A butterfly eye's view of birds

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Summary

The striking color patterns of butterflies and birds have long interested biologists. But how these animals see color is less well understood. Opsins are the protein components of the visual pigments of the eye. Color vision has evolved in butterflies through opsin gene duplications, through positive selection at individual opsin loci, and by the use of filtering pigments. By contrast, birds have retained the same opsin complement present in early-jawed vertebrates, and their visual system has diversified primarily through tuning of the short-wavelength-sensitive photoreceptors, rather than by opsin duplication or the use of filtering elements. Butterflies and birds have evolved photoreceptors that might use some of the same amino acid sites for generating similar spectral phenotypes across ~540 million years of evolution, when rhabdomeric and ciliary-type opsins radiated during the early Cambrian period. Considering the similarities between the two taxa, it is surprising that the eyes of birds are not more diverse. Additional taxonomic sampling of birds may help clarify this mystery. ***BioEssays*** **30:1151–1162, 2008.**

Introduction

Butterflies and birds possess some of the most-spectacular color displays among terrestrial animals. Their color has inspired human artistic expression and made these animals prominent study systems in evolutionary biology. Butterflies and birds also provide examples of the role of color in sexual selection both in the context of mate choice,[1] and intraspecific male–male interactions.[2] Moreover, both groups employ color vision in foraging for food such as flowers or fruit,[3–5] raising the prospect of similar adaptations to a shared light environment. Birds also prey on butterflies and are thought to be the primary drivers for the evolution of crypsis, warning signaling[6] and mimicry[7] in butterfly wing coloration. This raises the intriguing possibility that the visual systems of birds may indirectly influence the evolution of butterfly vision. For example, wing color changes in response to avian predation may lead to selective pressures on butterfly visual systems for enhanced detection of conspecifics that bear novel, adaptive color patterns. However, studies trying to understand the significance of coloration and visually mediated behavior in either of these animal groups are often done in the absence of any specific information about the visual system of the species in question.

How do butterflies and birds see color? Do they all see color in the same way? To gain insight into their extraordinary visual worlds, we review the molecules underlying vision in these two groups and put these findings into a phylogenetic context. We focus primarily on the visual pigments, because they hold the key to understanding color vision, and offer superb examples of convergent evolution, the process by which animals that are distantly related independently evolve similar traits as a result of having to adapt to similar environments or ecological niches. We discuss the astonishing diversity of visual pigments among the different butterfly families and highlight an example of recent positive selection that has diversified the long wavelength-sensitive visual pigment in a group of mimetic butterflies. We contrast this diversity in butterflies with the apparently more invariant visual system of birds and conclude that the eyes of birds are probably more diverse than is presently evident. We postulate that future advances in understanding how eye evolution shapes the direction of morphological and behavioral trait evolution in these groups rests on exploring the behavioral consequences of the yet-to-be-characterized differences in their visual systems.

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Abbreviations: UV, ultraviolet; UVS, ultraviolet sensitive; VS, violet sensitive; B, blue; LW, long wavelength; RH, rhodopsin; SWS, short wavelength sensitive; MWS, middle wavelength sensitive; nm, nanometer; LWS, long wavelength sensitive; λmax, wavelength of peak absorption.

Rhabdomeric vs ciliary photoreceptors

To discover how butterflies and birds see the world, we first need to understand the molecular basis of vision. Vision is made possible by image-resolving eyes, which are present in cnidarians, annelids, mollusks, arthropods and chordates.[8] The image-resolving eyes of butterflies (arthropods) and birds (chordates) are examples of convergent evolution. Both butterfly and bird eyes possess functionally similar traits, retinae composed of arrays of photoreceptor cells, and all visual information comes from the capture of photons by these

cells, but the origins of these photoreceptor cells and the photoreceptor molecules that they contain are distinct. In image-forming eyes, photoreceptor cells generally contain visual pigments that comprise a light-sensitive, retinal-derived chromophore covalently bound to an opsin protein.[9] Photons reaching the chromophore cause its photoisomerization and induce a conformational change in the opsin protein, which, in turn, activates a messenger G-protein and initiates a biochemical cascade that results in a neural signal to the brain.[10]

Opsins are ancient proteins belonging to the G-protein-coupled receptor family and have a seven-transmembrane domain structure characterized by a lysine residue in the seventh helix, which is the chromophore-binding site.[9] The opsin family predates the emergence of the major groups of animals present today, and it is thought that opsin diversification into numerous subfamilies occurred much earlier in animal evolution than the deuterostome–protostome split.[11,12] The visual opsins of butterflies and birds belong to two different subfamilies that presumably couple to different G proteins in the visual transduction cascade, Gqα(butterflies) and Giα-(birds).[13–15] Butterfly and bird opsins are also associated with distinct photoreceptor cells, the rhabdomeric and the ciliary types,[13] respectively. Butterfly compound eyes contain ommatidial units composed of nine rhabdomeric photoreceptor cells (R1–9) that expand their apical side to express opsins in microvillous membranes known as rhabdomeres (Fig. 1). And, like epithelial cells, tight junctions seal adjacent photoreceptor cells in a narrow band just beneath their apical surface. The nine rhabdomeres are fused to form a central structure known as a rhabdom. Vertebrate lens eyes, by contrast, contain ciliary photoreceptor cells that expand their cell membrane around a protrusion known as a cilium to express opsins in the outer segment, a stack of membranous disks. In birds, the retina contains two types of ciliary photoreceptor cells—the rod cells, which express rod opsins, and the cone cells, which express cone opsins (See below) (Fig. 2). Phylogenetically, rod opsins (rhodopsins) are the product of cone opsin duplication, and produce extremely light-sensitive photoreceptors involved in dim-light vision. Cone opsins by contrast are less sensitive to light and usually function during the day in the context of color vision. Rhabdomeric and ciliary photoreceptors have very different evolutionary histories, yet they have been convergently recruited during evolution to facilitate color vision.

To distinguish different wavelengths of light that are perceived as distinct colors, the brain needs input from at least two types of photoreceptor cells.[16] Having more than two photoreceptor types confers an even greater capacity to see different colors, although to prove that a photoreceptor is being used in color vision, as opposed to achromatic or motion vision, requires behavioral testing.[17] Photoreceptor types vary primarily in the kind of visual pigments that they contain.

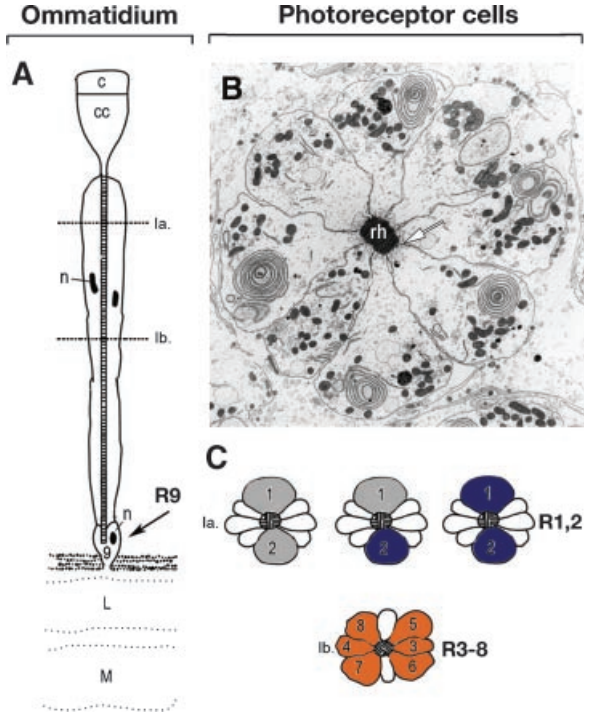

Figure 1. Diagram of an ommatidium and pattern of UV, B and LW opsin mRNA in the retina of a nymphalid butterfly. **A:** Longitudinal view of an ommatidium. In nymphalids, the photoreceptor cell bodies that contribute to the fused rhabdom are organized into two tiers, comprising the R1–8 cells (tier I) and the basal R9 cell. Dashed horizontal lines, Ia and Ib, indicate the approximate level from which the tangential sections of the R1–8 cells (shown to right) were taken. **B:**Transmission electron micrograph of an tangential section through an ommatidium of the butterfly *Vanessa cardui* (Courtesy of Richard White) showing the R1–8 photoreceptor cells and fused rhabdom. Arrow indicates tight junctions near the apical side of the photoreceptor cell body. **C:** Pattern of UV (grey), B (dark blue) and LW (orange) opsin mRNA expression in the cell bodies of the R1–8 photoreceptor cells. C, cornea; cc, crystalline cone; rh, rhabdom; n, photoreceptor cell nucleus; L, lamina; M, medulla.

The absorbance spectrum of a visual pigment depends on the interaction of the chromophore with critical amino acid residues in the opsin protein. The chromophore itself has a wavelength of peak absorption or λ_{max} value in the UV region at ~380 nm.[18] However, through interaction with key amino acid residues within the chromophore-binding pocket of the opsin, a diversity of λ_{max} values can be achieved,[19,20] a phenomenon called 'spectral tuning'. The chromophore used by butterflies is 11-*cis*-3-hydroxyretinal,[21] while the chromophore used by birds is 11-*cis*-retinal.

Although some animals can use two kinds of chromophores, it is primarily through spectral tuning of the opsin that

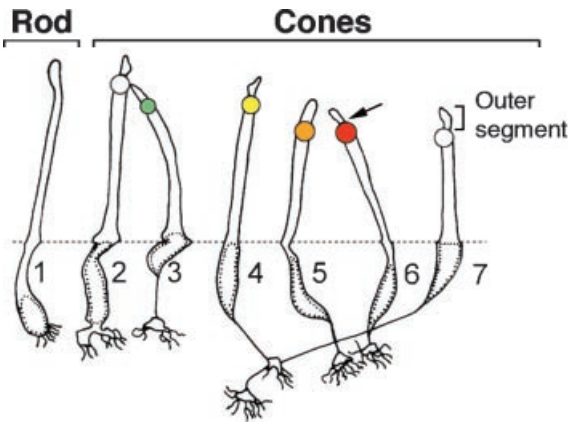

Figure 2. Diagram of the rod and cone photoreceptor cells of a chicken retina. Chicken retinas contain one kind of rod (*1*) and at least seven kinds of cones (*2–7*). Rod cells do not contain oil droplets. Chicken cones, comprised of double (*2,3*) and single cones (*4–7*), contain several kinds of colored oil droplets indicated by circles: pale green (accessory cones), yellow (principal cones [not shown] or single cones), orange (single cones), red (single cones), colorless (principal or single cones) and transparent (single cones [not shown]). The two photoreceptors of the double cones, principal (*2*) and accessory (*3*), are drawn separately here. Dotted lines indicate nuclei. Horizontal dashed line indicates outer limiting membrane. Arrow indicates location of cilium that connects the visual pigment-containing outer segment with the cell body. Adapted from López-López R, López-Gallardo M, Pérez-Álvarez MJ, Prada C 2008 Cell Tissue Res 332:13–24.

visual pigment sensitivities can be adaptively matched to the ambient light environment. The clearest evidence of this comes from aquatic environments, in which photoreceptor λ_{max} values in a variety of fish appear to be optimized for specific habitats: the deeper the dweller, the more blue-shifted are the visual pigments.[22,23] The absorbance spectra of butterfly and bird visual pigments vary widely, with λ_{max} covering a broader part of the light spectrum in butterflies (340–600 nm; $n = 35$; Table 1) than in birds (355–571 nm; $n = 31$; Table 2). No clear example of adaptation of the visual pigments of a butterfly or bird to the ambient light environment has yet been identified, however. This raises the possibility that spectral tuning of the visual pigments of butterflies and birds may be driven more by the particular needs of social signaling and foraging than by some general constraint of the environmental lighting.

Evolution of the visual pigments in insects

The diversity of butterfly wing colors has long been appreciated by biologists. A more-recent surprise, however, has been the extraordinary radiation of their visual systems, compared to other insects like bees.[24] Insect vision in general is based on three major classes of photoreceptors, with λ_{max} in the ultraviolet (UV, 300–400 nm), blue (B, 400–500 nm) and long wavelength (LW, 500–600 nm) parts of the spectrum. The visual pigments present in these classes of photoreceptors roughly correspond to three classes of opsin protein encoded by distinct UV, B and LW rhabdomeric opsin genes.

The most-current arthropod opsin gene phylogenies suggest insect UV/B and 'blue-green' opsins originated prior to the Chelicerate–Pancrustacean split.[25] Distinct insect UV and B opsin loci subsequently evolved via duplication of the ancestral UV/B opsin gene. Similarly, the insect LW opsin evolved from a duplication of the blue–green opsin, followed by loss of the blue–green opsin in all insects except *Drosophila* and the blow fly, *Calliphora*. Character-mapping of the extant holometabolous insect visual pigments onto a species tree further supports the scenario that the ancestral eye of bees, moths and butterflies expressed one UV, one B and one LW opsin, and insects like the red flour beetle, *Tribolium*, lost their B opsin, perhaps as a result of a nocturnal lifestyle.[26]

Lineage-specific B and LW opsin gene duplications in butterflies

The number of visual pigments (P) in the eyes of butterflies, however, varies from three to five among the five major butterfly families (Table 1). This variation in pigment number is due to lineage-specific gene duplications evident when mapped onto a butterfly family phylogeny[27] (Fig. 3). Duplications of the B opsin gene have occurred independently in two of the five butterfly families (Pieridae and Lycaenidae)[28,29] and duplications of the LW opsin gene have occurred independently in three of the families (Papilionidae, Nymphalidae and Riodinidae),[30–32] leading to a diversity of photoreceptor λ_{max} values in the middle—(425–500 nm) and long-wavelength ranges (505–600 nm; Table 1). For example, in the pierids, duplicate blue opsins have diversified into violet (P425) and blue (P453) absorbing visual pigments.[28] In lycaenids, independently duplicated blue opsins have diversified into blue (P437) and blue-green (P500) visual pigments.[29]

In papilionid butterflies, three LW opsin genes are expressed in the eye, each the result of a round of duplication.[27,31] These duplicated opsins now encode three different visual pigments with λ_{max} values ranging from 515 nm to 575 nm.[33] While nearly all nymphalids that have been looked at to date using a combination of anatomical, physiological and molecular methods,[34,35] contain eyes with one UV, one B and one LW opsin, we recently discovered that LW opsin gene duplications have also occurred in two species of nymphalids out of 39 surveyed.[32] The most-red-shifted butterfly visual pigment known, present in the riodinid butterfly *Apodemia mormo*, has a $\lambda_{max} = 600$ nm, which arose through an independent gene duplication of the LW opsin gene followed by accelerated rates of amino acid evolution.[32] In total, of the >50 species of butterflies studied, 7 LW opsin gene duplicates

Table 1. Approximate λ_{max} of visual pigments, where known, in different butterfly species.

Family	Subfamily	Species	UV	B	LW	References
Lycaenidae	Lycaeninae	*Lycaena rubidus*	**360**	**437, 500**	**568**	85[a]
		Lycaena heteronea	360	437, 500	568	85[a]
		Lycaena dorcas	360	437, 500	568	85[a]
		Lycaena nivalis	360	437, 500	575	85[a]
Nymphalidae	Apaturinae	*Asterocampa leilia*			530	32[a]
		Sasakia charonda	345	425, 440	540	86[b]
	Charaxinae	*Archaeoprepona demophon*			**565**	32[a]
	Danainae	*Danaus plexippus*	**340**	**435**	**545**	87[b]
	Heliconiinae	*Agraulis vanillae*			**555**	32[a]
		Heliconius charithonia			**550**	32[a]
		Heliconius erato	**370**	**470**	**555**	32[a,c]
		Heliconius hecale			**560**	32[a]
		Heliconius sara			**550**	32[a]
	Limenitidinae	*Limenitis archippus archippus*			**514**	41[a]
		Limenitis archippus floridensis			**514**	41[a]
		Limenitis arthemis astyanax			**545**	41[a]
		Limenitis lorquini			**530**	41[a]
		Limenitis weidemeyerii			**530**	41[a]
	Nymphalinae	*Aglais urticae*	380	460	530	88[c]
		Anartia jatrophae			**530**, **565**	32[a]
		Euphydryas chalcedona			**565**	32[a]
		Inachis io			**530**	89[a]
		Junonia coenia			**510**	89[a]
		Nymphalis antiopa			**534**	89[a]
		Polygonia c-album			532	90[a]
		Polygonia c-aureum	350	450	540, 565	86[b]
		Siproeta stelenes			**522**	89[a]
		Vanessa cardui	**360**	**470**	**530**	34[a]
	Satyrinae	*Hermeuptychia hermes*			**530**	32[a]
		Neominois ridingsii			**515**	32[a]
		Oeneis chryxus			**530**	32[a]
		Pararge aegeria	360	460	530	91[b,c]
Papilionidae	Papilioninae	*Papilio xuthus*	**360**	**460**	**530, 515, 575**	33[b]
Pieridae	Pierinae	*Pieris rapae*	**360**	**425, 453**	**563**	92[b]
Riodinidae	Riodininae	*Apodemia mormo*	**340**	**450**	**505, 600**	32,93[a]

Pigments where partial or complete opsin sequence is available are shown in bold.
[a]Results obtained from epi-microspectrophotometry
[b]Results obtained from intracellular recording
[c]Results obtained from electroretinogram

have been identified, not all of which encode known spectral variants.

What are butterflies doing with so many visual pigments? *Papilio* butterflies use two of the duplicated LW opsins to see in the green part of the light spectrum when ovipositing[36] and foraging,[37,38] and the lycaenid butterfly *Polyommatus icarus* uses one of the duplicate B opsins together with the LW opsin to see green up to 560 nm when foraging.[39] The pattern suggests that opsin gene duplications in butterflies have allowed two different solutions to the problem of discriminating colors in the long wavelength part of the visible light spectrum (important for discriminating foliage and some flowers), although further experiments (e.g. RNAi knockdown) are needed to test how better color discrimination in this range confers an adaptive advantage.

Positive selection at the LW opsin locus in mimetic *Limenitis* butterflies

While gene duplications, followed by spectral tuning at one or both gene copies, allow an expansion of the number of visual pigments present in the eyes of butterflies an additional mechanism generating spectral diversity is positive selection on single opsin loci. A particularly striking example of how positive selection may generate spectral diversity among closely related species comes from the butterfly genus *Limenitis*, members of which have radiated across the North American continent from a European ancestor during the past 3–4 million years.[40] This genus is most well known for containing wing color pattern mimics such as the viceroy *Limenitis archippus*, which mimics the monarch *Danaus plexippus,* and *L. arthemis astyanax,* which mimics the

Table 2. Approximate λ_{max} values of cone visual pigments, where known, in different bird species.

			Visual pigment λmax (nm)				
Order	**Species name**	**Common name**	**SWS1**	**SWS2**	**RH2**	**LWS**	**References**
Anseriformes	*Anas platyrhynchos*	Mallard duck	415	452	**506**	567	51
Anseriformes	*Anas platyrhynchos dom.*	Aylesbury duck	415	449	501	570	51
Anseriformes	*Anas platyrhynchos dom.*	Khaki Campbell duck	426	456	501	570	51
Ciconiiformes	*Puffinus pacificus*	Wedge-tailed shearwater	406	450	503	566	94
Ciconiiformes	*Puffinus puffinus*	Manx shearwater	402	452	—	—	95
Ciconiiformes	*Phalacrocorax carbo*	Common cormorant	**405***				68
Ciconiiformes	*Spheniscus humboldti*	Humboldt penguin	**403**	450	—	543	79
Columbiformes	*Columba livia*	Pigeon	***404 (393)**	**452**	**506**	**566**	95,96
Galliformes	*Coturnix coturnix japonica*	Japanese quail	418	450	505	567	97
Galliformes	*Gallus gallus dom.*	Chicken	***418 (419)**	**453**	**507**	**571**	68,95
Galliformes	*Meleagris gallopavo*	Turkey	420	460	505	563	98
Galliformes	*Pavo cristatus*	Peafowl	424	458	505	567	99
Passeriformes	*Amadina fasciata*	Cutthroat finch	370	447	500	563	100
Passeriformes	*Corvus frugilegus*	Rook	—	—	497	565	101
Passeriformes	*Dolichonyx oryzivorus*	Bobolink	372, 403		505	564	83
Passeriformes	*Erythrura gouldiae*	Gouldian finch	370	440	500	562	100
Passeriformes	*Leothrix lutea*	Red-billed leothrix	355	454	499	568	102
Passeriformes	*Lonchura maja*	White-headed munia	373	446	500	562	100
Passeriformes	*Neochmia modesta*	Plum-headed finch	373	442	500	565	100
Passeriformes	*Parus caeruleus*	Blue tit	**372**	449	502	563	103
Passeriformes	*Passer domesticus*	House sparrow	—	445	503	563	59
Passeriformes	*Serinus canaria*	Canary	**363**	**440**	**501**	**567**	61
Passeriformes	*Sturnus vulgaris*	Starling	362	449	504	563	104
Passeriformes	*Taeniopygia guttata*	Zebra finch	***359 (359)**	***427 (440)**	***505 (505)**	***566 (560)**	57,95
Passeriformes	*Turdus merula*	Blackbird	373	454	504	557	103
Psittaciformes	*Melopsittacus undulatus*	Budgerigar	***371 (360)**	440	**499**	566	68,95
Strigiformes	*Strix aluco*	Tawny owl	—	463	503	555	105
Struthioniformes	*Rhea americana*	Rhea	—	447	506	571	106
Struthioniformes	*Struthio camelus*	Ostrich	405	445	506	570	106
Tinamiformes	*Nothoprocta cinerascens cinerascens*	Brushland tinamou	—	—	—	564	107
Tinamiformes	*Nothoprocta perdicaria sanborni*	Chilean tinamou	—	—	—	566	107

Pigments where partial or complete opsin sequence is available are shown in bold
*In parentheses, obtained using spectrophotometric measurements of reconstituted, in vitro expressed opsin protein.

pipevine swallowtail, *Battus philenor*. Using epi-microspectrophotometry, we found unprecedented levels of spectral diversity among the LW visual pigment of four *Limenitis* species, with the LW visual pigment $\lambda_{max} = 515$, 530 or 545 nm in these species[41] (Table 1). By reconstructing phylogenetic relationships, we inferred that a blue-shift in LW visual pigment λ_{max}, had occurred from an ancestral λ_{max} ~545 nm to $\lambda_{max} = 515$ nm.[41] Importantly, using several approaches, we found the signature of positive selection at several amino acid sites in *Limenitis* LW opsins. Using homology modeling of *Limenitis* LW opsin against the bovine rhodopsin crystal structure, we determined that several positively selected sites were located in the chromophore-binding pocket; these are prime candidate spectral tuning sites.

Remarkably, we also found that at least one of the amino acid sites under positive selection contains a Ala64Ser substitution correlated with blue shifts in the butterfly LW opsins that at a homologous site (amino acid 164 using bovine rhodopsin numbering) in the ciliary cone opsin of humans is responsible for a 5–7 nm blue shift[42,43] and which is under balancing selection in New world monkeys.[44] While no site-directed mutagenesis data yet exist proving the spectral effects of these substitutions in the butterfly pigments, our findings suggest a common molecular basis for spectral shifts in both insects and vertebrates, across more than 540 million years of evolution.

What is the mechanism that has driven the recent radiation of the LW visual pigments in *Limenitis* butterflies? One hypothesis is that spectral diversification was an indirect consequence of visual predator (bird)-mediated selection on the wing color in these butterflies. Evolving a wing color pattern to resemble another unrelated butterfly species might create a selective pressure on the eye of the mimic to detect subtle differences in the wing colors of both models and mimics,

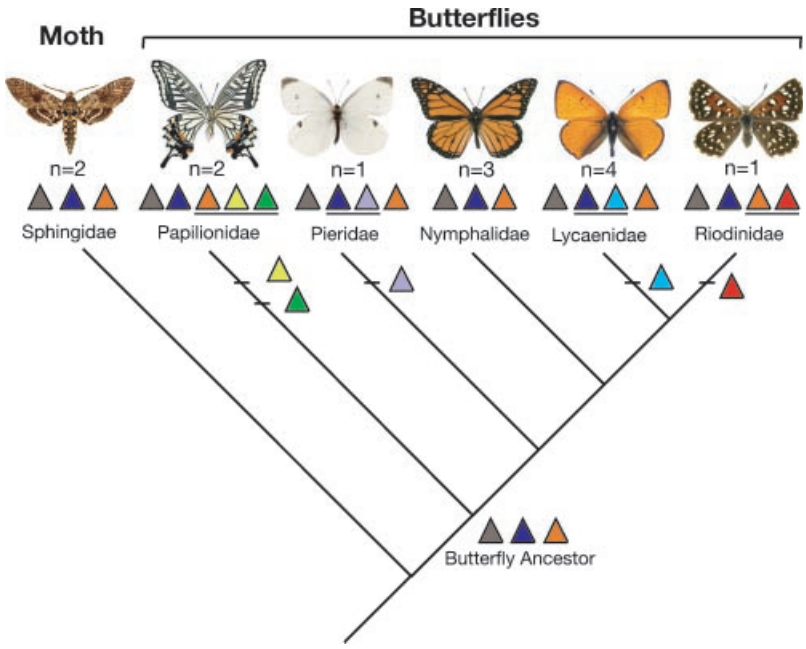

Figure 3. Phylogeny of the butterfly families, character mapping of visual pigments expressed in the eyes and proposed ancestral butterfly visual pigments involved in vision. Triangles in indicate UV (grey), B (dark blue) or LW visual pigments (orange). N = number of studies where opsin DNA sequences are correlated by in situ hybridizations with physiological measurements (intracellular recordings, epi-microspectrophotometry, electroretinograms) of visual pigment λ_{max}. Underlines indicate duplicated pigments.

thereby minimizing time spent chasing after the wrong potential mate. Determining the λ_{max} of the UV and B visual pigments in these butterflies is crucial for examining this hypothesis since wavelength discrimination requires at least two spectrally distinct photoreceptors, and it is possible that the other pigments in these butterflies have also diversified. Lastly, determining how shifts in λ_{max} relate to changes in wing reflectance spectra in *Limenitis* butterflies, coupled with behavioural tests, will help resolve the issue.

Lateral filtering pigments

In addition to the visual pigments, butterfly photoreceptor cells can also contain lateral filtering pigments that are red, orange or yellow and absorb short wavelength light. Changes in the color and distribution of these pigments (as well as other UV-absorbing filtering pigments) have also led to shifts in the peak spectral sensitivity of photoreceptors in some ommatidia. For example, red filtering pigments have been shown to shift photoreceptor sensitivities towards longer wavelengths in *Papilio xuthus* and *Pieris rapae*.[45] In the butterfly *Heliconius erato*, LW photoreceptors express the same opsin pigment but differ in sensitivity as a result of the presence of a red filtering pigment.[46] The two resulting photoreceptors are then used in color vision in the context of foraging.[46] Although the evolution of filtering pigments is poorly understood at present, their presence in the butterfly eye may be the ancestral condition.

Filtering pigments are interesting from an evolutionary perspective because they diversify photoreceptor sensitivities, and they may also play a role in the co-evolution of photoreceptors and mating signals. Some of the pigments found in the brightly colored areas of butterfly wings, possibly used in mate signaling, are evolutionarily derived from and molecularly similar to eye pigments studied in flies.[47] However, despite the potential importance of filtering pigments to butterfly eye function, their genetic basis, evolution and behavioral significance remain poorly understood at present.

Evolution of color vision in birds

Like butterflies, birds are also able to see in color. In fact, among vertebrates, birds possess one of the most complex eyes and are capable of perceiving and discriminating a greater diversity of colors than any mammal.[48] Vision in birds is mediated by at least seven types of photoreceptor cells: one type of rod receptor, four types of single cone receptors and two types of photoreceptor cells, the principal and the accessory, that are fused together forming the double cone[49] (shown separately in Fig. 2). Only the single cone classes are thought to be involved in color vision, but this is not entirely clear since only an handful of behavioral tests have been conducted on birds.[50] As in butterflies, the photoreceptor cells of birds contain the visual pigments that determine the wavelengths of light to which the receptors are maximally

sensitive. The avian eye possesses one of the richest complements of visual pigments among vertebrates, with five classes of visual pigments present.[51] These classes are distinguished based on pigment spectral sensitivity and amino acid sequences of their respective opsins. They are: ultraviolet (UV)- or violet-sensitive (VS) cone pigments (SWS1, $\lambda_{max} = 355–426$ nm; $n = 25$); short wavelength-sensitive type 2 cone pigments (SWS2, $\lambda_{max} = 427–460$ nm; $n = 26$); middle wavelength-sensitive, (RH1 and RH2, rod and cone pigments, respectively $\lambda_{max} = 497–507$ nm); and long wavelength-sensitive cone pigments (LWS, $\lambda_{max} = 543–571$ nm; $n = 29$; Table 2).

In addition to visual pigments, avian cone photoreceptors also contain spherical organelles called oil droplets, which are heavily pigmented by carotenoids and function to filter the spectrum of light received by the visual pigment. In chicken, principal cones have a yellow (not shown) or colorless droplet, accessory cones contain a pale green droplet, and single cones have a red, orange, yellow, colorless or transparent (not shown) droplet[49] (Fig. 2). Except for a handful of studies,[52,53] there has not been direct association of the opsin protein with a specific oil droplet and photoreceptor type. One early study using monoclonal antibodies localized the LWS opsin of chicken to the outer segments of the double cone (both principal and accessory cone photoreceptor cells) and to the outer segments of a single cone with a red oil droplet, but the oil droplet color of the identified double cones was contradicted by later reports.[53] Nonetheless, it has been assumed that, in birds, colored droplets do not increase the number of spectral types of photoreceptors as filtering pigments do in butterflies, but instead act as cut-off filters.[54] The absorption of short wavelengths by the pigmented oil droplets narrows the range of wavelengths that the photoreceptor is sensitive to and reduces the overlap between adjacent spectral classes, potentially improving the discrimination of natural reflectance spectra.[55]

Early vertebrate cone opsin gene duplications form the basis of bird color vision

The LWS, SWS1 and SWS2 cone opsins present in birds are ancient and existed before the divergence of the jawed and jawless vertebrates in the early Cambrian, ~540 mya.[56] They evolved through two successive rounds of duplication from an ancestral LWS opsin gene.[57] This early jawless vertebrate probably also had homologs of the modern jawed vertebrate rod opsin, RH1, and cone opsin, RH2. Whether or not the early early jawless vertebrate had exact copies of these opsin classes is somewhat unclear since the modern jawless lamprey contains two RH genes, RHA and RHB, that appear to be about equally related to the RH1 and RH2 genes of jawed vertebrates (See discussion in Bowmaker 2008).[58] Unlike in most eutherian mammals, which have lost two cone pigments, all five opsin lineages have been retained in birds (Fig. 4).

Diversification of bird color vision via spectral tuning of the short wavelength visual pigments

To our knowledge, the most-extensive diversification of avian visual systems has occurred in the SWS1 class as a consequence of spectral tuning, and has resulted in gain or loss of UV sensitivity.[59] In fact, bird SWS1 visual systems could be divided into two types: the UVS type, which has a UV-biased visual system with λ_{max} between 355 and 373 nm and a violet-sensitive, VS, type, with λ_{max} between 402 and 426 nm.[60] Unlike the other cone pigments, SWS1 pigments are present in cones that contain transparent oil droplets with no significant light absorption throughout the light spectrum, meaning that the λ_{max} of those photoreceptors is solely determined by the visual pigment.[61,62]

Phylogenetic inference and ancestral reconstruction of opsin proteins in vitro have proved a useful tool in correlating SWS1 opsin gene sequences with SWS1 visual pigment spectra, and understanding the evolution of ultraviolet vision in birds. The combination of approaches has shown that, in most extant vertebrates, UV vision was directly inherited from the early vertebrate ancestor and lost in some groups afterwards (e.g. mammals)(Fig. 4). Although the SWS1 opsin gene was never lost in birds, the ancestral bird SWS1 pigment was most likely violet- (VS)[63] rather than ultraviolet—(UVS) sensitive. The UVS visual system is most likely produced by a serine-to-cysteine substitution at site 90 (bovine rhodopsin numbering), resulting in a spectral blueshift of ~35 nm in λ_{max}.[64,65] (Intriguingly, Asn90Lys or Glu90Lys substitutions are also responsible for UV vision in insects[66]). This 'reinvention' of UV sensitivity in birds is of special interest because it appears to have occurred multiple times in independent lineages, possibly occurring not just at the level of orders but within bird families, although this conclusion is based solely on DNA sequence data limited to a very small portion of the SWS1 gene.[67]

In addition, in some bird groups, a UV-sensitive pigment evolved from a violet-sensitive ancestral pigment via another amino acid substitution, serine to phenylalanine at site 86.[68] The exact evolutionary pathway and spectral tuning of violet sensitive pigments remain to be investigated; however, because DNA sequence and spectral data are available from only four bird species with violet-sensitive pigments: Humbold penguin, domestic pigeon, chicken and common cormorant.[65,68,69] The shifts between the VS to UVS types of visual system were most likely driven by selection, although the exact agents of selection remain unclear. Indeed the SWS1 opsins have evolved the fastest of all the avian visual pigments,[70] but no formal evidence of adaptive evolution at the DNA sequence level yet exists. What has also been suggested is that the SWS2 class of visual pigments may have evolved in tandem with the SWS1 class.[71] Bird UVS systems are accompanied by SWS2 visual pigments whose λ_{max} is more UV-light shifted, whereas in VS systems the SWS2 pigment λ_{max} is

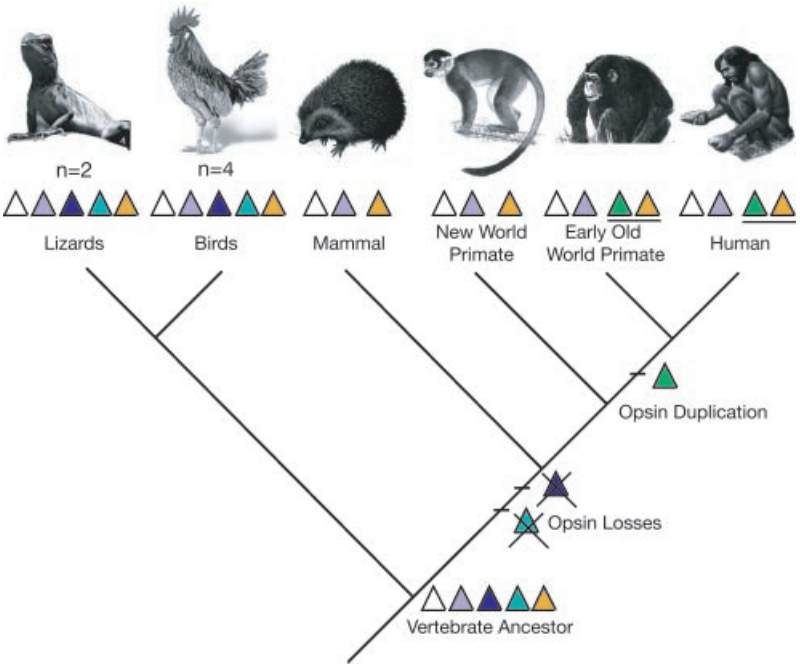

Figure 4. Phylogeny of the vertebrates, character mapping of rod and cone visual pigments expressed in the eyes, and proposed ancestral vertebrate visual pigments involved in vision. Triangles indicate RH1 (white) rod visual pigments, SWS1 (violet), SWS2 (dark blue), RH2 (light blue) or LWS (light orange) cone visual pigments. N = number of studies in lizards or birds where opsin DNA sequences are correlated with physiological measurements (microspectrophotometry or in vitro expression, reconstitution and biochemical characterization) of visual pigment λ_{max}. Underlines indicate duplicated pigments.

blue-shifted. (SWS2 pigments are found in single cones with 'colorless' oil droplets that filter light below 450 nm, thereby sharpening their sensitivity to light). A potential explanation for this correlated evolution is that reduction in overlap of spectral classes improves color constancy[72] and increases chromatic contrast.[55]

UV vision has a range of functions in birds including foraging, conspicuousness of nestling mouths during begging, and signaling in mate choice.[73,74] Recent debate in the literature has been focused on the hypothesis that UV represents an adaptive 'private communication' channel in animals that use it, although the evidence remains mixed.[75] For example, by using a retinal model, Håstad et al (2005)[76] found that collar feather badges, which function as sexual signals in male passerines (which have a UVS type visual system), are much more conspicuous to conspecifics than to potential avian predators (which have a VS type system). Alternatively, UVS vision may confer an adaptive advantage when foraging for bird-dispersed fruits or on insects such as butterflies that have wings with a strongly UV-reflecting component. The UVS and VS types of vision differ in their ability to discriminate color at short wavelengths, with objects such as fruits that reflect in the 410–420 nm range being more contrasting to birds possessing the former type of visual system.[77]

Conservation of middle- and long-wavelength photoreceptors across birds

Unlike butterflies, where extensive spectral diversity is observed in the middle- and long-wavelength photoreceptor classes, in bird species (n = 29), λ_{max} in this range is remarkably conserved.[59] Rod (RH1) peak sensitivities vary remarkably little (λ_{max} = 501–509 nm) across, birds and within a given species, match that of the RH2 cone visual pigments (λ_{max} = 497–509 nm).[71] Rod photoreceptor cells (that are not involved in color vision) lack colored oil droplets so their spectral sensitivity is solely determined by the λ_{max}. In contrast, the effective λ_{max} of cells containing RH2 visual pigments is shifted towards longer wavelengths by about 40 nm because of associated colored oil droplets.[78] Unlike in butterflies, there is no evidence to date of lineage-specific LWS opsin duplication. In the bird species that have been studied to date (n = 31), LWS visual pigment λ_{max} values cluster in three spectral categories: 543 nm (Humbold penguin), 555 nm (owls) and 562–571 nm (e.g. blue tit and chicken, λ_{max} = 563 and 571 respectively) (See Table 2 for references). Although it

is generally assumed that avian LWS pigments are spectrally tuned through the same amino acid sites as those of mammals and that some of the same amino acid residues are seen across both groups, definitive tests are missing due to a paucity of DNA sequence data for this pigment. In fact, our BLAST searches of GenBank yielded only four bird LWS opsin sequences, chicken (*Gallus gallus*), canary (*Serinus canaria*), pigeon (*Columba livia*) and zebrafinch (*Taeniopygia guttata*), which are nearly identical in terms of the λ_{max} values of the pigments they encode (Table 2). (By way of comparison, there are >50 LW opsin gene sequences currently available for butterflies). It would be very interesting to sample this gene in species for which spectral data are also available for the LWS pigment (e.g. Table 2, un-bolded species). For example, although most birds possess LWS visual pigments with λ_{max} values between 560 and 570 nm, a few species have pigments that are shortwave-shifted. The Humbold penguin has a λ_{max} of 543 nm, thought to be an adaptation to increased visual sensitivity at depths in the ocean in which longer wavelengths of light are attenuated more rapidly than the rest of the spectrum.[79] It is not clear how spectral tuning may have occurred in the Humbold penguin, because its LWS opsin gene has yet to be sequenced and spectral tuning sites identified.

Spectral tuning of avian LWS pigment λ_{max} for most birds is thought to reflect selection on the double cones involved in achromatic tasks and movement detection rather than on the single LWS cone involved in color vision.[59] Double cones, the principal and accessory cone cells that they are comprised of, express LWS pigments[53] and are the most-abundant type in the avian retina.[80] In chicken, principal cones have yellow or colorless oil droplets, while accessory cones contain a pale green droplet.[49] In the single cones that also express the LWS opsin, red-colored oil droplets act to shift the peak spectral sensitivity towards longer wavelengths λ_{max} >600 nm). Changes in oil droplet expression may be the primary means of matching the spectral sensitivity of LWS single cones to the light environment; however further anatomical work correlating specific LWS opsin mRNAs and/or proteins to particular cone cells is needed to verify this hypothesis.

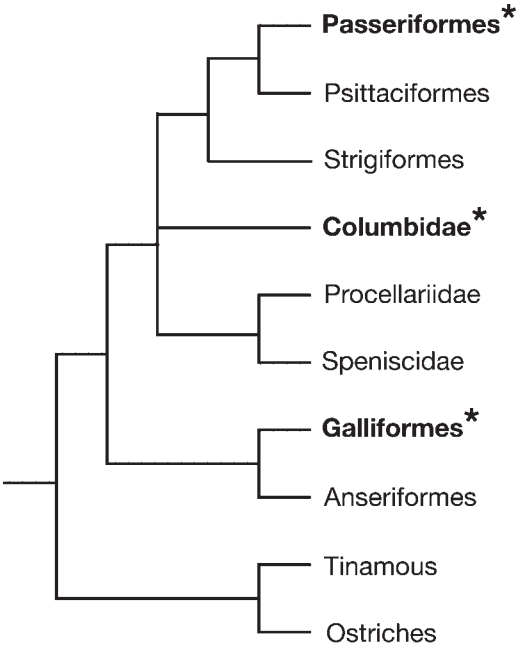

Figure 5. Phylogeny of bird groups in which visual pigment spectral data are available. Clades in which opsin gene sequences are also available for all five visual pigments are indicated by an asterisk.

Why are the eyes of birds not more diverse?

Both butterflies and birds contain comparable number of species (an estimated 15,000 butterfly species versus an estimated 10,000 bird species), use color in the context of both natural and sexual selection, and have evolved over a similar time period in evolutionary history (~160 mya for birds)[81] and (+140 mya for butterflies).[82] No doubt the wings of butterflies bear witness to the visual abilities of birds, for example through the evolution of aposematic or cryptic color patterns that exploit avian sensory systems and learning abilities. Furthermore, molecular studies have made it amply clear that opsin gene duplication is common in fish, primates and arthropods in general. Thus, there is no intrinsic reason why the visual pigment (opsin gene number) complement of birds should be conserved, nor is it clear why the oil droplets matched to the visual pigments should be tightly conserved either (in butterflies, filtering pigments are not matched to visual pigments). A hint of things to come may be found in a recent study of the Bobolink, a New World migratory Passerine.[83] Instead of the expected LWS visual pigment in both members of the double cones, a violet-sensitive pigment ($\lambda_{max} = 403$ nm) was found in the accessory member. It is not yet clear whether this represents a duplicated SWS1 opsin gene or an extremely blue-shifted SWS2 opsin gene, as no violet- or blue-sensitive single cone was found and no opsin gene sequence data are yet available. We suspect that, with only four species of birds studied for both opsin gene sequences and visual pigment spectra (Fig. 5), representing three avian families out of >70,[84] the jury is still out on the extent of diversity of bird visual systems. The eyes of birds, like those of butterflies, have many surprises yet to be revealed.

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References

1. Hill GE, McGraw KJ. 2004. Correlated changes in male plumage coloration and female mate choice in cardueline finches. Animal Behav 67:27–35.
2. Lederhouse R, Scriber J. 1996. Intrasexual selection constrains the evolution of the dorsal color pattern of male black swallowtail butterflies, *Papilio polyxenes*. Evolution 50:717–722.
3. Kelber A, Vorobyev M, Osorio D. 2003. Animal colour vision—behavioural tests and physiological concepts. Biol Rev Camb Philos Soc 78:81–118.
4. Ham AD, Osorio D. 2007. Colour preferences and colour vision in poultry chicks. Proc Biol Sci 274:1941–1948.
5. Koshitaka H, Kinoshita M, Vorobyev M, Arikawa K. 2008. Tetrachromacy in a butterfly that has eight varieties of spectral receptors. Proc Biol Sci 275:947–954.
6. Endler JA, Mappes J. 2005. Predator mixes and the conspicuousness of aposematic signals. Am Nat 163:532–547.
7. Langham G. 2004. Specialized avian predators repeatedly attack novel color morphs of *Heliconius* butterflies. Evolution 58:2783–2787.
8. Land MF, Fernald RD. 1992. The evolution of eyes. Annu Rev Neurosci 15:1–29.
9. Terakita A. 2005. The opsins. Genome Biol 6:213.
10. Menon ST, Han M, Sakmar TP. 2001. Rhodopsin: structural basis of molecular physiology. Physiol Rev 81:1659–1688.
11. Kojima D, Terakita A, Ishikawa T, Tsukahara Y, Maeda A, Shichida Y. 1997. A novel Go-mediated phototransduction cascade in scallop visual cells. J Biol Chem 272:22979–22982.
12. Velarde RA, Sauer CD, Walden KK, Fahrbach SE, Robertson HM. 2005. Pteropsin: a vertebrate-like non-visual opsin expressed in the honey bee brain. Insect Biochem Mol Biol 35:1367–1377.
13. Arendt D. 2003. Evolution of eyes and photoreceptor cell types. Int J Dev Biol 47:563–571.
14. Hisatomi O, Tokunaga F. 2002. Molecular evolution of proteins involved in vertebrate phototransduction. Comp Biochem Physiol B Biochem Mol Biol 133:509–522.
15. Lee YJ, Shah S, Suzuki E, Zars T, O'Day PM, Hyde DR. 1994. The *Drosophila dgq* gene encodes a G alpha protein that mediates phototransduction. Neuron 13:1143–1157.
16. Goldsmith T. 1990. Optimization, constraint, and history in the evolution of eyes. Q Rev Biol 65:281–322.
17. Yamaguchi S, Wolf R, Desplan C, Heisenberg M. 2008. Motion vision is independent of color in *Drosophila*. Proc Natl Acad Sci USA 105:4910–4915.
18. Han M, Groesbeek M, Smith SO, Sakmar TP. 1998. Role of the C9 methyl group in rhodopsin activation: characterization of mutant opsins with the artificial chromophore 11-*cis*-9-demethylretinal. Biochemistry 37:538–545.
19. Honig B, Greenberg AD, Dinur U, Ebrey TG. 1976. Visual-pigment spectra: implications of the protonation of the retinal Schiff base. Biochemistry 15:4593–4599.
20. Nathans J. 1990. Determinants of visual pigment absorbance: role of charged amino acids in the putative transmembrane segments. Biochemistry 29:937–942.
21. Seki T, Vogt K. 1998. Evolutionary aspects of the diversity of visual pigment chromophores in the Class Insecta. Comp Biochem Physiol 119:53–64.
22. Bowmaker JK, Govardovskii VI, Shukolyukov SA, Zueva LV, Hunt DM, et al. 1994. Visual pigments and the photic environment: the cottoid fish of Lake Baikal. Vision Res 34:591–605.
23. Hunt D, Fitzgibbon J, Slobodyanuk S, Bowmaker J. 1996. Spectral tuning and molecular evolution of rod visual pigments in the species flock of cottoid fish in Lake Baikal. Vision Res 36:1217–1224.
24. Peitsch D, Feitz A, Hertel H, de Souza J, Ventura D, Menzel R. 1992. The spectral input systems of hymenopteran insects and their receptor-based colour vision. J Comp Physiol A 170:23–40.
25. Koyanagi M, Nagata T, Katoh K, Yamashita S, Tokunaga F. 2008. Molecular evolution of arthropod color vision deduced from multiple opsin genes of jumping spiders. J Mol Evol 66:130–137.
26. Jackowska M, Bao R, Liu Z, McDonald EC, Cook TA, Friedrich M. 2007. Genomic and gene regulatory signature of crytozoic adaptation: Loss of blue sensitive photoreceptors through expansion of long wavelength-opsin expression in the red flour beetle *Tribolium castaneum*. Frontiers in Zoology 4.
27. Briscoe AD. 2008. Reconstructing the ancestral butterfly eye: Focus on the opsins. J Exp Biol 211:1805–1813.
28. Arikawa K, Wakakuwa M, Qiu X, Kurasawa M, Stavenga DG. 2005. Sexual dimorphism of short-wavelength photoreceptors in the small white butterfly, *Pieris rapae crucivora*. J Neurosci 25:5935–5942.
29. Sison-Mangus MP, Bernard GD, Lampel J, Briscoe AD. 2006. Beauty in the eye of the beholder: the two blue opsins of lycaenid butterflies and the opsin gene-driven evolution of sexually dimorphic eyes. J Exp Biol 209:3079–3090.
30. Briscoe AD. 1998. Molecular diversity of visual pigments in the butterfly *Papilio glaucus*. Naturwissenschaften 85:33–35.
31. Kitamoto J, Sakamoto K, Ozaki K, Mishina Y, Arikawa K. 1998. Two visual pigments in a singe photoreceptor cell: identification and histological localization of three mRNAs encoding visual pigment opsins in the retina of the butterfly *Papilio xuthus*. J Exp Biol 201:1255–1261.
32. Frentiu FD, Bernard GD, Sison-Mangus MP, Van Zandt Brower AVZ, Briscoe AD. 2007. Gene duplication is an evolutionary mechanism for expanding spectral diversity in the long wavelength photopigments of butterflies. Mol Biol Evol 24:2016–2028.
33. Arikawa K. 2003. Spectral organization of the eye of a butterfly, *Papilio*. J Comp Physiol A 189:791–800.
34. Briscoe AD, Bernard GD, Szeto AS, Nagy LM, White RH. 2003. Not all butterfly eyes are created equal: Rhodopsin absorption spectra, molecular identification and localization of ultraviolet-, blue-, and green-sensitive rhodopsin-encoding mRNAs in the retina of *Vanessa cardui*. J Comp Neurol 458:334–349.
35. Sauman I, Briscoe AD, Zhu H, Shi D, Froy O, et al. 2005. Connecting the navigational clock to sun compass input in monarch butterfly brain. Neuron 46:457–467.
36. Kelber A. 1999. Ovipositing butterflies use a red receptor to see green. J Exp Biol 202:2619–2630.
37. Kelber A, Pfaff M. 1999. True colour vision in the orchard butterfly, *Papilio aegeus*. Naturwissenschaften 86:221–224.
38. Kinoshita M, Shimada N, Arikawa K. 1999. Colour vision of the foraging swallowtail butterfly, *Papilio xuthus*. J Exp Biol 202:95–102.
39. Sison-Mangus MP, Briscoe AD, Zaccardi G, Knuttel H, Kelber A. 2008. The lycaenid butterfly *Polyommatus icarus* uses a duplicated blue opsin to see green. J Exp Biol 211:361–369.
40. Mullen SP. 2006. Wing pattern evolution and the origins of mimicry among North American admiral butterflies (Nymphalidae: *Limenitis*). Mol Phylogenet Evol 39:747–758.
41. Frentiu FD, Bernard GD, Cuevas CI, Sison-Mangus MP, Prudic KL, Briscoe AD. 2007. Adaptive evolution of color vision as seen through the eyes of butterflies. Proc Natl Acad Sci USA 104:8634–8640.
42. Asenjo AB, Rim J, Oprian DD. 1994. Molecular determinants of human red/green color discrimination. Neuron 12:1131–1138.
43. Merbs SL, Nathans J. 1993. Role of hydroxyl-bearing amino acids in differentially tuning the absorption spectra of the human red and green cone pigments. Photochem Photobiol 58:706–710.
44. Surridge AK, Osorio D, Mundy NI. 2003. Evolution and selection of trichromatic vision in primates. Trends Ecol Evol 18:198–205.
45. Stavenga DG, Arikawa K. 2006. Evolution of color and vision of butterflies. Arthropod Structure and Development 35:307–318.
46. Zaccardi G, Kelber A, Sison-Mangus MP, Briscoe AD. 2006. Color discrimination in the red range with only one long-wavelength sensitive opsin. J Exp Biol 209:1944–1955.
47. Reed RD, Nagy LM. 2005. Evolutionary redeployment of a biosynthetic module: expression of eye pigment genes vermilion, cinnabar, and white in butterfly wing development. Evol Dev 7:301–311.
48. Osorio D, Vorobyev M, Jones CD. 1999. Colour vision of domestic chicks. J Exp Biol 202:2951–2959.
49. López-López R, López-Gallardo M, Pérez-Álvarez MJ, Prada C. 2008. Isolation of chick retina cones and study of their diversity based on oil droplet colour and nucleus position. Cell Tissue Res 332:13–24.

50. Goldsmith TH, Butler BK. 2005. Color vision of the budgerigar (*Melopsittacus undulatus*): hue matches, tetrachromacy, and intensity discrimination. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 191:933–951.
51. Jane SD, Bowmaker JK. 1988. Tetrachromatic colour vision in the duck (*Anas platyrhynchos L.):* microspectrophotometry of visual pigments and oil droplets. J Comp Physiol 162:225–235.
52. Wilkie SE, Vissers PM, Das D, Degrip WJ, Bowmaker JK, Hunt DM. 1998. The molecular basis for UV vision in birds: spectral characteristics, cDNA sequence and retinal localization of the UV-sensitive visual pigment of the budgerigar (*Melopsittacus undulatus*). Biochem J 330:541–547.
53. Oishi T, Kawata A, Hayashi T, Fukada Y, Shichida Y, Yoshizawa T. 1990. Immunohistochmical localization of iodopsin in the retina of the chicken and Japanese quail. Cell Tissue Res 261:397–401.
54. Hart NS, Vorobyev M. 2005. Modelling oil droplet absorption spectra and spectral sensitivities of bird cone photoreceptors. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 191:381–392.
55. Vorobyev M. 2003. Coloured oil droplets enhance colour discrimination. Proc Biol Sci 270:1255–1261.
56. Collin SP, Knight MA, Davies WL, Potter IC, Hunt DM, Trezise AE. 2003. Ancient colour vision: multiple opsin genes in the ancestral vertebrates. Curr Biol 13:R864–R865.
57. Yokoyama S, Blow NS, Radlwimmer FB. 2000. Molecular evolution of color vision of zebra finch. Gene 259:17–24.
58. Bowmaker JK. 2008. Evolution of vertebrate visual pigments. Vision Res In press.
59. Hart NS, Hunt DM. 2007. Avian visual pigments: Characteristics, spectral tuning, and evolution. Am Nat 169:S7–S26.
60. Endler JA, Mielke PW. 2005. Comparing entire colour patterns as birds see them. Biol J Linn Soc 86:405–431.
61. Das D, Wilkie SE, Hunt DM, Bowmaker JK. 1999. Visual pigments and oil droplets in the retina of a passerine bird, the canary *Serinus canaria*: microspectrophotometry and opsin sequences. Vision Res 39:2801–2815.
62. Bowmaker JK, Knowles A. 1977. The visual pigments and oil droplets of the chicken retina. Vision Res 17:755–764.
63. Shi Y, Yokoyama S. 2003. Molecular analysis of the evolutionary significance of ultraviolet vision in vertebrates. Proc Natl Acad Sci USA 100:8308–8313.
64. Yokoyama S, Radlwimmer FB, Blow NS. 2000. Ultraviolet pigments in birds evolved from violet pigments by one amino acid change. Proc Natl Acad Sci USA 97:7366–7371.
65. Wilkie SE, Robinson PR, Cronin TW, Poopalasundaram S, Bowmaker JK, Hunt DM. 2000. Spectral tuning of avian violet- and ultraviolet-sensitive visual pigments. Biochemistry 39:7895–7901.
66. Salcedo E, Zheng L, Phistry M, Bagg E, Britt S. 2003. Molecular basis for ultraviolet vision in invertebrates. J Neurosci 23:10873–10878.
67. Ödeen A, Håstad O. 2003. Complex distribution of avian color vision systems revealed by sequencing the SWS1 opsin from total DNA. Mol Biol Evol 20:855–861.
68. Carvalho LS, Cowing JA, Wilkie SE, Bowmaker JK, Hunt DM. 2007. The molecular evolution of avian ultraviolet- and violet-sensitive visual pigments. Mol Biol Evol 24:1843–1852.
69. Okano T, Kojima D, Fukada Y, Shichida Y, Yoshizawa T. 1992. Primary structures of chicken cone visual pigments: vertebrate rhodopsins have evolved out of cone visual pigments. Proc Natl Acad Sci USA 89:5932–5936.
70. Yokoyama S. 2000. Molecular evolution of vertebrate visual pigments. Prog Retinal Eye Res 19:385–419.
71. Hart NS. 2001. The visual ecology of avian photoreceptors. Prog Retinal Eye Res 20:675–703.
72. Osorio D, Marshall NJ, Cronin TW. 1997. Stomatopod photoreceptor spectral tuning as an adaptation for colour constancy in water. Vision Res 37:3299–3309.
73. Bennett ATD, Cuthill IC, Partridge JC, Maier EJ. 1996. Ultraviolet vision and mate choice in zebrafinches. Nature 380:433–435.
74. Church SC, Bennett ATD, Cuthill IC, Partridge JC. 1998. Ultraviolet cues affect the foraging behavior of blue tits. Proc R Soc Lond B 265:1509–1514.
75. Stevens M, Cuthill IC. 2007. Hidden messages: Are ultraviolet signals a special channed in avian communication? BioScience 57:501–507.
76. Håstad O, Victorsson J, Ödeen A. 2005. Differences in color vision make passerines less conspicuous in the eyes of their predators. Proc Natl Acad Sci USA 102:6391–6394.
77. Schaefer HM, Schaefer V, Vorobyev M. 2007. Are fruit colors adapted to consumer vision and birds equally efficient in detecting colorful signals? Am Nat 169:S159–S169.
78. Bowmaker JK, Knowles A. 1977. The visual pigments and oil droplets of the chicken retina. Vision Res 17:755–764.
79. Bowmaker JK, Martin GR. 1985. Visual pigments and oil droplets in the penguin *Spheniscus humboldti*. J Comp Physiol A 156:71–77.
80. Hart NS. 2001. Variations in cone photoreceptor abundance and the visual ecology of birds. J Comp Physiol A 187:685–698.
81. Sereno PC. 1999. The evolution of dinosaurs. Science 284:2137–2147.
82. Wahlberg N. 2006. That awkward age for butterflies: Insights from the age of the butterfly subfamily Nymphalinae (Lepidoptera). Syst Biol 55:703–714.
83. Beason RC, Loew ER. 2008. Visual pigment and oil droplet characteristics of the bobolink (*Dolichonyx oryzivorus*), a new world migratory bird. Vision Res 48:1–8.
84. Mayr G. 2007. Avian higher-level phylogeny: well-supported clades and what we can learn from a phylogenetic analysis of 2954 morphological characters. J Zool Syst Evol Res 46:63–72.
85. Bernard G, Remington C. 1991. Color vision in *Lycaena* butterflies: spectral tuning of receptor arrays in relation to behavioral ecology. Proc Natl Acad Sci USA 88:2783–2787.
86. Kinoshita M, Sato M, Arikawa K. 1997. Spectral receptors of nymphalid butterflies. Naturwissenschaften 84:199–201.
87. Stalleicken J, Labhart T, Mouritsen H. 2006. Physiological characterization of the compound eye in monarch butterflies with focus on the dorsal rim area. J Comp Physiol A 192:321–331.
88. Steiner A, Rudiger P, Gemperlein R. 1987. Retinal receptor types in *Aglais urticae* and *Pieris brassicae* (Lepidoptera), revealed by analysis of the electroretinogram obtained with Fourier interferometric stimulation (FIS). J Comp Physiol A 160:247–258.
89. Briscoe AD, Bernard GD. 2005. Eyeshine and spectral tuning of long wavelength-sensitive rhodopsins: No evidence for red-sensitive photoreceptors among five Nymphalini butterfly species. J Exp Biol 208:687–696.
90. Vanhoutte KJA, Stavenga DG. 2005. Visual pigment spectra of the comma butterfly, *Polygonia c-album*, derived from in vivo epi-illumination microspectrophotometry. J Comp Physiol A 191:461–473.
91. Paul R, Steiner A, Gemperlein R. 1986. Spectral sensitivity of *Calliphora erythrocephala* and other insect species studied with Fourier Interferometric Stimulation (FIS). J Comp Physiol A 158:669–680.
92. Wakakuwa M, Stavenga DG, Kurasawa M, Arikawa K. 2004. A unique visual pigment expressed in green, red and deep-red receptors in the eye of the small white butterfly, *Pieris rapae crucivora*. J Exp Biol 207:2803–2810.
93. Bernard G, Douglass J, Goldsmith T. 1988. Far-red sensitive visual pigment of a metalmark butterfly. Investigative Ophthamology 29: 350.
94. Hart NS. 2004. Microspectrophotometry of visual pigments and oil droplets in a marine bird, the wedge-tailed shearwater *Puffinus pacificus*: topographic variations in photoreceptor spectral characteristics. J Exp Biol 207:1229–1240.
95. Bowmaker J, Heath L, Wilkie S, Hunt D. 1997. Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. Vision Res 37:2183–2194.
96. Yokoyama S, Radlwimmer F, Kawamura S. 1998. Regeneration of ultraviolet pigments of vertebrates. FEBS Letters 423:155–158.
97. Bowmaker JK, Kovach JK, Whitmore AV, Loew ER. 1993. Visual pigments and oil droplets in genetically manipulated and carotenoid deprived quail: a microspectrophotometric study. Vision Res 33:571–578.
98. Hart NS, Partridge JC, Cuthill IC. 1999. Visual pigments, cone oil droplets, ocular media and predicted spectral sensitivity in the domestic turkey (*Meleagris gallopavo*). Vision Res 39:3321–3328.
99. Hart N. 2002. Vision in the peafowl (*Pavo cristatus*. J Exp Biol 205:3925–3935.

100. Hart NS, Partridge JC, Bennett AT, Cuthill IC. 2000. Visual pigments, cone oil droplets and ocular media in four species of estrildid finch. J Comp Physiol A 186:681–694.
101. Bowmaker JK. 1979. In: Granda AM, Marxwell JH, editors. Neural mechanisms of behavior in the pigeon. NY: Plenum.
102. Maier EJ, Bowmaker JK. 1993. Colour vision in the passeriform bird, *Leiothrix lutea:* correlation of visual absorbance and oil droplet transmission with spectral sensitivity. J Comp Physiol A 172:295–301.
103. Hart NS, Partridge JC, Cuthill IC, Bennett AT. 2000. Visual pigments, oil droplets, ocular media and cone photoreceptor distribution in two species of passerine bird: the blue tit (*Parus caeruleus* L.) and the blackbird (*Turdus merula* L.). J Comp Physiol A 186:375–387.
104. Hart N, Partridge J, Cuthill II. 1998. Visual pigments, oil droplets and cone photoreceptor distribution in the european starling (*Sturnus vulgaris*). J Exp Biol 201:1433–1446.
105. Bowmaker JK, Martin GR. 1978. Visual pigments and colour vision in a nocturnal bird, *Strix aluco* (tawny owl). Vision Res 18:1125–1130.
106. Wright MW, Bowmaker JK. 2001. Retinal photoreceptors of paleognathous birds: the ostrich (*Struthio camelus*) and rhea (*Rhea americana*). Vision Res 41:1–12.
107. Sillman AJ, Bolnick DA, Haynes LW, Walter AE, Loew ER. 1981. Microspectrophotometry of the photoreceptors of palaeognathous birds—the emu and the tinamou. J Comp Physiol A 144:271–276.