# Single nucleotide substitutions and diverse ecological contexts enable multiple re-emergences and fixation of ultraviolet sensitive vision in birds

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Characters: 17,987

Visual systems in animals play a major role in both environmental and interspecific interactions. Vertebrates use vision to efficiently forage, evade predators, and select mates, among other functions (Kelber and Jacobs 2016). Variation in the spectral sensitivity of vertebrate eye pigments reflect strong selection exerted by many environmental and biotic interactions in which vision plays a central role. The spectrum of light reception in the vertebrate retina is primarily mediated by the absorptive properties of retinal opsin proteins (Yokoyama et al. 2000), with carotenoid expression in pigmented oil droplets from retinal cone cells playing a secondary role in some vertebrate groups (Stavenga and Wilts 2014; Toomey et al. 2015). Vertebrate opsin proteins are divided into five evolutionarily distinct groups: rhodopsin (RH1), RH1-like (RH2), short-wavelength sensitive (SWS1), SWS1-like (SWS2) and long/middle wavelength sensitive (LWS/MWS; Kelber and Jacobs 2016). Each group is responsible for the reception of specific wavelengths of light. Short-wavelength sensitive (SWS1) opsins typically have maximum absorption ($\lambda_{max}$) of wavelengths at 430 nm, but in some cases absorb wavelengths in the violet and ultraviolet regions of the electromagnetic spectrum ($\lambda_{max}$= 310-400). Violet-sensitive (VS) and ultraviolet sensitive (UVS) cone cells containing VS/UVS SWS1 opsins are especially common in many species of birds, which are strongly dependent on vision. Sensitivity to ultraviolet light is demonstrated to effect sexual signaling (Barreira et al. 2012), foraging (O'Daniels et al. 2017), and common species communication (Carvalho et al. 2011). UVS vision has arisen by single nucleotide alterations to the SWS1 opsin sequence (Yokoyama et al. 2000) in most birds, and has experienced a complicated series of gains and losses throughout avian adaptive radiation (Odeen and Hastad 2013).

The motivation for evolving UVS vision in birds must be analyzed on a species by species basis due to the extreme diversity in ecological roles served by bird species and potential functionality of UVS vision in these roles. However, UVS vision appears to have an unusually complicated evolutionary history. The evolutionary mechanism for this complexity is not well understood. I propose the complex phylogenetic distribution of modern avian UVS vision is likely due to small physiological barriers to switching between vision types, a diverse array of scenarios in which UVS vision may be beneficial, and the proximity of light-receiving proteins to strong environmental selective pressures as a result of avian dependence on vision. The resulting phylogenetic distribution of UVS and VS pigments represents many switches between

UVS and VS forms of SWS1 opsin pigments among bird phyla as a result of successful environmental pressure to diversify and tune avian vision to species-specific circumstances.

### *Single nucleotide substitutions induce ultraviolet sensitivity in avian SWS1 opsins*

Color-sensitive vision, including UVS vision in birds, begins with light reception by visual pigments in retinal cone cells. Cone cell visual pigments are composed of the chromophore 11-*cis*-retinal or 11-*cis*-3,4-dehydroretinal covalently bonded to an opsin protein. The G-coupled opsin protein stimulates light perception pathways upon *trans*-isomerization of the associated chromophore induced by absorbing photons with specific wavelengths (Yokoyama et al. 2000). The range of wavelengths absorbed by opsin proteins is dependent on the sequence and structure of the opsin protein; this relationship is used to reconstruct evolutionary shifts between vision types (Odeen and Hastad 2003). In birds, a single nucleotide substitution in SWS1 opsin sites 86, 90, or 93 is sufficient to blue-shift spectral sensitivity of SWS1 from violet to ultraviolet. These residues form a tuning site located on the SWS1 opsin near the interior of a retinal binding pocket. Non-conservative nucleotide substitutions effect protonation of the associated chromophore, blue-shifting the opsin protein $\lambda_{max}$. The residues in the SWS1 tuning site accurately predict the vision type (ultraviolet sensitive vs. violet sensitive) for most bird species (Odeen and Hastad 2013). Superimposition of predicted vision type from SWS1 opsins on established bird phylogenies evolutionary history of UVS vision in birds has been reconstructed.

### *Evolutionary history of UVS vision*

SWS1 sequence analysis indicate that nucleotide substitutions at SWS1 opsin residues 84-94 are responsible for reversals between UVS and VS vision types. The specific identity of these substitutions is frequently shared among phyletic groups, but experimental confirmation of vision type is frequently conducted through retinal microspectrophotometry (MSP) of visual pigments. A phylogenetic assignment of VS/UVS vision conducted by Odeen and Hastad (2013) concluded the common ancestor of birds was VS because VS vision was experimentally identified a single member of the Paleognathae basal group, the common ostrich (*Struthio camelus;* Wright and Bowmaker 2001). Hastad et al. (2013) acknowledge residue C90 found in the paleognath tuning site is known to blue-shift SWS1 absorption and is common to all birds with UVS vision. They hypothesize a second base conserved to paleognaths (M93) was responsible for stabilizing protonation of the Schiff base linking retinal and the SWS1 opsin in the presence of C90, preventing blue-shift. A later analysis of a second paleognath, the emu (*Dromaius novaehollandiae*) determined that emus likely have UVS vision (Hart et al. 2016). Despite contradicting experimental results, a VS common ancestor is generally accepted as it is a more parsimonious explanation for modern distribution of UVS vision in the class Neognathae, which contains the vast majority of bird species (Odeen and Hastad 2013). The evolutionary history of SWS1 in the remainder of birds has a large number of convergent events and reversals (Odeen and Hastad 2003).

The relative ease of VS-UVS and UVS-VS shifts by nucleotide substitution result in a complicated history with variation even among closely related species. Sequencing of SWS1 opsins demonstrate that at least nine orders of birds exhibit UVS vision (Figure 1). Of these orders, Charadriiformes, Ciconiformes, Coraciiformes and Passeriformes, which is the largest and most diverse order of birds, have a combination of UVS and VS vision as a result of repeated gains and losses of UVS from the VS common ancestor (Hart et al. 2016). UVS vision arose once via a single substitution (S90C) in Pteroclidiformes (sandgrouses), Trogoniformes (trogons and quetzals), Coraciiformes (kingfishers, bee-eaters, rollers, motmots, and todies) and Psatticiformes (parrots). Although relatively few samples exist, MSP measurements in paleognaths indicate that a similar VS to UVS occurred in emus (Hart et al. 2016). The absence of UVS vision in closely related orders indicate that these shifts occurred after the evolutionary divergence at the order level. Coraciiformes is the largest and most diverse order to experience a single shift from VS to UVS, and one of several single-shift orders where only a family (motmots: Momotidae) shifted to UVS, despite general similarity in habitat preference, plumage, and mating type for coraciiform birds. Psatticiformes is the only order in which UVS vision appears to be ubiquitous, where it may play a role in sexual dichromatism (Barreira et al. 2012).

The remaining orders that express ultraviolet sensitivity have a more complicated history. Charadriiformes, the shorebirds, and Ciconiformes, the order including storks, shoebills, herons, bitterns, and flamingos, each have experienced two VS to UVS shifts and one reversal to VS, resulting in a combination of VS and UVS vision in these groups. The evolution of passerines, which includes more than 5000 species, is far more complex, with at least eight shifts between VS and UVS vision (Odeen et al. 2011). Many UVS passerines belong to the monophyletic parvorder Passerida as a consequence of a shift from VS to UVS shift in the common ancestor of Passerida and the superfamily Corvoidea. (Figure 2). In non-Passerida species of the superfamily Corvoidea, two UVS to VS shifts occurred, followed by a single shift back to UVS in the family Vireonidae. As a result, many species in the Corvoidea group are VS, while close relatives in Passerida are UVS. Shifts between UVS and VS in passerines occur due to substitutions between cysteine and serine at SWS1 opsin residue 90 in the spectral tuning site. Despite well-supported phylogenetic reconstructions in the majority of passerines based on opsin sequences, early shifts between UVS and VS remain ambiguous. Passerines also exhibit a complex evolutionary history in SWS1 opsins relative to closely related groups, such as Psatticiformes, which experienced only a single shift from VS to UVS. This is hypothesized by Odeen and Hastad (2013) to be a result of passerines experiencing the largest adaptive radiation of any avian order and occupying a large variety of ecological niches relative to other orders, such as Psatticiformes.

Despite a well-established order of evolutionary events in UVS vision, the precise geological timing and location of these events remains unclear. This is primarily due to a lack of complete fossil records for modern birds making the determination of a geological timescale and location difficult. Attempts to reconcile DNA evidence and a sparse fossil record have been contentious for some years (Brown et al. 2007; Field 2017). Some fossil evidence suggest that many archaic birds lived alongside ancient dinosaurs, but members of the class Aves were the only birds that survived the Cretaceous-Paleogene (K-T) mass extinction (Clarke et al. 2005), implying significant Cretaceous divergence of bird species. A dearth of fossils from the Mesozoic makes the timing of pre K-T divergences difficult to establish. Analysis of mitochondrial DNA sequences places ordinal level divergence in the Cretaceous just before the K-T boundary for some birds, but in the Eocene-Oligocene (E-O) boundary for others (van Tuinen et al. 2006). It is likely birds were widespread in the beginning of the Cenozoic, and experienced adaptive radiation similar to other animals to occupy niches vacated by extinct non-avian dinosaurs. Because shifts for VS to UVS appear in individual orders (Figure 1), it is possible that UVS shifts occurred within this adaptive radiation between ordinal and familial-level divergence, placing the first instances of UVS vision arising between 65 and 33 million years ago in a wide variety of ancient climates.

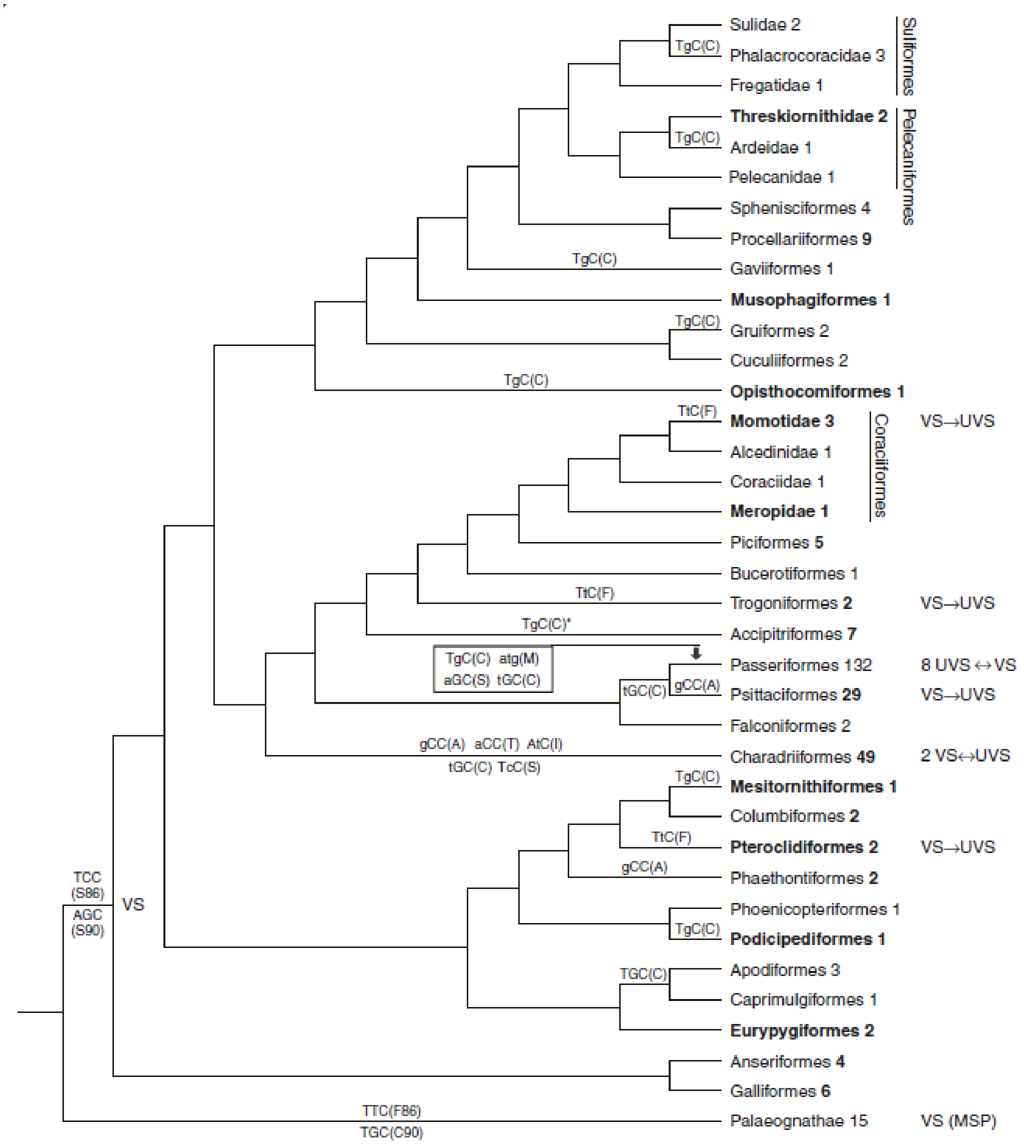

**Figure 1** – Tree taken from Hacket et al. (2008) showing changes from UVS SWS1 opsin pigments to VS opsin pigments. Bold taxa are introduced in Odeen and Hastad (2013). Codons and corresponding amino acids for tuning site 86 and 90 are show above and below lines. Substitutions to tuning site nucleotides are indicated in lowercase in the most likely codon in the tree. Number of species analyzed per clade is shown next to taxon names. Number and direction of VS/UVS shifts are indicated next to each clade.

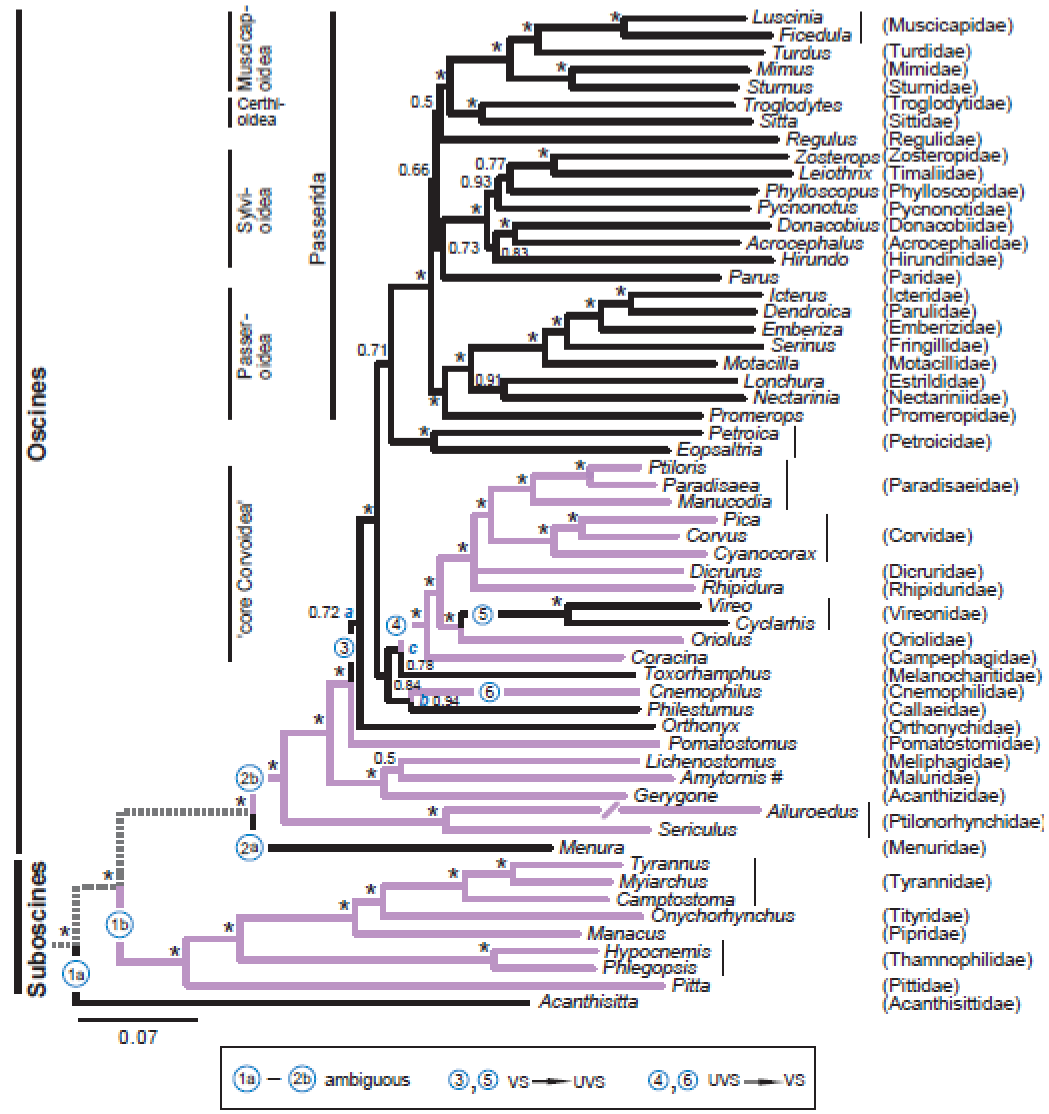

**Figure 2** – Majority rule consensus gene of single copy universal marker genes for passerines with 2 falconiform and 2 psatticiform outgroups from Odeen et al. (2011). Asterisks (*) indicate node posterior probability of > 0.95. VS optimized vision is indicated by violet, and UVS optimized vision is indicated by black. Transitions between states are indicated by 1a, 2a, 3, etc. with 1a-2b representing unknown shifts between VS and UVS vision.

### *Evolutionary causation for emergence and expansion of UVS vision*

The complicated evolution of SWS1 opsins is enabled by the physiology of UV photoreceptive cells in the retinas of birds and ease of switching vision types exclusive to avian SWS1 opsins. Most long, mid and shortwave sensitive photoreceptors determine spectral tuning with a combination of chromophore isoform and pigmented cone oil droplets acting as spectral filters in addition to specific opsin protein sequences (Toomey et al. 2015; Toomey et al. 2016). Modeling of this three-component avian spectral tuning system indicates that coordinated changes in opsin sequences, cone oil carotenoid palette, and chromophore isoform are required to produce discernable differences in wavelength sensitivity (Lind et al. 2017). Therefore, physiologically realistic changes in a single character (such as single amino acid changes in opsin sequence due to mutation) are unlikely to produce a large increase in fitness mediated by enhanced clarity or color perception in vision. The resulting adaptive landscape has broad phenotypic optima for vision-based fitness, where phenotypes varying in single characters may have identical vision-mediated fitness. Selection must act on several visual components consistently to induce a coordinated shift in wavelength sensitivity, and because selection on visual systems in vertebrates occurs with respect to many behaviors and not towards a single phenotype, the general result in vision systems is usually a compromise that does not strongly resemble a phenotype optimized to a particular environmental or organismic interaction.

However, many well-studied examples of birds and other vertebrates exist where UVS vision seems to be suited to a particular, ecologically determined function (reviewed in Owens and Rennison 2017). Ultraviolet sexual dichromatism is observed in a variety of UVS birds with and without dichromatism in the normal visual spectrum (Barreira et al. 2012; Fidler et al. 2016; Hunt et al. 1998). UVS vision in pollinating passerine birds aids in distinguishing preferred targets of pollination (Odeen and Hastad 2010), and UV emission and absorption signals are used by a variety of species to identify specific forage (O'Daniels et al. 2017). It is clear that the emergence of UVS vision in major tuning site substitutions is strongly effected by particular environmental conditions. Studies of colored oil droplet and lens characteristics in birds with UVS vision show that short wave light is selectively filtered from long wave light by the oils and lenses, enabling the retinal photoreceptor to more easily discriminate between short wavelengths of light (Carvalho et al. 2011; Stavenga and Wilts 2014). The origin of this discriminatory ability is in SWS1 and SWS2 opsin sequence-mediated sensitivity. The SWS1 opsins are highly sensitive to a narrow range of wavelengths, particularly in tetrachromatic birds, whose visual system allows for dedicated SWS1 opsin cone cells (Odeen and Hastad 2010). Any shifts in the spectral sensitivity induced by tuning site single nucleotide substitutions in the SWS1 opsin can seriously affect the resulting vision type, especially given the large blue shift of SWS1 opsins (~90 nm) due to substitution (Yokoyama et al. 2000). Therefore, SWS1 opsin sequence and single nucleotide shifts are exceptional in their ability to create discernable differences in vision by a physiologically realistic shift in a single element of the visual system.

The unique physiology of short wave light reception in UVS birds and the mutability of the SWS1 opsin informs the complicated evolutionary history of the SWS1 opsin with respect to VS and UVS vision. It provides an evolutionary explanation for multiple instances of regained UVS vision in species where it had been gained and lost before, particularly in passerine and charadriiform birds, which exist in diverse habitats and serve a variety of ecological roles (Jønsson et al. 2015). Single nucleotide substitutions are responsible for both blue- and red-shifting SWS1 opsins and alterations to SWS1 opsin sequences are the primary mechanism for inducing UVS vision. UVS and VS variants may arise more frequently than variants in non-SWS1 opsins that require coordinated character shifts or other polygenic traits, where variants are masked by opposing additive genetic effects. Studies have demonstrated that UVS variants experience a selective benefit from UVS vision enhancing the ability to discriminate between colors and enhance visual resolution (Vorobyev et al. 1998), as well enhance fitness in species-specific interactions. For example, the New Zealand yellowhead (*Mohoua ochrocephala*) is susceptible to an obligate brood parasite, the pacific long-tailed cuckoo (*Urodynamis taitensis*). The yellowhead is VS and unable to identify kin-specific UV cues used by its UVS relative, the whitehead (*Mohoua albicilla*) to remove cuckoo eggs from its nest and save its offspring. This demonstrates a clear fitness advantage conveyed by expanding the visual range into the UV. The importance of vision in birds' environmental interactions implies selection would act strongly on UVS variants to either fix. It remains unclear why some species shift back to VS vision once UVS vision is gained, but VS to UVS shifts are far more common that the reciprocal shift (Odeen and Hastad 2013). This occurs most often in passerines, the largest and most diverse order of birds. The variety of ecological roles, habitat distribution, and morphological traits of passerines and the class Aves more generally provide ample opportunities for selective conditions to favor fixation of UVS variants once they are induced from VS ancestors by single nucleotide substitutions.

### *Conclusions*

The evolutionary causation of UVS vision for specific avian species is dependent on the benefit of increased color discrimination, resolution, and perception of UV light in the species' ecological role. This is demonstrated by UVS vision performing differing functions in morphologically and behaviorally distinct bird species. The unique susceptibility of the SWS1 opsin-mediated visual system to dramatic shifts in spectral sensitivity arising from single nucleotide substitutions allow UVS-sensitive birds to appear in VS-sensitive populations with the change of a single base pair. Intense selective pressure on the visual systems of birds combined with substantial differences in wavelength sensitivity for UVS variants may strongly favor fixation of modified opsin genotypes once they occur if UVS vision provides a benefit to fitness. The ease of emergence and selective benefits of UVS vision may help explain the unusually complicated evolutionary history of UVS vision in birds, where UVS lost and regained multiple times. The order with the most species and diversity, passerines, is also the most complex in terms of UVS/VS shifts. Habitat and morphological diversity in passerines could increase the chances a UV variant arose in a habitat that favors fixation of UVS SWS1 opsin sequences.

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