Complex Distribution of Avian Color Vision Systems Revealed by Sequencing the SWS1 Opsin from Total DNA

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To gain insights into the evolution and ecology of visually acute animals such as birds, biologists often need to understand how these animals perceive colors. This poses a problem, since the human eye is of a different design than that of most other animals. The standard solution is to examine the spectral sensitivity properties of animal retinas through microspectophotometry—a procedure that is rather complicated and therefore only has allowed examinations of a limited number of species to date. We have developed a faster and simpler molecular method, which can be used to estimate the color sensitivities of a bird by sequencing a part of the gene coding for the ultraviolet or violet absorbing opsin in the avian retina. With our method, there is no need to sacrifice the animal, and it thereby facilitates large screenings, including rare and endangered species beyond the reach of microspectrophotometry. Color vision in birds may be categorized into two classes: one with a short-wavelength sensitivity biased toward violet (VS) and the other biased toward ultraviolet (UVS). Using our method on 45 species from 35 families, we demonstrate that the distribution of avian color vision is more complex than has previously been shown. Our data support VS as the ancestral state in birds and show that UVS has evolved independently at least four times. We found species with the UVS type of color vision in the orders Psittaciformes and Passeriformes, in agreement with previous findings. However, species within the families Corvidae and Tyrannidae did not share this character with other passeriforms. We also found UVS type species within the Laridae and Struthionidae families. Raptors (Accipitridae and Falconidae) are of the violet type, giving them a vision system different from their passeriform prey. Intriguing effects on the evolution of color signals can be expected from interactions between predators and prey. Such interactions may explain the presence of UVS in Laridae and Passeriformes.

Introduction

Insights into color perception are often crucial to understanding animal behavior, ecology, and speciation. The sensitivity maxima of color receptors (single cones) are located in different spectral positions among animals, so that individual colors may be perceived very differently, even among related species. Unfortunately, the human eye is of an uncommon type, only shared by Old World monkeys and apes (Jacobs 1993) and therefore unfit to mirror the color perception of most other animals. The human eye is trichromatic, as our color vision involves three distinct classes of cones. Retinas with four classes of cones involved in color perception (tetrachromatic vision) have been reported in birds (Goldsmith 1990), fish (Palacios et al. 1998), and reptiles (Fleishman, Loew, and Leal 1993). Due to an additional class of cones, tetrachromats have the theoretical ability to see twice the number of colors compared with trichromats. Humans may hence be blind to many critical aspects of animal coloration and perception (Losey et al. 1999). We may not only perceive slightly different hues compared with other animals but also are possibly missing major components of animal coloration.

Compared with humans, birds have an additional color channel located in the ultraviolet (UV) to near ultraviolet range. The UV waveband is unperceivable by humans, but it has been shown to be ecologically important to birds. Experimental alterations of the UV component in the plumage have significantly affected sexual signals in many bird species (Maier and Bowmaker 1993; Bennett et al. 1996, 1997; Andersson and Amundsen 1997; Hunt et al. 1997, 1998, 1999), and it has been demonstrated a number of times that UV plays an important role in prey detection and foraging (Goldsmith 1980; Viitala et al. 1995; Church et al. 1998; Siitari, Honkavaara, and Viitala 1999). Still, UV does not seem to be more important to birds than does other parts of the spectrum (Hunt et al. 2001; Maddocks, Church, and Cuthill 2001). The focus on UV as a separate communication channel that has imbued behavioral studies in recent years ignores potentially important differences in color perception arising from tetrachromacy.

An important step towards an understanding of how animals perceive color is knowledge of their chromatic ocular disposition (COD), meaning the composite effect of the cone visual pigments' (opsin's) wavelength of maximum absorbance (λ-max), the filtering by the ocular media (including lens and cornea) and the oil droplets of the cones, and the relative abundance of different cone types. There appears to be two main CODs in birds. The most pronounced difference is in the λ-max of the opsin in the UV/violet (SWS1) and short-wavelength sensitive (SWS2) cones. One large group (violet sensitive, or VS [Hart et al. 2000*b*]) possesses SWS1 cones with a λ-max ranging from 403 to 426 nm (Hart, Partridge, and Cuthill 1999). A systematically more restricted group (ultraviolet sensitive, or UVS [Hart et al. 2000*b*]) has a more UV-biased SWS1 with a λ-max between 355 and 380 nm (Hart, Partridge, and Cuthill 1999). The VS system has been demonstrated throughout the avian phylogeny, in *Anas platyrhyncos* (Jane and Bowmaker 1988), *Gallus gallus* (Bowmaker et al. 1997), *Spheniscus humboltii* (Bowmaker and Martin 1985), *Coturnix coturnix japonica* (Bowmaker et al. 1993), *Meleagris gallopavo* (Hart, Partridge, and Cuthill 1999), *Pavo cristatus* (Hart 1998),

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Table 1
Type of Color Vision in Examined Bird Species

Order	Family	Name	Common Name	Type	Amino Acid Sequence	Calc. λ-max[a]	Meas. λ-max[b]	Reference
Anseriformes	Anatidae	Anas platyrhynchos	Mallard duck	VS	FV**S**CIF**S**VF**I**V	405[c]	420	Jane and Bowmaker 1988
Ciconiiformes	Accipitridae	*Accipiter gentilis*	Northern goshawk	VS	FI**X**CIF**S**VF**T**V	406		
Ciconiiformes	Accipitridae	*Accipiter nisus*	European sparrow hawk	VS	FI**S**CIF**S**VF**T**V	405		
Ciconiiformes	Accipitridae	*Buteo buteo*	Common buzzard	VS	FI**S**CIF**S**VF**T**V	405		
Ciconiiformes	Accipitridae	*Circus aeruginosus*	Marsh harrier	VS	FI**S**CIF**S**VF**T**V	405		
Ciconiiformes	Accipitridae	*Pandion haliaetus*	Osprey	VS	FI**S**CIF**S**VF**T**V	405		
Ciconiiformes	Ardeidae	*Ardea cinerea*	Grey heron	VS	FI**C**CIF**S**VF**T**V	406[d]		
Ciconiiformes	Charadriidae	*Charadrius dubius*	Little ringed plower	VS	FI**A**CIF**S**VF**T**V	406		
Ciconiiformes	Charadriidae	*Haematopus ostralegus*	Common pied oystercatcher	VS	FI**A**CIF**S**VF**T**V	406		
Ciconiiformes	Charadriidae	*Himantopus himantopus*	Black-winged stilt	VS	FV**A**CIF**S**VF**T**V	406		
Ciconiiformes	Falconidae	*Falco peregrinus*	Peregrine falcon	VS	FI**S**CIF**S**VF**T**V	405		
Ciconiiformes	Gaviidae	*Gavia stellata*	Red-throated diver	VS	FI**C**CIF**S**VF**T**V	406[d]		
Ciconiiformes	Laridae	*Alca torda*	Razorbill	VS	FV**A**CIF**S**VF**T**V	406		
Ciconiiformes	Laridae	*Larus argentatus*	Herring gull	UVS	FI**I**CVF**C**IS**I**V	371[c,d]		
Ciconiiformes	Laridae	*Larus fuscus*	Lesser black-backed gull	UVS	FI**I**CVF**C**IS**I**V	371[c,d]		
Ciconiiformes	Laridae	*Larus marinus*	Greater black-backed gull	UVS	FI**I**CVF**C**IS**I**V	371[c,d]		
Ciconiiformes	Laridae	*Uria aalge*	Common murre	VS	FL**A**CIF**S**VF**T**V	406		
Ciconiiformes	Phalacrocoracidae	*Phalacrocorax carbo*	Common cormorant	VS	FY**C**CLF**S**VF**T**V	406[d]		
Ciconiiformes	Phoenicopteridae	*Phoenicopterus sp.*	Greater flamingo	VS	FV**S**CVL**S**VF**V**V	408		
Ciconiiformes	Procellariidae	*Oceanodroma leucorhoa*	Leach's storm-petrel	VS	FI**S**CIF**S**VF**T**V	405		
Ciconiiformes	Procellariidae	*Puffinus puffinus*	Manx shearwater	VS			402	(unpublished in Bowmaker et al. 1997)
Ciconiiformes	Spheniscidae	Pygoscelis adeliae	Adelie penguin	VS	FV**S**CIF**S**VF**T**V	405		
Ciconiiformes	Spheniscidae	Spheniscus humboldti	Humboldt penguin	VS			403	Bowmaker and Martin 1985
Columbiformes	Columbidae	*Columba livia*	Domestic pigeon	VS	FI**S**CIF**S**VF**T**V	405	409	Bowmaker et al. 1997
Coraciiformes	Alcedinidae	*Alcedo atthis*	Kingfisher	VS	FI**S**CIF**S**VF**T**V	405		
Coraciiformes	Coraciidae	*Coracias garrulus*	Common roller	VS	FI**S**CIF**S**VF**T**V	405		
Galbuliformes	Bucconidae	*Nystalus maculatus*	Spot-backed puffbird	VS	FI**S**CIF**S**VF**T**V	405		
Galliformes	Phasianidae	Coturnix japonica	Japanese quail	VS	FV**S**CVL**S**VF**V**V	408	419	Bowmaker et al. 1993
Galliformes	Phasianidae	*Gallus gallus*	Chicken	VS	FV**S**CVL**S**VF**V**V	408	415, 418	Okano et al. 1992, Bowmaker et al. 1997
Galliformes	Phasianidae	*Meleagris gallopavo*	Domestic turkey	VS			418	Hart, Partridge, and Cuthill 1999
Galliformes	Phasianidae	*Pavo cristatus*	Common peafowl	VS			421	Hart 1998
Gruiformes	Gruidae	*Balearica pavonina*	Crowned crane	VS	FI**C**CIF**S**VF**T**V	406[d]		
Gruiformes	Rallidae	*Fulica atra*	Common coot	VS	FL**W**CIF**S**VF**T**V	406[d]		
Passeriformes	Corvidae	*Corvus corone cornix*	Hooded crow	VS	FM**C**CIF**S**VF**T**V	406[d]		
Passeriformes	Corvidae	*Corvus monedula*	Jackdaw	VS	FL**C**CIF**S**VF**T**V	408[d]		
Passeriformes	Fringillidae	*Serinus canaria*	Canary	UVS	LM**C**CVF**C**IF**T**V	371[d]	369	Das et al. 1999
Passeriformes	Muscicapidae	*Turdus merula*	Eurasian blackbird	UVS			373	Hart et al. 2000*b*
Passeriformes	Paridae	*Parus caeruleus*	Blue tit	UVS			371	Hart et al. 2000*b*
Passeriformes	Passeridae	*Amadina fasciata*	Cut-throat finch	UVS			370	Hart et al. 2000*a*
Passeriformes	Passeridae	*Erythrura gouldiae*	Gouldian finch	UVS			370	Hart et al. 2000*a*
Passeriformes	Passeridae	*Lonchura maja*	White-headed munia	UVS			373	Hart et al. 2000*a*
Passeriformes	Passeridae	*Neochmia modesta*	Plum-headed finch	UVS			373	Hart et al. 2000*a*
Passeriformes	Passeridae	*Taeniopygia guttata*	Zebra finch	UVS	LM**C**CVF**C**IF**T**V	371[d]	360–380	Bowmaker et al. 1997
Passeriformes	Sturnidae	*Sturnus vulgaris*	Common starling	UVS	LM**C**CIF**C**IF**T**V	371[d]	359	Hart et al. 1998

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Table 1
Continued

Order	Family	Name	Common Name	Type	Amino Acid Sequence	Calc. λ-max[a]	Meas. λ-max[b]	Reference
Passeriformes	Sylviidae	*Leiothrix lutea*	Pekin robin	UVS			355	Maier and Bowmaker 1993
Passeriformes	Sylviidae	*Phylloscopus trochilus*	Willow warbler	UVS	LM**M**CIF**C**IF**T**V	371[d]		
Passeriformes	Tyrannidae	*Manacus manacus*	White-bearded manakin	VS	FI**S**CIF**S**VF**T**V	405		
Passeriformes	Tyrannidae	*Myiarchus tyrannulus*	Brown-crested flycatcher	VS	FM**C**CIF**S**VF**T**V	406[d]		
Piciformes	Picidae	*Dendrocopos major*	Great spotted woodpecker	VS	FL**S**CIF**S**VF**T**V	405		
Psittaciformes	Psittacidae	*Melopsittacus undulatus*	Budgerigar	UVS	FL**A**CII**C**IF**T**V	371	371	Bowmaker et al. 1997
Psittaciformes	Psittacidae	*Psittacus erithacus*	Grey parrot	UVS	FL**A**CIF**C**IF**T**V	371		
Strigiformes	Caprimulgidae	*Caprimulgus europaeus*	European nightjar	VS	FL**C**CVF**S**VF**T**V	406		
Struthioniformes	Rheidae	*Rhea americana*	Common rhea	UVS	FI**F**CFF**C**VF**M**V	371[d]		
Struthioniformes	Struthionidae	*Struthio camelus*	Ostrich	VS	FI**S**CIF**S**VF**T**V	405		
Trogoniformes	Trogonidae	*Trogon curucui*	Blue-crowned Trogon	VS	FI**F**CVF**S**VF**T**V	406[d]		
Upupiformes	Upupidae	*Upupa epops*	Hoopoe	VS	FM**S**CIF**S**VF**T**V	405		

NOTE.—Scientific and common names were retrieved (May 23, 2002) from the Integrated Taxonomic Information System online database, http://www.itis.usda.gov. Amino acids in bold represent tuning sites 86, 90, and 93 (Wilkie et al. 2000). Approximate λ-max values were calculated from these sites. Measured λ-max values were taken from published MSP studies.

[a] Calculated λ-max.

[b] Measured λ-max.

[c] The mutations in position 93 are new to this study and their effects are unknown.

[d] The mutations in position 86 are new to this study and their effects are unknown.

Puffinus puffinus (Bowmaker et al. 1997), *Struthio camelus* (Wright and Bowmaker 2001), and *Taeniopygia guttata* (Bowmaker et al. 1997). The UVS system has so far been found only in birds of the orders Passeriformes and Psittaciformes: *Leiotrix lutea* (Maier and Bowmaker 1993), *Melopsittacus undulatus* (Bowmaker et al. 1997), *Sturnus vulgaris* (Hart, Partridge, and Cuthill 1998), *Serinus canaria* (Das et al. 1999), *Parus caeruleus* (blue tit) (Hart et al. 2000*b*), *Turdus merula* (Hart et al. 2000*b*) and four species of estrildid finches (Hart et al. 2000*a*) (for common names, see table 1).

The type of SWS1 opsin possessed by a bird indicates its COD. The λ-max of the SWS2 cone covaries with that of SWS1 (Bowmaker et al. 1997; Hart et al. 2000*a*) in all species studied so far. The λ-max of the remaining two single-cone types (medium-wavelength sensitive [MWS] and long-wavelength sensitive [LWS]) differ only little between species, barring a few species (reviewed by Hart 2001). The oil droplets of the cones, which narrow spectral sensitivity (Kawamuro, Irie, and Nakamura 1997), fall into conserved classes, each associated with a particular cone type (Bowmaker et al. 1997), and hence do not confound the functional segregation of the two avian CODs. The T-class oil droplet associated with SWS1 has no detectable absorption between 330 and 800 nm (Hart et al. 2000*a*), making the SWS1 opsin gene sequence an accurate predictor of the spectral tuning of the SWS1 cone.

Microspectrophotometry (MSP) has been the standard method used to examine the COD of animals. To prepare retinas for MSP, the live subjects are held in darkness for several hours before being sacrificed and having their eyes dissected (Hart, Partridge, and Cuthill 1999). Due to the complexity of the method, the absorbance of visual pigments has only been examined in a limited number of species. From in vitro examination, Wilkie et al. (2000) was able to determine the shift in λ-max that results from typical between-species amino acid substitutions in five spectral tuning sites in the SWS1 amino acid sequence. Shi, Radlwimmer, and Yokoyama (2001) identified five additional tuning sites in a study on mammals. Of all amino acid changes identified, those in positions 86, 90, and 93 (following the amino acid numbering of bovine rhodopsin) are of particular importance to the spectral tuning in birds (Shi, Radlwimmer, and Yokoyama 2001). Substitutions in four of the sites described by Wilkie et al. (2000) lead to minor or no shifts in λ-max (A86S: −1 nm; T93V: +3; A118T: +3; S298A: 0), but a change from cysteine (C) to serine (S) in position 90 leads to a substantial change in λ-max (35 nm). Hence a C in position 90 characterizes the UVS group, whereas the VS group has an S in the same position (Yokoyama, Radlwimmer, and Blow 2000). Based on Wilkie et al. (2000) we have developed a molecular method that can be used to quickly, easily, and cheaply assess the approximate COD in almost any bird by sequencing part of the SWS1 opsin from small samples of total DNA.

Materials and Methods

We isolated total DNA from blood, muscle tissue, or quill bases with chelex extractions and using the DNeasy

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Tissue Kit (QIAGEN). Standard procedures were applied, except for DNA isolated from feather with the DNeasy Tissue Kit, where the DNeasy minicolumn loaded with 35 ml of preheated water was incubated 5 min at 70°C to increase the DNA yield. Other DNA material was obtained as phenol-chloroform extractions from colleagues. We designed degenerate PCR primers based on the sequences coding for the UVS, VS, or SWS1 (synonyms) opsin gene from *Serinus canaria* (GenBank accession number AJ277922), *Melopsittacus undulatus* (Y11787), *Columbia livia* (AH007798), and *Gallus gallus* (M92039) using Primer3 (Rozen and Skaletsky 1998) and the EMBOSS (Rice, Longden, and Bleasby 2000) package. The primer pair SU193a/SU396b: 5′-CCSCTYAAYTACATCCT-GGT-3′/5′-RACRATGTARCGCTCRAA-3′ (beginning at bovine rhodopsin amino acid positions 70 and 137) amplified an approximately 800 bp-long sequence in *Serinus canaria*, including a long intron. This intron is probably the reason for the lack of product in the other samples tested. Aligning this product with the above mentioned opsin sequences allowed us to identify the position of the intron and design a new primer pair, SU149a/SU306b: 5′-CCRTSGTSCTSDKSGTCAC-3′/5′-SYBCTTSCCGAAGAY RAAGT-3′ (beginning at positions 55 and 107). SU149a, positioned 44 bp upstream from SU193a, is located outside the focus exon in some species. Therefore, SU193a was used as the forward primer in species where PCR failed with SU149a/SU306b. To overcome problems with amplifications in raptors, we also designed a third forward primer, SU161a (beginning at position 59), 5′-KSGTCACCRTYMRKTACAA-3′, partially overlapping SU149a.

Combining the forward primers SU149a, SU161a, and SU193a with the reverse primer SU306b, we conducted PCR on an Eppendorf Mastercycler Gradient. Each 25 μl reaction volume contained 30 to 50 ng total DNA extracts, 0.125 μl 5 U Taq-polymerase (Applied Biosystems), 2.5 μl 10X reaction buffer, 10 pmol of each primer, 0.2 mM of each dNTP, and 50 mM $MgCl_2$. Reaction conditions were 90 s at 94°C, 5 × (30 s at 94°C, 30 s at 54°C and, 1 s at 72°C), 38 × (15 s at 94°C, 30 s at 54°C, and 5 s at 72°C), and 10 min at 72°C. The extension time was kept very short to minimize nonspecific amplification of longer fragments.

We performed double-stranded sequencing of the PCR product with Big-Dye Terminator Cycle Sequencing v2.0 kit on an ABI-prism 310 automated sequencer following the user's manual. The same primers were used in cycle sequencing as in the PCR. PCR products for sequencing were prepared using Microcon YM-100 and YM-50 centrifugal filter devices (MILLIPORE). In case of amplification of multiple products, we purified the product from a 2% agarose gel using QIAquick Gel Purification kit (QIAGEN).

To translate our sequences we used the published amino acid sequence from *Melopsittacus undulatus* UV-sensitive opsin (Wilkie et al. 1998) as a template. From the alignment of amino acid sequences, we identified the spectral tuning sites 86, 90, and 93 (Wilkie et al. 2000) and calculated λ-max values from the tuning sites following Wilkie et al. (2000). We assumed the effect of these sites on spectral tuning to be additive. Although this assumption disregards interactions between sites (see Shi, Radlwimmer, and Yokoyama 2001), addition should provide a reasonable approximation of λ-max.

Results

We amplified the target sequence in a total of 45 species of which the spectral tuning was previously unknown in 37 (table 1). The results of the remaining eight and comparisons between closely related species were consistent with MSP examinations (see table 1) and in vitro observations of cloned genes (Wilkie et al. 2000). The length of the amplified coding fragment was 74 bp with primer pair SU193a/SU306b, 107 bp with SU161a/SU306b, and 119 bp with SU149a/SU306b. All amino acid sequences presented in table 1 are translated from sequences produced in this study. Because of an intron after amino acid position 121, we could not design a primer pair to amplify tuning site 118. Hence, our calculations disregard the potential upward shift in λ-max of 3 nm that a potential A118T mutation would produce. Calculated and measured λ-max values differed with 15 nm in *Anas platyrhyncos* and 11 nm in *Sturnus vulgaris*. Still, these differences are much smaller than that between the VS and UVS vision systems, which is at least 23 nm (Hart et al. 1999).

We found five new mutations at position 86 and one at 93, that is, mutations not described in Wilkie et al. (2000). However, since these positions only marginally contribute to the spectral tuning with their previously reported amino acids (Wilkie et al. 2000), we do not expect the new mutations to have any drastic effects on the spectral tuning of the SWS1-opsin. Nevertheless, these new findings call for further investigations using in vitro studies or MSP examination.

Our results confirm that the UV-tuned COD is present in passeriform and psittaciform birds and that most other bird taxa are violet-tuned. However, we found UVS also in the Laridae (genus *Larus*) and Rheidae families of the orders Ciconiiformes and Struthioniformes, respectively, and VS in the passeriform families Corvidae, Trogonidae, and Tyrannidae, as well as in the Struthioniform family Struthionidae.

For unknown reasons, we failed to amplify the SWS1 opsin sequence from the following species: *Branta bernicla* (brant), *Anas crecca* (green-winged teal), *Apus apus* (common swift), *Aquila chrysaetos* (golden eagle), *Podiceps cristatus* (great crested grebe), *Mommotus mommota* (blue-crowned motmot) and *Strix aluco* (tawny owl).

Discussion

Our results support the notion that the VS type of color vision is the most common among birds, but it is also apparent that the avian distribution of vision systems is more complex than what has previously been shown. All studies to date have indicated that the VS color system is the dominating among birds and that the only bird species with a clear-cut UV-biased vision belong to the orders Psittaciformes and the Passeriformes. No previous study

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shows both UVS and VS in the same taxonomic order. Although we confirm the presence of the UVS type in Passeriformes and Psittaciformes and the VS type in Anseriformes, Columbiformes, and Galliformes (table 1), we have also found species with the UVS-type vision in Ciconiiformes and Struthioniformes and species with VS-type vision within Passeriformes and Struthioniformes. The variation of CODs is not restricted to high-level taxa such as orders, but varies at least within families.

The distribution of the UVS/VS character in the avian phylogeny has been considered to reflect the degree of relatedness of avian taxa and to be most parsimoniously explained by a single evolutionary split of the passeriform and psittaciform lineages from the anseriform and galliform lineages (Hart et al. 2000*a*). However, that UVS is present in at least nine families from four orders (table 1), interdispersed with VS taxa (fig. 1) strongly indicates that the UVS character has been acquired independently in each of these groups and that its distribution does not reflect the degree of relatedness between avian species. The vast majority of vertebrate animals studied have the amino acid serine in position 90, and this has lead Yokoyama, Radlwimmer, and Blow (2000) to suggest that having cystein in the same position is a derived state in birds. Indeed, the exclusive presence of serine in position 90 in the majority of families examined suggests that VS is the primitive state. This is also indicated by molecular and morphological phylogenies (fig. 1). However, the closest relatives to birds in which the SWS1 opsin is known are chameleon and mammals (Yokoyama, Radlwimmer, and Blow 2000), and these taxa are probably too distant relatives to provide phylogenetic resolution, as this character state varies even within avian families. Furthermore, the character state (UVS/VS) is controlled by a single-nucleotide mutation (Wilkie et al. 2000). One should therefore be careful not to draw too far-reaching conclusions from the character state in any extant outgroup. The closest living relatives to birds are the crocodilians, with which they share a common ancestor no younger than 250 Myr (Benton 1997). This provides ample time for multiple character changes.

It is more likely that the distribution of CODs in the class Aves has adaptive rather than phylogenetic explanations. The difference in peak sensitivity between UVS and VS is quite dramatic and changes not only the perception of objects that reflect light solely in the UV or violet ranges but also the perception of objects that reflect both UV/violet and longer wavelengths. This should have important consequences for foraging, habitat use, social signaling, and mate choice. We can expect intriguing effects on the evolution of color signals from interactions

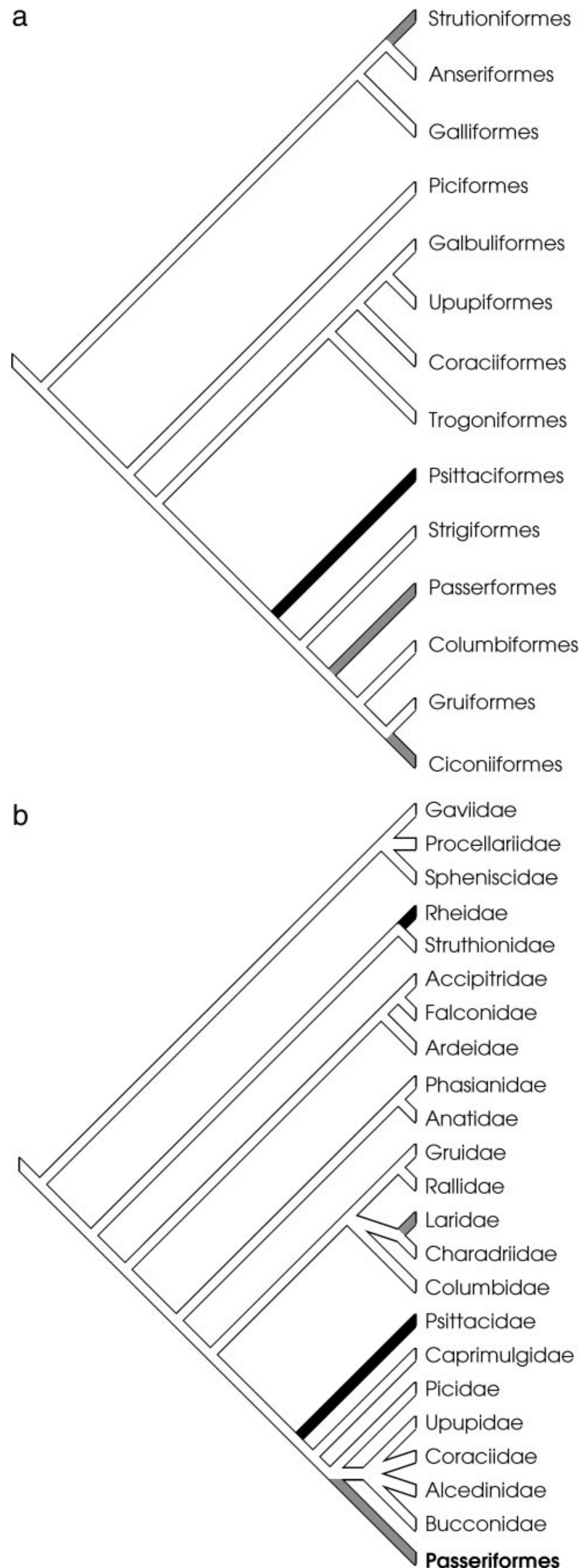

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FIG. 1.—Type of vision system mapped onto phylogenetic relationship among avian taxa: (*a*) Phylogeny of orders based on DNA–DNA hybridization analysis (Sibley and Ahlquist 1990), and (*b*) phylogeny of families based on morphology (Cracraft 1981). The passeriform families are combined (in bold). White denotes violet sensitive (VS), black indicates ultraviolet sensitive (UVS), and gray is taxa, including both systems.

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between predators and prey. Such interactions may explain the presence of UVS in Laridae and Passeriformes.

Since UV scatters more under water than longer wavelengths, UV coloration and vision are only effective at short distances (< 5 m). UV may hence be useful in sexual and social signaling between fish of the same species to reduce the risk of detection by predators (Losey et al. 1999), such as other fish and swimming birds. That fish are able to make use of this private communication channel is implied by the facts that UV pigments of teleost cone receptors peak at around 360 nm (Losey et al. 1999) and that many fish species reflect UV. However, for birds like gulls (*Larus* spp.), which prey on fish just below the water surface, underwater UV scattering will be negligible and their UVS COD could be an adaptation to more effectively spot prey.

All six raptors examined are of the VS type, giving them a vision system different from many of their passeriform prey. This could enable perching birds to signal with colors that are conspicuous to members of their own species but dull or cryptic to raptors. That advantage would be common to all UVS prey species and should facilitate diversification of sexual and social signals and hence reproductive isolation and speciation. Signaling with colors that are inconspicuous to predators should reduce the cost of signaling. Selection should then favor stronger signals in the wavelengths to which predators are insensitive, that is, favor higher plumage reflectance in the SWS1 and SWS2 ranges or higher sensitivity to those parts of the spectrum.

An animal's response to a color signal depends on the signal's fit on the COD of that particular species, rather than what properties a human observer considers the signal to have. Evolutionary biologists and behavioral ecologists need to acknowledge the COD of their study animal to ask relevant questions and design experiments correctly. Indeed, the distribution of CODs is such a complex one that, when studying animal signaling, it may be necessary to verify the CODs even if they are known from related species. In bird studies, our method offers a considerably more practical tool for that purpose than does MSP. However, we do not imply that our method should replace the latter; MSP is undeniably more direct and informative. It is worth noting that some species carrying the SWS1 opsin gene might not express it, posses a very low proportion of SWS1 cones in the retina, or have ocular media absorbing ultraviolet light. So far, all our results are in agreement with those from MSP, although our λ-max approximations deviate by up to 15 nm, supporting a fine-tuning role for other sites (see Shi, Radlwimmer, and Yokoyama 2001). Our method can be used to quickly estimate a COD from total DNA, without the need to keep or sacrifice the animal. It thereby facilitates large screenings, including rare and endangered species, making it possible to find species with an aberrant COD suitable for MSP examination.

New Sequences

The new sequences reported in this paper are available from GenBank with accession numbers AY227147 to AY227191.

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