CROTCHETS & QUIDDITIES

How the Eye Got Its Brain

KENNETH WEISS

Some interesting Just So stories about evolution that, O Best Beloved, may not be just so.

Evolution provides an explanatory framework for biology. Our usual formula is to explain a trait by equating its present function with its adaptive reason for being, invoking natural selection as the causal force. But since there is no trait that we won't try to explain by such necessarily *post hoc* scenarios of deterministic molding by selection, evolutionary explanations are often derided by critics as Just So stories. This is irritating, because we think we are going Kipling one better by saying How the Leopard *Really* Got His Spots (see Murray, 1997). But if we examine our stories more closely, it is difficult to know when we have the true ones right. The evolution of eyes illustrates this in many ways.

A WORLD OF EYES

Eyes have attracted evolutionary interest for a long time. They are a classic case of improbability cited to support arguments from design. There appear to be 65 or so phylogenetically independent forms of eyes (e.g., Salvini-Plawen and Mayr, 1961). This diversity is exemplified in the outer circle of Figure 1. Systematics is a tangled bank on which I'm happy to let others fathom the diversity of nature (e.g., Wagner, 1989; Lockwood and Fleagle, 1999) but one doesn't have to be a cladist to see why eyes have become classic instances of evolutionary analogy rather than homology. Indeed, every reader of this column has probably answered a question about this correctly on an exam. Or was that answer *wrong?*

On *his* tangled bank, Darwin shuddered that the independent evolution of such remarkably complex structures as eyes would be so unlikely as to present a serious challenge to the credibility of natural selection. He hypothesized an original prototype eye consisting of a photoreceptor, asymmetric light shield, and a covering, from which "the difficulty ceases to be very great in believing that natural selection may have converted the simple apparatus" into the diversity of "optical instruments" seen today. It is not clear how widely he thought this applied, but seems to have been suggesting homology as an escape valve from implausibly improbable parallelism. So the recent discovery of a genetic mechanism widely shared in animal eye development had a striking impact on evolutionary Just So stories, by appearing to be remarkably consistent with a homology scenario. Maybe the Just So story about eyes being analogous not homologous structures, isn't. You should have been marked off on your exam!

The location where a structure will form in an embryo can be specified by the action of "selector" genes that induce cascades of gene expression and differentiation. A gene called *pax6* is involved in the cascade that initiates eye development. Initially called *eyeless* in flies (because it explains How a Fly Lost Its Way), homologous genes were discovered and found to play a similar inductive role for eyes throughout the animal kingdom. Trans-species experiments show the protein and its gene expression properties retain significant functional similarity between vertebrates and invertebrates. *Pax6* also is expressed in many tissues of developing eyes, including lens placode, cornea, iris, retinal and/or photoreceptor cells in at least some taxa, the *crystallin* gene products that give lens and cornea their optical properties, and *pax6* may induce some *opsin* (photoreceptor) genes.

Yet how can we reconcile this fact with the clear evidence from phylogeny and morphology that diverse eyes developed independently? Basically following Darwin's logic, Walter Gehring (2002) hypothesizes a Precambrian proto-eye (Figure 1, center) that evolved out of basic photoreceptor capability in ancestral cells, and then diversified through gain or loss of structures the diverse eyes that selection demanded for each circumstance (focusing, following motion, etc.). He calls this "intercalary" evolution because his idea is that in the evolution of eyes various already-existing genes were recruited in different lineages at different stages of eye development between induction by *pax6* at the top, and photoreceptor differentiation at the bottom of the cascade.

Illustrating this kind of evolution are the crystallins (Piatigorsky, 1998a,b). Despite their name, these proteins did not evolve specifically as window panes the way that, say, hemoglobins evolved to carry oxygen (but see below!). Instead, crystallins typically have evolutionarily prior functions unrelated to vision, but their properties made them suit-

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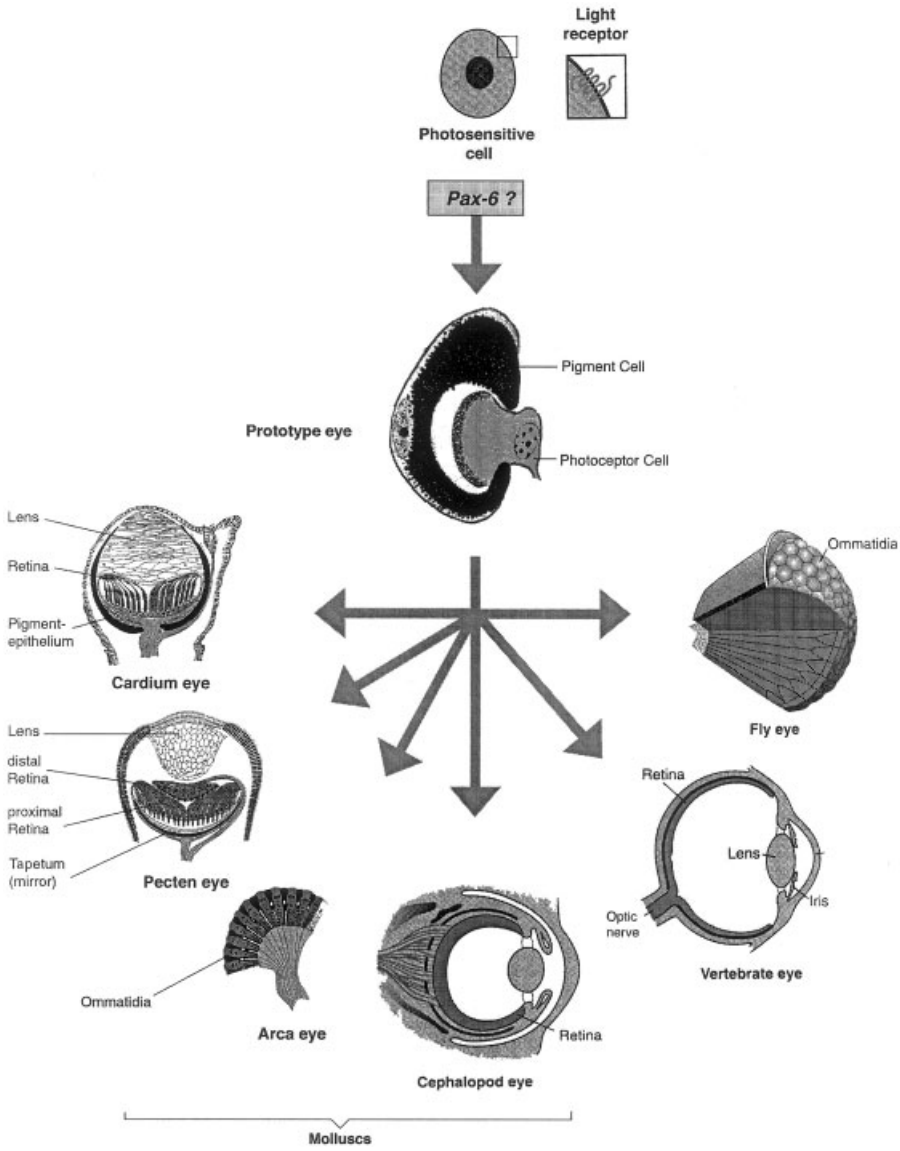

Figure 1. Intercalary evolution of the eye. A primitive photoreceptor pathway cell leads to a prototype eye from which today's eyes evolved. A range of very different types of eyes are found within mollusks alone. (modified from Gehring 2002, by permission from Int. J. Dev. Biol.)

able to be opportunistically recruited by selection for intercalation into eye development. In its search for panes, different proteins were picked for the lenses and corneas of different lineages. *Pax6* protein binds the regulatory region and may induce the expression of most crystallin genes in which this has been tested. In this sense crystallins have clear-cut developmental and functional homology, but the genes themselves are analogous, not homologous.

NOT-SO, OR AT LEAST NOT-SO-FAST

This makes a nice story but there are several cautionary elements. Gehring suggests intercalation by recruitment of available genes into various stages of eye development, but that's a far cry from intercalating all the individual complex *morphological* bits and pieces of compound eyes, eyes with focusing lenses, enclosed orbit, and the like. How evolution could do this over and over again is what perplexed Darwin. Is this merely another Just So story dressed in modern genetic clothing, or is it really plausible?

If we think of patterning *processes* rather than genes in the abstract, the story indeed seems plausible. An organism can be viewed as a set of developmental gene-regulating circuits rather than of genes each evolved for a specific use (e.g., see my earlier column, Weiss, Volume 11:3, 2002). *Pax6* is but one example. Other regulatory or inductive genetic mechanisms widely shared across the animal spectrum are responsible for gene-expression cascades that serve functions that are relevant to eye development: localizing structures, inducing placode formation (lens, cornea), tissue invagination (optic cup, retina), or repetitive patterning (ommatidia, the 100+ eyes along a scallop's mantle). These circuits are parts of an ancient animal developmental genetic Toolbox that can be induced under various circumstances, of which eyes are but one. The same pathway is used in making fly bristles, bird feathers, and your teeth. There is almost every imaginable kind of eye, but they are assembled by mixing—intercalating—these ready made processes into eye development.

Such mechanisms may not be so difficult to induce rapidly in a new evolutionary context, as shown within species by the occurrence of spontaneous ectopic wings, antennae, legs, eyes, nipples, teeth, or hair. This may just require the mutational production of short regulatory-gene enhancer sequences in the DNA upstream of a gene that then becomes recruited. As an example, the protein coded by *pax6* recognizes the sequence **TAATGCGATTA** (with some tolerance for variation), and this sequence can be found in variable number and location flanking crystallin genes in the chick and various mammals. There are enhancer sequences for numerous other regulatory factors in the same region, that vary from crystallin to crystallin (Cvekl and Piatigorsky, 1996). The number and location of these enhancers could affect timing or quantity of expression. In this way eye homology may extend well beyond *pax6* itself, but it is an Amish quilt homology. When it was

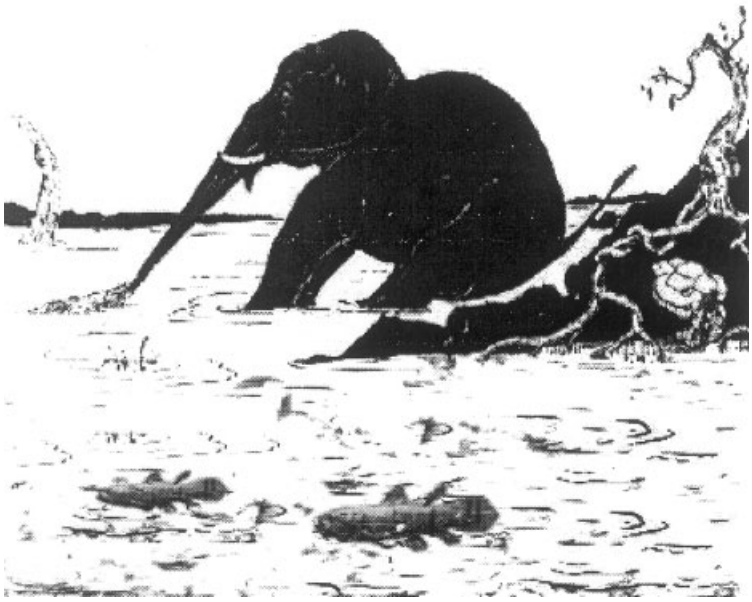

Figure 2. Coel-A-Canth out of its depth in the great grey-green Limpopo River (Modified from Kipling, 1902; fish from *http://www.unmuseum.org/coelacan.htm,* copyright Lee Krystek 2001.)

time to see the light, each creature grabbed different bits off the developmental shelf, even if they all used *pax6* to make it happen.

This explanation makes a plausible story for eye evolution. But such stories must be viewed with circumspection. For example, when crystallin genes have been experimentally inactivated in mice, there have been little or no changes in their eyes. If eyes don't need crystallins, or don't need any particular ones, then perhaps *pax6* enhancers arose in the DNA flanking many genes, by chance or for unrelated cellular functions, generating a statistical distribution of relative expression (and hence concentration levels of proteins) in eye tissue. We call the most abundant of these "crystallins" and feel obligated to explain their presence in terms of opportunistic recruitment by natural selection for their refractive properties.

We should at least entertain the possibility that the *random* intercalation of developmental cascades explains the repeated independent evolution of diversity of complex eyes as much as specific adaptive forces. An eye that was good enough, was good enough.

DARKLY THROUGH A LENS

The frequency of the light spectrum that a given opsin responds to is largely determined by a few critical amino acids, and many an adaptation tale has been told about how the response spectrum of the opsins has been mutationally "tuned" by selection to the living circumstances of various creatures (Mollon, 1989; Yokoyama, 1999, 2000). For example, opsins in coelacanths (*Latimeria*) living below 200 M under water perceive the dim, blue-shifted light that penetrates that deep. This leads us to assume this is the result of submarine warfare in which fish with essentially X-ray vision snapped up all the food, starving less optically perceptive compatriots into extinction. But might there be a different Story. . . .

> ***O****nce upon a most High and Far-Off time, Beloved, all creatures in the great grey-green, greasy Limpopo River had eyes like you and me. Alligator dozed on the banks all set about with fever-trees, and Fish swam lazily in the light of the High Sun, fearing the deeps, where only the dim-light goes, lest he bump his nose in the dark. One day in that very old time, Coel-A-Canth strayed down, down in the O So Murky and Lo, he could see! He and his mates frolicked in the empty waters. Time passed, and they were long forgotten at the Top. But if you go down today, you'll find them still at play, with their Eyes-for-the-deep-deep-blue.*

In this alternative tale (Note 1), behavior sorts existing variation with little or no need for adaptive selection for dim-light vision. Fish go where they can see, and stay to fish those waters (so to speak), associating and mating with each other. If you can't see in the dark you don't go there. As mutations conferring better dim-light vision arose, the fish moved even deeper until they had basically no contact with the upside world. Mutations that eliminate color vision could accumulate because they have no harmful effect.

This scenario would lead today's coelacanths to be as "adapted" as if the telegenic blood-and-guts melodrama of classical darwinian selection were responsible. The genetic signature of reduced variation in the opsin genes relative to general variation in the coelacanth genome would also be found, because only a subset of the variation in the population ventures deep. An inverted alternative could be that coelacanth vision is primitive in vertebrates, but that gets us in too deep for this column.

An anthropologically interesting opsin story is that of primate color vision. Old World monkeys, and great apes (including humans) have two X-linked opsin genes, one sensitive to red and one to green light. This, along with blue opsin generates our trichromatic vision. However, most New World monkeys—and most other primates—have only a single X-linked opsin gene (see Boissinot et al., 1998). In many species this gene has a multiple-allele polymorphism, so that heterozygous females are trichromats, but homozygous females and all males are dichromats. The number in each category depends on the local allele-frequencies, which vary among species and populations. Did a multiallelic system evolve many times in different species? Perhaps. Howler monkeys are a New World exception

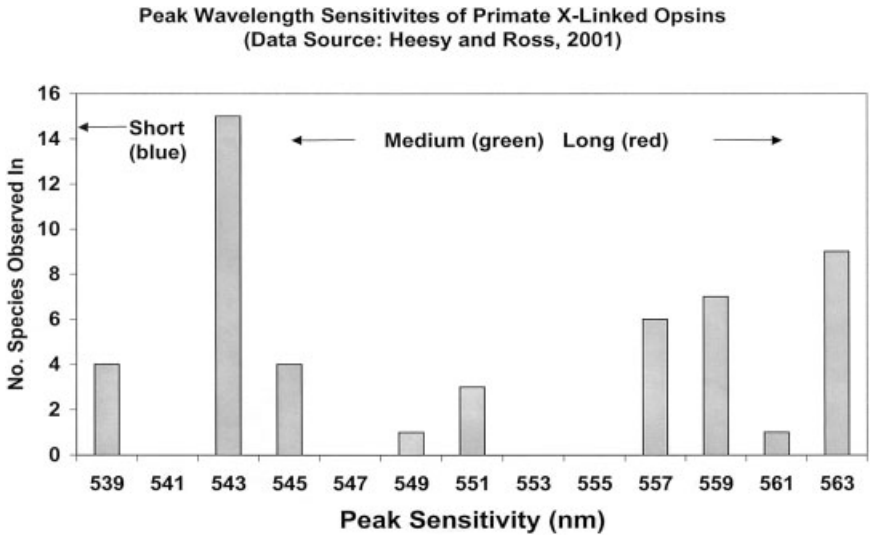

Figure 3. Peak wavelength sensitivites of primate X-linked opsins.

that have true trichromacy (two X-linked opsins). Did that evolve independently? Probably.

These questions relate to whether primates were originally polymorphically trichromatic and diurnal, or dichromatic and nocturnal (Tan and Li, 1999; Heesy and Ross, 2001). Detailed analyses of opsin light-frequency sensitivities have shown the functional value or adaptive use of color vision by various vertebrates, including primates for whom color vision is valuable for finding fruit in dappled forest visual backgrounds (Dominy et al., 2001; Heesy and Ross, 2001; Jacobs, 1995; Mollon, 1989). However, such explanations essentially equate present function with past natural selection and this can require *ad hoc* contortions. Explaining the strange patterns of polymorphism-based color vision by adaptive scenarios might be a challenge even for Kipling, and simpler explanations may be tenable.

The diversity of spectral peak sensitivities observed in the single X-linked opsins of many primates suggests that selective pressure maintains *some* ability to use light in this part of the spectrum, but that what we see in most primates today may just be the range generated by random genetic drift in the opsin gene, consistent with that constraint (Figure 3). It is not necessary to invoke repeated Hobbesian wars of all against all, with special color requirements consistent over long time periods for each species, to explain variable patterns of dichromat males and a subset of trichromat females.

The samples tested have generally been small, and bigger samples might reveal variation in all these species. It would be rather arbitrary to use an allele frequency- or sample-dependent definition of a species' vision, so we may be over-interpreting the limited data. Once gene duplication generated two X-linked opsins in anthropoid ancestry, selection or behavior could play around with the greater sensitivities made possible, with one gene sensitive to red and the other to green parts of the spectrum, but we should keep in mind that red-green color vision is highly variable in humans, too, and not all of us are true trichomats.

A VISUAL MIRAGE

There is another way in which our adaptive scenarios may be literal mirages. Humans have trichromatic color vision, but in important ways we don't need it. The loss of an opsin gene can lead to colorblindness, but James McCann and Edwin Land (inventor of Polaroid photography) discovered with clever filtering experiments, that humans can perceive colors that are not there. That and much other work has shown that what we do is compare the signals received by our different opsins from the same visual "pixel." Comparative processing enables images to retain their perceived properties under varying light intensity, shading, and so on. The response spectra of different opsins also overlap to some extent, and there are various post-photoreceptor neural processing mechanisms (that could themselves evolve adaptively). Even flies can adapt to images by taking their context into account. We might say that color is in the mind rather than the eye of the beholder, and should be careful speculating about what organisms can see (Figure 4).

JUST SO!

Kipling's creatures got *their* traits by chance and circumstance, and their own silly behavior. Biologists are kept busy writing our own stories about the Once Upon a Times. In the real world, behaviorally driven selection of local environments can lead to adaptation just as classical darwinism does, but where environment is selected by behavior to fit the genes, rather than the darwinian other way round. Perhaps we should broaden our explanatory search space (Note 2).

Part of the problem is that there is no one mandate: not even all creatures in the same environment use vision in the same way. Not all see the same colors. Not all predators have similar vision. Humans are at risk in the dark but can't see in the dark. Pineal response does not require "eyes." Even among mollusks there are diverse types of eyes (Figure 1, left). Median eyes may have evolved multiple times as a separate visual sense in arthropods, but ocelli do not use *pax6* though they do use (their own) opsins. Some nematodes even use *hemoglobin* for light perception (Burr et al., 2000).

Pax6 is highly conserved and important in eye-precursor tissue. It remains expressed in many tissues in developing eyes. Its enhancers are found around many eye-related genes;

(Virtual) Figure 4. Believing is seeing. See http://people.msoe.edu/~taylor/eisl/land.htm

but it is not the universal Monarch of the Sees atop all animal vision. At the other developmental end, opsins are also conserved. But some of the links in the evolutionary reconstructions have been challenged. For example, planarians express, but do not require, *pax6* in producing eyes. In flies, *pax6* regulates the rhodopsin *rh1,* and *pax6* enhancer sequences are found flanking most vertebrate opsin genes, but that use of *pax6* is not necessary for eyes in an actual fly. Even the original *eyeless* story was not so categorical; the flies often had eyes (sometimes small or imperfect), and eyes can reappear after some generations because of other genes or pathways. Another gene, *eyes absent,* is expressed early in development in flies and mutations variously affect either the ocelli or the compound eyes.

In the face of this patchwork evolution, Darwin's queasiness about eye evolution should sober our efforts to explain our own favorite traits. We properly invoke the contingent nature of evolution in trying to explain adaptive diversity: creatures evolve to use what is there to be used. How, if, or why natural selection in a particular setting chose a particular option was at the time it happened truly *ad hoc,* but in our time we must assess it *post hoc.* Not every possible explanation may be equally plausible and various kinds of tests may eliminate some contenders. But because we can always find a plausible adaptive explanation for *any* observation we make today, evolutionary reconstructions really *are* just-so stories in important ways. Trying to understand when we've got the true one is one of the most serious challenges in evolutionary biology.

My title? Light by itself is useful, but its pattern can be more So. Single-celled organisms already possessed basic photoreception Very Long Ago. But though they could *detect* light, complex organisms (Note 3) had to evolve a brain so they could *see.* Now *that* is a pretty good Story!

NOTES

I would welcome any comments on this column: *kenweiss@psu.edu.* I maintain *Crotchety Comments* on my web page: www.anthro.psu.edu/rsrch/weiss_lab.htm

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1. Do we tend inadvertently to think like Kipling? "[My purpose is to] elucidate how the coelacanth has modified its visual pigments to adapt to its natural habitat." Yokoyama, 1999.

2. Behavioral adaptation has long been considered under sometimes-controversial rubrics such as the Baldwin effect or sympatric speciation.

3. Well, even some single-celled algae have eyes that without a brain interpret light in regard (at least) to locomotion.

TO READ

Many things discussed here can be profitably explored by web searching.

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