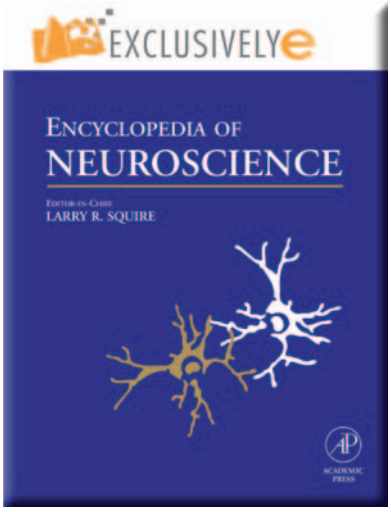

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Vertebrate Eyes: Evolution

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Introduction

Light is the premier source of information for many species, and the selective pressure to use this information led to the evolution of eyes. How did eyes evolve? Darwin, the great English naturalist who first brought the systematic explanatory power of evolution to bear on the bewildering biological complexity of our planet, believed that eyes offered a special challenge to evolutionary thinking because they are such "organs of extreme perfection and complication."

Nearly a century and a half later, new discoveries ranging from molecular to macroscopic levels of analysis now provide an answer to the question of how eyes evolve. The evolution of photodetection led to eyes that show natural selection acting at both the organ and the molecular levels. The repeated use of key regulatory gene sequences in eye development and lens formation raises the question of why some transcription factors are regularly recruited to build eyes. Because we can analyze the evolution of structural gene sequences relatively easily, many phyolgenetic relationships among the components of eyes are known. However, the difficulty of tracing exact selective forces that shaped the regulation of gene expression in eyes and elsewhere has made it difficult to understand the evolution of such complex organs.

Structural and Functional Adaptations

Despite the great variety of eyes in the animal kingdom, the laws of physics have constrained optical solutions for collecting and focusing light to just eight types of eyes. To be useful, animal eyes cannot be simple photon detectors but, rather, produce an image by comparing light arriving from different directions.

The likely sequence of evolutionary steps leading to eyes began with simple eyespots in the early Cambrian period, 570–500 Ma. Such photosensitive spots would discriminate between light and dark but could not record complex light patterns. Eyespots that invaginated would add the capacity to detect the direction of incident light and additional receptors could enhance usefulness. Duplication of an existing pit may have led to compound eyes, whereas duplication of receptors may have led to a chambered eye. Adding an optical system in front of the photosensitive elements would increase collection of light and, if it produced an image, would dramatically improve the usefulness of an eye.

The addition of optical systems to eyes led to the evolution of strikingly similar ocular structures in distinctly unrelated animals, such as fishes and cephalopods. The chambered or camera-like eyes in these two lineages are similar in many details, despite the fact that their owners are phylogenetically quite distant. Both evolved spherical lenses to achieve sufficient refractive power for focusing light underwater, but the inverted retinal layers of fishes (and all vertebrates) are distinctly different from the noninverted, somewhat simpler retinae of cephalopods. Both eye types have photoreceptor cells that use related opsin molecules to detect light but very different transduction cascades. Thus, macroscopically, these eye types and the animals bearing them are not remotely homologous, but there are some similarities in the molecules used during development. The exploitation of homologous genes to build nonhomologous eyes may lie at the heart of understanding eye evolution and evolutionary processes more generally.

Of approximately 33 animal phyla, approximately one-third have no specialized light-detecting organ, one-third have light-sensitive organs, and the remaining third are animals with eyes. Image-forming eyes exist in six of 33 extant metazoan phyla (Cnidaria, Mollusca, Annelida, Onychophora, Arthropoda, and Chordata) which account for 96% of known species alive today, meaning that eyes must be useful. Eyes have many features in common because their optical features ultimately depend on the physical properties of light, which travels in straight lines, consists of electromagnetic radiation, varies in wavelength, etc. For example, eyes have evolved to be sensitive within a narrow range of wavelengths, relative to the broad spectrum of energy produced by sunlight, because early evolution occurred in water, which selectively filters what we know as light. Selection for biochemical mechanisms sensitive to this limited range of wavelengths set the sensitivity that emerged during subsequent evolution. Although nonaquatic species moved to land where they were exposed to a significantly broader spectrum of electromagnetic radiation from the sun, animal eyes are limited to vision within the narrow band dictated by our origins in water. Some insect and fish species evolved additional receptor types for ultraviolet light, but the more common restricted sensitivity reflects just how profound early evolutionary solutions persist in evolved organs.

Photon Capture

Both vertebrate and invertebrate eyes transduce photons into cellular signals using seven-tramsmembrane

spanning opsin proteins (30–50 kDa) that combine with a retinal chromophore based on vitamin A. Opsins arose before eyes and evolved into at least seven distinct families. Opsin gene duplications gave rise to the independent evolution of three-color vision in Old and New World primates. Wavelength sensitivity in photoreceptors is modulated by amino acid side groups adjacent to the chromophore binding site in the seventh transmembrane domain of opsins, and evidence of natural selection can be seen in the arrangements of these residues.

The two primary photoreceptor types use different families of opsins packed into different membrane specializations and use different transduction cascades. Vertebrate photoreceptors use members of the ciliary opsin family, so called because these are incorporated into specialized cilia. In contrast, invertebrate photoreceptors use members of the rhabdomeric opsins formed into rhabdoms, hence the name. In vertebrates, the photoreceptors produce hyperpolarizing potentials using a phosphodiesterase cascade which provides amplification. In invertebrates, photoreceptors are depolarizing, using a phospholipase C cascade with amplification provided as the TRP channels open.

We have detailed knowledge about the evolutionary relationships among opsin molecules, and in particular, the adaptive radiation of pigment types due to natural selection for particular wavelength responses has been described for some special cases (e.g., east African cichlids and squirrelfish). However, there is a great deal of variance in spectral sensitivities arising from specific selective advantages for one solution over another. Detailed comparisons between terrestrial vertebrates and insects, for example, revealed that there are not unique solutions to encoding both spatial and spectral information. Mammals and bees use long wavelength receptors for luminance and color vision, whereas flies and birds have evolved separate sets of photoreceptors for the two purposes.

Capacities of eyes vary greatly depending on their ultimate structure. For example, resolution of an image (e.g., subtended degree) differs by approximately 13-fold among vertebrates and even more between vertebrates and invertebrates, and it is a function of photoreceptor size and spacing. Eagles have the greatest acuity, which is approximately 10^5-fold greater than that found in planaria. Similarly, a comparison of relative sensitivities among vertebrates reveals a range of 4×10^5 between highly sensitive deep-sea animal vision and human foveal vision. Animals clearly have evolved eyes with resolution, sensitivity, and wavelength detection to match their needs, even as those needs change during their life history.

The greatest variety of eyes exists among invertebrates, which have both camera eyes (e.g., cephalopods) and compound eyes (e.g., *Drosophila*). Moreover, invertebrates also have the greatest variety of eye number and location on given species. Whereas vertebrates settled on paired, chambered eyes with lenses on the head, invertebrate species may have multiple, nonpaired eyes and eyes in remarkable locations. For example, certain butterflies have light-detecting organs located such that darkness signals successful copulation. A visual system in the planula of a box jellyfish, *Tripedalia cystophora*, has been described that has eyecups directly connected to motor cilium. In this case, there is no nervous system to process visual information because the eyes are a complete sensorimotor system unto themselves.

Lenses: Multiple Protein Types and Gene Sharing

All biological optical systems fall into one of three classes based on image formation: images formed via shadows, via refraction (e.g., lens and/or cornea), or via reflection. The most common eyes collect light through an aperture and focus it with a lens onto photoreceptor cells specialized to convert photons into neural signals. Some eyes exist without pupils and even without lenses (*Nautilus*), but eyes that evolved to give their owners a clear view of the environment on a short timescale do have lenses. Could lenses that consist of tightly packed proteins provide some insight about how eyes evolved?

In vertebrates, lenses are formed from modified epithelial cells with high concentrations of soluble proteins, known as 'crystallines' because of their organized packing into arrays. In contrast, in most invertebrates, the lens proteins are secreted by specialized cells of the eye. Lenses of mitochondrial origin have been found in the two pairs of eyes of the parasite *Neoheterocotyle rhinobatidis*. Despite their distinct origins, both vertebrate and invertebrate lenses must have their constituent proteins distributed to produce a radial gradient of refractive index that is low at the edge of the lens and high in the center. A particular gradient of refractive index is essential for vision in animals living in water, but gradients are also found in terrestrial vertebrates and invertebrates.

Until quite recently, approximately ten crystalline proteins found in lenses were thought to be unique to lens tissue having evolved for this function. Of the large number of crystallines, α, β, and γ crystallines are specialized lens proteins in vertebrates although α and β are related to heat shock protein and schistosome egg antigen, respectively. However, the remaining vertebrate although α and β are lens proteins are not conserved but, rather, comprise a diverse group, many of which are used as enzymes elsewhere in the

body. Surprisingly, most of these taxon-specific lens proteins are actually products of the same genes as the enzymes, and this double use has been termed 'gene sharing.' For example, a crystalline protein in the duck lens is similar to a metabolic enzyme, argininosuccinate lyase, and both the lens protein and metabolic enzyme are encoded by the same gene, not by duplicated genes, although such sharing may have been a prelude to gene duplication. This molecular opportunism is so effective that it has also occurred both in cephalopods and in *Drosophila*. One possibility is that since lenses need the production of a relatively large amount of protein, genes that can be easily upregulated in other tissues might be preferentially selected.

Perhaps the most remarkable example of a lens from an unusual source is found in the brittlestar (*Ophiocoma wendtii*), which forms crystal lenses as a part of its skeletal armor from calcite crystals. The crystals, oriented to bring light onto the photoreceptive surfaces in the body, focus the light much as corrective lenses might, and they effectively concentrate the light by approximately 50 times.

The common cellular strategy of assembling lenses from diverse proteins seems to be a convergent evolutionary solution that has occurred in many vertebrates independently. The exquisite gradient of the refractive index which evolved in vertebrates and invertebrates alike resulted because it is the only way known to make an optically useful lens. What remains unknown is how diverse proteins are assembled through folding and organized to preserve key properties of transparency and suitable refractive index gradient along the axis of the lens. The challenge for understanding lens development is to identify the mechanisms responsible for organizing diverse proteins into a functioning lens. This knowledge could provide useful insights about eye evolution from the perspective of lens assembly.

Origins of Eyes

Logically, eyes might be monophyletic, having evolved from a single progenitor, or polyphyletic, having arisen more than once during evolution, and views on this have switched back and forth throughout the years. A comparison of overall structure, photoreceptor types, developmental origins of eye tissue, position of receptor axons, and other anatomical markers among eyes using current fauna suggested that eyes evolved not once but at least 40 different times, and possibly many more. This 'multiple-origins' hypothesis, based on morphological evidence, was later challenged by results from molecular experiments. A single, well-conserved 'master' gene, *Pax6*, can initiate eye construction in diverse species, suggesting that eyes might have arisen from a single ancestor. Since this original debate, there have been several salient discoveries that suggest eyes arose more than once and we carry the evidence within our own eyes.

Eye development requires morphological transformations of newly generated tissue regulated by multiple genes with expression patterns overlapping in time and space. Eyes develop from the prospective forebrain, beginning in the eyefields, which are made up of cells of the anterior neural plate. As the prosencephalon grows, this region moves forward until the optic groove forms and the neuroectoderm of the groove locally contacts the surface ectoderm, inducing the lens placode. As the placode invaginates to form the lens vesicle, the optic vesicle forms the bilayered optic cup, which ultimately becomes the eye. The interaction between the optic vesicle and the lens placode was identified as the 'organizer of the lens' by Spemann. The presumptive lens arises from the lens placode, a thickening of the ectoderm in contact with the optic vesicle. Coincident with this change is the onset of expression of proteins that will form the lens. Other structures of the eye are formed by large- and small-scale tissue movements, caused and accompanied by the expression of tissue-specific genes at that site. The cornea arises from the surface ectoderm over the lens and from migrating mesenchyme derived from the neural crest. Many of the original observations about the role of specific tissue bits in these processes resulted from exquisite embryonic manipulations related to transplantation experiments. With well-described macroscopic change in hand, the next challenge is to synthesize the phenomenological, macroscopic morphological observations with molecular explanations of eye development and understand what this tells us about evolution.

The morphological process of eye development has been viewed as a set of steps toward a final tissue arrangement. Underlying this apparently straightforward sequence of large-scale events, however, are distributions of gene expression with substantial overlap in both time and space. Gene expression is closely regulated, and specific gene products are used repeatedly, which makes the causal relationships difficult to conceptualize. Nonetheless, progress in characterizing the genes responsible for particular steps in eye development has been quite rapid. Functions for at least 15 transcription factors and several signaling molecules have been described in human and mice eyes, based on developmental disorders and/or molecular manipulations. As with other molecular actors, both the transcription factors and the signaling molecules expressed during ocular development are also developmentally important in a wide range of other tissues, meaning that combinatorial expression patterns are important.

As is now well known, the paired box gene 6 (*PAX6*), a member of the family of genes that encode transcription factors with a homeodomain and a paired domain, appears to be important in eye formation across many species. The remarkable demonstration that *PAX6* can induce eyes where they should not be ('ectopic') in *Drosophila*, and similar subsequent demonstration in vertebrates, led to the suggestion that there might be 'master control genes' responsible for development and differentiation of ocular tissue in many species. Subsequent work has shown that the idea of a master control gene is a misnomer, however, since a suite of genes are required, collectively, to initiate eye development, and transcription factors are a necessary part of the initiation process. Nonetheless, it is remarkable that some of the same genes appear in the context of eye development, despite great evolutionary distance among the owners of the eyes. How this might have occurred is discussed later.

In *Drosophila*, development of the photoreceptor array in the eye is known to require seven genes which work in combination with particular signaling systems through a complex regulatory network. Deletion of any one of the seven key genes causes loss or radical reduction of the *Drosophila* eye, and deletion of all but one can cause formation of an ectopic eye. Moreover, many of these genes play a role in developmental cascades used in formation of the kidney, muscle, and other organs. Thus, the master gene hypothesis is not supported because the whole collection of genes is needed to produce a reasonable eye. Eye development appears to require new ways of thinking about how complex tissues are made and how such organs arose in evolution. The widespread and redundant activities of specific genes during ocular development suggest that building eyes requires the orchestrated activity of a suite of molecular actors.

As described previously, the diversity of eyes confirms their dynamic evolutionary past. Explosive speciation, or the 'big bang' of animal evolution, happened during the Cambrian, when existing eye types appear to have improved radically, coincident with the onset of carnivory and predation. Many selective forces were likely at work, including perhaps the first instances in which light enabled behavioral signals, so no predominant selective force can be claimed. The rapidity of eye evolution has always been a question, but computer simulations show that approximately 2000 sequential changes could produce a typical image-forming eye from a light-sensitive patch. With reasonable estimates, this suggests that an eye could evolve in less than half a million years, making the virtual explosion of eyes during the Cambrian seem reasonable. After the Cambrian, three phyla emerged: arthropods, mollusks, and chordates. Although these groups all use the opsin molecule to capture light, details of the structure and function of their eyes differ considerably.

Functional Evidence about Eye Evolution

Until recently, the photo detection systems we understood well were localized primarily to eyes and pineal glands and a few other sites in the body such as the skin. For each of these, a canonical opsin and related transduction cascade were known. Specifically, ciliary structures associated with specific G-proteins are known from vertebrate eyes, and microvilli associated with inositol phosphate signaling cascades are known from invertebrate eyes. However, recently each of these phototransduction cascades was found in unexpected organisms. The polychete ragworm (*Platynereis dumerilii*), in addition to the rhadomeric photoreceptors in its eyes, has ciliary photoreceptors in the brain and typical types of opsins associated with each photoreceptor type were localized only with that type (e.g., vertebrate opsin in the brain and invertebrate opsin in the eye). This means that the two main types of 'eyes' exist in a worm.

Correspondingly, in vertebrates, a small population of intrinsically photosensitive retinal ganglion cells have been discovered that play key roles in the regulation of nonvisual photic responses. These rely on melanopsin, first identified in vertebrate melanophores, brain, and eyes. Melanopsin is in photosensitive ganglion cells which comprise a non-image-forming system that can detect the presence or absence of light and are required for normal light-induced circadian phase shifting. Subsequent functional analyses showed that retinal melanopsin functions via a phototransduction cascade that resembles invertebrate opsins and, similar to invertebrates, has intrinsic photoisomerase activity. Therefore, not only do vertebrates carry a version of the invertebrate visual transduction system with them but also it is used in a variety of ways, including to provide information to the 'image-forming' visual system.

Taken together, these findings show that at least two kinds of photoreception existed in the Urbilateria, before the split into three Bilateria branches at the Cambrian, and, importantly, each of these branches still carries versions of these two systems. Considering that seven families of opsin have been described in humans, we can expect more surprises in the detection of light. The additional opsins discovered recently have not been functionally characterized, but the evidence suggests that there are no more opsins to be discovered. Even so, determining how all the existing opsins work together is a daunting challenge.

How Did Eyes Evolve?

Eyes exist in a variety of shapes, sizes, optical designs, and locations on the body, but they all provide similar information about wavelength and intensity of light to their owners. Different tissues have been recruited to build lenses and retinas across the phyla. In contrast, all eyes share the same mechanism of absorbing photons which has been conserved across phylogeny, namely the opsin–chromophore combination. Despite new findings yielded by powerful molecular techniques, all evidence still suggests that eyes have a polyphyletic origin, as underscored by the discovery that two photodetection systems had evolved prior to the split of the Urbilateria into three families. Clearly, nonhomologous eyes contain homologous molecules responsible for many structural, functional, and even developmental features. Given a growing list of homologous gene sequences among molecules in the eye across vast phylogenetic distances, the challenge is to discover what makes the eyes of *Drosophila*, squid, and mouse so different. Understanding these differences is probably more difficult than determining what they have in common.

It seems increasingly evident that as eyes have evolved, different functional mechanisms have been generated by recruiting existing gene programs. From genome sequencing, we know that there are far fewer genes in organisms than previously thought, so the use and reuse of genes and their products in combinatorial assemblies as reported for known genomes makes sense. In the development of eyes, this seems to be the rule, not the exception. Specifically, in the evolution of eyes, it seems likely that light sensitivity evolved early in the Cambrian in the form of a proto-opsin molecule in association with the chromophore retinal. This molecular combination, sensitive to light, became associated with genes such as *pax6*, *eya*, and others. It seems likely that this combination was recruited and worked well in early evolution of eyespots and other light-sensing organs.

Important insights about how regulatory gene networks might have evolved come from what is called the 'hox paradox.' During development, orthologous genes are expressed in superficially similar domains during embryonic development of very different organisms (e.g., *Drosophila* and mouse), yet these embryos produce adults that are anatomically quite distinct, having very few structures with common ancestors. Although not completely resolved, one resolution of this paradox is that there has been evolutionary convergence in the use of some genes and hence apparent homology, which seems to be the likely scenario for the evolution of eyes. Some genes have been recruited into regulatory gene networks repeatedly, possibly committed early in evolutionary history and kept because they simply work well.

As different eye types evolved over time, there was probably repeated recruitment of particular gene groups, not unlike improvisational groups of actors, interacting to produce candidates for selection. The evolutionary fiddling through which various combinations or routines were tried could have led to numerous parallel evolutionary paths for eyes as we now envisage.

From this, two different mechanisms for transmitting the photic information to surrounding cells were selected for – one in ciliary and one in rhadomeric photoreceptors. These two systems are likely present in all organisms as described previously for worms and mice. The major surprise is that both of these transduction systems persisted with each selected as the primary visual system for a major branch of animals. In vertebrates, these independently evolved phototransduction systems now collaborate in eyes to deliver information about light to our brains. Therefore, the answer to the question of whether eyes evolved from a single prototypical eye (monophyletic), or whether they evolved repeatedly (polyphyletic), appears to be that quite evidently eyes arose at least twice and probably many times.

See also: Activity in Visual Development; Contextual Interactions in Visual Perception; Contextual Interactions in Visual Processing; Photoreceptor Adaptation; Photoreceptors: Physiology; Visual System Development: Invertebrates; Visual System: Invertebrates; Visual Development; Visual System: Adaptive Regression and Progression in Subterranean Mammals.

Further Reading

Fernald RD (2000) Evolution of eyes. *Current Opinion in Neurobiology* 10: 444–450.

Fernald RD (2004) Eyes: Variety, development and evolution. *Brain Behavior and Evolution* 64: 141–147.

Fernald RD (2006) Casting a genetic light on the evolution of eyes. *Science* 313: 1914–1918.

Land MF and Fernald RD (1992) The evolution of eyes. *Annual Review of Neuroscience* 15: 1–29.

Land MF and Nilsson D-E (2002) *Animal Eyes. Oxford Animal Biology Series*. Oxford: Oxford University Press.

Nilsson DE (1996) Eye ancestry: Old genes for new eyes. *Current Biology* 6: 39–42.

Nilsson DE (2005) Photoreceptor evolution: Ancient siblings serve different tasks. *Current Biology* 15: R94–R96.

Salvini-Plawen LV and Mayr E (1977) On the evolution of photoreceptors and eyes. *Evolutionary Biology* 10: 207–263.

Terakita A (2005) The opsins. *Genome Biology* 6: 213.

Walls GL (1942) *The Vertebrate Eye and Its Adaptive Radiation.* Bloomfield Hills, MI: Cranbrook Institute of Science.

Wistow G (1993) Lens crystallins: Gene recruitment and evolutionary dynamism. *Trends in Biochemical Sciences* 18: 301–306.