevented from seeing ultraviolet light by the absorption of the pre-retinal media, especially the lens (Wyszecki & Stiles, 1967).

Because we humans are limited in our spectral range we have to rely on other evidence, sometimes supported by photography and video, to give us an idea how much visual information we miss in our normal lives. Photographic colour film is available which allows us to visualize the visual scene in the near infrared at wavelengths below about 1000 nm, where it is to be expected that many freshwater fish can see quite well. The film shows the very strong reflectance of foliage at these long wavelengths, but has so far not shown any extra information. Perhaps freshwater fishes are sensitive to these wavelengths because they penetrate furthest through water stained brown by dissolved organic matter. It has been suggested recently that the deep-sea crustacean *Rimicaris exoculata*, which congregates around the very hot (350°C) volcanic vents to feed, can use the rhodopsin (λ_{\max} 500 nm) contained in special organs in their cephalothorax to detect the black-body radiation from the hot water (van Dover *et al.* 1989; Pelli & Chamberlain, 1989). These authors suggest that the water is just sufficiently hot to give enough light at 600 nm to isomerize rhodopsin molecules at a just detectable level. Not taken into account, however, is the significant absorption of 600 nm light by even very clear waters, or the problems of distinguishing infrared radiation from visible blue-green light from bioluminescent animals to which the rhodopsin is very sensitive.

Using an ultraviolet-pass filter in conjunction with a standard camera lens and monochrome film, it is possible to visualize patterns that are only visible in the ultraviolet. This approach has been particularly useful in seeing 'pollen guide' patterns on flowers (Lythgoe, 1979) and, in one example, patterns on the flanks of a fish (Harosi & Hashimoto, 1983). Using monochrome photography it is often difficult to decide which objects are dark because they are in shade and which are dark because they absorb ultraviolet light. By substituting an ultraviolet-sensitive tube for the blue-sensitive tube in a colour video camera, it has been possible to overcome the difficulty of ambiguity due to shading. Despite difficulties due to incorporating an ultraviolet channel into an optical system for which it is not designed, it is evident that there is other visual information in the ultraviolet (Loew & Lythgoe, 1985). Leaves reflect different amounts of ultraviolet light according to species and age, and some that are exposed to high levels of ultraviolet light appear to have ultraviolet-absorbing pigments which would be visible to an animal with ultraviolet receptors. In general, any object that owes its colour to scattering is likely to reflect ultraviolet light strongly. Clouds and blue sky both fall into this category, as do the white feathers of birds, snow and many light-coloured rocks. Iridescent interference colours often do not reflect ultraviolet light, even when they appear deep blue in colour. There is really no way of

telling by eye whether an object reflects or absorbs ultraviolet light and the reality is often counterintuitive. For example, most violet and blue flowers are ultraviolet-absorbers, whereas red poppies reflect very strongly in the ultraviolet.

It is likely that the shapes, patterns and colours of flowers have co-evolved with the visual system of their pollinators. The presence of 'pollen guides' in flowers is quoted as an example of this co-evolution. Sometimes these pollen guides are visible to humans and take the form of marking the area where the nectar and pollen are located. Sometimes, however, the pollen guides are only revealed by ultraviolet video or photography.

Birds, like insects, are exposed to ultraviolet light and it is not surprising that they too have ultraviolet receptors. Fishes living at shallow and moderate depths also possess ultraviolet receptors and one can only suppose that useful amounts of ultraviolet light penetrate through the water. Many fishes feed very close to the water surface, or even at the surface itself, and may only need to see their prey from a distance of a few centimetres. It may be that objects like small plankters suspended very near the surface reflect the ultraviolet light that has travelled the very short distance from the surface, whereas light from the background spacelight which has travelled further through the water is poor in ultraviolet light (Lythgoe, 1988). Conversely, Bowmaker & Kunz (1987) suggest that the plankters may be net absorbers of ultraviolet light, whereas the background is likely to be bright because it scatters this short-wavelength light.

Particles of molecular size scatter short-wavelength light more than long-wavelength light and this is the reason why clear sky is blue and part of the reason why optically pure water is blue. Scattered light is also plane-polarized with the plane of maximum polarization at right-angles to the light ray. For this reason skylight and underwater spacelight are plane-polarized. Many animals are able to use the plane of polarization for navigation (Waterman, 1981). In bees it is the ultraviolet receptors that are used to detect the plane of polarized light. However, in the more nocturnal cricket *Gryllus bimaculatus*, which has receptors maximally sensitive at 332 nm, 445 nm and 515 nm, it is the blue-sensitive 445 nm receptors that are used to scan the sky above and are the polarization detectors (Zufall *et al.* 1989). Differential sensitivity to the plane of polarized light is not necessarily confined to one photoreceptor class: the ultraviolet cones in the goldfish are most sensitive to light when the e-vector is vertical, whereas the red- and green-sensitive cones are most sensitive when it is horizontal (Hawryshyn & McFarland, 1987). The blue-sensitive cones in the goldfish show no differential sensitivity to polarized light.

Colour vision variation within a species

Some lower vertebrates have the ability to alter their visual pigments at different stages of their life history (Beatty, 1984; Shand *et al.* 1988). As a general rule, it appears that colour vision polymorphism is the result of having different opsins in the visual pigment, whereas most seasonal or developmental changes in visual

pigment through life are the result of substituting one chromophore group for another. It is often possible to recognize environmental reasons why particular visual pigments should appear at different stages of life but, so far, there have been few convincing proposals why different animals sharing the same environment should be polymorphic for visual pigments. It can only be supposed that there is some specialization for particular visual tasks. It should be said, however, that no one has firmly proposed a set of visual tasks that a human deuteranope or protanope can do better than someone who has 'normal' colour vision (Dartnall *et al.* 1983).

Chromophore substitution

As was mentioned earlier, the substitution of dehydroretinal for retinal as the chromophore group in the visual pigment molecule results in a shift in spectral absorbance to longer wavelengths. The effect is negligible at short wavelengths but is as much as 60 nm at long wavelengths (Dartnall & Lythgoe, 1965; Whitmore, 1988). This is significant when it is considered that only 40 nm separates our own long-wave-sensitive rhodopsins. Freshwater animals, including crustaceans (Suzuki & Eguchi, 1987) that live more than a few centimetres below the surface, tend to have porphyropsins because of the long-wave bias in the environmental light (Lythgoe, 1979). Most other animals have rhodopsins. Many animals that move from fresh water onto the land or migrate between the oceans and fresh water show chromophore substitution (Beatty, 1984). Thus tadpoles, of both frogs and toads, have mainly porphyropsins, whereas the adults have rhodopsins. Migrating salmon and trout have rhodopsins when living in the sea, which they substitute for porphyropsins when they move up the rivers to breed. Freshwater eels living their adolescent lives have porphyropsins but, in anticipation of their sexual migration to the sea, substitute rhodopsin for porphyropsin (Carlisle & Denton, 1959).

Opsin changes

Eels appear to take one further step beyond the substitution of chromophore groups for, in reaching their breeding grounds in the tropical west Atlantic, they experience the same homochromatic blue light as deep-sea fishes, and adopt a 'deep-sea' blue-sensitive pigment with similar spectral absorption characteristics. Until recently the eel was the only animal known to substitute or change rhodopsins, but Shand *et al.* (1988) have shown that the pollack *Pollachius pollachius*, which is a marine coastal fish of northwest Europe, appears to change the rhodopsin in the blue-sensitive class of cone from a λ_{\max} of about 420 nm to one of about 460 nm when they have grown to 50–70 mm standard length (Fig. 7). The other cone pigments do not change. The change occurs at about the time when the juvenile pollack changes from a diet of small plankters, usually caught near the surface, to one of small fish, usually captured in rather deeper water. The trout also shows a change in the short-wave-sensitive cones. Up to the age of 2 years the trout has a class of ultraviolet-sensitive cones that are lost as it grows older and

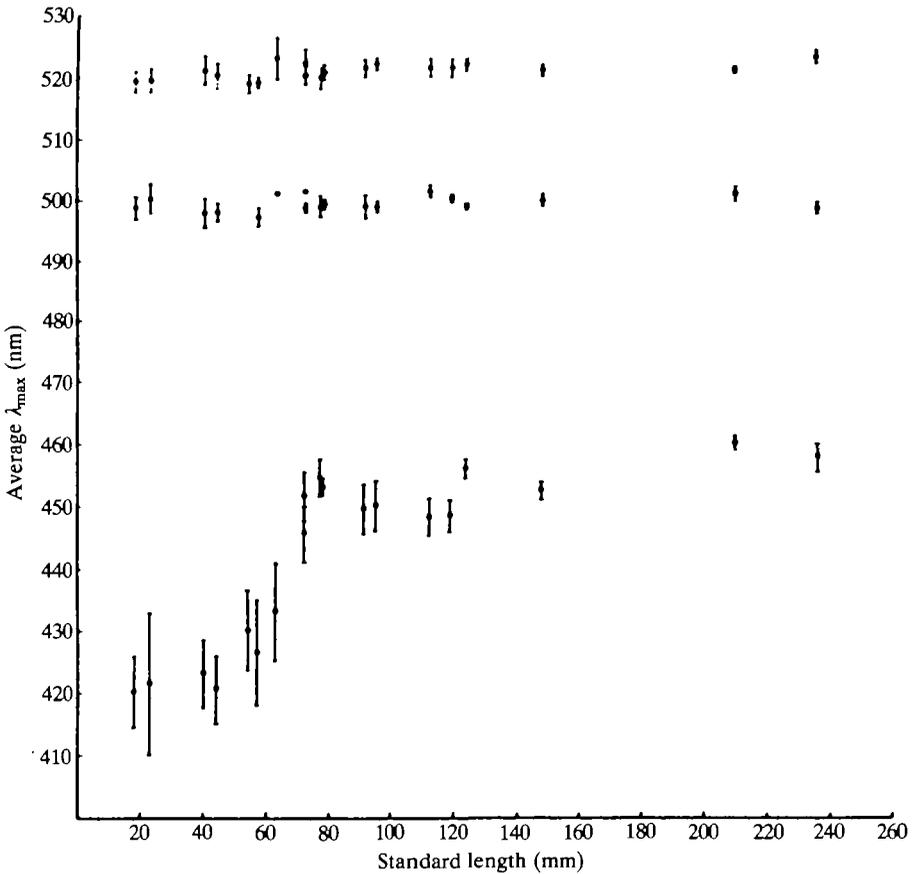


Fig. 7. The visual pigments in the cones of the pollack during its transition from a plankton eater in shallow water to a predator on small fish in generally deeper water. The change in λ_{\max} of the blue-sensitive pigment coincides with the change in lifestyle. The standard deviation and the means of the data are plotted.

changes from a shallow feeder on plankton to a more predatory feeder on fish in somewhat deeper water (Bowmaker & Kunz, 1987).

Opsin polymorphism

From our human perspective it is the norm for each individual to retain the same colour vision mechanism through life, and for each individual to have the same mechanism as his neighbour. However, it is now becoming clear that many animals change photopigments according to season, age or lifestyle, whereas others are polymorphic for visual pigments. Anomalous red-green colour vision in human males appears to be the result of a type of visual pigment polymorphism that is more strongly established in non-human primates, particularly the New World monkeys (Bowmaker *et al.* 1983, 1985; Dartnall *et al.* 1983).

Cone polymorphism has also been described in a fish, the guppy (*Poecilia*

reticulata) (Archer *et al.* 1987; Archer, 1988). These, in common with other very shallow-living fishes, have a pure rhodopsin retina and are only slightly more sensitive to long-wave light than are primates. Guppies appear to have a class of ultraviolet-sensitive cones, a blue-sensitive cone at 410 nm and another at 465 nm, a rod at 503 nm, and the polymorphic group of cones of λ_{max} 533, 548 and 572 nm. Analysis of the shape of the spectral absorption curves suggests that the 548 nm cone probably contains a mixture of the 533 nm and 572 nm rhodopsins. Phenotypes have been identified that have all three polymorphic cones, the 572 nm cone alone, the 533 nm and the 548 nm cone, and the 533 nm and the 572 nm cone. Archer (1988) failed to find the 533 nm cone class on its own, but more recent work suggests that this class is also present. Unlike in the primates, there is no evidence of cone polymorphism being sex-linked, despite the fact that guppies show strong sex differences in both size and coloration. The females are always brown, whereas the males are smaller and have striking patterns which vary from fish to fish.

Visual pigments and the discrimination of natural objects on land

In the past we have considered that the ordinary terrestrial scene is much too complicated to analyse why different animals have the visual pigments that they do. Instead we have concentrated on the underwater visual scene, where the wavelength-selective absorption by water limits the light available for vision to a relatively narrow waveband. Recently, however, we have begun to question whether many visual scenes on land are really so complicated that they defeat analysis. Animals living in environments where vegetation is plentiful experience a world that is predominantly green and brown. In such an environment many important visual tasks are likely to involve distinguishing between objects of very similar colour, such as finding cryptically coloured insects which is, for example, an important visual task for foraging squirrel monkeys. By contrast, colours used in display are very different in spectral radiance from their background, and almost any set of visual pigments that absorb in the same general region of the spectrum will serve to distinguish them.

A single photoreceptor can give information about the amplitude (brightness) of a radiance, but two photoreceptors of different spectral absorbance are required to give any information about the shape of a spectral radiance curve; i.e. about colour. The signals from two different photoreceptors can be combined additively $V_1 + V_2$ to give unambiguous information about the amplitude of the radiance; or one of them can inhibit the other $V_1 - V_2$ to give information about the shape of the spectral reflectance curve which is ambiguously combined with its amplitude. The brightness element can be stripped from $V_1 - V_2$ by dividing it by $V_1 + V_2$ and there is good neurophysiological evidence for the presence of $V_1 + V_2$ and $V_1 - V_2$ channels in vertebrates (Ingling & Martinez, 1983; Derrington *et al.* 1984). The comparison between the two channels to give unambiguous information about

colour, as distinct from brightness, apparently occurs at a high level in the brain (Livingstone & Hubel, 1988).

We have chosen to express the chromaticity (C) of a single object as:

$$C = (V_1 - V_2)/(V_1 + V_2) \quad (1)$$

When the spectral radiances of similarly coloured objects are measured, the spread in the values of C will be greater for objects that differ more in colour. Objects are likely to differ more at some wavelengths than at others, and visual pigments that collect most photons at wavelengths where the objects are most different are likely to give the greatest variations in C . We set ourselves the task of finding out which pairs of visual pigments give the greatest range of values of C for different classes of naturally occurring objects. The range in C , for each pair of visual pigments, was measured conventionally as the standard deviation of the values of C for the chosen class of objects.

All the samples were collected in summer (August) from natural woodland near Bristol, England. The collections were (a) mature healthy green leaves, (b) fallen leaves and (c) items of forest litter, such as rotten twigs, brown fungi and rotten wood, but not including fallen leaves or anything that was in any way tinged green with chlorophyll. The specimens were taken into the laboratory and their spectral reflectance measured with a scanning spectroradiometer and the data acquired by computer. We made the simplifying assumption that they would be viewed by photon-white light that was sufficiently bright to avoid problems with stochastic variation of photon flux. The spectral absorptions of the visual pigments in the individual photoreceptors were calculated by assuming an optical density of 0.05 and Dartnall's 'nomogram' spectral absorption curve for rhodopsin, transformed through the spectrum using the Mansfield transform (MacNichol, 1986). The values of C were computed for each object for every combination of 26 rhodopsins (λ_{\max} range 350–600 nm at 10 nm steps in λ_{\max}). Finally, the standard deviations of the values of C for each of the possible pairs of visual pigments 'viewing' the different collections was computed and the results are displayed in the contoured 'varygrams' shown in Fig. 8.

The higher the standard deviations for C , the greater the number of objects that can be distinguished on the basis of chromaticity. It is clear that the best pair of visual pigments for distinguishing green leaves is not the same as that which is best for distinguishing between items of brown forest litter. However, in all the cases that we considered, the best pair includes a blue-sensitive rhodopsin with λ_{\max} in the 420–450 nm range. For green leaves the best long-wave pigment is in the 510–520 nm range and for forest litter the best long-wave pigment is 570 nm or longer. So far, the longest known rhodopsin λ_{\max} is 572 nm. For mammals the most red-sensitive rhodopsin has a λ_{\max} at about 565 nm.

In their present form these calculations do not consider trichromatic vision, and they do not take into account statistical uncertainty due to low numbers of photons in dim light, a consideration which is likely to be most important at short wavelengths. Nevertheless, it is interesting to compare the pigment pairs that

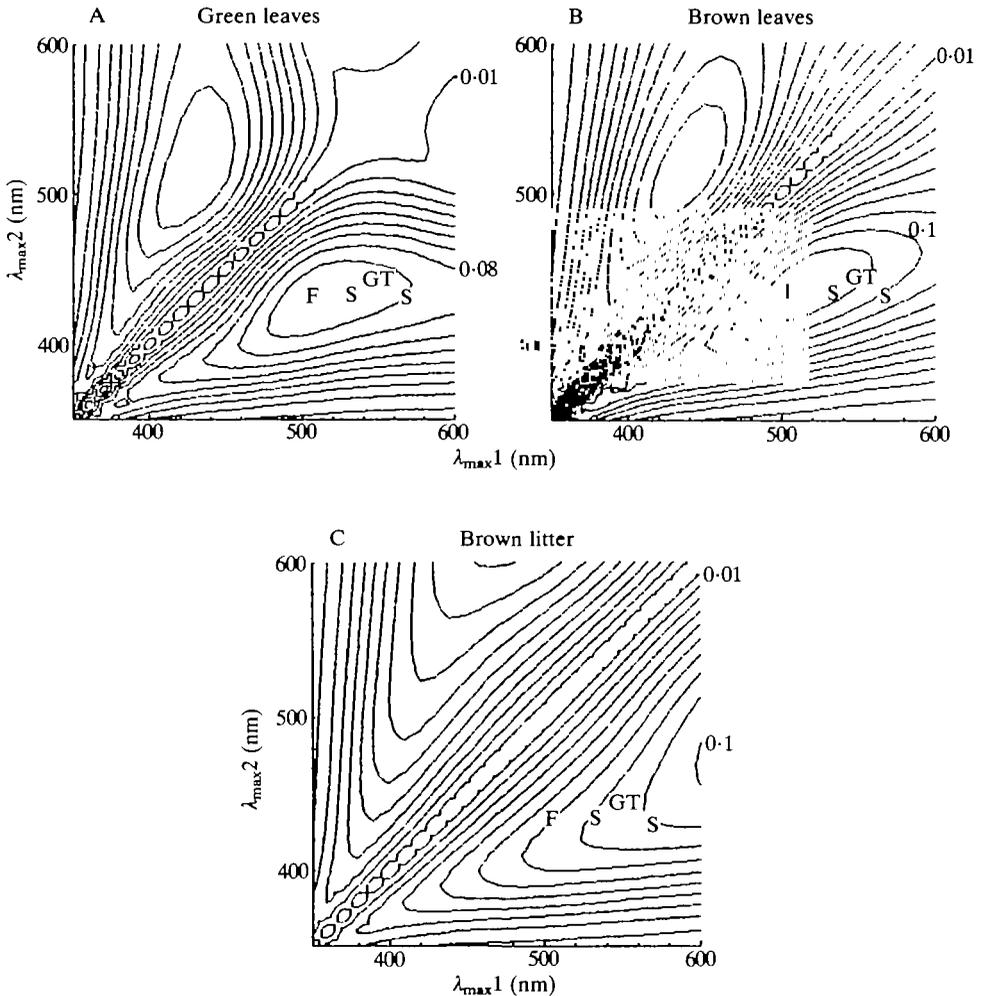


Fig. 8. 'Varygrams' showing the amount of variation in the value of C , a measure of chromaticity, for a retina containing different pairs of rhodopsins. The rhodopsins are 'looking at' (A) various green leaves, (B) fallen dead deciduous leaves and (C) items of brown forest litter. The contours represent the standard deviation of the values of C , the higher the value, the more different chromaticities can be distinguished. The letters represent the rhodopsins known to be present in the two types of cone of various dichromatic animals. G, grey squirrel; T, tree shrew; S, two dichromatic phenotypes of the squirrel monkey; F, the rhodopsins in the two types of rod in the adult frog.

actually occur in some known dichromats (Mollon *et al.* 1984; Jacobs & Neitz, 1986; Jacobs *et al.* 1985; Blakeslee *et al.* 1988) with the 'best' pigment pairs as computed here. For example, tree shrews and the squirrel monkeys that lack the green pigment would be best at distinguishing between twigs, dead leaves and rotten wood, whereas those squirrel monkeys that lack the red pigment would be best at distinguishing between different items of green foliage. It is also interesting

that two species of frog, *Rana pipiens* and *R. temporaria*, have two classes of rod: one with a λ_{\max} at about 432 nm, and another with a λ_{\max} varying between 500 and 510 nm (Bowmaker *et al.* 1975). If the signals from the two rod types were compared in the neural network to give a form of colour vision, they would be well-adapted for distinguishing between different shades of green. The importance of a blue-sensitive cone in colour discrimination was not something we had anticipated, but it is interesting that the molecular structures of the four (human) rhodopsins give some reason for believing that the blue-sensitive rhodopsin separated from 500 nm rhodopsin in ancient evolutionary time, but that the separation of the green- and red-sensitive pigments was a comparatively recent evolutionary event (Nathans *et al.* 1986).

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