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OPTIMIZATION, CONSTRAINT, AND HISTORY IN THE EVOLUTION OF EYES

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ABSTRACT

Several features of the evolution of eyes and photoreceptors are examined in an effort to explore the relative roles of adaptation and historical and developmental constraints. Optical design shows clear evidence of adaptation, which in some respects approaches optima predictable from physics. The primate fovea, on the other hand, illustrates how adaptation can be channeled by developmental heritage.

The primary structures of opsins reveal multiple evolutionary lineages within both Drosophila *and humans. The pigments of vertebrate rods comprise a subset of opsins whose evolutionary relationships map onto the phylogeny of the parent species. The evolutionary reasons for why most rod pigments absorb maximally at 500 ± 10 nm are obscure, as there is no convincing explanation based on adaptation alone.*

Rods are appropriately distinguished from cones on the basis of which opsin gene is expressed. This criterion is likely to be in conflict with other definitions in phyletic lines (e.g., geckos, snakes) that have long diurnal or nocturnal histories accompanied by loss of one or more opsin genes, followed by a secondary adaptation to life in a different photic environment.

Color vision—a generalizable perception associated with the spectral composition of light—is usefully distinguished from wavelength-specific behaviors. The latter are also based on multiple visual pigments and more than one spectral class of receptors but cannot be altered by learning. The distinction is particularly forceful in bees, which exhibit both kinds of behavior.

The evolution of primate color vision has been shaped by historical factors involving an extensive period of early mammalian nocturnality. Birds, by contrast, have more elaborate cones and a richer set of visual pigments. Avian color space can be represented in a tetrahedron.

To suppose that the eye, with all its inimitable contrivances . . . could have been formed by natural selection, seems, I freely confess, absurd in the highest degree.

THESE WORDS from *The Origin of Species by Natural Selection* were Darwin's introduction to a general outline of the selective forces that could have led to the evolution of eyes. Perhaps partly because Darwin pointed to the eye in this fashion, the eye has continued to perplex those who are determined to be confused by evolution (for both historical and contemporary examples, see Dawkins, 1986). Recent work on eyes, however, provides a number of

interesting insights about the evolution of photoreceptors and visual systems in both the vertebrates and the invertebrates. Because little of this work was either performed or presented with an explicit evolutionary perspective, the present account may be useful both to biologists who work on eyes but tend not to think about explanations involving evolutionary cause, as well as to biologists who are conversant with evolutionary theory but are less familiar with recent findings on visual systems.

EVOLUTIONARY ASPECTS OF OPTICAL DESIGN

Optimization and Optical Performance

The idea that evolution optimizes is subject to a sizeable caveat, but it remains a useful hypothesis if one is going to be able to identify the relevant conditions and constraints (Maynard Smith, 1978). In analyses of behavior this identification can present a daunting challenge, for unanticipated circumstances in the real world of animals have a way of confounding the assumptions and boundary conditions in hypothesized models of optimal behavior. In dealing with the interrelated sensory tasks of maximizing spatial acuity and contrast sensitivity, however, both the "camera" eyes of Old World primates and birds, as well as the compound eyes of diurnal insects, present clear examples of evolutionary optimization. The examples are clear for the simple reason that the things that should be optimized are determined by some straightforward rules of physics. The investigator's task in examining the hypothesis of optimization is therefore to ask how closely the optical performance of eyes of different optical design approaches the limits set by physics. This question has been posed and answered in several cases, albeit without direct reference to optimization theory.

Eyes represent images of the external world on two- dimensional sheets of photoreceptors, analogous to pieces of film. It is useful to recognize that evolution has produced two fundamentally different optical systems, depending on whether the retinal sheet is *concave* or *convex* (Kirschfeld, 1969; Wehner, 1981). When the photoreceptor surface is concave, an optical image can be produced with a single refractile lens suitably placed in front of the retina (Fig. 1A). This is the optical design of the vertebrate eye as well as of the large eyes of cephalopod molluscs. On the other hand, when the retinal surface is convex, it can form an image if the individual photoreceptor elements are sensitive only to a narrow cone of light incident perpendicularly to the retinal surface (Fig. 1B). This is the optical principle on which the compound eyes of arthropods is based.

Despite the very different modes of design that underlie the construction of the single-lens eyes of vertebrates and the compound eyes of arthropods, similar considerations determine their capacities to resolve images (Kirschfeld, 1976; Snyder, 1979; Land, 1981; Wehner, 1981). An important factor in visual acuity (angular resolution) is the *angular separation* (Φ) of the individual receptors. Resolution is proportional to $1/\Phi$. For a single-lens eye, $1/\Phi = f/s$, where f is the focal length of the lens and s is the separation between receptors (Fig. 1C). The longer the focal length, the greater the magnification of the image in the focal plane. For a diurnal compound eye (apposition type), on the other hand, simple geometrical considerations show that $1/\Phi = r/d_l$, where r is the radius of curvature of the eye and d_l is the diameter of the ommatidial lens (Fig. 1E).

In either case, angular resolution can be improved by increasing the numerator (f or r) or decreasing the denominator (s or d_l). In single-lens eyes, decreasing s means making the receptors narrower (decreasing d_r in Fig. 1D) and packing them closer together. But there are limits to how far this process can be carried to advantage, for a second physical consideration intrudes. In resolving spatial detail, the responses of adjacent photoreceptors must be distinguished not only from each other but also from the noise that is due to the random nature of the absorption of small numbers of photons. Put another way, receptors must be able to detect reasonably small differences in intensity, i.e., they must possess adequate *contrast sensitivity*. When only a few quanta are absorbed by a photoreceptor, the signal-to-noise ratio (S/N) is poor. From physical principles, S/N should improve in proportion to the square root of the number of quanta absorbed. The probability of photon capture (sensitivity) is proportional to the cross sectional area presented by the photoreceptor, so making the diameters of the receptors (d_r in Fig. 1D) very small leads to a decrease in S/N and loss of contrast sensitivity, particularly disadvantageous if the animal must optimize performance in conditions

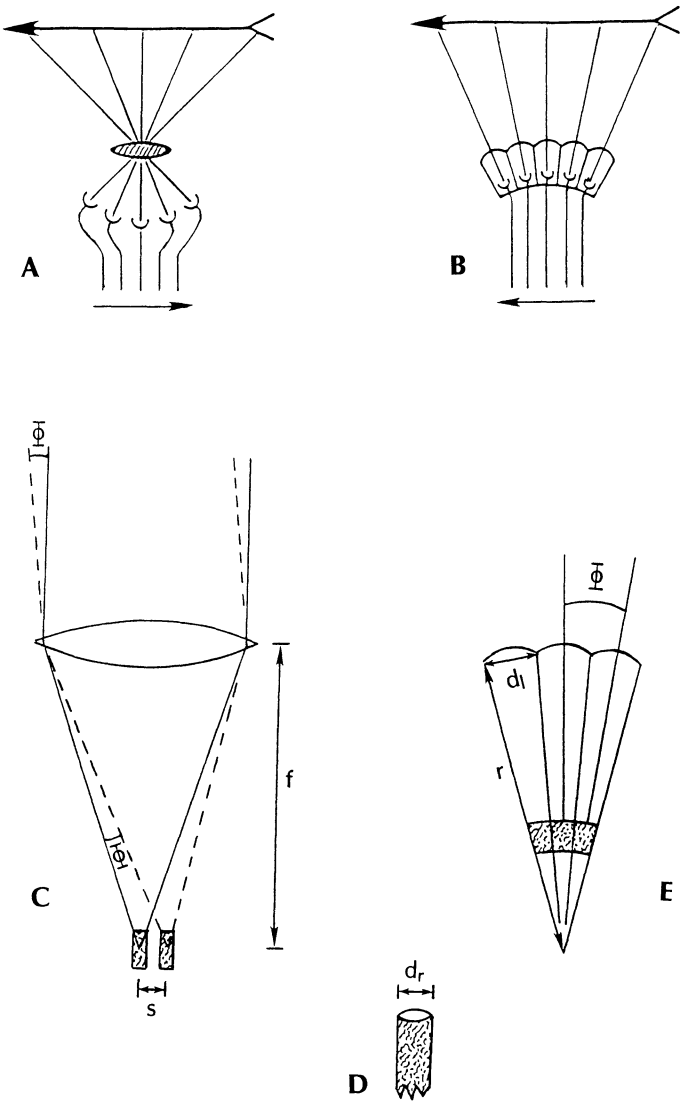

FIG. 1. THE TWO OPTICAL SYSTEMS OF IMAGE-FORMING EYES

Image-forming eyes differ, depending on whether the retina is concave (A, single-lens eyes, as for example in vertebrates) or convex (B, compound eyes of arthropods). After Kirschfeld, 1969. C-E: As described in the text, several common parameters contribute to the optical performance of eyes. Φ, angular separation of receptors; *f*, focal length; *s*, spacing of receptors; d_r, diameter of receptor; d_l, diameter of lenslet; *r*, radius of curvature.

of dim light. Furthermore, receptors act as wave-guides for light propagating along their length. For waveguides less than about a μm in diameter, a significant fraction of the energy (of wavelengths in the visible region of the spectrum) is in a boundary region external to the photoreceptor. Consequently, if the spacing of receptors is too close there will be optical crosstalk between near neighbors. For these reasons the outer segments of vertebrate receptors are not smaller than about 1 μm in diameter and are spaced at least 2 μm apart.

The obvious way to increase the focal length is to increase the diameter of the eye, but the

size of the animal imposes an upper limit on the size of eye that can be accommodated in the skull. Larger eyes also permit a larger entrance pupil, which is also necessary to preserve sensitivity, because the quantum flux on the image plane (the retina) is inversely proportional to the square of the focal length.

The size of the entrance pupil is important for another reason. The *acceptance angle* of an individual receptor (the angle through which the receptor collects light) is as important for visual acuity as the *angular separation* between receptors. If diffraction-limited, the acceptance angle ϱ is proportional to λ/d, where d is the diameter of the pupil, and λ is the wavelength of light (Snyder, 1979). In other words, if the pupil diameter becomes too small the image is broadened by diffraction effects.

In the human eye the size of the entrance pupil can adjust so that optical performance approaches what is theoretically possible. Fig. 2 shows the modulation transfer function (MTF) for the human eye with pupil diameters of 1.5 and 2.0 mm, compared with the theoretical performance of a lens of the same aperture. Imagine an eye viewing a grating (the object) whose stripes modulate sinusoidally in intensity. The MTF is the ratio of image contrast to object contrast, plotted as a function of spatial frequency of the grating. For the ideal lens, the fall in MTF with spatial frequency is due to diffraction, and at its best, the human eye approaches the diffraction limit. Making the pupil smaller increases diffraction, and if the pupil dilates under conditions of dim light, the image is degraded by lens aberrations.

How well is the retina designed to take advantage of this optical performance? The finest sinusoidally modulated gratings that can be detected by the human eye have a frequency of about 50 cycles/degree, which requires sampling intervals at about 36 sec of arc. The spacing of cones in the central fovea (see below), where visual acuity is highest is about 2 μm, which corresponds to a sampling interval of 25 sec if all cones participate or 35 sec for cones of one spectral class. The density of receptors in the fovea is therefore appropriate to utilize fully the optical performance of the front of the eye (Woodhouse and Barlow, 1982).

Similar considerations determine the optical performance of compound eyes. If the facet diameter d_f is made too small, the visual field

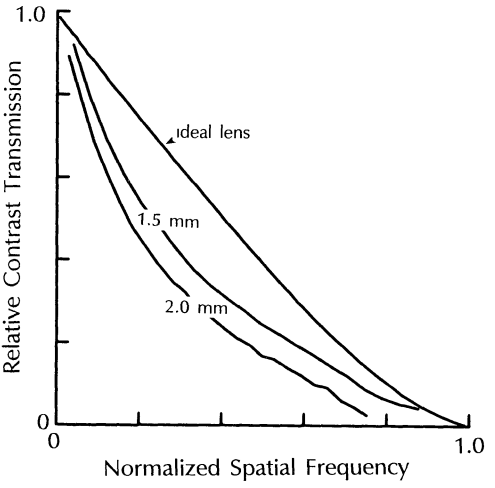

FIG. 2. OPTICAL PERFORMANCE OF THE HUMAN EYE APPROACHES THE DIFFRACTION LIMIT

The modulation transfer function describes the degradation of contrast in the image with increasing spatial frequency. In this figure, measurements for two pupil diameters are compared with a diffraction-limited lens of the same aperture (Campbell and Gubisch, 1966). The objects were series of light and dark stripes of different spatial frequency, each varying sinusoidally in intensity. Frequencies are normalized to the cut-off, about 50 cycles/degree. In an ideal lens the loss of contrast that is described in this way is due to diffraction. See the text for further details.

of the ommatidium is broadened by diffraction. In practice, however, facets are not diffraction-limited. The need for absolute sensitivity and the preservation of S/N and contrast sensitivity imposes a lower limit of facet diameter of about 10 μm, significantly larger than the diffraction limit. Absolute sensitivity and the concomitant maintenance of contrast sensitivity has therefore been the determining factor in the evolution of facet size.

The obvious way to increase angular resolution is therefore to increase the radius of curvature of the eye. As with the single-lens eyes of vertebrates, however, there are anatomical limitations to the size of eye that can be carried on an insect's head. This restriction is partially circumvented in a very interesting way. Many insects have local regions of the eye in which the cornea is quite flat and the radius of curvature of the eye is relatively large. The ommatidial axes in such a "foveal region" are

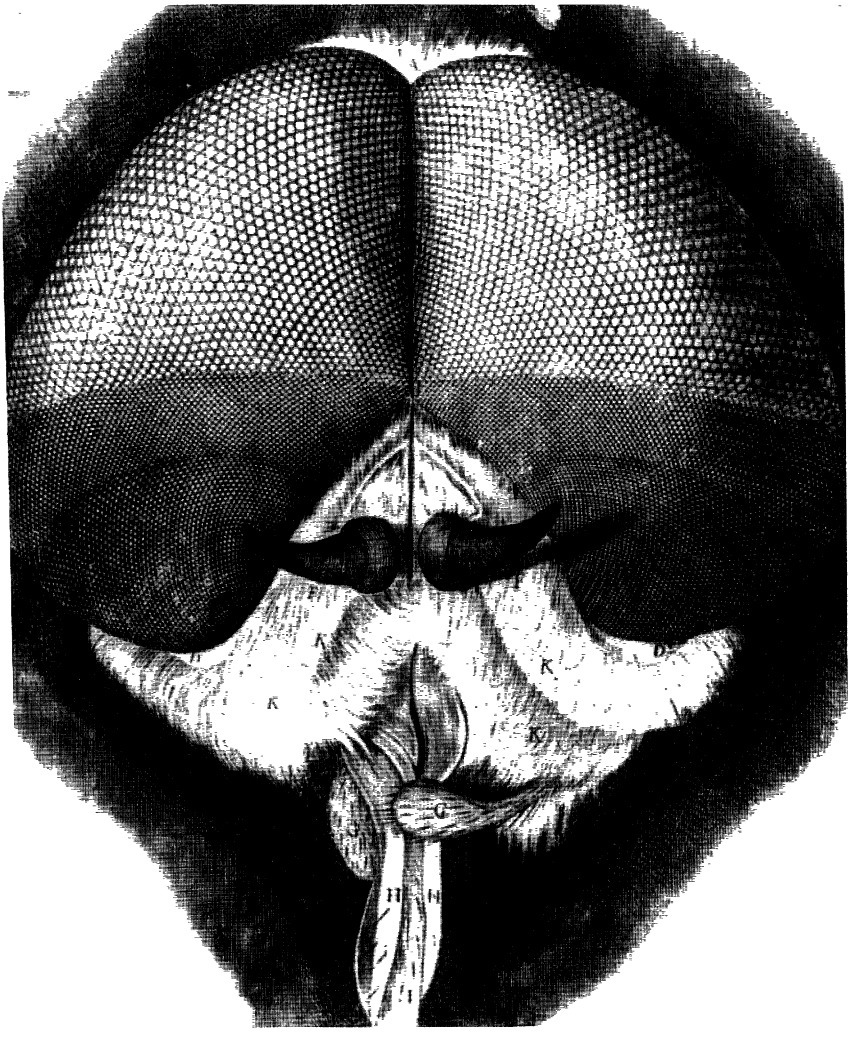

FIG. 3. THE OPTICAL PROPERTIES OF COMPOUND EYES VARY LOCALLY

This drawing from Hooke's 1665 *Micrographia* shows the head of what appears to be a tabanid fly (Wehner, 1981). Note the variation in facet diameter and radius of curvature in different regions of the eye. Large facets are found where the radius of curvature is greatest; these are regions of small interommatidial angle, small acceptance angle, high spatial acuity, and high contrast sensitivity. See the text for further details.

therefore separated by small angles. The visual field therefore approaches in size the (small) interommatidial angle, and visual acuity is locally high. But because of the large radius of curvature, facet diameters are also large. Absolute sensitivity and contrast sensitivity are thus also kept high, and small objects that lie within the visual field of a single ommatidium are more likely to generate a detectable signal. Fig. 3 is Robert Hooke's 1665 drawing of a tabanid fly with a so-called divided eye in which in the dorsal region both the facet diameter and the radius of curvature are relatively large. Fig. 4 shows that in a large number of arthropods facet diameter is about twice as large as the diffraction limit.

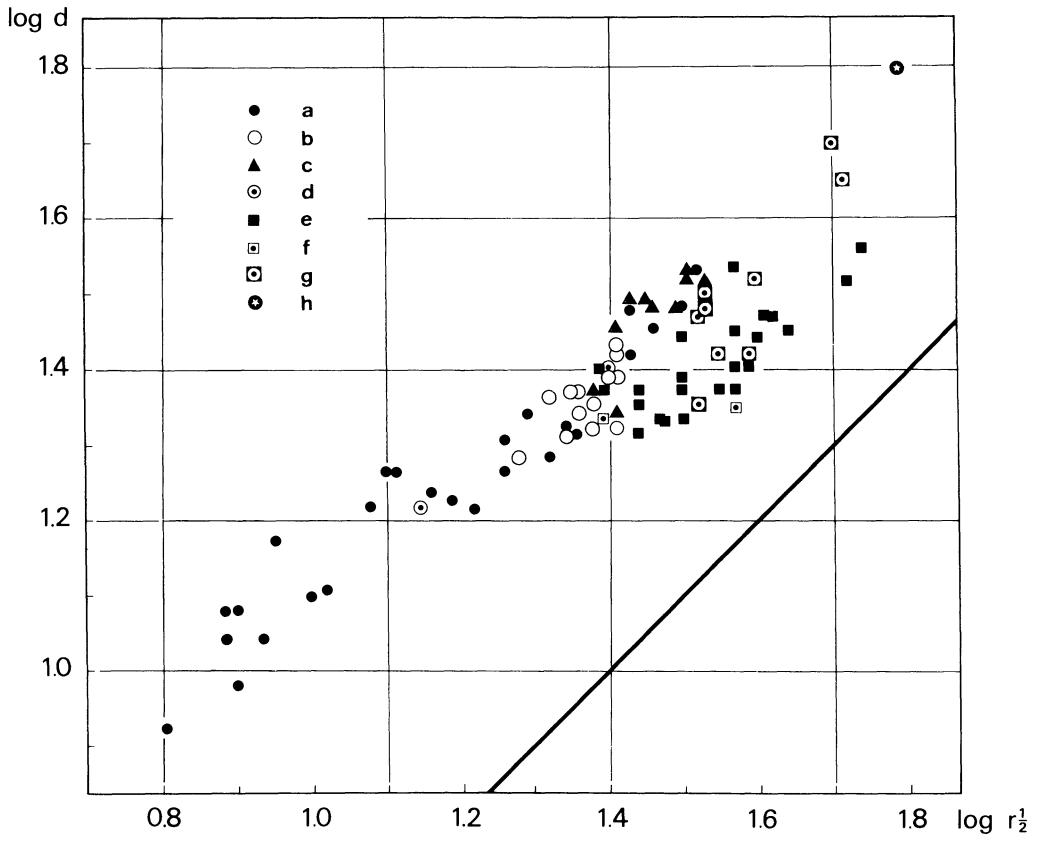

FIG. 4. FACET DIAMETER AND RADIUS OF CURVATURE REVEAL ONE FOCUS OF NATURAL SELECTION IN THE EVOLUTION OF COMPOUND EYES

In a number of insects, facet diameters are about twice as large as they would need to be at the diffraction limit, indicating that absolute quantum catch and contrast sensitivity are critical parameters in natural selection. Axes: *d*, facet diameter; *r*, radius of curvature. Other symbols indicate species from which the measurements were made. Solid line: diffraction limit. From Wehner, 1981.

Optimization in the Face of Embryological Constraint

From optical considerations, the vertebrate retina has a curious design. Because the eye arises from the invagination and collapse of an outpocketing of the brain (Fig. 5), light must pass through the neural retina before it strikes the photoreceptor cells. This is equivalent to placing a thin diffusing screen directly over the film in your camera; it can only degrade the quality of the image. The eyes of cephalopod molluscs are not so compromised in design; the vertebrate eye is burdened with this feature because of the mechanics of its ontogeny.

Birds and primates have regions of the retina specialized for high visual acuity called foveas in which the cone cells occur in high density and converge little if at all on retinal interneurons. In foveal regions the optical problem of scatter has been alleviated by lateral displacement of the cell bodies of the neural retina, insofar as possible clearing an optical path in front of the foveal cones (Fig. 6). This morphological example shows that evolutionary history can shape both the need for and the reach of adaptation, and it thereby sets the stage for what follows.

EVOLUTION OF VISUAL PIGMENTS

Opsins Have Several Ancient Evolutionary Lineages

The visual pigments are intrinsic membrane proteins — opsins — located in the photoreceptor organelles in the receptors. Opsins bind

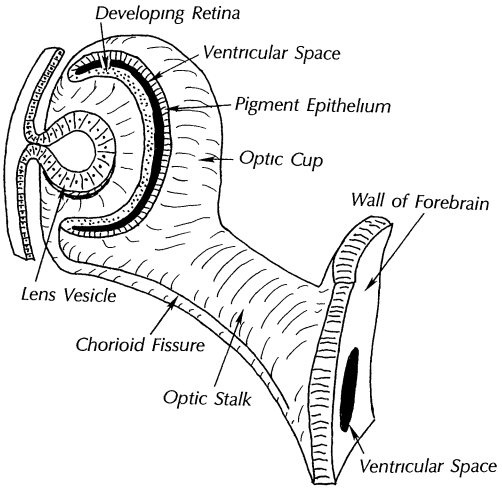

FIG. 5. REVERSAL OF THE VERTEBRATE RETINA ARISES IN DEVELOPMENT

The vertebrate eye develops by invagination of the optic vesicle, forming the optic cup. The cells lining the ventricular space along the back wall of the optic cup become the pigment epithelium. The front wall differentiates into the retina, with the covalently with 11-*cis* retinoids to form the visual pigments. The retinoid chromophores and the nature of the attachment to opsin are shown in Fig. 7. Light causes an isomerization from the 11-*cis* to the all-*trans* configuration, and ensuing changes in the conformation of the opsin are instrumental in triggering a biochemical cascade that leads to excitation of the photoreceptor cell.

At this writing, the primary structures of 13 opsins have been reported, based, in 12 cases, on the nucleotide sequence in the encoding gene. With the exception of the opsin of cephalopod molluscs, the polypeptide chains

receptors facing the old ventricular cavity, and the neural retina facing the vitreal surface. Light must therefore pass through the retinal interneurons before reaching the receptors. The chorioid fissure is an extension of the original invagination of the optic vesicle along the optic stalk. It eventually pinches closed within the stalk, housing the optic nerve (axons of the retinal ganglion cells) and the blood supply to the retina.

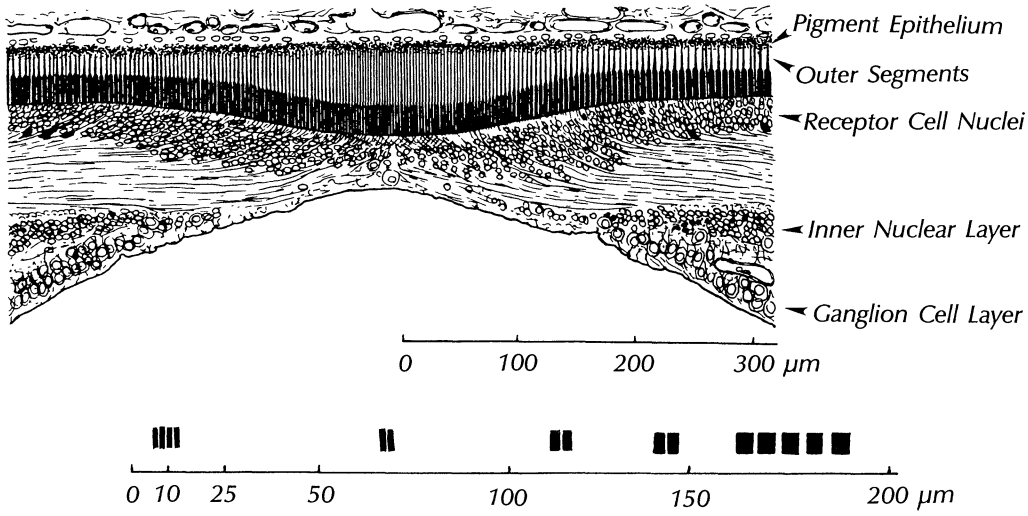

FIG. 6. THE CENTRAL FOVEA OF THE HUMAN EYE SHOWS ADAPTATIONS TO AMELIORATE THE DISADVANTAGE OF A REVERSED RETINA

In this view of the fovea, incident light would come from below. The inner nuclear layer and ganglion cell layer of the neural retina are evident at the edges of the fovea; in the center of the fovea the cell bodies are displaced in order to provide a clearer path for light to reach the photoreceptor cells. The fibers running from the central fovea to make synaptic connection with bipolar cells and horizontal cells in the neural retina are the basal ends of the foveal cones. Xanthophylls deposited in the cell membranes of these fibers are responsible for the *macula lutea*, the yellow filter that covers the foveal region of the human retina. Note that the receptors become wider and less densely packed with distance from the center of the fovea (lower, expanded scale). Modified from Polyak, 1941.

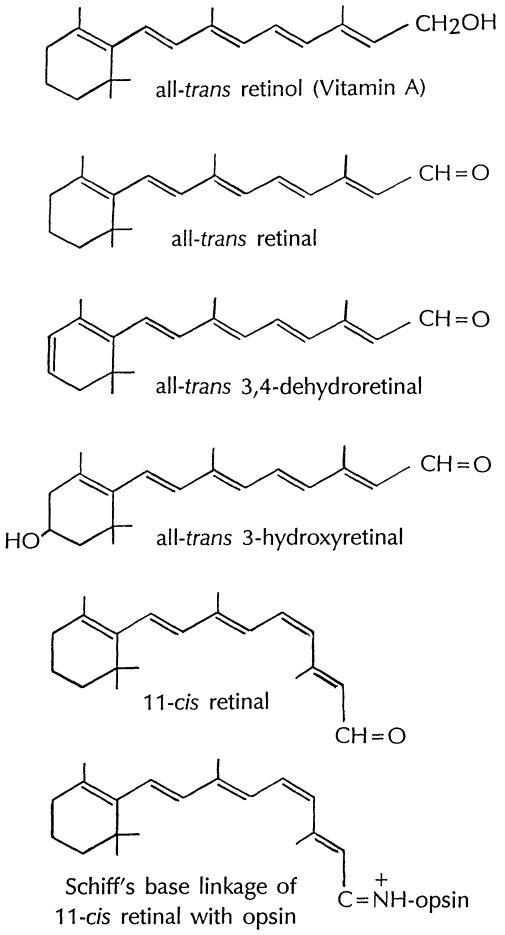

FIG. 7. STRUCTURES OF THE RETINOIDS INVOLVED IN VISUAL EXCITATION

Three aldehydes are common as chromophores of visual pigments; in each, light causes an isomerization of the eleven-*cis* isomer to the all-*trans* configuration. The retinoids are attached by a Schiff's base linkage to the ε-amino group of a lysine in helix 7 of the opsin, as shown by the structure at the bottom of the figure.

are similar in length, varying between 348 and 382 amino acid residues. Analyses designed to identify hydrophobic regions of the chain and originally applied to bovine rhodopsin (e.g., Argos et al., 1982) suggest that all these visual pigments fold so as to form seven membrane-spanning helices (Fig. 8). The octopus protein fits this model as well as the other opsins, but it differs in having about 90 additional amino acids associated with the C-terminal, cytoplasmic tail.

When the polypeptide chains of the pigments are aligned—with judicious recourse to hypothetical insertions and deletions (O'Tousa et al., 1985; Applebury and Hargrave, 1986), a process facilitated by recognition of the helical domains—it is possible to see similarities between pigments at the amino acid level. Identities of codon or of amino acid are frequently referred to casually as homologies; however, homology refers to descent from a common ancestor, and the assertion of homology in this situation requires additional evidence (Reeck et al., 1987).

Two analyses have been performed on these sequence data. First, from the comparisons of amino acids at corresponding positions, a matrix was constructed of the number of differences between each pair of opsins, and the matrix used to calculate evolutionary distances between opsins. Fig. 9 is an unrooted tree, calculated using FITCH, one of J. Felsenstein's programs in PHYLIP. The sum of the lengths of the internodes between any pair of opsins is proportional to the number of amino acid substitutions required to make the transition between opsins, and the nodes represent hypothetical ancestral proteins.

Starting with this analysis we can begin to discuss homology. (a) The vertebrate rod pigments (human, mouse, cow, sheep, chicken) form a cluster whose branching pattern reflects the phyletic relatedness of the animals themselves. (b) Evidence to be discussed further below indicates that the long- and middle-wavelength human cone pigment genes stem from a gene duplication within the last 60 million years. Within each of these two branches of the tree (rod opsins, long- and mid-wavelength human cones), opsins are clearly homologous.

There are reasons to believe that the long branches in Fig. 9 also reflect evolutionary divergence within a larger family of homologous proteins. (c) If the C-terminal tail of all the opsins is "excised" and the tree recalculated, the branch to the octopus opsin seems quite unremarkable (Fig. 10A). The extra divergence in Fig. 9 is therefore due largely to the addition of several score amino acids to the C-terminal end.

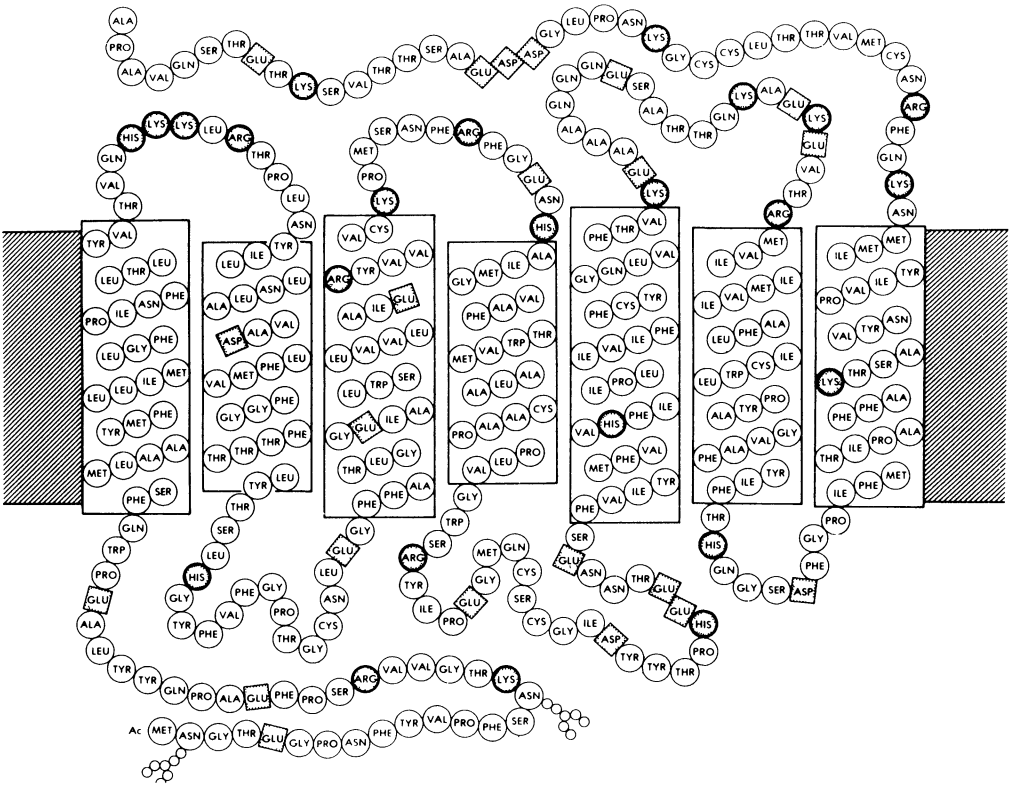

FIG. 8. ALL OPSINS SHARE A SIMILAR STRUCTURE CONTAINING SEVEN HELICES
The primary structure of opsins suggest a common secondary structure consisting of seven α-helices spanning the membrane. This amino acid sequence is for cattle opsin and is from Hargrave et al., 1983. A version of the model that indicates similarities and differences between species and is published in color can be found in Applebury and Hargrave, 1986.

(d) There are local regions of particularly high similarity between proteins which suggests conservation of homologous domains; for example, the cytoplasmic loop between helices 1 and 2 and within helix 7 (which contains the binding site of the chromophore) (Fig. 11). Applebury and Hargrave (1986) have summarized extensively the evidence for local similarity in the subset of opsins that had been sequenced at that time. But can we argue that these regions of high similarity do not reflect convergent evolution? If evolutionary distances are calculated using just the amino acids associated with helix 7 (Fig. 10B), the relationships between opsins are qualitatively similar to those in the bulk of the molecule (Fig. 10A). Helix 7 therefore appears to be a region of the molecule that has been relatively conserved during a process of divergent evolutionary change.

(e) The trees in Figs. 9 and 10 were obtained with an algorithm that minimizes the sum of $(d - d')^2/d^2$, where d is the observed and d' is the expected distance between opsins. Alternatively, one can utilize information in the *sequences* of amino acids and calculate the minimum number of nucleotide changes in the genetic code required to generate the most parsimonious phylogenies (Felsenstein, 1982). Presented with the sequences (less the C-terminal amino acids that extend into the cytoplasm) for all 13 opsins, the PHYLIP program PROTPARS finds that there are two equally parsimonious trees whose topologies differ only in whether the branch to Cbh arises closer to

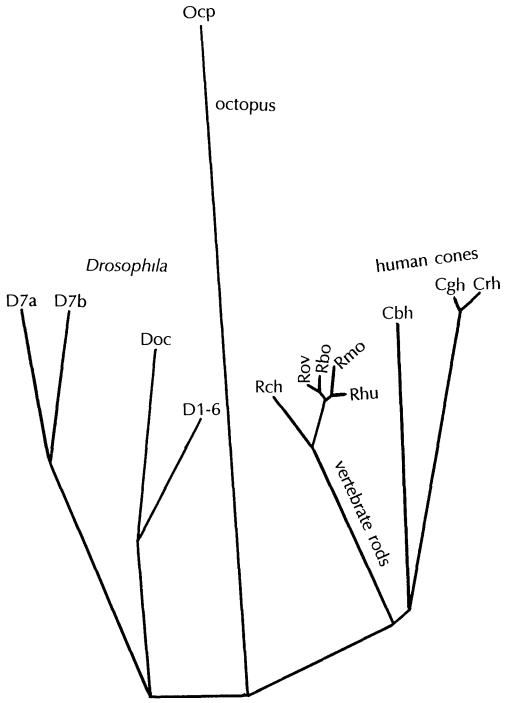

FIG. 9. EVOLUTIONARY DISTANCES BETWEEN THIRTEEN OPSINS

This tree was calculated from numbers of differences in amino acids between pairs of opsins by using the distance matrix program FITCH (an implementation of the method of Fitch and Margoliash, 1967) in the Phylogeny Inference Package (PHYLIP, version 3.1) (Felsenstein, 1985). D1, Doc, D7a, and D7b are the pigments of retinular cells 1 to 6, the ocellus (Feiler et al., 1988; Pollock and Benzer, 1988), and two subsets of retinular cell 7 of *Drosophila*; Ocp, the opsin of octopus; Rch, chicken rod pigment; Rbo, Rov, Rmo, Rhu are mammalian rod pigments from cattle, sheep, mouse, and human retinas; Cbh, Cgh, and Crh are the human cone pigments. [These three cone pigments are sometimes referred to as blue, green, and red; later in the body of the text they are called short (S), middle (M), and long (L), referring to their relative spectral positions.]

Original references to the sequences are as follows: O'Tousa et al., 1985; Zuker et al., 1985 (D1-6); Cowman et al., 1986 (Doc); Zuker et al., 1987 (R7a); Montell et al., 1987 (R7b); Ovchinnikov et al., 1988 (Ocp); Takao et al., 1988 (Rch); Ovchinnikov et al., 1982; Hargrave et al., 1983; Nathans and Hogness, 1983 (Rbo); Findlay, 1986 (Rov); Baehr et al., 1988 (Rmo); Nathans and Hogness, 1984 (Rhu); and Nathans, Thomas, and Hogness, 1986 (human cone pigments).

The vertebrate and most of the invertebrate opsins differ in length by several percent and were aligned for maximal similarity. The opsins of cephalopod molluscs have about 90 more amino acids on the C-terminal end, which accounts for the long branch to Ocp (compare with Fig. 10A).

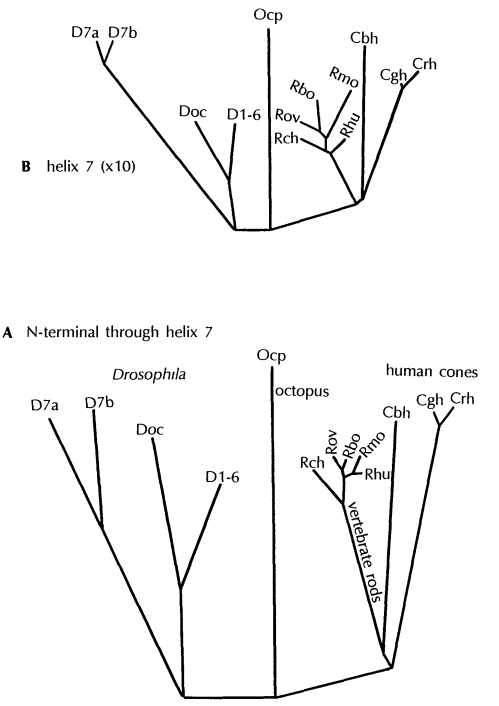

FIG. 10. EVOLUTIONARY DISTANCES FOR TWO REGIONS OF THE OPSIN MOLECULE

(A) Evolutionary relationships of 13 opsins calculated as for Fig. 9, but without including the C-terminal, cytoplasmic end of the molecule. This makes the mollusc (octopus) opsin seem less different from the arthropod and vertebrate pigments, and emphasizes that the opsin of the short-wavelength sensitive human cone (Cbh) is about as closely related to the opsins of rods as it is to the other human cone pigments.

(B) Evolutionary tree calculated for the stretch of amino acids in helix 7 and displayed on a 10× expanded scale relative to the tree in A. The relationships of the opsins are approximately the same in A and B, suggesting that helix 7 is a conserved region in a family of homologous proteins, and that the similarities between opsins in Fig. 11 are due to conservation rather than convergence.

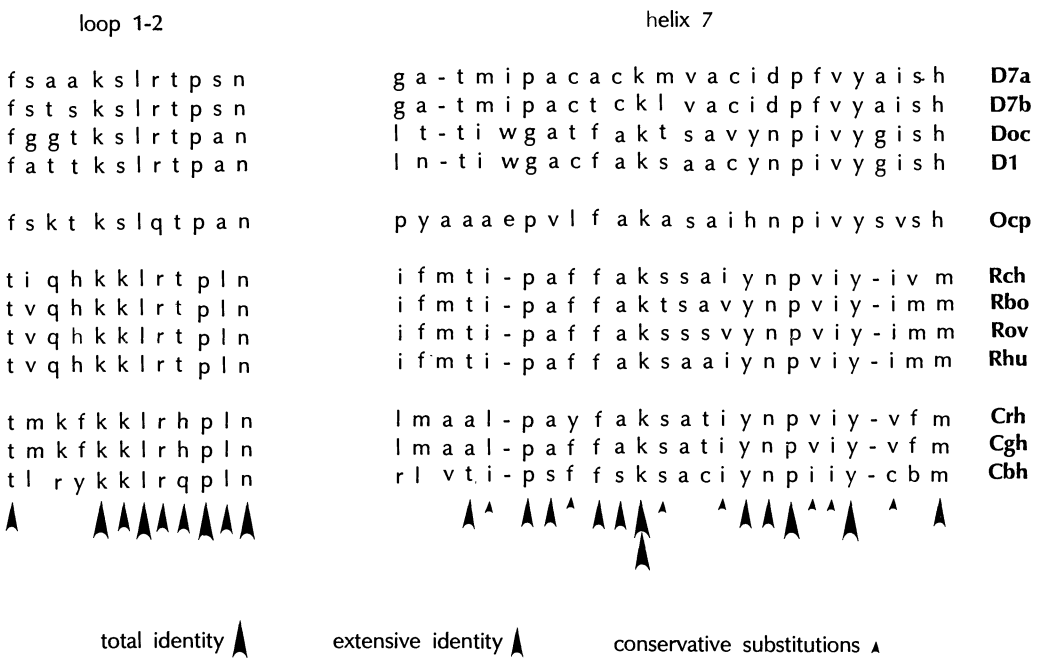

FIG. 11. TWO REGIONS OF HIGH SIMILARITY IN 12 OPSINS

Two regions that appear to be highly conserved are loop 1-2, the cytoplasmic loop between helices 1 and 2, and helix 7, the region in which the chromophore binds. The arrowheads indicate sites at which there are many amino acid identities and numerous functionally conservative substitutions. The amino acids are indicated by the single-letter code. The double arrowhead under helix 7 indicates the lysine (code k) to which the chromophore binds.

rod opsins or to the other cone pigments (Fig. 12). In all other respects the branching patterns of these two trees are the same as those in Figs. 9 and 10A. This parsimony method therefore agrees with the analysis based on pairwise difference matrices. In both methods the question of whether the opsin of blue-sensitive human cones is closer to rod or other human cone pigments remains unresolved by the data currently available.

(f) There is a relative conservation of gene structure implied by the pattern of introns and exons between otherwise distantly related proteins (Applebury and Hargrave, 1986), although this pattern is not conserved between all members of the ensemble.

(g) Recent work on the β-adrenergic receptor (Dixon et al., 1986) and the muscarinic acetylcholine receptor (Kubo et al., 1986) suggest that opsins are part of a still larger group of related proteins that function by activating second messenger cascades. This interesting observation also indicates the need for caution in concluding, solely on the basis of hybridization of genomic fragments with cDNA for bovine rhodopsin (Martin et al., 1986), that organisms such as the alga *Chlamydomonas* or the bacterium *Halobacterium halobium* have rhodopsins engaged in sensory transduction. Other evidence for *Halobacterium* does exist, however (Spudich and Bogomolni, 1984).

The well-known "bacteriorhodopsin" from *Halobacterium* raises an interesting evolutionary issue. This molecule is a proton pump rather than a visual pigment, the retinal cycles between all-*trans* and 13- rather than 11-*cis* (Stoeckenius and Bogomolni, 1982), and there is no significant similarity with cattle rhodopsin at the amino acid level (Hargrave et al, 1983). On the other hand, bacteriorhodopsin shares some conformational features with the visual pigments, making seven helical traverses of the membrane (Henderson, 1977; Engelman and Zaccai, 1980). The question therefore arises

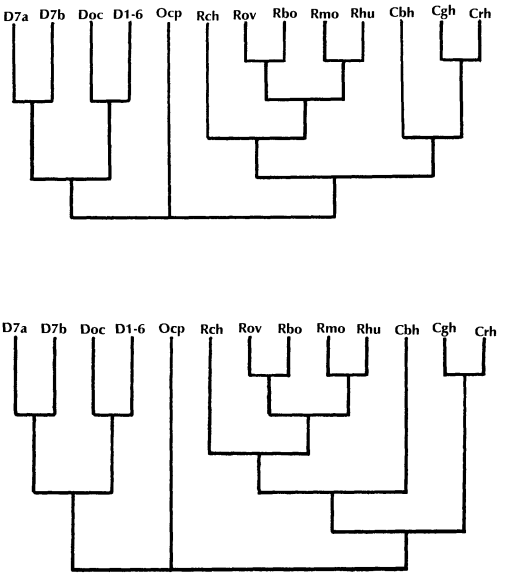

FIG. 12. THE TWO CLADISTIC TREES GENERATED BY A PARSIMONY ANALYSIS

The analysis (PROTPARS) takes account of amino acid sequence data. The two trees differ only in the placement of the pigment for human blue-sensitive cones.

whether similarities in conformation are an example of convergent evolution, or whether the secondary and perhaps some aspects of tertiary structure have been the only common foci of natural selection, the divergence of visual opsins and bacteriorhodopsin having taken place so long ago that there is no trace of homology remaining in the primary structures. Lysozymes from several sources are thought to share structural similarities through divergent evolution from a common precursor despite a loss of sequence homology (Weaver et al., 1985), but convincing examples of convergent evolution of protein conformations (not just catalytic sites) are harder to identify (Creighton, 1984).

The separation of human and bovine rhodopsins has been used to suggest a rate of divergence of about 1 percent every 10^7 years (Nathans, Thomas, and Hogness, 1986), but the divergence within the family Bovidae indicates the rate can be about twice as fast. Such rates are well within the range of evolutionary rates of change of other proteins (Creighton, 1984). Furthermore, fish of the genus *Salvelinus* have rhodopsins with maximal absorbance (λ_{max}) at 503, 508, and 512 nm (Bridges, 1972). Each of these forms must involve at least one amino acid substitution, and the evolutionary changes have almost certainly taken place in post-Pleistocene time. In fact, two of the populations are interfertile, and hybrids make both parent pigments (McFarland and Munz, 1965). These observations suggest that under the proper circumstances evolutionary changes in visual pigments can be ten times as rapid as inferred from the data on mammalian rhodopsins.

It is easy to make too much of any estimation of rate of evolutionary change based on these numbers. First, the database is thin. Second, members of a family of proteins may diverge in evolutionary time at a rate that corresponds to the average rate of accumulation of mutations that produce little or no alteration in function ("neutral" mutations). The divergence of vertebrate rod opsins may be largely of this nature. On the other hand, some subset of the differences between the red- and green-sensitive cone pigments is due to changes that have altered function (i.e., at least the absorption spectrum). Mutations that alter function can be the object of vigorous selection at rates far different from the passive accumulation of functionally neutral substitutions. The long- and middle-wavelength cone pigments of the human retina nevertheless seem to have diverged from each other sometime during the past 65 million years, during the adaptive radiation of mammals. But of this, more below.

In summary, and regardless of how bacteriorhodopsin fits into the picture, the visual pigments seem to represent a single family of proteins. We now have data on two organisms, ourselves and *Drosophila*, that synthesize more than one kind of opsin. In each of these species there are opsins that diverged from each other before the appearance of contemporary classes, if not phyla, and still other opsins that diverged at about the same time as the separation of extant orders or families. And as was mentioned above, there is spectral evidence for the evolution of opsin at the level of species. The evolutionary history of opsins is thus multidimensional, and each of the major branches on the trees in Figs. 9, 10 and 12 (as well as other major branches that remain to be drawn) will each have its own phyletic substructure awaiting ex-

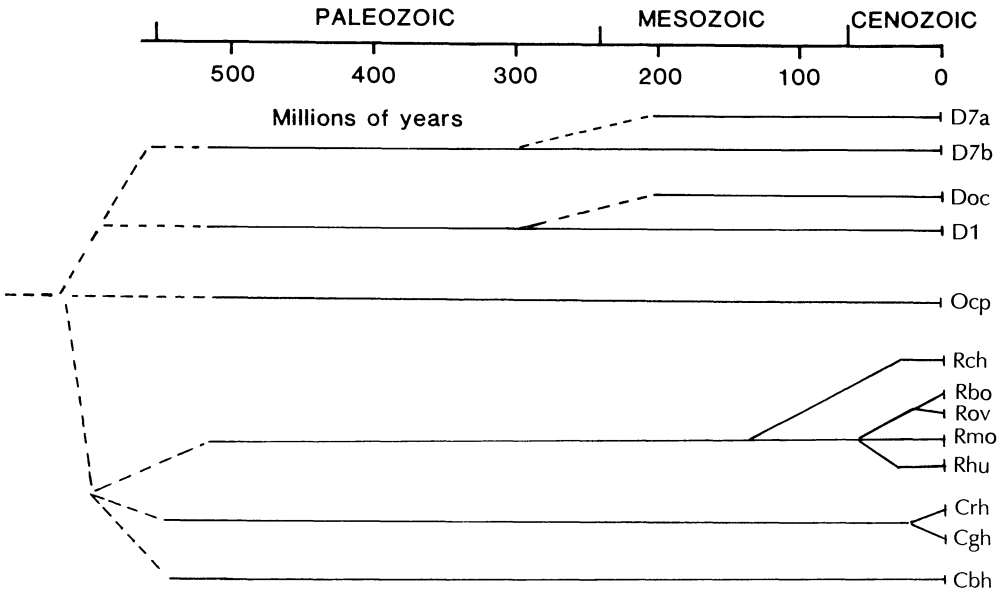

FIG. 13. INFERRED TEMPORAL RELATIONSHIPS OF THE SEVERAL VISUAL PIGMENTS WHOSE PRIMARY STRUCTURES ARE KNOWN
See the text for discussion of the times of divergence.

ploration. A tentative and very rough chronology for the relationships between opsins is shown in Fig. 13.

Phylogeny and Chromophores

In addition to retinal, visual pigments utilize 3,4-dehydroretinal, 3-hydroxyretinal, or 4-hydroxyretinal as the chromophore. Dehydroretinal is found in certain fish, amphibia, reptiles (Bridges, 1972; Beatty, 1975a; Knowles and Dartnall, 1977), and very occasionally invertebrates (Suzuki and Eguchi, 1987; Matsui et al., 1988; Zeiger and Goldsmith, 1989). Because of their color, the rod pigments that utilize dehydroretinal are called porphyropsins rather than rhodopsins. The terms porphyropsin and rhodopsin have also been used to refer to invertebrate pigments, but historically they are not applied to vertebrate cone pigments.

3-hydroxyretinal is known from certain insects, notably (but not exclusively) Diptera and Lepidoptera (Vogt, 1983, 1984, 1987; Vogt and Kirschfeld, 1984; Goldsmith et al., 1986; Seki et al., 1986, 1987; Smith and Goldsmith, 1990), and because of the relationship of the chromophore to xanthophyll, the visual pigments are referred to by some writers as xanthopsins (Vogt, 1983, 1988). 4-hydroxyretinal has been found in a bioluminscent squid, where it occurs together with retinal and dehydroretinal (Matsui et al., 1988).

A few years ago the question of whether rhodopsin or porphyropsin was the more primitive loomed large in discussions of the evolution of visual pigments (e.g., Wald, 1960; Crescitelli, 1972). Although more comparative biochemical information about photoreceptors in the little-explored classes and phyla of animals would clearly be useful, at this time the evolutionary choice of chromophore seems largely an epiphenomenon. This is because the enzymes for modifying the β-ionone ring of retinal do not have either the same pervasive phyletic presence or the same degree of historical continuity as opsin. The histories of porphyropsin and xanthopsin are therefore largely evolutionary sagas about the enzymes of chromophore metabolism, and the names porphyropsin and xanthopsin in most cases carry no evolutionary implications about the protein moiety of the visual pigment.

The presence of 3-dehydroretinal is generally thought to be an adaptation for shifting the wavelength of maximal absorption of the visual pigments to longer wavelengths (see below). In vertebrate species in which retinal and

dehydroretinal occur simultaneously, rhodopsin and porphyropsin usually employ the same opsin. There is, however, at least one interesting exception. The eel *Anguilla* appears to have two genes for rod opsins, one of which is adapted for the part of the life cycle that is spent in deep water. Both opsins can occur together in the retina (Beatty, 1975b), but whether both genes can be expressed simultaneously in the same rod cell remains to be determined. The extent of evolutionary divergence of these two opsins is also unknown.

The evolutionary significance of 3-hydroxyretinal is unknown. The hydroxyl group on the ring does not shift the spectrum of the retinoid and has no known implications for changing the absorption spectrum of the visual pigment. At the time of its discovery this retinoid seemed to be a phyletic marker (Vogt, 1983; Vogt and Kirschfeld, 1984), but more recent work (Vogt, 1987; Seki et al., 1987; Smith and Goldsmith, 1990) has revealed an irregular distribution among insects that defies any simple historical or adaptive explanation (Fig. 14).

Phylogeny and Photoreceptor Organelles

The relatively large eyes of vertebrates, arthropods, and cephalopod molluscs are so different from one another that they must represent independent evolutionary accomplishments. All eyes, however, contain photoreceptor organelles that provide an increased surface area for the deposition of photopigment. These fall into two general classes, depending on whether or not they form in association with the basal body of a cilium. The distinction drew its original interest from the observation that the photoreceptors of the vertebrate eye are derived from cilia whereas the photoreceptors of the major invertebrate phyla are not. Early studies suggested that, in general, Deuterostomes have ciliary photoreceptors, whereas Protostomes do not (Eakin, 1968, 1972, 1982). As more invertebrate species have been examined, so many exceptions to this generalization have appeared that the original hypothesis of two major evolutionary lines has lost its force. The extensive morphological diversity of photoreceptor cells and of their modes of association in various invertebrate phyla has been interpreted as polyphyletic, with perhaps as many as 40 to 65 evolutionary lines (von Salvini-Plawen, 1982). This view, however, draws attention to the independent evolution of photoreceptor *organs* at the expense of features of photoreceptor *organelles* that may be homologous (Vanfleteren, 1982). As we have seen, opsins from three phyla have now been sequenced and appear to be homologous proteins (Figs. 9-12); it is therefore doubtful that the utilization of retinal-based proteins as photoreceptor molecules in cephalopods, arthropods, and vertebrates is polyphyletic, as previously hypothesized (Wald, 1960). It remains an interesting question which other characters of photoreceptor organelles and photoreceptor cells may be monophyletic. The eyes of cephalopods, arthropods, and vertebrates are not homologous, yet at the molecular level, some of their constituent elements are.

What Evolutionary Causes Determine the Wavelengths at Which Vertebrate Rhodopsins Absorb Maximally?

"This is now an old story, and grown tedious."
Wald (1960)

Why do visual pigments absorb where they do? I do not mean the proximate cause of the absorption spectrum, which will be found in terms of the fine details of tertiary structure and the distribution of charges within the molecule. That question may be answered within the next few years as altered opsin molecules are crafted at will (Sakmar et al., 1989). I am posing instead the question of ultimate cause, which involves natural selection and evolutionary history.

The problem may be somewhat simplified by focusing on a subset of visual pigments, the rhodopsins of vertebrate rods. First, there is now a substantial database to consider. And second, most vertebrates have only a single rod pigment. Unlike the cones which must work as a team, the properties of rods may have been shaped by natural selection in a more direct manner.

The spectral distribution of λ_{max} of vertebrate rod pigments, based on 274 measurements tabulated by Lythgoe (1972b), is shown by the histogram in Fig. 15. Omitted from this tally are pigments based on 3-dehydroretinal, as well as the pigment of the green rods of frogs, and the reptilian pigments that come from transmuted cells (Crescitelli, 1972, 1977) and whose affinities with rhodopsin are therefore suspect (see below). Most of the vertebrate

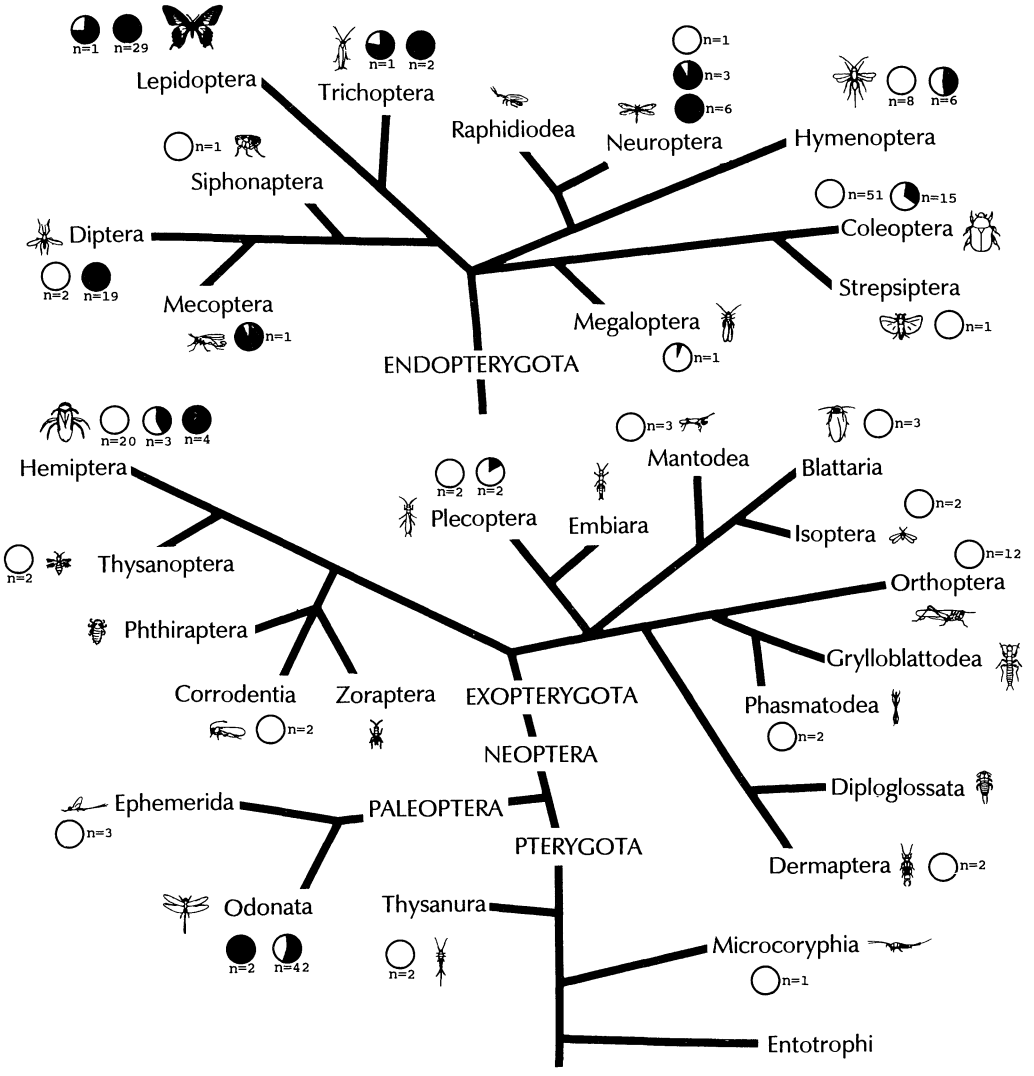

FIG. 14. PHYLOGENETIC RELATIONSHIPS OF THE ORDERS OF INSECTS, COMPARED WITH THE KNOWN DISTRIBUTION OF VISUAL PIGMENT CHROMOPHORES

Open circles, number of species (n) with only retinal; filled circles, species with only 3-hydroxyretinal; partially shaded circles, species with both chromophores in the same eye (fraction shaded indicates average proportions of 3-hydroxyretinal). From Smith and Goldsmith, 1990.

rhodopsins have λ_{max} close to 500 nm. The few exceptions at longer wavelengths are mostly from fish inhabiting waters in which the spectral transmission has been shifted to longer wavelengths by dissolved and suspended organic material. The somewhat larger number of exceptions at shorter wavelengths all come from fish or mammals that inhabit or dive to depths in relatively clear oceanic water.

The correlation of blue-shifted rhodopsins with life in deep water, where the window of available light is centered at about 480 nm, has been noted by a number of workers. Lythgoe (1972a, 1979) and Knowles and Dartnall (1977) have reviewed the subject and provide quantitative estimates of how sensitivity in an aquatic environment might be expected to vary with the spectral position of the visual pigment.

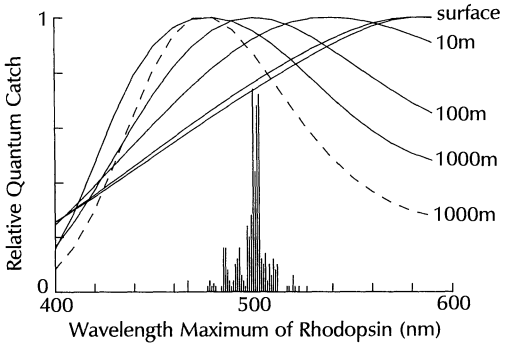

FIG. 15. SPECTRAL DISTRIBUTION OF VERTEBRATE ROD PIGMENTS AND OF AVAILABLE LIGHT

Histogram: Spectral distribution of λ_{max} of 274 vertebrate rod pigments, based on the compilation of data by Lythgoe (1972b). There are 10 pigments per scale division on the ordinate.

Curves: Relative effectiveness of rhodopsin in trapping available photons in different light environments, plotted as a function of λ_{max} at different depths of clear water, calculated as the integral of $Q(\lambda)\cdot\exp[-k(\lambda)m]\cdot A(\lambda)$ between 320 and 650 nm for rhodopsins with λ_{max} from 400 to 600 nm, where $Q(\lambda)$ is the solar energy distribution for a color temperature of 5500° K, after quantizing (Judd et al., 1964), $k(\lambda)$ is the spectral dependence of the absorption coefficient of water (Sverdrup et al., 1942), m is the depth in meters, and $A(\lambda)$ is the absorptance spectrum (i.e., fraction absorbed) of rhodopsin at the specified λ_{max}, calculated from a polynomial expression designed to replicate rhodopsin spectra (Bernard, unpubl.). For each depth the quantum-catch integrals were normalized to that of the pigment with maximum sensitivity. Solid curves are for an axial absorbance of rhodopsin in the rod of 1.0; the dashed curve for an axial absorbance of 0.1.

Fig. 15 shows calculations of the relative quantum catch (i.e., effective sensitivity) as a function of λ_{max} of rhodopsin for several depths of clear, deep water. These calculations show that although there is an advantage to deep-dwelling animals in having their rod pigments absorb maximally near 480 nm, the dependence of sensitivity on λ_{max} is surprisingly small. With axial absorbance in the rods of 1 (high values are characteristic of deep-sea fish), the difference in sensitivity between 480 and 580 nm rhodopsins is less than a factor of two. And on going from 500 to 480 nm, the sensitivity increases by only about 10 percent. This small improvement, perhaps in conjunction with contrast considerations (Lythgoe, 1979), has nevertheless been sufficient to drive the λ_{max} of deep-sea rhodopsins to wavelengths shorter than 500 nm in both teleosts and mammals (seals, cetaceans).

As Lythgoe has pointed out, however (and as is also shown by Fig. 15), there is a poorer sensitivity match between the rhodopsins of terrestrial and shallow-water animals and the spectral distribution of either sunlight or moonlight in those environments. If a small selective advantage has served to pull the pigments of deep-sea creatures to shorter wavelengths, why is the center of distribution of λ_{max} (Fig. 15) not found at wave-lengths longer than about 500 nm?

Three adaptational hypotheses have been proposed. Munz and MacFarland (1973, 1977) pointed out that, particularly in aquatic environments, the solar spectrum is blue-shifted at twilight and suggested that rods have evolved for maximum sensitivity at that time of day. Calculations by Govardovskii (1976) similar to those of Fig. 15, however, do not show the necessary match with pigment absorption. Furthermore, the placement of λ_{max} near 500 nm is characteristic of all rod pigments, not just those of fish. And for many nocturnal animals, moonlight and starlight, which are not blue-shifted, are likely to be more important than the relatively brief intervals at twilight.

The second hypothesis is that natural selection for vision in dim light has occurred in environments dominated by reflections from green plants. The problem with this idea is that the window of light that is reflected from green plants is centered closer to 550 nm than 500 nm (Fork, 1977; Lythgoe, 1979).

The third adaptive explanation is of a different nature. Because dark-adapted rods are sensitive to single photons of light, Barlow (1956, 1957) built on the idea that thermally activated isomerizations of the chromophore could cause noise that the rod would be unable to distinguish from low levels of light. Such dark noise would clearly be undesirable in a photoreceptor otherwise capable of detecting single photons. He made the additional suggestion that the absorption maximum of the pigment is an index of the energy barrier that must be surmounted for isomerization, concluding that the longer the wavelength of maximal absorption, the greater will be the proportion of the mole-

TABLE 1

Thermodynamic parameters for isomerization of retinal and "quantum events"

	Thermal isomerization of 11-*cis* retinal to all-*trans*	Dark occurrence of "quantum events" in toad rods
Energy of activation	22.4 to 26.2 kcal/mole*	21.9 kcal/mole**
Entropy of activation	−10 to −21.4 kcal/mole*	−35.3 kcal/mole**
Absorption maximum	380 nm	500 nm
Corresponding energy to the first excited singlet state	75.3 kcal/mole	57.2 kcal/mole

* depending on solvent; Hubbard et al. (1966)

** Baylor et al. (1980)

cules that have obtained the requisite energy from chance thermal events. This hypothesis predicts an enormous effect of spectral position on dark noise — nearly 2 log units equivalent light intensity over a 50 nm shift in λ_{max}.

Spontaneous dark activations of the excitation cascade have been measured in the photocurrents of single amphibian rods (Baylor et al., 1980). On the basis of power spectra, these single shots of current are identical to the quantum events observed at low light levels. It therefore seems likely that both are triggered by the interaction of rhodopsin with G-protein, the next molecular participant in the phototransduction cascade. The activation energy for the pulses of dark current is 22 kcal/mole (corresponding to a wavelength of 1300 nm) and the entropy of activation is negative. The cause therefore appears to be different from the thermal denaturation of rhodopsin that has been measured in solution and at higher temperatures (Hubbard, 1958-59) and which involves a large increase in entropy. On the other hand, the thermodynamic activation parameters are more nearly like those measured for the dark isomerization of 11-*cis* to all-*trans* retinal in solution (Hubbard et al., 1966). These numbers are summarized in Table 1.

This coincidence of thermodynamic parameters does little, however, to support the hypothesized relation between dark noise and λ_{max}. First, the two reported activation energies are nearly the same and bear no relation to the energy difference between the ground and first excited state for either molecule, which is obviously much smaller for rhodopsin than for free retinal. Second, both entropies of activation are negative, and larger for the initiation of the shots of membrane current than for isomerization of free retinal, implying that the rates of reaction are determined by conformational parameters. Taken together, these observations are not readily compatible with the assumption that the pathway of thermal isomerization involves the same excited state that is produced by the absorption of a photon, or that the λ_{max} of rhodopsin is an indication of the relative rate of thermal isomerizations. In fact, theoretical considerations suggest that thermal isomerizations around C = C bonds involve excited vibrational levels in the ground state and perhaps a low-lying triplet state (Cundall, 1964; Abrahamson and Japar, 1972).

As a final consideration, it is not yet demonstrated that isomerization of the chromophore is involved in the initiation of the shots of dark current that are observed in rod outer segments. Despite the very suggestive energy of activation, the conformation of opsin required to activate the cyclic nucleotide cascade could conceivably be achieved by occasional chance thermal events without the retinal changing shape. If this were the mechanism, however, the lifetime of the "activated" rhodopsin would probably be shorter than when the retinal isomerizes, fewer G-proteins would be activated, and the individual pulses of dark current might therefore be smaller. With either mechanism, however, the hypothesis that the Purkinje shift is an adaptation to reduce retinal noise caused by thermal activations of rhodopsin stands on very shaky ground.

The tight clustering of λ_{max} of vertebrate rhodopsins near 500 nm might have a completely different kind of explanation. Although retinal-based (and 3-hydroxyretinal-based) visual pigments are found with λ_{max} anywhere from 340 to at least 570 nm, rhodopsins in the narrow definition of the term (i.e., vertebrate rod pigments) do not exhibit this variety. In fact

only a very small number of rhodopsins have been reported with λ_{max} longer than 512 nm (Fig. 15). And rhodopsins, as we have seen, seem to have diverged from cone pigments and invertebrate pigments several hundred million years ago. It is therefore appropriate to consider whether, for some considerable evolutionary time, an improbable sequence of mutational events would have been required in order to produce functional rod pigments absorbing at wave-lengths longer than 512 nm. When we know more about the relation between structure and function in the rhodopsin molecule, it should be possible to address this question. For the present, however, it stands as a possible explanation for the compact distribution of λ_{max} (Fig. 15), involving evolutionary inertia in addition to adaptation by natural selection.

The capacity to make 3-dehydroretinal allows the visual pigments to absorb at longer wavelengths, but the extra double bond in the chromophore does not shift the absorption spectrum of rod pigments far enough to optimize quantum absorption in turbid water. Lythgoe (1979) has suggested that the principal adaptive advantage of 3-dehydroretinal is found in the relation of the cone pigments, rather than of the rod pigments, with the environment. On the other hand, the broader significance of 3-dehydroretinal may be that it reflects a difficulty in shifting the spectra of vertebrate rod pigments to longer wavelengths solely through changes in the opsins.

This discussion has not provided a very satisfying explanation for the spectral distribution of rod pigments and, so far, it has raised more questions than it has settled. The choice between an untested hypothesis of evolutionary constraint and shaky explanations based on adaptation should leave any reader uncomfortable. But perhaps we have not exhausted the store of possibilities. We certainly have not addressed all of the paradoxical questions. For example, if fitting the absorption spectrum of rhodopsin to the ambient light to maximize absolute sensitivity is important (as deep-sea animals suggest is the case), and there are evolutionary difficulties in producing a large shift to longer wavelengths from 500 nm, why not achieve a better match with the environmental light by expressing the gene for the long wavelength cone pigment in rods as well as in a subset of cones? If the spectrum of rhodopsin is currently maladapted and is constrained (in an example of Dollo's Law) in shifting from 500 nm, why was the spectrum centered at 500 nm much earlier in evolutionary time? Was that an adaptation to an ancient environment? If the porphyropsin of tadpoles really represents an improvement, why do frogs revert to rhodopsin on metamorphosis? And why do 27 species of anomuran and brachyuran decapod crustaceans—whose main rhabdoms contain but a single visual pigment—have rhodopsins with λ_{max} at 493 nm $\pm$ 9 nm s.d. (range 473-515 nm), virtually identical to the distribution of vertebrate rod pigments (Cronin and Forward, 1987; Forward et al., 1988)? Vertebrate and crustacean opsins are unlikely to be closely related, but do they share a common adaptation that has not been recognized? We are clearly missing something important.

There are functional differences between rods and cones involving, at the very least, absolute sensitivity and time course of response. Most important, some of these differences may be dependent on features of the opsins we do not yet fully recognize. Is it thus not possible that if a cone pigment were expressed in a rod, more than the spectral sensitivity would be different, and we would simply not recognize the cell as a rod? The absorption spectrum is how we ordinarily distinguish one visual pigment from another, but natural selection clearly has a richer set of criteria on which it works. Moreover, the various functional adaptations of opsin are unlikely to be totally independent of each other. Consequently, in the rhodopsin molecule of vertebrate rods an absorption maximum between 480 and 515 may be baggage, so to speak, carried along as part of a critical complex of adaptations. This remains a hypothesis of evolutionary constraint, but more subtle and difficult to test than the first. Yet it has the merit of inviting us to think more deeply about why rods and cones are different.

Transmutation Revisited, Or When Is a Rod a Rod?

In his seminal treatise on the adaptive radiation of the vertebrate eye, Walls (1942) developed the hypothesis that rods, once lost in diurnal lines, could be refashioned from cones (or vice versa) by a process he termed *transmu-*

tation. He was led to this view by comparing photoreceptors in phylogenetically and ecologically diverse groups, particularly reptiles. For example, most lizards are strongly diurnal and have only cones in their retinas. Most of the geckos, however, are secondarily nocturnal, and their photoreceptors have become rod-like, with relatively massive outer segments and (usually) no oil droplets. Their affinities to cones are apparent in the light microscope, because some of these "rods" have paraboloids and some are double, with principal and accessory members to the pair. At the ultrastructural level the synaptic terminals are more cone-like than rod-like (Pedler and Tilly, 1964; Hedden, cited by Kleinschmidt and Dowling, 1975). Photoreceptors intermediate in form between the rod-like cells of nocturnal geckos and typical cones of diurnal lizards are found in some species, and there is agreement that on morphological criteria these gecko photoreceptors are derived from cones (Underwood, 1951, 1968, 1970; Crescitelli, 1972, 1977).

Walls believed that the process of transmutation had occurred frequently and in many phyletic lines, and he hypothesized that the rods of both birds and mammals were derived independently from cones. A rod, in this view, is identified by morphological criteria, supplemented by knowledge of whether the animal is more active during the day or at night. But Walls also asserted (reasonably) that a rod, by definition, contains rhodopsin. As he had no way to measure rhodopsin, he concluded (incorrectly) that rhodopsin and rods had been reinvented many times.

Because of the evolutionarily ancient divergence of rod and cone pigments, rhodopsin genes should have been expressed in continuous and unbroken lines of descent from the appearance of the first vertebrates to contemporary birds and mammals. In fact, the relatively close relationship between avian and mammalian rod opsins is consistent with a common ancestral opsin sometime in the Mesozoic (Figs. 9, 13). This realization spells trouble for the orthodox version of transmutation that is central to Walls's ideas on the evolution of vertebrate photoreceptors. Furthermore, it emphasizes the need to think clearly about what we mean by rods and cones. Here is the definitional dilemma: in addition to functional and morphological criteria for distinguishing between rods and cones, criteria that can be ambiguous (Underwood, 1968, 1970), we now have at our disposal a simple molecular definition as well—expression of the gene for rod opsin (rhodopsin) or a cone opsin. Moreover, with the tools of molecular biology, this criterion is in principle independent of the first two. For the sake of emphasis I shall adopt the molecular definition in what follows.

Geckos, which present the classical example of transmutation, illustrate how the two definitions can be in conflict. The "rods" of *Gekko* are so called because the majority of outer segments are not only large, but most of the disks are unconnected to the plasma membrane—the single morphological feature considered by most to be diagnostic of rods (but see Underwood, 1970). The two known visual pigments of *Gekko* photoreceptors, however, are clearly not typical rhodopsins; the absorption maxima are at 521 nm and 467 nm (Liebman, 1972; Crescitelli, 1977; Govardovskii et al., 1984). Furthermore, the 521 nm pigment, which is present in most of the receptors, reacts with a monoclonal antibody that in birds and other vertebrates is specific for cone pigments absorbing at long wavelengths (540-570 nm) and does not react with rods (Szél et al., 1986). On the basis of both their pigment and their likely evolutionary history, most of these cells are therefore cones, albeit cones that are functioning under conditions for which rods are better suited. Have they crossed some arbitrary line in their evolutionary process of transmutation and *become* rods? To call them rods is to abandon the expectation that rods contain rhodopsin.

Given the all-cone retinas of most lizards, we can hypothesize that the ancestors of geckos were diurnal for such a long time that they lost their gene for rod opsin. Although the 467 nm pigment reacts with a polyclonal antibody that binds to rhodopsin in other animals (Szél et al., 1986), this in itself does not establish the ancestral affinities of the 467 nm opsin. Except for the curious 430 nm pigment of the "green rods" of frogs whose ancestry is equally mysterious, no other terrestrial vertebrate has a rod pigment absorbing at such short wavelengths. And the blue-sensitive cone pigment that has been sequenced is as closely related to human rod opsin as it is to the other cone pigments (Fig. 12). Transmutation of gecko cones may

thus be an adaptive response to nocturnal life, forced by the absence of rods. In this view, neither *Gekko* "rod" pigment closely resembles rhodopsin, either because of insufficient evolutionary time or a constraint imposed by the low probability of traversing the necessary mutational paths. If transmutation is generally triggered by the loss of one or more pigment genes, however, it is doubtful that it can lead to the total change of cellular personality that Walls supposed was possible. It should be seen as selection on one cell type (e.g., cones) to fill a functional void created by the absence of the other (e.g., rods).

In summary, loss of a pigment gene is a likely prelude to transmutation of photoreceptors, but this has probably not happened as frequently or extensively as Walls supposed. Nor does the loss of a pigment gene necessarily lead to transmutation, as we shall see in the discussion of mammalian color vision. Even if the extent of change is limited, however, transmutation poses some interesting and as yet unanswered questions. To what extent can a cell with cone pigments achieve the absolute gain usually associated with rods? Cones generally show a greater capacity to decrease their gain (i.e., adapt) than do rods, and in this respect *Gekko* photoreceptors are more cone- than rod-like (Kleinschmidt and Dowling, 1975). The rods of skates present a similar and, in this evolutionary context, even more puzzling capacity to adapt (Dowling and Ripps, 1972). Clearly, the evolutionary relationships of the pigment, the properties of the transduction cascade, the control of channel conductance, various morphological features of the cell, and the patterns of synaptic connection could fruitfully be explored further in phyletic lines where rods or cones have been put to unusual adaptive challenge.

EVOLUTION OF COLOR VISION

Color Vision Depends on Spectrally Different Visual Pigments

Different opsin genes code for pigments whose absorption spectra peak in different regions of the spectrum (Fig. 16). The absorption spectrum describes the relative probability that photons of specific energy—i.e., light of various wavelengths—will be absorbed. Once a photon is absorbed by a visual pigment, however, the probability that the retinaldehyde will isomerize to the all-*trans* configuration is approximately 0.7, whether the visual pigment is from a vertebrate (Dartnall, 1972) or an invertebrate (Cronin and Goldsmith, 1982). Thus, the only information that a photoreceptor cell can report to the central nervous system is that an isomerization has occurred; information about the wavelength is lost. Put another way, a photoreceptor cannot distinguish between a moderate flux of photons near the wavelength of maximal absorption and a higher flux at a wavelength that is poorly absorbed if both lights cause the same rate of isomerization. Wavelength and intensity are therefore confounded. The fact that visual pigments can signal only the rate at which photons are caught is frequently called the principle of univariance (Rushton, 1972).

Two conditions must be met for an animal to be specifically sensitive to color (wavelength). It must possess at least two spectrally different classes of receptor, and it must have the appropriate neural apparatus to compare the outputs of the different types of receptor. This formalism does not require the presence of two or more visual pigments because, in principle, two receptors might contain the same visual pigment but differ in spectral sensitivity because a colored filter in front of one of the receptors modified the effective absorption spectrum. At one time it was thought that the colored oil droplets in the cones of birds and reptiles might have such a function, and filters have been described in some arthropods (Bernard and Miller, 1968; Marshall, 1988, Cronin and Marshall, 1989). Nature, however, seems not to have chosen such a path, at least not frequently enough for it to have come to light. All known color vision systems are based on the presence of two or more visual pigments packaged in separate cells.

In principle, single receptors might contain different proportions of a mixture of two pigments, thereby creating the basis for tri- or tetrachromatic color vision from only two opsins. No such cases are known. There are no definitive cases of opsin mixtures in a single cell, and indirect evidence (e.g., in primates, see below) suggests that there may be developmental control mechanisms preventing the simul-

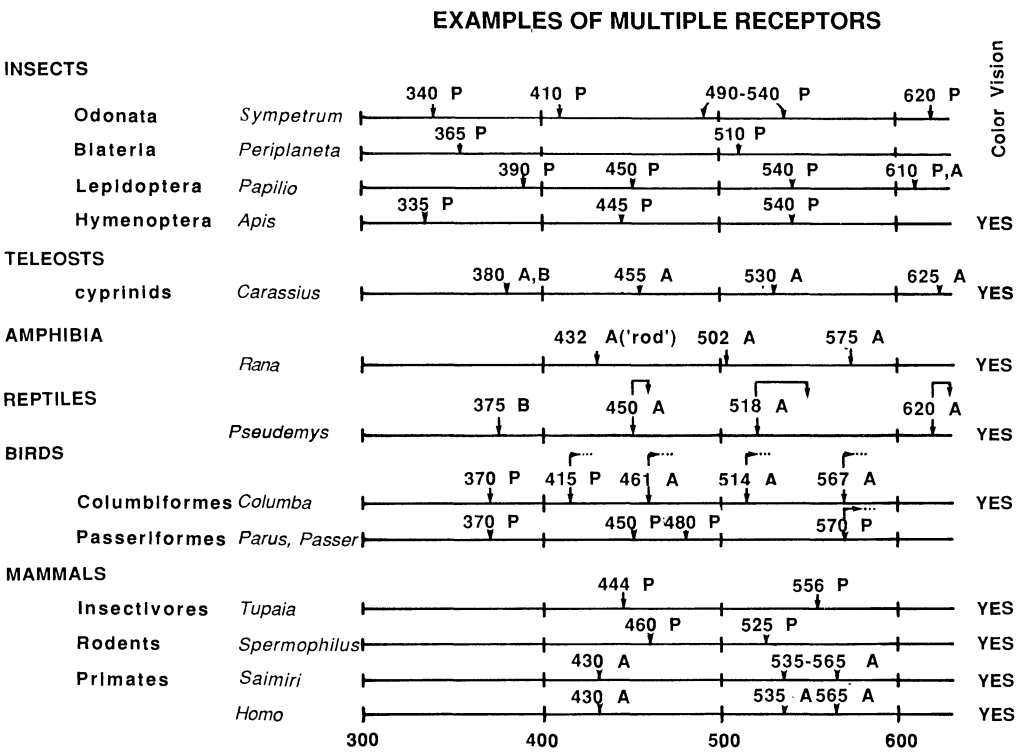

FIG. 16. EXAMPLES OF ANIMALS THAT HAVE MORE THAN ONE VISUAL PIGMENT

These are species that have been shown to have multiple visual pigments and/or different spectral classes of photoreceptors, prerequisites for color vision. The arrowheads above the wavelength scales indicate the positions of the λ_{max} of pigments (or receptors); horizontal limbs on long-stemmed arrows indicate shifts in spectral sensitivity caused by cone oil droplets. The presence of behavioral evidence for color vision is indicated in the last column. This compilation is not an exhaustive survey of the literature, but is intended to illustrate the wide taxonomic distribution of retinal capacity for color vision and wavelength-dependent responses and to indicate some of the better-studied examples. The superscripts indicate experimental technique: *A*, absorption spectra of pigments measured directly; *B*, behavior; *P*, physiological measurement.

For references see the reviews of Jacobs (1981) on vertebrates and Menzel (1979) on invertebrates as well as the following more recent citations: *Sympetrum*, Meinertzhagen et al., 1983; *Papilio*, Matic, 1983; *Carassius*, Neumeyer, 1985, 1986; *Pseudemys*, Arnold and Neumeyer, 1987; *Parus*, *Passer*, Chen and Goldsmith, 1986; *Tupaia*, Jacobs and Neitz, 1986; *Saimiri*, Bowmaker et al., 1987.

taneous expression of more than one opsin gene in any one cell.

What Do We Mean by "Color Vision"?

Suppose that a retina contained two or more kinds of receptors differing in spectral sensitivity. Because it can be argued that their presence might simply serve to broaden the wavelength band over which the animal is sensitive, the occurrence of multiple receptors is not a sufficient demonstration of color vision. Although the presence of interneurons with antagonistic input from two spectral classes of receptor is usually considered to be strong presumptive evidence, for reasons that will soon become clear this, too, does not demonstrate that the animal has color vision. The only criterion for establishing the presence of color vision is an appropriate behavioral test. What constitutes appropriate, however, is a matter that requires definition.

Traditionally, the problem has been formu-

lated as a need to demonstrate that wavelength and intensity can be processed by the animal as independent variables — to show that in making visual discriminations, hue and brightness are not confounded. In order to design such an experiment one must therefore show that choices between visual stimuli can be based on their differences in chromatic content, either regardless of their relative brightnesses or when matched for equal brightness. One kind of complication arises from the fact that estimations of the wavelength dependence of brightness depend to some extent on the method of measurement. Jacobs (1981) provides a more extensive discussion of this problem, with particular reference to comparative studies of vertebrate color vision.

A second complication appears when considering the evolution of color vision in the broadest biological context, where it is desirable to formulate hypotheses on wider bases than are suggested by the human visual experience. Our own experience tells us that to have color vision is to see colors. How do we translate that into an operational definition that can be applied to other animals?

The commonest technique in studying sensory capacities of vertebrates is operant conditioning, in which the animal learns to perform a simple task like pushing a bar when it sees the "correct" stimulus. The successful animal therefore must be able to extract from the stimulus some quality associated with wavelength composition; and although some chromatic discriminations will be easier for the animal to make than others, in principle the animal can be trained to accept light of any spectral composition as being "correct." What colors the animal learns will bring it reward are therefore quite arbitrary, implying that its perceptual capacities have at least this degree of flexibility and ability to generalize. In this respect the animal shares a feature of our own visual experience — it sees colors.

Although the key to success in applying this operational definition of color vision is the capacity of the animal to learn, this does not mean that the ability to learn is a prerequisite for color vision. The ability of the animal to learn simply allows the experimenter to probe the animal's sensory competence.

A definitional confusion arises when the animal must be studied solely by means of spontaneous behaviors. This theme can be developed by first considering some features of the visual systems of insects. Many Lepidoptera have multiple visual pigments. The butterfly *Pieris* exhibits several different behavioral responses to colored lights, each with a distinct action spectrum exhibiting maximum sensitivity at different wavelengths: escape (λ_{max} 370 nm), feeding (λ_{max} 450 nm with a secondary maximum at 600 nm), drumming (λ_{max} 560 nm), and egg laying (λ_{max} 540 nm) (Scherer and Kolb, 1987). Most of the spectral sensitivity curves are narrower than the absorption spectra of visual pigments, and with mixtures of 600 and 558 nm light, both feeding and drumming are inhibited by the presence of the inappropriate wavelengths. The neural wiring thus appears to be more complicated than if each behavior were driven by a single spectral type of receptor. The principle of univariance is not observed; more than one pigment must therefore be involved.

There is no learning involved in any of these behaviors, and efforts to train the butterflies to different colors have not been successful. In the absence of any demonstration of learning, there is no evidence for a perceptual dimension of color. The most parsimonious interpretation, and the one that is consistent with the fixed relation between behavior and color, is that this butterfly's several visually directed responses are triggered by specific configurations of sensory input. They are therefore examples of *wavelength-dependent behaviors*.

Color vision and wavelength-dependent behaviors are distinguished by behavioral tests. Both involve multiple receptors whose outputs can sum (not necessarily equally) or can inhibit each other in the afferent path. The lack of independence of receptors is therefore not necessarily an indication of color vision. To summarize, the behavioral criteria for color vision imply the presence of processes in which the quality of color has a perceptual identity. By contrast, the concept of wavelength-dependent behavior arises from behavioral experiments in which the relation between wavelength and motor response cannot be freely altered by training, implying a neural circuit with substantially less plasticity. Until our concepts are expanded by new experimental findings, this distinction should remain useful.

The distinction embodies more than a

semantic quibble, because color vision and wavelength-dependent behaviors can coexist in the same animal. Single-unit recordings show that there are three spectral classes of receptor in the eye of the bee (*Apis mellifera*); in order of decreasing relative numbers they have maxima at 540 nm, 335 nm, and 435 nm (Menzel and Blakers, 1976; Menzel et al., 1986). The "luminosity curve" of the electroretinogram (ERG) seems to reflect the relative numbers of each kind of receptor and has no deeper physiological significance.

Like most insects, dark-adapted bees are strongly attracted to near-ultraviolet light, and the action spectrum for this behavior has a major peak near 350 nm with a much smaller peak near 540 nm (Bertholf, 1931; Kaiser et al., 1977; but see Menzel and Greggers, 1985, for somewhat different results, obtained when phototaxis was observed during the foraging cycle). This action spectrum can be measured by determining the relative energies of spectral lights required for equal numbers of choices in a forked maze. There is no evidence for color vision in these experiments, and although all three receptor types participate, their contributions are unequally weighted.

On the other hand, the action spectrum for a threshold optomotor response of the bee to a moving striped drum is quite different. Here the maximum sensitivity lies at about 540 nm with no distinct peak in the UV (Kaiser and Liske, 1974). In this behavior, as in visual scanning (Lehrer et al., 1985), the green-sensitive receptors dominate the input, and there is again no evidence for color vision. Note that in studying spontaneous, color-specific behaviors the definition of "brightness" is context-dependent.

In these behaviors of the bee there appears to be summation of inputs from the several spectral types of receptors, although the contributions of each receptor depend on the biological function of the response. In the celestial orientation and navigation of bees, however, there is evidence for mutually inhibitory interplay between the outputs of UV receptors sensitive to orthogonally polarized light, and between receptors sensitive to UV and those sensitive to longer wavelengths. UV light (or more specifically the polarization pattern of UV light as detected by an array of specialized receptors on the dorsal rim of the eye) is attributed by the bee to the antisolar portion of the sky, while longer wavelengths are ascribed to the sun (Edrich et al., 1979; Brines and Gould, 1979; Rossel and Wehner, 1984).

Bees also have a trichromatic color vision, but as far as is known it is employed exclusively in feeding and in recognition of the hive. It has been extensively studied at feeding sites (Daumer, 1956; Menzel, 1967; von Helversen, 1972a,b), where foraging bees can be trained to associate food with particular spectral lights or other chromatic stimuli, and controls demonstrate that the choices of trained bees depend on wavelength and not intensity. The spectral "brightness" curve, based on spontaneous choices, is maximal in the UV (Daumer, 1956); the wavelengths showing the steepest learning curves fall in the violet (Menzel, 1967).

The closer bees have been examined, the richer their repertoire of wavelength-dependent behaviors appears to be (Menzel, 1985). It is probably unwise to assume, however, that wavelength-dependent behavior is the exclusive province of arthropods. There is a rich literature on the role of "releasers" in vertebrate ethology in which the sensory mechanisms have not been adequately pursued. There are, however, a few cases of presumptive wavelength-dependent behavior in vertebrates that have received attention. If the tendency of most species of frogs to jump towards a light is measured as in a forced choice experiment, short wavelengths (λ_{max} at 480 nm) stimulate positive phototaxis and longer wavelengths inhibit (Muntz, 1962; Chapman, 1966; Hailman and Jaeger, 1974; Fite et al., 1978). This result has been interpreted as an indication of the presence of color vision, but it is far from clear from this behavior that the underlying neural circuits subserve other, more general, kinds of chromatic discriminations. The data are consistent with the hypothesis that phototaxis is driven by some mixture of short wavelength receptors and inhibited by long wavelength-sensitive cones, even though the spectral response is a function of the state of adaptation (Muntz, 1966). Ganglion cells with appropriate antagonistic input have been seen in frog retina (Backstrom and Reuter, 1975).

In conclusion, wavelength-dependent behavior is likely present in both vertebrates and invertebrates, and it may or may not exhibit features that are traditionally assumed to de-

fine the presence of color vision. The role of color in our own visual experience does not prepare us to think in these terms. It is therefore useful to consider the circumstances in which such behaviors are most likely to be observed. They are examples of what ethologists call "species-specific behavior," which, of course, does not imply that they cannot occur across larger taxa than species. This sort of behavior is frequently characterized as "unlearned," but whether that is accurate in any particular case is a question that can only be answered by studying the animal's development and has usually not been of interest to investigators interested in visual performance. What may sometimes be meant by "unlearned" is that if there was any accumulated experience involved in the ontogeny of the behavior, the investigator was not responsible for providing it.

Wavelength-dependent behavior is likely a common feature of small nervous systems that employ a great deal of peripheral filtering of sensory information. Peripheral filtering and stereotyped behavior are not necessarily evolutionarily primitive. In insects they provide parsimonious adaptive solutions to the sensory needs for survival and reproduction of small-bodied, short-lived animals occupying narrow ecological niches (Wehner, 1981). Bernard and Remington (in press) have recently provided what appears to be an exquisite example in which two sympatric species of butterfly of the genus *Lycaena* show adaptive differences in the proportions of their visual pigments in a manner that is clearly related to species differences in the spectral reflectance of the wings.

A Great Variety of Animals Have Multiple Visual Pigments

Fig. 16 summarizes some of the better known examples of animals with multiple visual pigments, selected to illustrate the taxonomic diversity with which color vision or wavelength-dependent behavior is associated, and because, in most of these examples of vertebrates, there is independent behavioral evidence for color vision (last column). The frequent occurrence in birds of brightly colored plumages, often associated with sexual dimorphism, provides indirect evidence that color vision is a common feature of the avian visual system, even in species that have not been tested directly. Although there is little or no quantitative behavioral data on the color vision of most invertebrates, the Hymenoptera are a dramatic exception. The color vision of bees has been studied in at least as much detail as any species other than humans (Menzel, 1985; Backhaus and Menzel, 1987). In many other groups of arthropods a rich endowment of visual pigments indicates the presence of interesting visual capacities, regardless of how the central nervous system handles the incoming information.

What conclusions can be drawn from these comparative data? First, color vision is found in the major extant vertebrate classes, and color vision or wavelength-dependent processes are common among insects. (Color vision is absent in the large eyes of cephalopod molluscs, and the status of crustacea is not at all clear.) Further, examples of retinas with two, three, or four presumptive color vision pigments are known from both arthropods and vertebrates. Until very recently, visual pigments with maximal absorption in the near UV were known only from arthropods, but cones with peak sensitivity at 370 to 380 nm have now been found in fish, turtles, and birds (Fig. 16; see also Harosi and Hashimoto, 1983; Bowmaker and Kunz, 1987). The best-studied of the lower vertebrates therefore characteristically have four (or even five) cone pigments. Trichromacy is not the norm.

The mammals require special comment. As reviewed in Jacobs (1981), mammalian color vision is most highly developed in the primates. Most species of rodents (many squirrels excepted), carnivores, and ungulates are either nocturnal or arrhythmic, and probably have very poor capacity for color vision. Rats and cats, both of which have been examined with some care, do not learn to make color discriminations easily and possess few cells that seem to be involved in color vision. The primitive mammalian condition as exemplified by tree shrews appears to be dichromatic, and even squirrels with many cones and diurnal habits seem to have remained dichromatic.

The following evolutionary picture is consistent with the evidence now available. Perhaps as much as two thirds of the history of mammals occurred during the Mesozoic, prior to the great spurt of change that followed the extinctions of the dinosaurs. During this time the paleontological evidence indicates mammals were small and nocturnal in habit

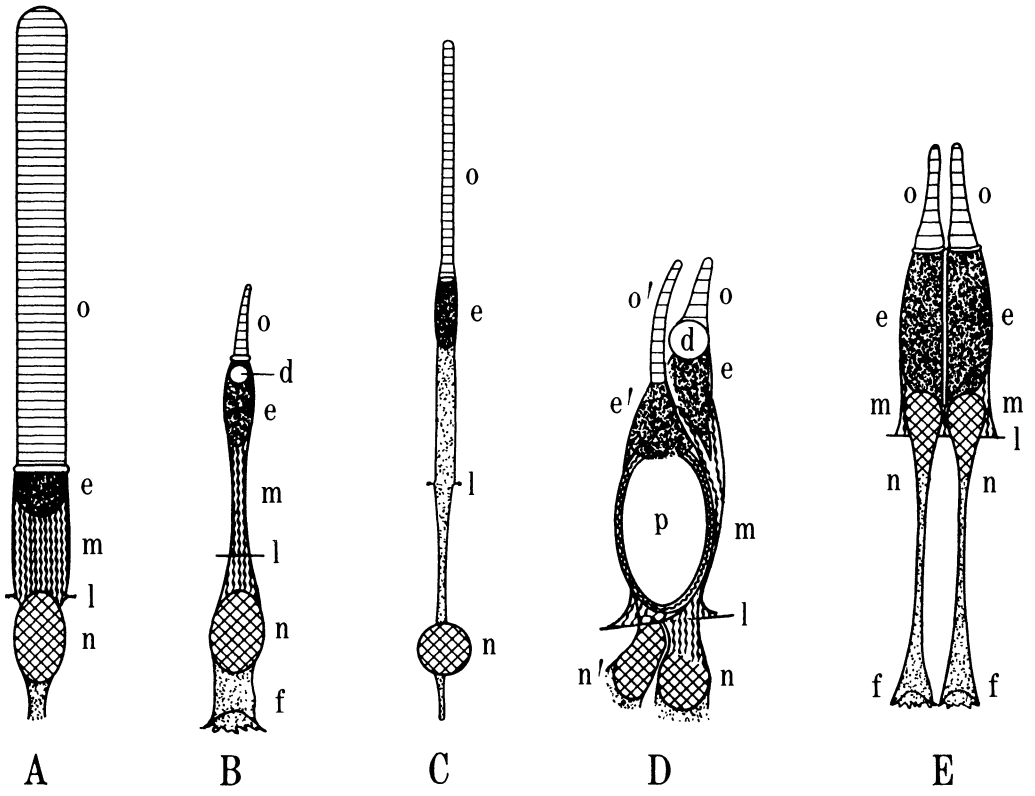

FIG. 17. VERTEBRATE RODS AND CONES ILLUSTRATING SOME FEATURES THAT ARE NOT PRESENT IN MAMMALIAN PHOTORECEPTORS

A, common or red rod of the frog *Rana pipiens*, dark-adapted with the myoid contracted. B, cone of *R. pipiens*, dark-adapted with the myoid elongated. C, human rod, lacking a contractile element. D, double cone of the painted turtle *Chrysemys picta* (note the large oil droplet in the principal member); E, twin cones of a teleost fish, the bluegill *Lepomis macrochirus*, light-adapted with the fused myoids contracted; *d*, oil droplet; *e*, ellipsoid (mitochondrial mass); *e'*, ellipsoid of the accessory member of the pair; *f*, footpiece or pedicel; *l*, external limiting membrane of the retina; *m*, myoid; *n*, nucleus; *o*, outer segment; *o'*, outer segment of the accessory cone; *p*, paraboloid (glycogen). Redrawn from Walls (1942).

(Lillegraven, 1979). As Walls (1942) pointed out, the cones of placental mammals are simplified from the versions of cones found in birds and reptiles, lacking both oil droplets and contractile myoids (Fig. 17), and the trichromatic color vision of Old World primates is the most elaborate color vision system known among mammals.

As the origins of primate visual pigments are ancient (Figs. 9, 13), the capacity for photopic vision probably declined seriously but was not lost during the long early history of mammals. In species of birds that live in ecological conditions where the retina is starved for light, the proportion of cone oil droplets that are pale or colorless increases (Peiponen, 1964; Muntz, 1972); consequently, total loss of oil droplets is a reasonable end point to expect if selection for photopic vision were relaxed for a sufficient time. In any event, oil droplets are not present in contemporary placental mammals. With a thoroughly nocturnal existence the need for contractile myoids would also have decreased, and with it the attendant pressure of natural selection to maintain these organelles.

The ancient lineage of cone pigment genes indicates that the cones, themselves, were not lost. Mammalian retinas like that of the rat, which were once thought to be all-rod, have on close examination revealed the presence of

cones (Sidman, 1958; Dowling, 1967; LaVail, 1976); in fact, as Walls (1942) recognized, cones occur in the retinas of most placental mammals. An unbroken line of cones and of two cone pigments would therefore seem to stretch farther back in evolutionary time than the fossil record allows us to trace the evolution of vertebrates.

As we have seen, birds and turtles appear to have a rich capacity for color vision, and in this respect they illustrate what might have been the future of other evolutionary lines. Such was not to be for mammals, for the adoption of nocturnal habits in the premammalian or early mammalian stock probably reduced the number of cone pigments to two, one absorbing in the blue in the neighborhood of 430 nm and the second absorbing at longer wavelengths, near 540 to 560 nm. With the adaptive radiation of mammals during the last 65 million years, several developments have occurred independently, few of which are understood in detail. In diurnal squirrels the retinas have become largely cone, but with no increase in the number of cone pigments. Thus these animals have a dichromatic color vision (Jacobs, 1981). Excepting the primates, in most other groups of mammals there persists only rudimentary capacity for color vision, which employs relatively few cones, which in many cases may be dichromatic, and which has generally proved hard to study by virtue of its modest contribution to overall visual capacity (Jacobs, 1981). The higher primates, however, justify Walls's contention that their color vision "is assuredly a law unto itself, genetically and historically speaking," although not for the reasons that he supposed.

Molecular Clues to the Evolution of Primate Color Vision

Two recent findings provide new insight into the evolution of primate color vision—the structure of the human genes for cone pigments (Nathans, Thomas, and Hogness, 1986; Nathans, Piantanida, Eddy, Shows, and Hogness, 1986), and the discovery of genetic polymorphisms (references below). We shall consider each of these briefly and in turn.

The differences in degree of identity of the human cone pigments was described above, indicating relatively recent divergence of the long- (L) and middle-wavelength (M) pigments. Moreover, the genes for these pigments are closely linked on the X chromosome, with one to several copies of the gene for M but a single copy of the gene for L. Inherited color deficiencies involving either absence of one pigment or the production of an altered gene product are well known. These relationships are summarized in Fig. 18. In the absence of L (*protanopia*) or M (*deuteranopia*) the individual is monochromatic at the long wavelength end of the visible spectrum, where normal observers are dichromatic. The two conditions of color deficiency can be distinguished from each other because at wavelengths longer than 500 nm the photopic luminosity curve tracks the absorption spectrum of the pigment (L or M) that is present.

When one of the gene products is altered, one of the pigments has an absorption spectrum intermediate between the normal positions of L (λ_{max} 560 nm) and M (λ_{max} 530 nm). The phenotypic expression of this condition is known as *anomalous trichromacy*. Anomalous trichromats are recognized because they do not accept the same Rayleigh matches (in which a mixture of monochromatic red and green lights is made indistinguishable from a monochromatic yellow) as do normal observers.

Color deficiencies are more frequent among males than females because the genes for L and M are carried on the X chromosome and the mutant phenotypes are expressed in the hemizygous state. The frequency of color deficiencies is high (several percent of males) because mutations arise continuously in the population from unequal crossover events, in which one X chromosome is left without a functional copy, or with a modified but functional copy, of one of the two genes (Fig. 18). Because individual X chromosomes usually have more than one copy of the gene for M, unequal crossovers, in principle, can also generate individual chromosomes containing genes for both normal pigments as well as a modified pigment.

A second important insight has come from studies of New World monkeys, particularly the squirrel monkey *Saimiri* (Bowmaker et al., 1987). Here the base condition is dichromacy, with two gene loci, one for a pigment maximally sensitive in the violet and a second for a pigment absorbing maximally in the green. As in Old World primates, the locus for the

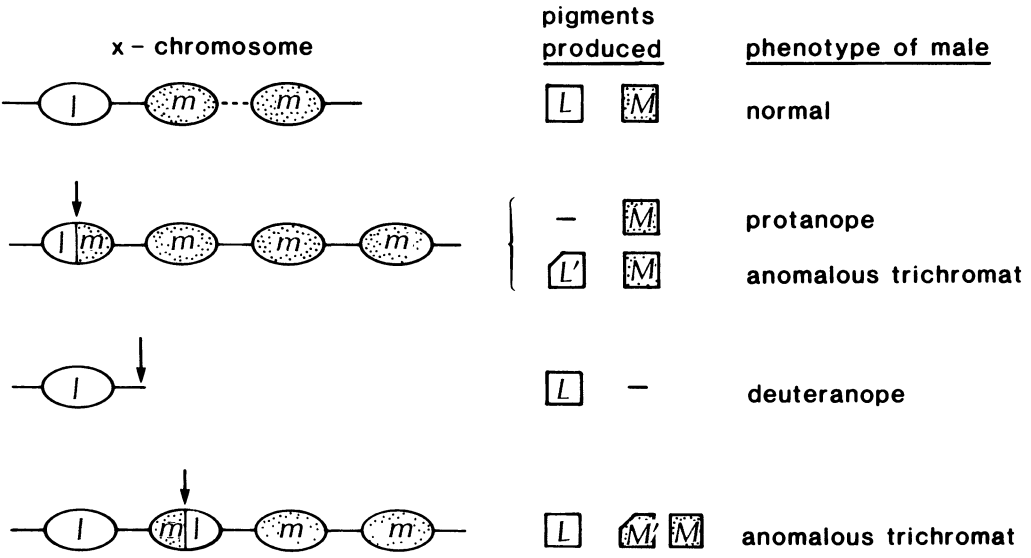

FIG. 18. MOLECULAR BASIS FOR THE COMMON (SEX-LINKED) FORMS OF HUMAN RED-GREEN COLOR DEFICIENCIES

As illustrated in the upper left of the diagram, the X chromosome normally contains a gene for the 565 nm cone pigment (*l*) and several copies of the gene for the 540 nm cone pigment (*m*). The gene products are the normal cone pigments, symbolized by L and M.

As a result of unequal crossover events the genes can be cleaved and repackaged in various ways, several of which are illustrated here. In the second row we see one result of a crossover that transected *l*. If *l* is thereby rendered nonfunctional, a male with this genotype lacks the cone pigment L, has no capacity for color vision in the red-green region of the spectrum, and is recognized in color vision tests as a protanope. If the gene containing coding regions from both *l* and *m* retains function, however, the resulting pigment (L′ or M′) characteristically has an absorption spectrum intermediate between that of L and M and the individual (male) with this chromosome is an anomalous trichromat.

In the third row the crossover has produced a chromosome containing *l* but not *m*. A male with this chromosome has no M pigment, exhibits the other form of red-green blindness, and is called a deuteranope. The bottom row shows that the molecular biology predicts that some individuals might have four functional color vision pigments (including the blue-sensitive pigment whose gene is not on the X chromosome). (Based on data of Nathans, Piantinida, Eddy, Shows, and Hogness, 1986.)

long-wavelength pigment is on the X chromosome. An interesting complication arises, however, because there are multiple alleles possible at this locus. That is, there are three different forms of the gene that can be present, coding for pigments with λ_{max} at 536, 549, or 564. This polymorphism leads to three different male phenotypes, all dichromatic (Fig. 19). Females can be homozygous for any of the three pigment genes, leading to the same three dichromatic phenotypes that are expressed in males. Females, however, can also have any one of three heterozygous genotypes, corresponding to the three possible combinations of pairs of these three alleles. Because in the somatic cells of female mammals, only one X chromosome is active (Lyon, 1974), individual cone cells of these heterozygous females express one or the other of the two pigments, but the retina contains both kinds of cone in addition to the S cones. Consequently, females that are heterozygous at this locus are trichromatic, and there are three different phenotypic expressions of this trichromacy (Fig. 19).

Armed with the example of squirrel monkeys, it is easier to understand some of the variation in color matching data on humans. Alpern (1979, 1981) came to the conclusion on the basis of differences between individual subjects that the human cone pigments come in three spectral "clusters." In the case of color-anomalous individuals, there are presumably

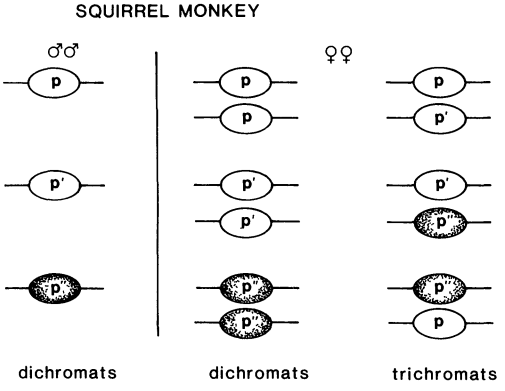

FIG. 19. GENETIC BASIS FOR COLOR VISION IN A NEW WORLD MONKEY

This figure shows the genetic configurations and phenotypic consequences of three alternative alleles located on the X chromosome of squirrel monkeys and coding for long wavelength cone pigments. Because there is an autosomal gene responsible for the violet-sensitive cone pigment, individual males have two cone pigments, but there are three different kinds of males, depending on which of the three sex-linked genes is present. Similarly, females homozygous at the X chromosome locus also have two cone pigments, and there are three classes of females. Females heterozygous at this locus, however, make three cone pigments. Color matching tests show animals with two pigments are dichromats; those with three pigments are trichromats. Moreover, there are three classes of trichomats, corresponding to the three possible X chromosome pigment pairs. It is most interesting that there is sufficient plasticity in the development of antagonistic synaptic processes in the visual centers to take advantage of the extra pigment present in the retinas of heterozygous females. See Bowmaker et al., 1987 for references.

several functional opsin genes resulting from crossover events involving the X chromosome. But there are also differences in the Rayleigh matches made by people with normal color vision. Neitz and Jacobs (1986) found a bimodal distribution in the color matches of a population of color-normal young males, which corresponds to a 3nm difference in the spectral maxima of their L pigments. The color matches of females, on the other hand, follow the binomial distribution predicted from the nearly equal frequencies of these two L opsins in the normal population. About half of the females should be heterozygous and contain both L opsins, albeit in different cones. As predicted on the further assumption that the two kinds of L cones are not distinguished by the nervous system, about half of the females exhibit color matches intermediate between the two groups of males.

In summary, rather than adopting a largely cone retina like the diurnal squirrels, the Old World primates have evolved a duplex retina containing a foveal region with high cone density. Looking at the higher primates, one is struck by a tendency of the ancestral gene on the X chromosome not to behave itself. The polymorphism in New World monkeys, multiple copies of the M pigment gene in the human genome, and the (presumed) duplication event that lies behind the human L/M gene pair have led to similar trichromatic phenotypes in two different groups of primates. We doubtless do not yet fully understand why this ancestral gene of primates has been such ready grist for the evolutionary mill, but it has enabled these animals to recover a richness of diurnal vision that was largely lost as their ancestors traversed the late Cretaceous. Nevertheless, the spectral range of λ_{max} of the pigments produced by this subset of primate genes spans but a modest 30 nm or so—just about the same spectral range that is occupied by the rhodopsins of the rods. In short, our own color vision was molded by adaptation to a diurnal life style, but within some very significant constraints imposed by the evolutionary history of mammals during the past 200 million years.

Why Primates and Other Mammals Do Not Have Ultraviolet Receptors

The cone oil droplets of sauropsids undoubtedly play a role in color vision (see below), but that is probably not their only function. In birds, most (but not all) of the droplets that appear colorless contain either a C_{27} apo-carotenoid with the trivial name of galloxanthin and a λ_{max} at about 400 nm (Fig. 20), or a carotenoid absorbing at even shorter wavelengths which is known only from microspectrophotometry (Goldsmith et al., 1984). In some droplets galloxanthin is found mixed with ε-carotene. These droplets remove much of the near ultraviolet and varying amounts of violet light. Only the small number of cones with truly transparent droplets (at least some of which are likely UV receptors) are not at least partially

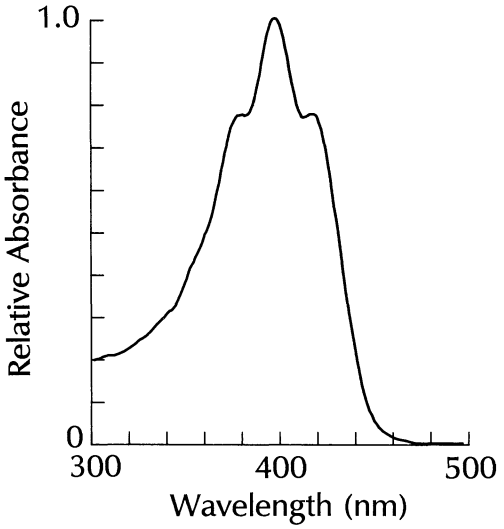

FIG. 20. ABSORBANCE SPECTRUM OF GALLOXANTHIN

This C_{27} apo-carotenoid is the principal pigment of the "pale" and "colorless" cone oil droplets of bird retinas. Like the more brightly colored oil droplets containing zeaxanthin and astaxanthin, galloxanthin-containing droplets transmit light effectively only on the long wavelength side of the absorption band. The concentration of galloxanthin in vivo is sufficiently high that most droplets absorb more than 80 to 90 percent of the incident light at 350 nm, and considerably more around 400 nm.

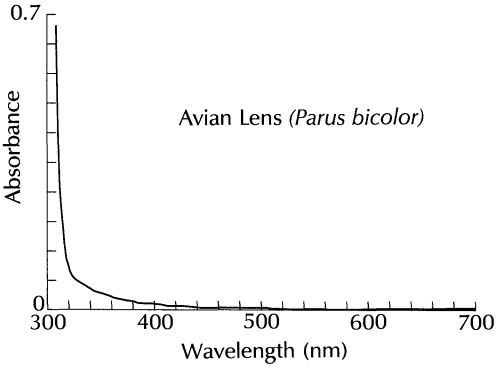

FIG. 21. AVIAN LENSES ARE TRANSPARENT TO NEAR UV LIGHT

Although birds differ in the relative abundance of UV-transmitting oil droplets, and therefore presumably also in the relative importance of UV sensitivity, the lenses of passerine birds that we have examined (7 species from 5 families) are transparent down to about 320 nm. Shown here is the absorbance spectrum of an isolated lens of the tufted titmouse (*Parus bicolor*).

screened from the shortest wavelengths in the terrestrial solar spectrum.

Diurnal animals without oil droplets tend to have yellow lenses to protect the photoreceptors from short wavelength photodamage, or to minimize chromatic aberration (Miller, 1979), or both. Lenses that absorb in the UV and violet are found, for example, in teleost fishes, whose ancestors may not have had any cone oil droplets, and mammals, whose ancestors lost theirs. But it is not just the placental mammals that "have been pulled through some sort of ancestral knot-hole" (Walls, 1942), losing their cone oil droplets on the way. The ancestors of present day snakes spent such a period underground that they lost their limbs and ears as well. The subsequent radiation of snakes has required a re-elaboration of retinal cells, but as in placental mammals, cone oil droplets remain absent. Like mammals, diurnal snakes have developed lenses that absorb at short wavelengths. In contrast to these diverse examples, birds characteristically have lenses that are transparent down to about 320 nm (Fig. 21; see also Govardovskii and Zueva, 1977; Emmerton et al., 1980).

The *macula lutea* of the primate retina appears to be a short wavelength filter put over the fovea to supplement the absorption by the lens (see the caption to Fig. 6). In the absence of oil droplets, which can alter the spectral sensitivity of individual cones, absorption by the lens (and *macula*) seem to be a relatively coarse adaptive solution to needs for short wavelength filtering.

One frequently hears the question, What does a UV receptor *do* for birds (or fish, or turtles)? The unspoken and usually unconscious assumption behind this question is that because humans lack a UV receptor, its presence in other animals is a biological curiosity demanding a particular adaptive explanation. Insects use UV receptors in special ways (described above), but those vertebrates that possess UV receptors may simply be taking full advantage of the available solar spectrum. A more appropriate question is therefore, Why don't primates (or mammals) have UV receptors like other vertebrates? The answer to *that* is easy: ultraviolet receptors are incompatible with lenses that

absorb UV light, and the rest, as they say, is history.

An Evolutionary Look at Color Vision through Pigment Triangles

We have seen that color vision is achieved by comparing the quantum catches of two or more spectrally different classes of receptor, and that in other animals the spectral distributions of those receptors can differ greatly from our own. Although the nervous systems of animals may manipulate the outputs of the receptors in different ways, the spectral positions of the pigments and the widths of their absorption bands must impose their character on the properties of the color vision system. Some of these properties can be explored experimentally by color mixing and color matching, but except for humans, such measurements have been undertaken on a significant scale only with other primates and honeybees. Because the results of color matching experiments depend on finding mixtures of lights that produce equivalent quantum absorptions, however, it is possible to approach the problem from the other direction and compare color vision systems from knowledge of the absorption spectra of the cone pigments. Although this theoretical approach cannot replace behavioral measurements, it can highlight predictions about performance.

From graphs of the absorption spectra, it is possible to read the relative absorbance of each pigment at any wavelength of choice. For the moment we shall refer to three pigments and call them for convenience L, M, and S (for long, middle and short wavelength regions), remembering that their spectral positions will vary from one species to another. The relative quantum absorptions of each pigment can be expressed as a fraction of the total quantum catch, as for example

$$m_\lambda = M_\lambda/(L_\lambda + M_\lambda + S_\lambda)$$

for the pigment M. For purposes of scaling, however, it is first convenient to plot the spectra so that there are equal areas under each curve (Rushton, 1972). With this scaling convention a white light consisting of equal numbers of quanta in each wavelength interval will produce the same quantum absorption in each pigment.

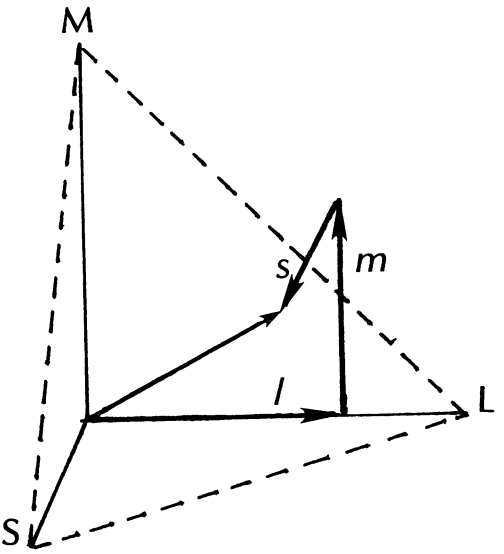

FIG. 22. GRAPHICAL CONSTRUCTION OF COLOR TRIANGLES

For any light, the relative absorptions (l, m, s) by the three pigments in a trichromatic system can be plotted on a set of orthogonal axes L, M, and S, each of unit length. In terms of its color, the light is thus characterized by a vector whose tip intersects the triangular plane LMS as shown by the small arrow. This point is reached by going l units along the L axis; m units up, parallel to the M axis; and s units out of the plane of the paper, parallel to the S axis. Modified from Rushton, 1972.

The effect of any colored light on the three pigments can be plotted on a three-dimensional coordinate system consisting of three orthogonal axes of unit length, corresponding to the three values of relative absorption, l_λ, m_λ, and s_λ. A vector from the origin of coordinates to any point in this pigment space can be extended so as to cut the triangular plane that is located with vertices at the ends of the three axes (Fig. 22). In terms of the relative pigment absorptions, any colored light can therefore be represented by a point in this plane, which is one representation of a *color triangle*. From simple geometrical considerations, if the triangle is scaled to have unit height, the length of a perpendicular from a point on the plane to one of the sides of the triangle is equal to the relative absorption associated with the receptor whose axis passes through the opposite vertex of the triangle. White light (as defined above)

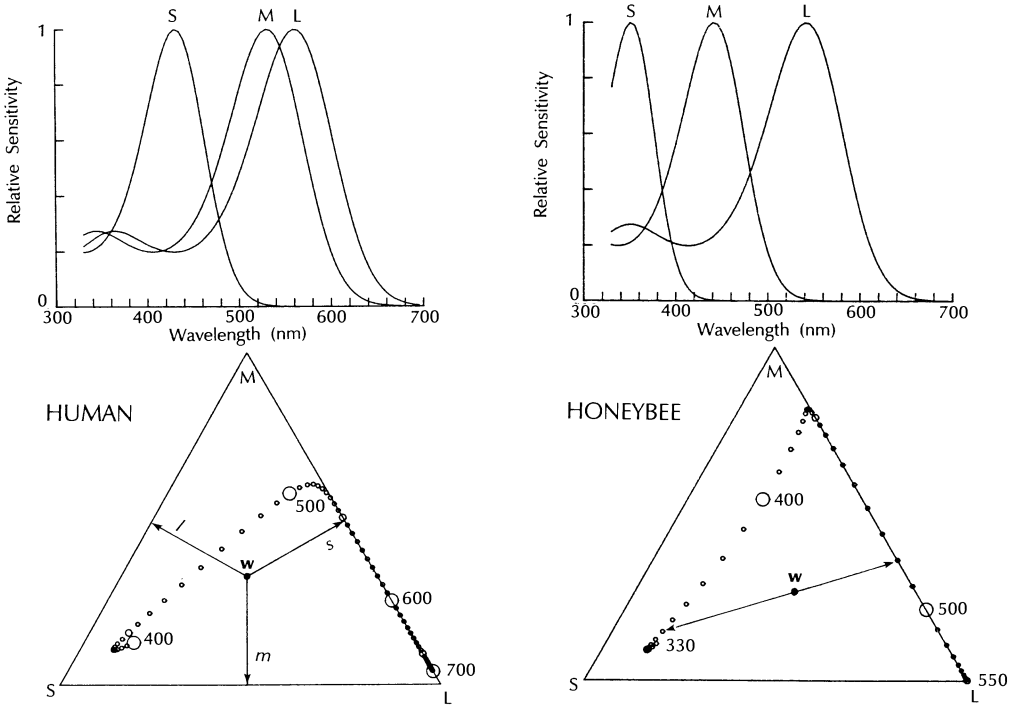

FIG. 23. COLOR SPACE OF MAN AND HONEYBEE

Above: absorbance spectra of the color vision pigments of humans and honeybees. Each species has three pigments, absorbing at short (*S*), middle (*M*), and long (*L*) wavelengths. Note that short wavelengths for the honeybee extend into the near ultraviolet. Below: color triangles. Each spectral locus was calculated by normalizing the pigment spectra for equal area (rather than equal height as shown in the upper part of the figure) and computing the relative quantum catch for each pigment as a function of wavelength. All other lights can be represented by points in the area under the spectral locus, as for example the two "white" (*w*) lights that are shown. Each vertex of the triangle represents one of the three pigments (*S*, *M*, and *L*). As illustrated in the left triangle, for any point on or under the spectral locus, one can draw perpendiculars to the three sides of the triangle; the length of each of these perpendiculars is proportional to the number of quanta absorbed by the pigment represented at the opposite vertex. This example is a light absorbed equally by the three pigments. The concept of complementary colors is illustrated by the double-headed arrow in the triangle on the right.

is represented by a point in the center of the triangle, equidistant from each side (Fig. 23, left).

In order to characterize a color vision system further, it is useful to plot the points corresponding to the wavelengths of the visible spectrum, the *spectral locus*. This has been done in Fig. 23 (left) at 5 nm intervals for a three-pigment system like that of Old World primates. All colors that the animal can see lie below the spectral locus. Keep in mind that although such pigment triangles are useful in describing color matches, they do not represent quantitatively the metrics of color space as determined psychophysically. Backhaus and Menzel (1987) describe a method for estimating just-noticeable differences based on pigment spectra and receptor noise.

It is well known that for Old World primates, the spectral locus does not approach the upper vertex of the triangle. This compression of pigment space results from the overlap in the spectra of M and L (Cgh and Crh in the terminology used in Figs. 9-13). From the point of view of design, greater independence of M and L would be achieved if the cone pigments were spaced more evenly in the spectrum. This point can be illustrated by plotting the spectral locus

for an animal like the honeybee, whose pigments are all separated by about 100 nm (Fig. 23, right).

This feature of human color vision has puzzled workers who have sought an explanation of the flattened color triangle in terms of evolutionary advantage, and the most plausible adaptational hypothesis in fact has nothing to do with color vision. In order to maximize acuity in the central fovea it is necessary to use all the cones. [Blue-sensitive cones (S) are absent from the central fovea and do not enter the argument.] If the outputs of both L and M cones feed a channel involved in discriminations of high spatial frequency, discrepant reports of luminance are unwanted, so there could be selection to minimize any differences in the spectral sensitivities of the participating cones (Barlow, 1982). In this view, the degree of overlap that is observed is therefore a compromise between the demands of spatial acuity and color vision.

This argument will appeal to proponents of optimization in evolution, and it is clearly based on an accurate understanding of the current state of the primate visual system. It may even be at least partly right. As we saw in the preceding section, however, the story also includes a haphazard history involving the vagaries of evolution and the unsettled properties of the ancestral gene that gave rise to the M/L pigment pair in the present system. Specifically, the range of λ_{max} that is covered by L, M, and their various mutant pigments—approximately 35 nm—is about the same spectral spread occupied by the rhodopsins of vertebrate red rods, raising again the question of how far and how fast λ_{max} is able to evolve in any one of the major branches of the opsin tree (Fig. 9). The spectral positions of primate M and L may therefore be a result of historical factors that have little or nothing to do with adaptation by natural selection.

A Graphical Representation of Tetrachromatic Pigment Space

We have seen that the familiar trichromatic system of Old World primates, on which virtually all theorizing about color vision is based, is not the biological norm, and moreover has some peculiar features that derive from the evolutionary history of mammals. In a number of fishes, reptiles, birds and perhaps insects there is reason to believe that color vision may be tetra- or even pentachromatic (Fig. 16). In order to explore further the implications of this observation, it therefore becomes useful to extend the graphical representation of pigment space to a hypothetical tetrachromatic visual system. Just as three quantum catches can be plotted on a surface in a triangle, four values can be plotted in the volume of a tetrahedron (Fig. 24), a conclusion reached independently by Neumeyer (1988, cited in Burkhardt, 1989). Each vertex of the tetrahedron represents a pigment (or more precisely, a spectral class of receptors), and for any point within the tetrahedron, the relative absorptions by each pigment are given by the lengths of the perpendiculars from the point to each of the four walls of the tetrahedron. If one of the receptors is absent, the system reduces to trichromacy, as all points project onto the opposite face, forming a color triangle (e.g., Fig. 27).

Fig. 25 shows the spectral locus for an ani-

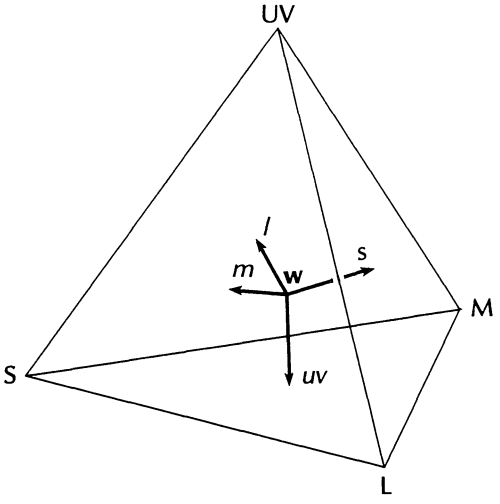

FIG. 24. TETRACHROMACY CAN BE DEPICTED WITH A COLOR TETRAHEDRON

Just as a trichromatic system can be portrayed graphically with a color triangle, a tetrachromatic system can be represented with a color tetrahedron. Any light can be represented by a point within the tetrahedron; in this example the point *w* is a white light absorbed equally by the four pigments (*L*, *M*, *S*, and *UV*). The relative absorptions by the four pigments (*l*, *m*, *s*, and *uv*) are given by the lengths of the perpendiculars from the point (in this case *w*) to the four sides of the tetrahedron.

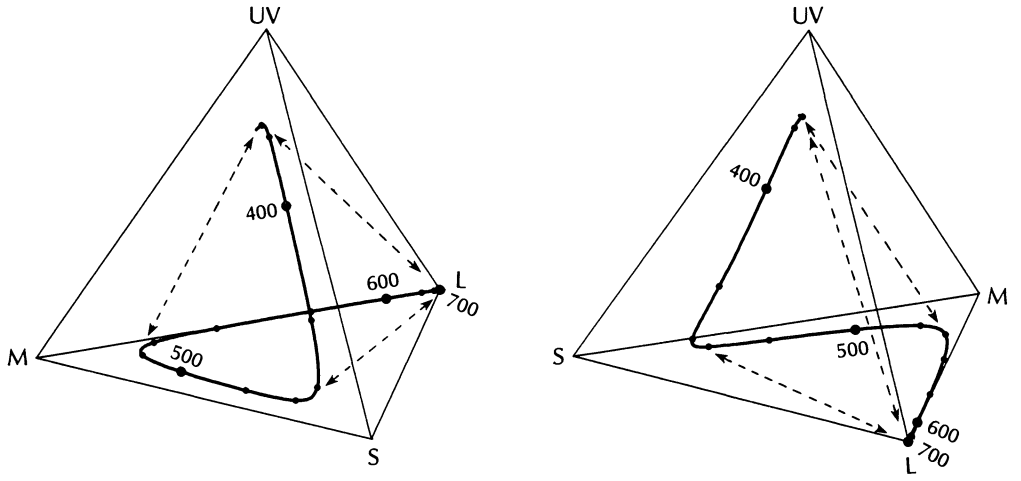

FIG. 25. TWO VIEWS OF A PIGMENT TETRAHEDRON FOR AN IMAGINARY TURTLE WITH NO CONE OIL DROPLETS

The visual pigments have λ_{max} at 370, 450, 520, and 620 nm; the spectral locus is shown with wavelength marks at 20 nm intervals. This kind of plot suggests that an animal with tetrachromatic color vision is likely to see three nonspectral color classes rather than the single nonspectral class of purples that is characteristic of trichromatic systems. These color mixtures would plot along the three dashed lines. All colors for this animal lie within the smaller tetrahedron defined by the spectral locus and the double-headed arrows.

mal with visual pigments with λ_{max} at 620 nm (L), 520 nm (M), 450 nm (S), and 370 nm (UV). (This is how the spectral locus of the turtle *Pseudemys scripta* would appear if the visual pigments were not filtered with cone oil droplets. The λ_{max} of L lies at particularly long wavelengths — 620 nm — because the chromophore of these pigments is 3-dehydroretinal.) This representation of receptor space leads to an interesting prediction that becomes evident as a result of the graphical exercise. In a trichromatic system, color mixtures plotting as points along the S-L gap in the spectral locus correspond to non-spectral hues that we see as purples and magentas. Fig. 25 suggests that in a tetrachromatic system there should be two additional sets of non-spectral hues plotting along UV-M and UV-L trajectories. Burkhardt (1989) has come to the same conclusion. Psychophysical experiments to demonstrate the existence of these three non-spectral hues have not been done.

The Effects of Cone Oil Droplets on Color Vision

Cone oil droplets, by narrowing the effective absorption bands of the cone pigments, decrease spectral overlap and increase independence of the cone channels (Barlow, 1982; Govardovskii, 1983). In Fig. 26 we see how filtering L and M with red and yellow oil droplets alters the spectral locus of the turtle. Color (pigment) space is expanded as the spectral locus clings to the L-M, M-S, and S-UV edges of the tetrahedron. Govardovskii (1983) suggested that if color discrimination is limited by the ability of central neurons to distinguish between the levels of photoreceptor excitation, the number of colors that can be discriminated should increase with the area under the spectral locus. The additional colors, lying further from the white point, should (in human terms) appear more saturated.

Oil droplets, however, can produce difficulties, as well. Obviously when spectral overlap of the pigments decreases too far, hue discrimination must suffer, a point also appreciated by Govardovskii. This is shown graphically in Fig. 26 by the fact that distance along the spectral locus is not directly proportional to wavelength. Specifically, note how the spectral locus is compressed around the L, M, and S corners of the tetrahedron. I have chosen this example partly because Arnold and Neumeyer (1987) have measured a wavelength discrimination func-

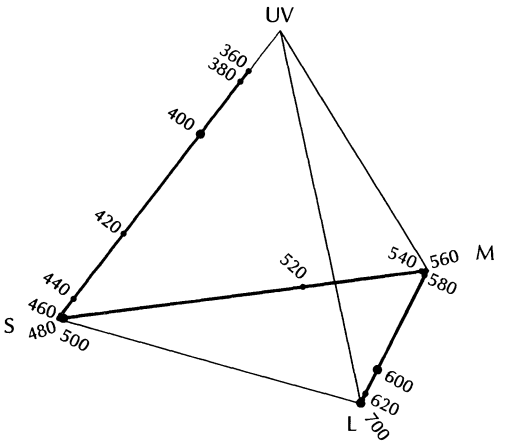

FIG. 26. SPECTRAL LOCUS FOR THE TURTLE WITH OIL DROPLETS PRESENT

This figure shows that when the 520 and 620 nm visual pigments are screened by yellow and red oil droplets, as occurs normally, the pigment space is expanded. Although cone oil droplets enlarge the space embraced by the spectral locus, their presence can also lead to local regions of poor wavelength discrimination. See the text for further details.

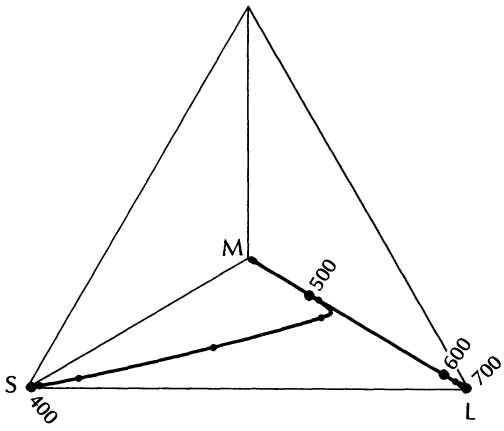

FIG. 27. FAILURE OF THREE VISUAL PIGMENTS TO SPAN THE SPECTRUM WHEN OIL DROPLETS ARE PRESENT

A hypothetical trichromatic system containing pigments with λ_{max} at 370 (*S*), 520 (*M*), and 570 (*L*), with the 520 and 570 nm pigments screened by yellow and red oil droplets with half-transmission at 520 and 590 nm. In this case, as there is not a fourth receptor the pigment space plots in the triangular *SML* plane. This particular combination of three pigments and associated oil droplets has a catastrophic effect on the pigment triangle, producing total loss of wavelength discrimination over a broad spectral region from about 490 to 520 nm. Such a calculation suggests dramatically why four or more cone pigments are commonly found in animals that are richly endowed with oil droplet filters and whose visible spectrum is broadened into the UV.

tion for the turtle *Pseudemys scripta*, and a fruitful comparison of theory and experiment is possible for this animal. They find minima in the $\Delta\lambda$ function (i.e., good discrimination) near 600, 510 to 530, and 400 nm, corresponding to the L-M, M-S, and S-UV limbs of the spectral locus. Likewise they find poor discrimination in wavelength bands 450 to 510 nm, at wavelengths longer than about 630 nm, and (to a lesser extent) 550 to 600 nm (with significant differences between two individual turtles in the latter). These regions of poor discrimination correspond to the compression of the spectral locus around the corners of the tetrahedron. The overall shape of the wavelength discrimination function is thus accounted for nicely by the effective (filtered) pigment spectra.

Pseudemys employs 3-dehydroretinal as chromophore; if it used retinal instead, the 518 and 620 nm pigments would have λ_{max} closer to 500 and 565 nm, respectively. How much of the turtle's problem with wavelength discrimination stems from having red-shifted cone pigments? Not much. Recalculating pigment space with 500 and 565 nm pigments (and assuming no change in the oil droplet filters or the 370 and 450 nm pigments) predicts a modest improvement in the 550 to 600 nm region of the spectrum, but the turtle's main difficulty with wavelength discrimination stems from the heavy screening of the short wavelength limb of the 518 nm (or 500 nm) pigment by oil droplets, greatly decreasing the spectral overlap with the 450 nm cone.

Why Many Vertebrates Have More Than Three Cone Pigments

Fig. 27 shows an extreme example of the difficulties that could be encountered by an animal with three retinal-based cone pigments, one of which (S) absorbs maximally in the UV, while M and L are also filtered by cone oil droplets. The spectral positions of M and L and the oil droplets in this example correspond to pigments that have been observed in the retinas

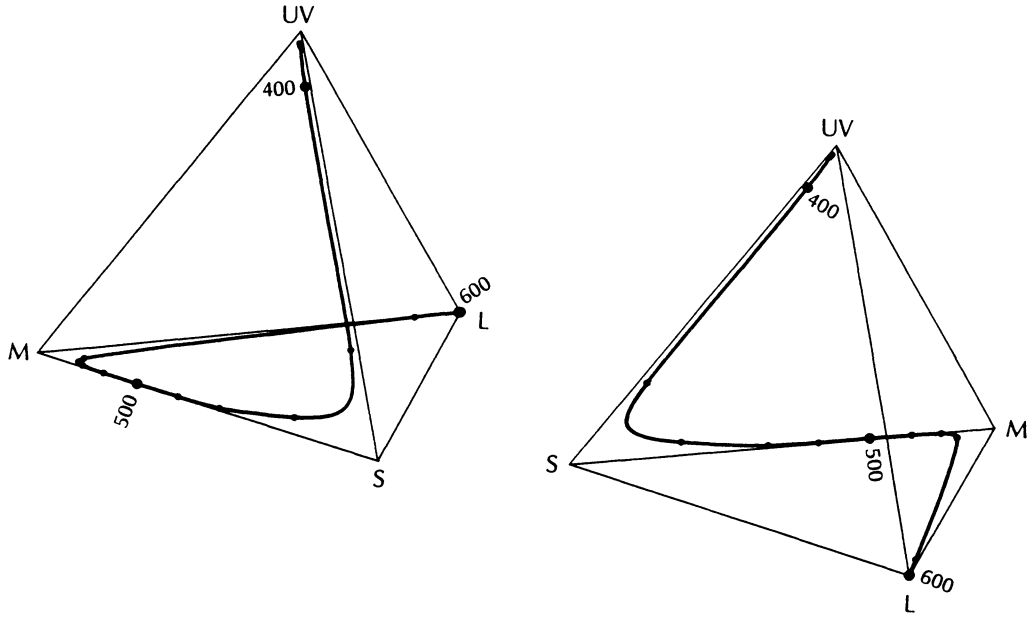

FIG. 28. AVIAN TETRACHROMACY

Two views of the pigment tetrahedron of a bird with pigments at 370, 450, 480, and 565 nm. The 370 nm pigment is assumed to lie behind transparent oil droplets, whereas the other pigments are screened at short wavelengths. As in Figs. 24, 26, and 27, both the pigments and oil droplet spectra were approximated by analytical functions for ease of computation. The 565 nm pigment lies behind droplets like those containing astaxanthin, the 480 nm pigment behind droplets similar to the "pale" droplets containing mixtures of galloxanthin and ε-carotene, and the 450 nm pigment behind "colorless" droplets like those containing galloxanthin (see Fig. 20; Goldsmith et al., 1984). This configuration of pigment and droplets has not been measured directly but is consistent with spectral sensitivity measurements during chromatic adaptation on a variety of passerine birds (Chen and Goldsmith, 1986; Goldsmith, 1986).

of some birds, but the calculations describe a hypothetical trichromatic retina. In this case, oil droplet filters placed in front of M and L have a disastrous effect on receptor space. The spectral locus sags along its left limb, then folds upon itself as it climbs and descends the right hand leg of the color triangle. In this spectral region there would thus be total ambiguity between pairs of widely spaced wavelengths.

We are now in a position to draw some tentative conclusions about the evolution of cone pigments in nonmammalian vertebrates. UV receptors appear to have a wide taxonomic occurrence and are probably ancient; but, like cone oil droplets, they have been secondarily lost in mammals. Oil droplets narrow the spectral bandwidths of cone pigments and, in principle, can increase the sensory dimension we associate with saturation. Since wavelength discrimination requires overlap of pigment spectra, however, narrowing spectral bandwidth too far can undermine color discrimination. Consequently, if the visible spectrum is to extend from 320 nm in the near UV to 650 nm in the red, and if spectral independence of cones is to be enhanced by oil droplet filters, it appears to be impossible to construct a color vision system with only three channels. Consequently, we should not be surprised to find four or more cone pigments in retinas of birds and reptiles that have brightly colored cone oil droplets.

The example of the turtle shows that in the presence of oil droplets, even four cone pigments do not necessarily rescue wavelength discrimination. If the cone pigments are optimally placed in the spectrum it is possible to narrow their absorption bands, enlarge color space, and still preserve a rich capacity for hue discrimination. For example, Fig. 28 shows a final set of calculations, illustrating how the pale

droplets containing apo-carotenoids (Fig. 20; Goldsmith et al., 1984) can mold the sensitivity of blue-sensitive cones so as to enlarge the graph of color space without the spectral locus collapsing locally as in Fig. 27.

This discussion of oil droplets and color spaces is largely theory. The color vision of birds is probably the most richly endowed of any vertebrates, but in no case do we have a total description of all pairs of pigments and droplets. Nor is it clear that the neural organization of the avian retina makes local regions more than trichromatic. Multiple foveas and regional differences in the distributions of oil droplets are warnings that general theoretical treatments of the retina such as I have initiated here may quickly prove inadequate. As with wavelength-specific behaviors of insects, the larger lesson is that theories of color vision developed for the human eye can be both misleading and confining when applied in broader biological contexts.

This having been said, there are some remarkable similarities shared by the trichromatic color vision systems of bees and humans (Menzel, 1985). These include the dimensions of hue and saturation, color contrast phenomena, the dependence of hue on intensity, the presence of spectral opponent neurons (but with very different receptive fields) and — most impressive — color constancy. This suggests that there is a limited number of useful ways nervous systems can exploit information from two or more spectral classes of receptor. That the nervous systems of Primates and Hymenoptera perform the same computational processes as a result of convergent evolution is a testimony to the power of adaptation by natural selection.

SUMMARY

(1) The idea that evolution optimizes must frequently be limited by qualification and assumption. In optical design, however, where it is possible to identify the physical principles against which performance should be measured, both vertebrate and invertebrate eyes present impressive examples of optimization. In the construction of foveas, on the other hand, the optimization is constrained by developmental heritage.

(2) Analysis of opsin sequences reveals homologies between opsins from three major phyla. These opsins likely diverged in Precambrian time. Both *Homo* and *Drosophila*, however, have at least four opsins. Within each of these species some opsins diverged more than 500 or 600 million years ago, whereas other opsins have separated more recently. With only very limited data available, it is thus already clear that opsins from different photoreceptors in the same animal (of which rods and cones are only the most familiar examples) will frequently have distinct and ancient evolutionary histories.

(3) Eyes of varying complexity and in different major groups of animals are generally considered to have polyphyletic origins. The fact that all photoreceptor cells use homologous proteins as their photoreceptor pigments, however, raises the question of which other genes may have homologous representatives in structurally diverse photoreceptor organs.

(4) Rods are characteristically distinguished by the fine structure of their outer segments. Logically, rods could also be defined as vertebrate photoreceptors in which the gene for rhodopsin, rather than a cone pigment, is expressed. Transmutation of photoreceptors is likely to have been triggered by the loss of one or more opsin genes, which could have led to situations in which the morphological and molecular criteria for telling rods from cones are in conflict. In the molecular definition, the "rods" of geckos are therefore cones, but cones that are attempting to fulfill the role of rods.

(5) Without recourse to dehydroretinal as the chromophore, most vertebrate rod pigments have λ_{max} very close to 500 nm ($\pm$10 nm). The only significant exceptions are deep-sea species whose rhodopsins have a hypsochromic (short wavelength) shift to about 480 nm. The evolutionary causes for this tight clustering around 500 nm are explored, and no persuasive explanation based on adaptation by natural selection is identified. Alternatives involving evolutionary constraints are considered, but the problem remains unsolved.

(6) All known color vision systems appear to be based on multiple opsins although, in principle, other possibilities are available. The presence of multiple visual pigments does not demonstrate color vision, nor does apparent violation of the principle of univariance. It is useful to distinguish wavelength-specific behaviors from color vision. The former do not entail any abstraction of a quality of stimulus

associated with wavelength and are frequently encountered in small-bodied animals occupying narrow ecological niches and living for short times. The presence of color vision implies that the animal can generalize some quality of stimulus based on differences in wavelength composition. We have no way to *detect* this capacity of the animal's nervous system, however, unless its behavior can be manipulated by learning. The distinction between wavelength-specific behavior and color vision is particularly interesting when both are exhibited in the same animal.

(7) Color vision is present in all classes of vertebrates and is probably most highly developed in birds. Mammals entered the Cenozoic with reduced diurnal capacity, and for the most part have remained that way. Cones are usually few, and lack myoids and oil droplets. Even where there are many cones, however, there are characteristically only two cone opsins. Against this evolutionary backdrop, the trichromacy of Primates is an exception, but the molecular biology of human cone pigments reveals that its origins are, evolutionarily speaking, relatively recent. Moreover, the capricious course of mammalian evolutionary history, rather than adaptation by natural selection, is probably primarily responsible for the spectral positions of the long- and mid-wavelength cone pigments.

(8) Receptors maximally sensitive in the near UV may well be present in all classes of vertebrates except mammals. To judge by the number of cone pigments, birds are possibly tetra- or pentachromatic. A tetrachromatic pigment system can be represented graphically as a tetrahedron; a plot of the spectral locus suggests there are three possible sets of non-spectral hues analogous to the purples of trichromacy. In mammals, the absence of UV receptors and cone oil droplets is a result of evolutionary loss and has consequences for primate color vision.

(9) The presence of cone oil droplets narrows the spectral bandwidth of the cone pigments. In principle this could increase the number of discernible hues; in practice it can also reduce wavelength discrimination in regions of the spectrum where only one cone is functioning. In animals with brightly colored cone oil droplets and a visible spectrum that extends into the near UV, the presence of four or more cone pigments seems a necessary prerequisite for maintaining good wavelength discrimination.

(10) The similarities between the "rules" of color vision exhibited by bees and humans are a result of convergent evolution. There thus appear to be only a few biologically useful ways in which nervous systems can manipulate information about color, and the same general position on the landscape of adaptive possibilities has been found independently by representatives of both vertebrates and arthropods.

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