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ORIGINAL PAPER

Ultraviolet visual sensitivity in three avian lineages: paleognaths, parrots, and passerines

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Abstract Ultraviolet (UV) light-transmitted signals play a major role in avian foraging and communication, subserving functional roles in feeding, mate choice, egg recognition, and nestling discrimination. Sequencing functionally relevant regions of the short wavelength sensitive type 1 (SWS1) opsin gene that is responsible for modulating the extent of SWS1 UV sensitivity in birds allows predictions to be made about the visual system's UV sensitivity in species where direct physiological or behavioral measures would be impractical or unethical. Here, we present SWS1 segment sequence data from representative species of three avian lineages for which visually based cues for foraging and communication have been investigated to varying extents. We also present a preliminary phylogenetic analysis and ancestral character state reconstructions of key spectral tuning sites along the SWS1 opsin based on our sequence data. The results suggest ubiquitous ultraviolet SWS1 sensitivity (UVS) in both paleognaths, including extinct moa (Emeidae), and parrots, including the nocturnal and flightless kakapo (*Strigops habroptilus*), and in most, but not all, songbird (oscine) lineages, and confirmed violet sensitivity (VS) in two suboscine families. Passerine hosts of avian brood parasites were included both UVS and VS taxa, but sensitivity did not co-vary with egg rejection behaviors. The results should stimulate future research into the functional parallels between the roles of visual signals and the genetic basis of visual sensitivity in birds and other taxa.

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Introduction

Color cues are ubiquitous features of avian orientation, foraging, and communication systems, and studies of (co)variation in visual signals and their associated sensory bases of vision in birds have provided a critical model system for the evolution of avian molecular, morphological, sensory, and behavioral diversity (Hill and McGraw 2006). In many bird species, color perception provides the behavioral basis for mate choice, predator avoidance, prey acquisition, and egg or nestling identification (Hubbard et al. 2010). Birds have highly complex visual systems, possessing five separate classes of cone photoreceptors, four of which directly contribute to color perception by absorbing maximally at wavelengths in the range of 360–570 nm of light, resulting in tetrachromacy (Hunt et al. 2009). In recent years, the role of avian visual signals outside of the human perceptual range, specifically in the ultraviolet (UV) wavelengths (<400 nm), has garnered increasing attention especially with respect to the evolution of private communication channels (Hauber et al. 2000, 2001; Hart 2001; Hauber and Sherman 2001; Ödeen and Håstad 2003; Goth and Evans 2004; Cuthill 2006; Underwood and Sealy 2008). Accordingly, many UV-based visual signals are known to play important roles in both the interspecific and intraspecific communication behaviors of many bird species (e.g., Bennett and Cuthill 1994).

Molecular basis of UV-sensitivity

Sequencing a short 'spectral tuning' region of the avian SWS1 opsin gene allows for accurate prediction of the degree of UV-sensitivity in diverse avian taxa (Ödeen and Håstad 2003; Carvalho et al. 2011; Machovsky Capuska et al. 2011). Site-directed mutagenesis, combined with in vitro expression work, has implicated a number of "spectral tuning" sites along the SWS1 photoreceptor, all of which are in the transmembrane (TM) II region of the protein (Hunt et al. 2009). For example, a single C90S substitution (following the bovine *Bos taurus* rhodopsin numbering) in the UV-sensitive SWS1 opsin of the budgerigar (*Melopsittacus undulatus*) produces a long-wave shift, consistently altering the SWS1 photoreceptor's maximal sensitivity by approximately 35 nm to light from 363 to 398 nm (Wilkie et al. 2000). Similarly, the same substitution in the UV-sensitive SWS1 opsin of the zebra finch (*Taeniopygia guttata*) shifts the SWS1 opsins peak spectral sensitivity from 359 to 397 nm, from UV towards the violet portion of the light spectrum (Yokoyama et al. 2000). The converse S90C shift in both the violet-sensitive pigeon (*Columba livia*) and chicken (*Gallus gallus*) SWS1 opsins produces a short-wave shift in spectral sensitivity from 393 nm towards the UV range of 359 nm and from 415 to 369 nm, respectively (Yokoyama et al. 2000). Shi et al. (2001) demonstrated five separate residues as important spectral tuning sites among mammals, though residues 86, 90, 93, and 118 appear to be the most important spectral tuning sites among avian species. Specifically, A86S, T93V, and A118T substitutions alter the maximal sensitivity of the SWS1 opsin −1 and 3 nm, and 3 nm, respectively, in the budgerigar SWS1 opsin (Wilkie et al. 2000). This suggests that residue 90 is singularly important in mediating the spectral tuning of the SWS1 opsin.

By convention, avian SWS1 opsins with a maximal sensitivity <400 nm are designated UV-sensitive (UVS), while those with a maximal sensitivity ≥400 nm are designated violet-sensitive (VS; Hart 2001; Ödeen and Håstad 2003; Hunt et al. 2009). Sequencing the short 'spectral tuning' region of the SWS1 photoreceptor may complement the need for terminal microspectrophotometry, intensive site-directed mutagenesis/in vitro protein expression, invasive physiological analyses and/or extensive behavioral experiments to assess the degree and function of UV-sensitivity in different bird species (Ödeen and Håstad 2003; Hunt et al. 2009; Aidala and Hauber 2010).

Avian UV-sensitivity and visual ecology

Previously, it was suggested that a VS SWS1 opsin is the ancestral state in birds (Yokoyama and Shi 2000; Hunt et al. 2001, 2009; Shi et al. 2001), with UVS SWS1 independently evolving at least four times among perching birds (Passeriformes), parrots (Psittaciformes), rheas (Struthioniformes), trogons (Trogoniformes), and gulls and terns (Ciconiiformes) (Mullen and Pohland 2008; Hunt et al. 2009; Ödeen et al. 2010; Machovsky Capuska et al. 2011).

Paleognaths

Paleognath birds are a diverse ancient lineage that includes both extant and extinct taxa with varied ecology and distribution (Davies 2002). This group encompasses ostriches (Struthionidae), rheas (Rheidae), cassowaries and emu (Casuariidae), kiwis (Apterygidae), tinamous (Tinamidae), and the extinct moa of New Zealand (Emeidae), with all except the tinamous being flightless. Being predominantly diurnal species (with the exception of the nocturnal kiwi), color vision is likely to play a major role in paleognath behavior, although the extent to which they are UV-spectra sensitive is not fully known. Interestingly, tinamou eggs are among the most colorful of all avian eggs with some reflecting in the UV range (Igic et al. 2010a). However, the

specific functions of tinamou egg color remain unknown in this group (Brennan 2010). Even less is known about the visual ecology of the extinct New Zealand moa, with most available information, gleaned from the fossil record, suggesting vision was important as these birds foraged mainly on low hanging branches, shrubs, and herbs in and on the margins of forests (Burrows 1989; Horrocks et al. 2004; Wood et al. 2008). The importance of visual sensory processing and perception in moa species has been confirmed in studies of cranial morphology (Ashwell and Scofield 2008; Corfield et al. 2008).

Microspectrophotometry studies of emu (*Dromaius novaehollandiae*), brushland tinamou (*Nothoprocta cinerascens*) and Chilean tinamou (*Nothoprocta perdicaria*) retinas did not detect any SWS1 cones (see Mullen and Pohland 2008), however earlier work by Wright and Bowmaker (2001) on ostrich (*Struthio camelus*) and common rhea (*Rhea americana*) isolated a SWS cone with maximal sensitivity around 400 nm, suggesting a VS state in both species. Sequencing the 'spectral tuning' region of the SWS1 opsin gene suggested that the ostrich and rhea are likely to possess VS SWS1 and UVS SWS1 opsins, respectively (Ödeen and Håstad 2003). Spectrophotometric measurements of rhea and ostrich plumage have failed to detect any UV-reflectance (Mullen and Pohland 2008) suggesting that variation in UV-sensitivity may not be driven by variation in UV-containing plumage color.

Parrots

Parrots (Psittaciformes) are widely known for their extravagant plumage and integument (scale and skin) coloration (Berg and Bennett 2010), and many parrot species possess highly UV-reflective/-fluorescent plumages (Hausman et al. 2003). The functional role(s) that coloration plays in parrot species remains unclear, although mate-choice studies have implicated UV-reflectance as a major factor. For example, female budgerigars prefer males with UV-reflecting plumage over those where UV-reflectance was removed (Pearn et al. 2001). Further, both male and female budgerigars prefer conspecifics with UV-fluorescent plumage over experimentally removed fluorescence (Arnold et al. 2002). Recently reported SWS1 opsin gene partial sequences from 14 parrot species spanning 3 families, support UVS SWS1 among all member families of the parrot order (Carvalho et al. 2011).

Passerines

Among perching birds (Passeriformes), UV signals play a major functional role for many species, aiding in foraging (Honkavaara et al. 2002), mate choice (e.g., Bennett et al. 1996), nest/nestling and egg discrimination (Jourdie et al. 2004; Avilés et al. 2006). For example, perceptual modeling work showed that frugivorous UVS birds possess an enhanced ability to detect fruits against their background color versus VS species (Schaefer et al. 2007). The mouth gapes of nestling passerines reflect UV light, especially in contrast to dark, non-UV-reflective nests, which is thought to facilitate nestling recognition (Hunt et al. 2003). Female European starlings (*Sturnus vulgaris*) may rank prospective mates based on the degree to which male plumage reflects UV spectra, apparently preferring males with higher UV-reflectance (Bennett et al. 1997). Male blue tits (*Cyanistes caeruleus*) possess sexually dichromatic ornamental crown patches which highly reflect UV-spectra and are likely informative in mate choice and acquisition (Andersson et al. 1998).

Egg coloration, recognition, rejection and host-parasite interactions

The UV-reflectance of the eggs of cavity-nesting species such as the spotless starling (*Sturnus unicolor*) is thought to aid in egg detection (Avilés et al. 2006). Blackcaps (*Sylvia atricapilla*) rely on UV signals to discriminate between their own and foreign parasitic common cuckoo (*Cuculus canorus*) eggs in their nest (Polačiková et al. 2007). Similarly, there is evidence that the eggs of some rejecter hosts of parasitic brown-headed cowbirds (*Molothrus ater*) differ in their degree of UV-reflectance from parasitic eggs, so that hosts may be able to attend to these differences in order to discriminate their own from parasitic eggs (Underwood and Sealy 2008). Further, the UV-matching hypothesis (Cherry and Bennett 2001), specifically predicts that the larger the difference in UV-reflectance between host and brood parasitic eggs, the better hosts can discriminate foreign vs. own eggs.

Hypothesis

Here we sequenced the SWS1 opsin gene 'spectral tuning' region, targeting the critical residue 90 (Wilkie et al. 2000) of representative members of the songbirds, parrots, and both extant and extinct paleognaths to test for UV-sensitivity. Using our generated sequences, we then conducted phylogenetic analysis followed by ancestral character state reconstructions for known spectral tuning sites in order to ensure sequence quality as well as to better assess the history of critical amino acid substitutions throughout evolutionary time. Among passerines, we focus on North American hosts of brood parasitic brown-headed cowbirds and endemic New Zealand hosts of brood parasitic cuckoos. We replicate and expand on previously published UVS states of parrot and passerine species (Ödeen and Håstad 2003; Carvalho et al. 2011), and include the New Zealand endemic nocturnal kakapo (*Strigops habroptilus*) (Gill 2010). We also broaden current knowledge of UV-

sensitivity among extant and extinct paleognath species, with an emphasis on endemic New Zealand taxa. We expect a co-variation of UV-sensitivity in relation to the use of UV signals in species' ecology. We predict a VS state in nocturnal species such as the kakapo and in non-ejector passerine hosts of brood parasitic cuckoos and cowbirds, in keeping with predictions of the UV-matching hypothesis (Cherry and Bennett 2001).

Materials and methods

Taxon sampling

Our paleognath samples included 16 individuals representing 15 species over 5 families, including the extinct moa family Emeidae of New Zealand (Table 1). DNA or tissue samples were also obtained from 14 parrot species with representatives of each of the three recognized Psittaciformes families (Table 2). Finally, we sampled 17 individuals representing 16 Passeriformes species over 7 North American families (Emberizidae, Icteridae, Mimidae, Parulidae, Passeridae, Turdidae, and Tyrannidae), 3 Australasian families (Acanthizidae, Pachycephalidae, and Petroicidae), and 1 South American family (Pipridae) (Table 3).

SWS1 sequencing

Paleognaths

DNA from extinct moa species (Table 1) was extracted from moa bone following the procedures required for ancient material. We incubated approximately 20 mg of bone shavings with rotation overnight at 56 °C in 300 µl of 0.25 M EDTA and ~50 µg of proteinase K. The mix was extracted with one volume of phenol:chloroform:isoamyl alcohol (25:24:1) followed by one volume of chloroform. DNA was precipitated from the mix with 0.5 volumes of 7.5 M ammonium acetate, 10 µl of 0.25 % linear polyacrylamide (LPA), and 2.5 volumes of ethanol. The mix was incubated at −20 °C for 20 min and then centrifuged in a benchtop microfuge at full speed for 15 min. The resulting pellet was resuspended in 25 µl of Milli-Q H_2O and then desalted by passage through 300 µl of dry Sephacryl S200HR (GE Healthcare, Buckinghamshire, UK). Extant ostrich, rhea, emu, and cassowary (*Casuarius*

Table 1 Predicted VS/UVS visual sensitivity among sampled paleognath species based on DNA sequencing of the SWS1 photoreceptor

Scientific Name	Common Name	Amino Acid Sequence	Predicted Sensitivity	Previously Predicted Sensitivity
Struthioniformes				
Casuariidae		**90**		
Casuarius casuarius 1	Cassowary	SLGGFI **F**CVL**C**VF**M**	UVS	
Casuarius casuarius 2	Cassowary	SLGGFI **F**CVL**C**VF	UVS	
Dromaius novaehollandiae	Emu	SLGGFI **F**CVL**C**VF	UVS	
Emeidae				
Emeus crassus†	Eastern Moa	SLGGFI **F**CI**F**C**V**F	UVS	
Euryapteryx curtus†	Stout-legged Moa	SLGGFI **F**CI**F**C**V**F	UVS	
Euryapteryx gravis†	NI Broad-billed Moa	SLGGFI **F**CI**F**C**V**F	UVS	
Pachyornis elephantopus†	Heavy-footed Moa	SLGGFI **F**CI**F**C**V**F	UVS	
Pachyornis geranoides†	Mantells Moa	SLGGFI **F**CI**F**C**V**F	UVS	
Rheidae				
Rhea americana	Common Rhea	SLGGFI **F**C**F**F**C**VF	UVS	UVS^{s,m}
Struthionidae				
Struthio camelus	Ostrich	S**V**GGFI **F**CVF**C**VF	UVS	VS^{s,m}
Tinamidae				
Crypturellus undulatus	Undulated Tinamou	SLGGF **V**F**C**VF **C**VF**M**	UVS	
Nothoprocta ornata	Ornate Tinamou	SLGGFI **F**CVF **C**VF**M**	UVS	
Nothoprocta pentlandii	Andean Tinamou	SLGGFI **F**C**C**F**C**VF**M**	UVS	
Nothura boraquira	White-bellied Nothura	SLGGFI **F**CVF **C**VF**M**	UVS	
Rhynchotus rufescens	Red-legged Tinamou	SLGGFI **F**CVF **C**VF**M**	UVS	
Tinamus major	Great Tinamou	SLGGFI **F**C**C**F**C**VF**M**	UVS	

Target amino acid sites 86, 90, and 93 are in bold. Previously predicted sensitivities were compiled from SWS1 sequencing (^{s}) and/or λ-max (^{m}) values reported in Ödeen and Håstad (2003). Intraordinal amino acid variations are shaded

† Denotes an extinct taxon

Table 2 Predicted VS/UVS visual sensitivity among sampled parrot species based on DNA sequencing of the SWS1 photoreceptor

Scientific Name	Common Name	Accession Number	Amino Acid Sequence	Predicted Sensitivity	Previously Predicted Sensitivity
Psittaciformes					
Psittacidae			**90**		
Agapornis roseicollis	Rosy-faced Lovebird	HM222559	SFCGFL**A**CIF**C**IF**T**	UVS	
Eclectus roratus	Eclectus Parrot	HM222563	SFCGFL**A**CIF**C**IF**T**	UVS	
Lorius garrulus	Chattering Lory	HM222555	SFCG FL**A**CIF**C**IF**T**	UVS	
Psittacula derbiana	Lord Derby's Parakeet	HM222561	SFCGF L**A**CIF**C**IF**T**	UVS	
Platycercus elegans	Crimson Rosella	HM222560	SFC GFL**A**CIF**C**IF**T**	UVS	UVS^{s,m}
Platycercus eximius	Eastern Rosella	HM222558	SFC?FL?CIF **C**IF**T**	UVS	
Psittacula krameri manillensis	Rose-ringed Parakeet	HM222562	SFCGFL **A**CIF**C**IF**T**	UVS	
Trichoglossus haematodus	Rainbow Lorikeet	HM222553	SFCGFL **A**CIF**C**IF**T**	UVS	
Cacatuidae					
Cacatua galerita	Sulphur-crested Cockatoo	HM222556	SFCGF? **A**CIF**C**IF**T**	UVS	UVS^{s}
Eolophus roseicapillus	Galah	HM222554	SFCGFL **A**CIF**C**IF**T**	UVS	UVS^{s}
Nymphicus hollandicus	Cockatiel	HM222557	SFCGFL **A**CIF**C**IF**T**	UVS	
Strigopidae					
Nestor meridionalis 1	New Zealand Kaka	HM222548	SFCGFL **A**CIF**C**IF**T**	UVS	
Nestor meridionalis 2	New Zealand Kaka	HM222549	SFCG FL**A**CIF**C**IF**T**	UVS	
Nestor notabilis 1	Kea	HM222550	SFCGF L**A**CIF**C**IF**T**	UVS	UVS^{s}
Nestor notabilis 2	Kea	HM222551	SFCG FL**A**CIF**C**IF**T**	UVS	UVS^{s}
Strigops habroptilus	Kakapo	HM222552	SFCG FL**A**CIF**C**IF**T**	UVS	

Target amino acid sites 86, 90, and 93 are in bold. Previously predicted sensitivities were compiled from SWS1 sequencing (^{s}) and/or λ-max (^{m}) values reported by Carvalho et al. (2011). Intraordinal amino acid variations are shaded

casuarius 1: see Table 1) tissue samples were extracted using DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

The SWS1 gene was then amplified using the forward primer 5′-agtcgacgcttctagcttTACATCCTGGTGAACAT CT-3′ and reverse primer 5′-catgctacctgctactgtTATCCCT GsGAGCTGGmGAT-3′. Lower case letters at the 5' end of each primer are generic sequences that allow direct sequencing of short PCR products. Amplification reactions were conducted in 10 µl reaction volumes consisting of 50 mM Tris–Cl pH 8.8, 20 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 1 mg/ml BSA, 200 µM of each dNTP, 0.5 µM of each primer, and approximately 1.5 µl extracted DNA. Thermal cycling was conducted in an ABI GeneAmp 9700 (Applied Biosystems, Foster City, CA, USA) with an initial denaturation step of 94 °C for 2 min, then 40 cycles of: 94 °C for 20 s and 54 °C for 1 min. Amplified products were then checked by gel electrophoresis in 2 % agarose in 0.5 × TBE, and purified by centrifugation through dry Sephacryl S200HR (GE Healthcare, Buckinghamshire, UK). PCR products were sequenced using ABI BigDye Terminator v3.1 chemistry, then edited and aligned in Sequencher v4.10.1 (Genecodes, Ann Arbor, MI, USA). Predicted protein sequences were derived using Geneious v. 5.1 (Drummond et al. 2010).

We extracted total DNA from six species of tinamou using the DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany). For all but one of these, DNA was extracted from frozen tissue following the standard protocol for tissue. For *Casuarius casuarius* (*Casuarius casuarius 2*: see Table 1), preserved tissue was unavailable, so DNA was extracted from a sliver of toepad collected from the hallux of a museum skin at the Yale Peabody Museum (YPM 86855). For this extraction, we modified the standard tissue protocol by adding 19 µl of 1 M dithiothreitol to the initial lysis reaction and reducing the final elution to 50 µl to ensure adequate DNA concentration.

We amplified a 119 base pair fragment of the SWS1 opsin gene using the degenerate primer pair SU149a/SU306b (Ödeen and Håstad 2003) and GoTaq Hot Start Polymerase (Promega, Fitchburg, WI, USA) following the manufacturer's instructions. We used a thermocycler touchdown protocol similar to Groth and Barrowclough (1999), but with an initial annealing temperature of 58 °C. Correct amplicon size was verified on agarose gels and the PCR product was prepared for sequencing by enzymatic digestion with Exonuclease 1 and Shrimp Alkaline Phosphatase (Werle et al. 1994). Sequencing was performed on an ABI 3730 Sequencer and alignment was conducted by eye in Sequencher v4.10.1 (Genecodes, Ann Arbor, MI, USA).

Parrots

All parrot samples were supplied as either purified DNA or blood samples stored in Queen's Lysis buffer (Seutin et al. 1991). DNA was extracted from pure blood samples using

Table 3 Predicted VS/UVS visual sensitivity among sampled songbirds and pigeon species based on DNA sequencing of the SWS1 photoreceptor

Scientific Name	Common Name	Parasite	Rejecter Status	Accession Number	Amino Acid Sequence	Predicted Sensitivity	Previously Predicted Sensitivity
Passeriformes							
Acanthizidae					90		
Gerygone igata 1	Grey Warbler	SBCU	A	HM159130	SFSG FMCCIFSVFT	VS	
Gerygone igata 2	Grey Warbler	SBCU	A	HM159131	SFSG FMCCIFSVFT	VS	
Emberizidae							
Melospiza melodia	Song Sparrow	BHCO	A		SVSGLM CCVFCIFT	UVS	
Icteridae							
Agelaius phoeniceus	Red-winged Blackbird	BHCO	A		SVSGLM CCVFCIFT	UVS	
Molothrus ater	Brown-headed Cowbird	NP	NP		SVSGLM CCVFCIFT	UVS	
Quiscalus quiscula	Common Grackle	BHCO	A		SVSGLM CCVFCIFT	UVS	
Mimidae							
Dumetella carolinensis	Gray Catbird	BHCO	R		SVSGLM CCIFCIFT	UVS	
Mimus polyglottos	Northern Mockingbird	BHCO	I		SVSGLM CCIFCIFT	UVS	
Pachycephalidae							
Mohoua albicilla	Whitehead	LTCU	A		SVSGLM CCIFCLFT	UVS	
Parulidae							
Setophaga petechia	Yellow Warbler	BHCO	A		SVSGLM CCVFCIFT	UVS	
Passeridae							
Passer domesticus	House Sparrow	NP	NP		SVSGLM CCVFCIFT	UVS	
Petroicidae							
Petroica sp.	Australasian robin sp.	NP	NP		SVSGLM CCIFCLFT	UVS	
Pipridae							
Manacus manacus	White-bearded manakin	NP	NP		SVSG FMCCIFSVFT	VS	$VS^{s,m}$
Turdidae							
Hylocichla mustelina	Wood Thrush	BHCO	A		SVSGLM CCVFCIFT	UVS	
Turdus migratorius	American Robin	BHCO	R		SVSG FMCCVFCIFT	UVS	
Tyrannidae							
Sayornis phoebe	Eastern Phoebe	BHCO	A		SVSG FMCCIFSVFT	VS	
Tyrannus tyrannus	Eastern Kingbird	BHCO	R		SVSG FMCCIFSVFT	VS	

Target amino acid sites 86, 90, and 93 are in bold. Parasitic species (*BHCO* brown-headed cowbird, *LTCU* long-tailed cuckoo, *SBTU* shining bronze cuckoo, *NP* not parasitized) and rejecter status (*R* rejecter, *I* intermediate rejecter, *A* accepter, *NP* not parasitized) are also shown. Previously predicted sensitivities were compiled from SWS1 sequencing (^{s}) and/or λ-max (^{m}) values reported in Ödeen and Håstad (2003). Intraordinal amino acid variations are shaded

a DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. We amplified the SWS1 opsin gene using previously published forward primers SU149a or SU193 paired with the reverse primer SU306b (Ödeen and Håstad 2003), modified to include M13-tails. We conducted PCR amplifications in 25 µl reaction volumes containing 60 mM Tris–HCl pH 8.5, 15 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.3 mM of each dNTP, 0.2 µM of each primer and 0.5 U of Platinum *Taq* polymerase (Invitrogen, Carlsbad, CA, USA). Thermal cycling reactions were performed using an ABI GeneAmp 9700 thermocycler following the protocol published by Ödeen and Håstad (2003): an initial denaturation at 94 °C for 2 min, followed by 5 cycles at 94 °C for 30 s, 54 °C for 30 s and 72 °C for 1 s, then 38 cycles where the extension time was lengthened to 5 s and a final 10 min extension at 72 °C.

PCR products were then purified using Exo/SAP treatment. We added 5 µl PCR product to 0.2 µl Exo I (GE Healthcare, Buckinghamshire, UK), 0.1 µl Shrimp Alkaline Phosphatase (GE Healthcare, Buckinghamshire, UK) and 1.7 µl UltraPure water (Invitrogen, Carlsbad, CA, USA). Mixtures were incubated for 30 min at 37 °C, then for 15 min at 80 °C in order to inactivate the enzymes. We sequenced samples in both directions using BigDye Terminator Cycle Sequencing kit v3.1 (Applied Biosystems, Foster City, CA, USA) with M13 forward and reverse primers. Each sequencing reaction contained 1 µl BigDye Terminator Mix, 3.5 µl 5× sequencing buffer, 0.2 µM primer, 1 µl DMSO and 2 µl PCR product. Sequencing reactions were purified using Agencourt CleanSeq (Beckman Coulter, Brea, CA, USA) according to manufacturer's instructions and analyzed using an ABI 3100 automated sequencer. Sequences were edited using Chromas Pro (Technelysium Pty. Ltd.) and exported to BioEdit (Hall 1999), where they were aligned, translated and compared to other avian opsin sequences downloaded from GenBank. Amino acid sequences were then aligned with our paleognath sequences (see below) using Geneious v. 5.1 (Drummond et al. 2010).

Passerines

All songbird samples were provided either as frozen tissue samples (North American species) or blood samples stored in Queen's Lysis buffer (Australasian species) (Seutin et al. 1991). DNA was extracted from samples using DNeasy kits (Qiagen, Hilden, Germany) following standard protocols. Initial attempts to amplify the whitehead (*Mohoua albicilla*) SWS1 gene sequence with the primers of Ödeen and Håstad (2003) were unsuccessful. We then designed a new set of primers based on alignments of SWS1 sequences from the zebra finch and the chicken. We designed two forward primers (SWS1_F1: 5′-CSCCCACGTGGGCCTTCTACC-3′; SWS1_F2: 5′-GTACCACATCGCSCCCATGTG-3′) and two reverse primers (SWS1_R1: 5′-CTGACCATGTGCCACCCGTG-3′; SWS1_R2: 5′-CGACCAGCASCRCCGGTGSAC-3′).

Amplification reactions contained a total volume of 10 µl and consisted of 1 µl undiluted genomic DNA (10–50 ng/µl concentration), 10 µM Tris–HCl (pH 8), 50 µM KCl, 4 µM $MgCl_2$, 0.25 mM of each nucleotide, 0.25 mM of each primer, and 0.025 U Jumpstart Taq polymerase (Sigma, St. Louis, MO, USA). Thermal cycling reactions were conducted in PTC-220 Dyad Thermal Cyclers (MJ Research, Waltham, MA, USA). Cycling profiles followed an initial denaturing at 95 °C for 4 min 30 s; 30–35 cycles of denaturing at 95 °C for 45 s, annealing at 54 °C for 1 min, an extension at 72 °C for 1–2 min 20 s, and a final extension at 72 °C for 5 min.

Amplification and fragment size confirmation was performed on PCR products via electrophoresis in 1.5 % agarose TAE gels. We then added 0.5 U each of Shrimp Alkaline Phosphatase (USB) and Exonuclease (USB) to each remaining 7 µl PCR product and incubated for 30 min at 37 °C followed by 10 min at 90 °C in order to digest unincorporated nucleotides and primers. Cycle sequencing reactions were conducted using the amplification primers as well as the previously published forward primer SU193a and reverse primer SU306b (Ödeen and Håstad 2003) which is internal to our designed amplification primers. Cycle sequencing reactions were performed using a BigDye 3.1 (Applied Biosystems, Foster City, CA, USA) sequencing kit using recommended cycling conditions. Sequences were then read using Applied Biosystems model 3100 or 3730 automated Genetic Analyzers. Both strands were sequenced in order to verify fragments, and sequences were checked and assembled used Sequencher 4.5 (Genecodes, Ann Arbor, MI, USA). The North American brown-headed cowbird, house sparrow (*Passer domesticus*), eastern phoebe (*Sayornis phoebe*), American robin (*Turdus migratorius*), red-winged blackbird (*Agelaius phoeniceus*) and the Australasian robin (*Petroica sp.*) samples were successfully sequenced using these primers. However, the whitehead sample still failed to sequence successfully using these new primers.

We then designed a "passerine-specific" primer using these sequences as well as previously published SWS1 sequence from the zebra finch (Ödeen and Håstad 2003) in order to target the *Mohoua* genus. We designed one forward primer, SWS1_F4: 5′-CTACCTGCAGACCATCTTCATGG-3′, which we successfully combined with previously published reverse primer SU306b (Ödeen and Håstad 2003). We then used this combination of primers with the SU306b reverse primer to sequence song sparrow (*Melospiza melodia*), gray catbird (*Dumetella carolinensis*), common grackle (*Quiscalus quiscula*), wood thrush (*Hylocichla mustelina*), northern mockingbird (*Mimus polyglottos*), yellow warbler (*Setophaga petechia*), and eastern kingbird (*Tyrannus tyrannus*) species. The same protocol was followed for all amplification, purification, and sequencing reactions described in this section. Finally, the white-bearded manakin (*Manacus manacus*) and gray warbler (*Gerygone igata*) samples were sequenced following protocols described for tinamou SWS1 sequencing in "Paleognaths" and parrot SWS1 sequencing in "Parrots", respectively.

SWS1 phylogenetic analysis and ancestral character state reconstruction

We aligned all SWS1 DNA sequences, including a SWS1 DNA sequence from *Gallus gallus* for use as the outgroup (Accession NM205438) using ClustalW (Thompson et al. 1994) implemented in Geneious v. 5.1 (Drummond et al. 2010). The alignment was then inspected visually and edited to match the maximum length of our sample sequence alignment (a gapless 226 bp sequence) for use in phylogenetic analysis. We used jModeltest v. 0.1.1 (Guindon and Gascuel 2003; Posada 2008) to determine the appropriate model of DNA evolution using Akaike Information Criteria (AIC) calculations to select the best model of evolution (Posada and Buckley 2004). Bayesian inference analysis was conducted using MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), estimating all model parameters during the analysis. The analysis was run for 10 million generations sampled every 100 generations employing Markov Chain Monte Carlo (MCMC) tree searches comprised 2 runs of 3 heated chains at a temperature of 0.5 and 1 cold chain each. The first 25 % of samples were discarded as burn-in (e.g. Nyári et al. 2009). By this point, all average standard deviations of split frequencies were $\leq$0.1 (0.004) all log likelihood values fluctuated within a stable range, suggesting that convergence had been reached. The majority rules consensus tree was edited using TreeGraph2 (Stöver and Müller 2010) and Adobe Creative Suite 5.0 (Adobe

Systems Inc. 2010). Ancestral character state reconstructions of amino acids were conducted using the majority rules consensus tree in Mesquite v. 2.75 (Maddison and Maddison 2011) under a parsimony model with unordered character states.

Results

Paleognath SWS1 opsin sequences

In families where more than one species was represented (Casuariidae, Emeidae, and Tinamidae), only the Tinamidae contain intrafamily variation in the SWS1 amino acid sequence (Table 1; complete DNA sequences and amino acid translations for all species are available in Supplementary Tables S1 and S2, respectively). While our common rhea sample was identical to a previously published SWS1 amino acid sequence of the same species, our ostrich sample contained numerous substitutions differing from a previously published sequence (Ödeen and Håstad 2003). Most importantly, our ostrich sample contained C90, while Ödeen and Håstad (2003) reported S90. Our ostrich C90 result has been replicated using a different sample than the one included here and was conducted in a separate laboratory (A Fidler, unpublished data; Tables S1 and S2). All paleognath samples in our study, both extant and extinct, possess C90, strongly suggesting a ubiquitous UVS SWS1 opsin among paleognaths.

Parrot SWS1 opsin sequences

SWS1 sequencing of our 16 parrot individuals resulted in a 74 base pair (bp) sequence including the codon for target amino acid site 90. The amino acid translations among the parrots were highly conserved across all three families studied. We observed little variability in the SWS1 amino acid sequences for all parrot samples (Table 2; complete DNA sequences and amino acid translations are available in Tables S1 and S2, respectively). The presence of C at site 90 for all parrot species studied here suggests a UVS SWS1 opsin (Table 2; Ödeen and Håstad 2003) for all members of the Psittaciformes Order.

Passerine SWS1 opsin sequences

The predicted SWS1 spectral tuning sequences (residues 80–92) showed intra-ordinal variation at residues 84, 85, and 88–92 (Table 3; complete DNA sequences and amino acid translations are available in Tables S1 and S2, respectively). Unlike either the parrots or paleognaths, we observed intrafamily variability within the target amino acid sequence of perching birds, although only among the suboscine family Tyrannidae. No intrafamily variation was detected at residue 90 (Table 3). Among our samples, only the two suboscine tyrant flycatchers (Tyrannidae) and the white-bearded manakin (Pipridae), and the New Zealand oscine gray warbler (Acanthizidae) are predicted to possess VS SWS1 opsins. All other species sampled are predicted to possess UVS SWS1 opsins (Table 3). Our white-bearded manakin sample possessed three amino acid differences relative to a previously published SWS1 sequence (Ödeen and Håstad 2003); our sample possessed V instead of F at position 81, M instead of I at position 85, and C instead of S at position 86 (See Table 3).

Among our passerine brood parasite hosts (Table 3), we did not observe a clear relationship between acceptance/rejection of parasitic eggs and predicted SWS1 opsin sensitivity. Of the eight accepter species, six were predicted to possess UVS SWS1 opsins. Of the three rejecter species, two were predicted to possess UVS SWS1 opsins. The one intermediate rejecter (northern mockingbird) was predicted to have a UVS SWS1 opsin. There was also no obvious relationship between parasitic egg acceptance/rejection and predicted SWS1 sensitivity by geographical location. The two Australasian host species, the accepter whitehead and the accepter gray warbler, were assigned UVS and VS SWS1 opsins, respectively. Similarly, among the North American brown-headed cowbird hosts, accepter and rejecter hosts varied in their predicted SWS1 opsin sensitivities (Table 3).

SWS1 phylogenetic analysis and ancestral character state reconstruction

Model selection analysis in jModelTest proposed a general time-reversible evolutionary model following a gamma rate distribution (GTR + Γ). Here, we show the majority rules consensus tree produced by Bayesian inference (Fig. 1). Despite the relatively short DNA sequence, our analysis produced three distinct, basally well-resolved clades of Passeriformes, Pstittaciformes, and paleognath species. The only samples falling outside of the three clades were, incidentally, the Passeriformes species predicted to possess VS opsins (Table 1; Fig. 1). However, these samples showed strong intra-species (*G. igata*) and moderate intra-familial posterior probability support (Tyrannidae).

Within the paleognath clade, members of both the Casuariidae and Tinamidae grouped strongly while the rhea, ostrich, and the extinct moas were not as well resolved. The lack of resolution among these species is most likely due to their being the shortest of the SWS1 sequences in this study. Nonetheless, the analysis firmly placed them within the paleognath clade with a posterior probability of 0.92. The parrot clade, despite including all parrot species in this

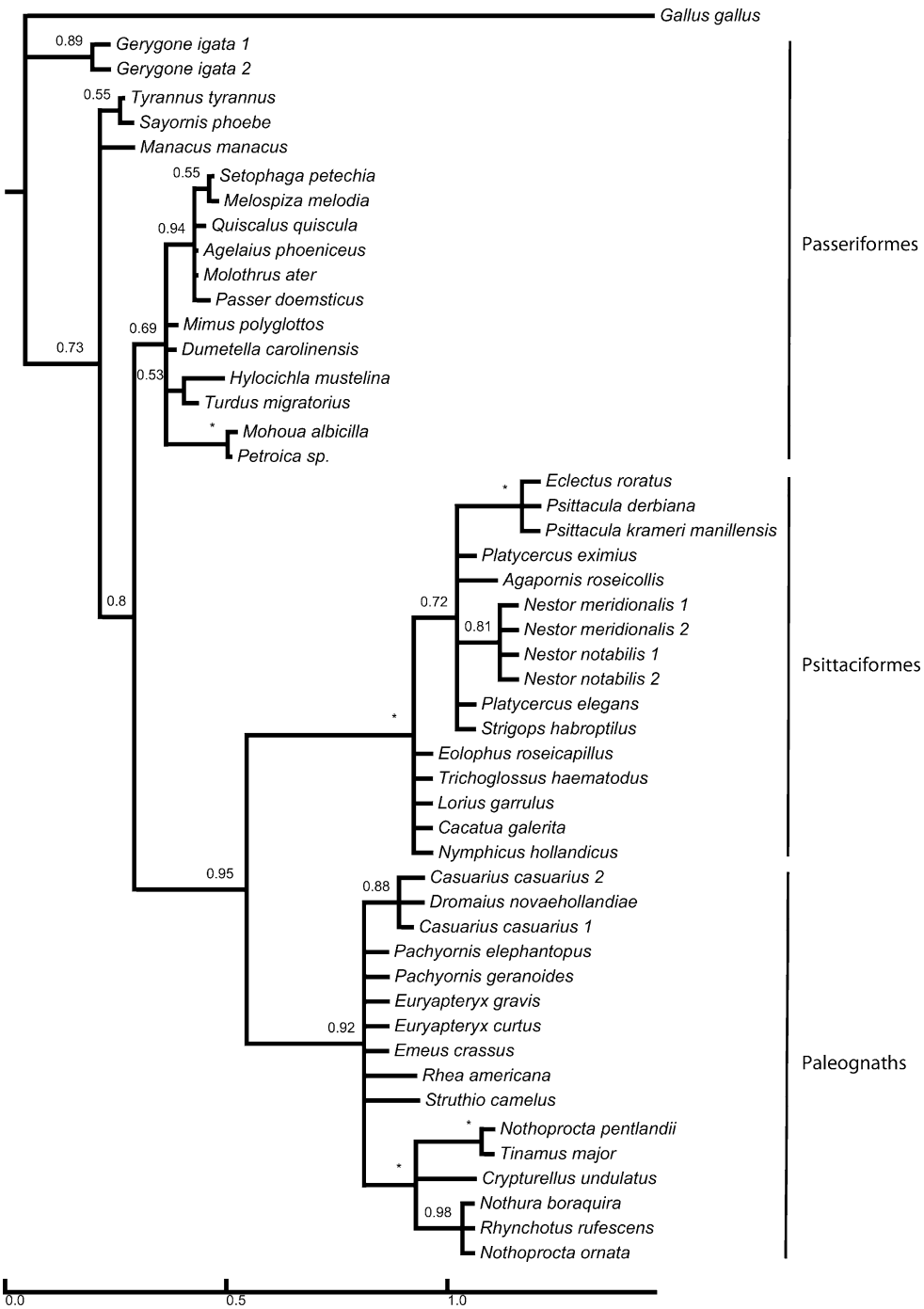
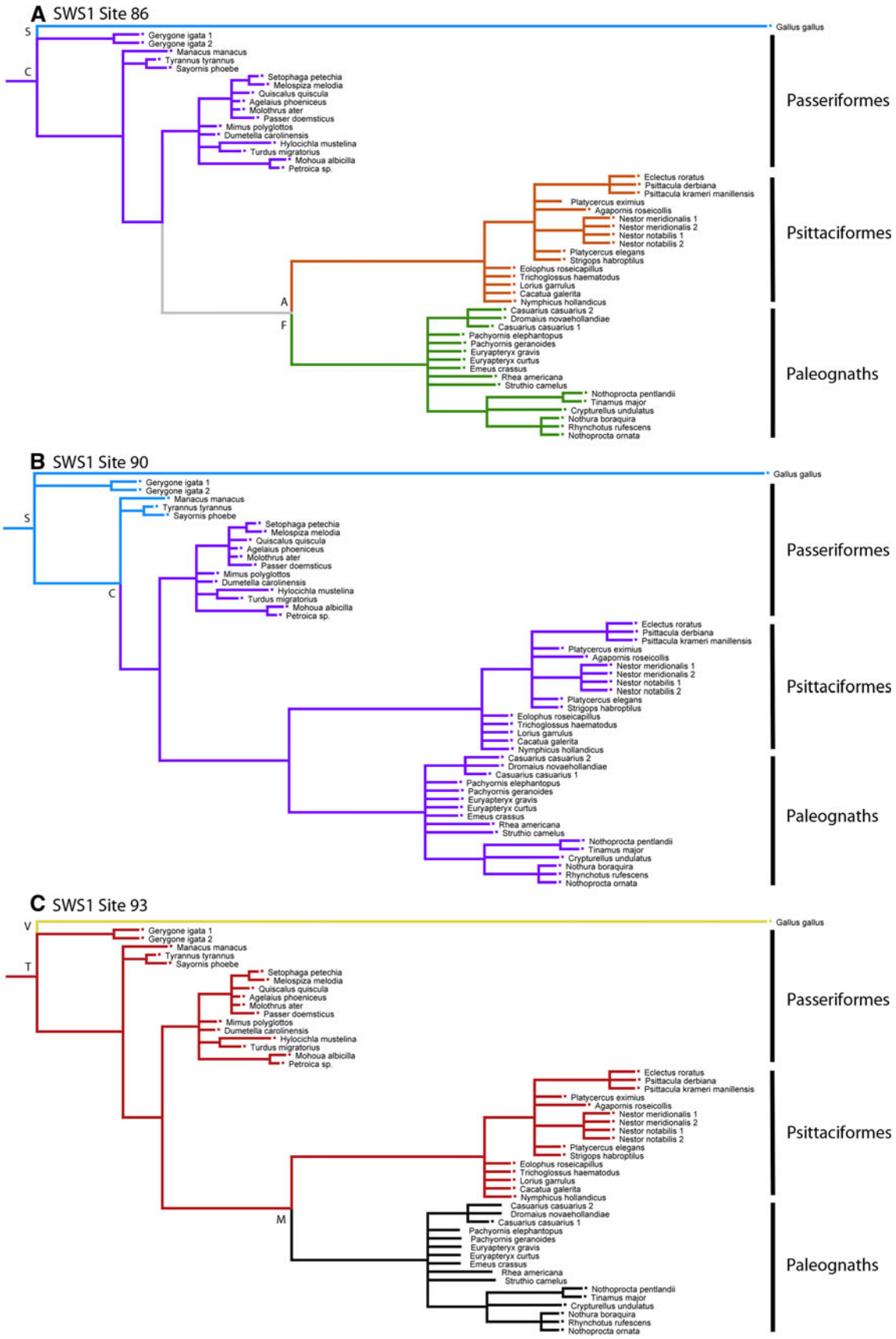


Fig. 1 Phylogram based on SWS1 nucleotide sequences produced by Bayesian inference in MrBayes (average standard deviation of split frequencies = 0.003) with posterior probabilities shown. *Asterisk* denotes posterior probabilities of 1.00

study with a posterior probability of 1.00, was less well resolved at the family and genus levels than the paleognath clade. However, three members of the Psittacidae (*E. roratus, P. derbiana, P. krameri manillensis*) grouped together as did the kea and New Zealand kaka samples (Nestoridae). The majority of the Passeriformes also formed a distinct basal clade in which the two Turdidae species had moderate support for each other, as did our Emberizidae and Parulidae species. The Australasian whitehead and *Petroica sp.* samples also grouped strongly with each other. The Icteridae all grouped into the same clade with a high (0.94) posterior probability but did not definitively resolve with one another, instead forming a polytomy with the house sparrow. Outside of the larger Passeriformes clade, the Tyrannidae grouped together as did the two Australasian warbler samples, all of which were predicted to be VS taxa.

All samples in this study possessed either C90 or S90. Ancestral character state reconstruction for this site under the produced phylogeny suggests C90 as the ancestral state



◀ **Fig. 2** Ancestral character state reconstruction of key SWS1 spectral tuning sites 86 (**a**), 90 (**b**), and 93 (**c**) produced in Mesquite using the phylogram from the Bayesian analysis. Each amino acid is color coded and labeled (*S* blue, *C* purple, *A* orange, *F* green, *V* yellow, *T* red, *M* black). *Gray* indicates an equivocal state

for both Psittaciformes and paleognaths. Assuming that S is the ancestral state of all birds (Yokoyama et al. 2000) it appears that C evolved at site 90 some time later. The Passeriformes lineage is divided seemingly on the basis of the amino acid present at site 90 in which the larger clade possesses C90 and the less resolved species possess S90 (Fig. 2). Interestingly, site 90 is the only amino acid that is definitively distributed in that manner among our sequences. Amino acid residue 86, varied across, but not within all orders. All Passeriformes possessed C86, all Psittaciformes possessed A86, all paleognaths possessed F86, while the outgroup Galliformes species possessed S86 (Fig. 2).

At amino acid residue 93, all Passeriformes and Psittaciformes possessed T93. The paleognaths possessed M93, though this is predicted by the character state reconstruction as the most parsimonious state for the extinct moas, rhea, emu, and ostrich because the sequence did not extend to site 93 (Table 2). Given that all the tinamous and the cassowary possessed M93, the character state reconstruction at this site (Fig. 2) is likely accurate. The Galliformes outgroup possessed V93. Based on the phylogenetic and ancestral character state reconstruction analyses of amino acid residues 86, 90, and 93 (Fig. 2), both the paleognath and Psittaciformes clades appear to retain their phylogenetic history while the Passeriformes clades are additionally split depending on which amino acid (C or S) is present at site 90.

Discussion

Predicted SWS1 opsin sensitivity

In this study, we expanded on previous work in which the 'spectral tuning' region of the SWS1 opsin gene was used to predict the degree of avian SWS1 sensitivity to UV spectra (<400 nm), and therefore infer the degree of several bird species' UV visual sensitivity (e.g., Ödeen and Håstad 2003). To date, all avian species examined prior to this study using direct SWS1 DNA sequencing (e.g., Ödeen and Håstad 2003; Carvalho et al. 2011) and/or site-directed mutagenesis followed by in vitro protein expression possess C90 or S90 (e.g., Yokoyama et al. 2000; Wilkie et al. 2000). All of our samples possessed either C90 or S90, allowing the predicted assignment of UVS or VS SWS1 opsins to be fairly straightforward in the current dataset. Our results suggest ubiquitous UVS SWS1 opsins in both the Psittaciformes and paleognaths, while the Passeriformes contain species with both VS and UVS SWS1 opsins (Tables 1, 2, 3).

In addition to showing both intra- and extra-ordinal variation at spectral tuning site 90, we also report extra-ordinal variation at two additional well-studied spectral tuning sites along the avian SWS1 opsin-residues 86 and 93 (e.g., Yokoyama et al. 2000; Wilkie et al. 2000). Our Passeriformes species invariably possessed C86 and T93, both of which have been reported elsewhere (Table 3; Ödeen and Håstad 2003). Similarly, other Psittaciformes species have also been shown to possess A86 and T93 (Table 2; Ödeen and Håstad 2003; Carvalho et al. 2011). The majority of paleognaths definitively possessed F86 and M93 (exceptions being the emu, rhea, ostrich, one cassowary, and the extinct moas, where the sequence did not encompass residue 93). Character state reconstruction analyses predicted M93 for our paleognath samples; (see Fig. 2), both of which have been previously documented in paleognath species (Ödeen and Håstad 2003). Variation at amino acid residues 86 and 93 does not covary well with the assignment of VS/UVS SWS1 opsins among avian species (e.g., Ödeen and Håstad 2003; Hunt et al. 2009; Tables 1, 2, 3; Fig. 2), although S86 never accompanies a UVS SWS1 opsin (Carvalho et al. 2007; Hunt et al. 2009). This is likely because the resultant changes in spectral sensitivity due to amino acid substitutions at sites 86 and 93 are much lower than that at residue 90 (Yokoyama et al. 2000; Wilkie et al. 2000).

Our findings largely coincide with and expand on the assigned VS/UVS avian SWS1 opsin states of earlier studies. Our Passeriformes samples demonstrated extra-familial variation at site 90, supporting recent findings that UVS/VS opsins have been acquired and lost at least eight times within the Passeriformes lineage (Ödeen et al. 2011). However, our white-bearded manakin SWS1 sequence differed from a previously published sample, possessing M85 and C86 instead of the previously reported I85 and S86 (Ödeen and Håstad 2003). Though the reasons for this discrepancy are not clear, our sample possessed a sequence identical to the brown-crested flycatcher (*Myiarchus tyrannulus*) reported in the same study (Ödeen and Håstad 2003) as well as to our own eastern phoebe and eastern kingbird Tyrannidae samples. In turn, our galah (*Eolophus roseicapillus*), sulfur-crested cockatoo (*Cacatua galerita*), crimson rosella (*Platycercus elegans*), and kea (*Nestor notabilis*) samples are identical to those reported by Carvalho et al. (2011), supporting their conclusion that UV-sensitivity is ubiquitous among all parrots.

We found F86, V88, and C90 in the ostrich, while an earlier study reported S86, I88, and S90 in the same species (Ödeen and Håstad 2003), leading us to predict a UVS

instead of the previously predicted VS SWS1 opsin. Our phylogenetic analysis places the ostrich firmly within the paleognath clade (Fig. 1). It is also a unique sequence in our data set (Table 1), making the discrepancy due to mislabeling of samples unlikely. However, earlier microspectrophotometry studies have reported a maximal absorbance of the ostrich SWS1 photoreceptor at 405 nm, which makes up 1.5 % of the total cones present in the ostrich retina, also suggesting violet sensitivity (Wright and Bowmaker 2001). Our sequence data suggest that the ostrich possesses F86 and C90, identical to our and an earlier published SWS1 sequence of the common rhea (Ödeen and Håstad 2003). Although maximal absorbance of the common rhea's SWS1 photoreceptor is not available, the SWS1 photoreceptor comprises 2.5 % of all the cone-types found its retina (Wright and Bowmaker 2001). This is comparable to the low-end of the SWS1 cone distribution in the UVS European starling, in which the SWS1 cone comprises 3–7 % of all cone-types (Hart et al. 1998). Given that UV plumage and integument (skin) signals play major roles in parrot behavior (Berg and Bennett 2010), even though in their retinas the SWS1 cone is again the least represented of the cone-types (9 % in the budgerigar; Wilkie et al. 1998), it is not unreasonable to suggest that UV signals likely possess behavioral relevance in paleognath species as well, though the reason for the discrepancy in the reported DNA sequences between our and Ödeen and Håstad's (2003) remains unknown.

Additional analyses such as microspectrophotometry, site-directed mutagenesis/in vitro expression, and/or behavioral experiments to determine the presence and/or functional relevance of UV-sensitivity would be ideal components to include in any study addressing UV-sensitivity at the molecular and/or behavioral levels. However, they are not available or practical for all avian species such as extinct or endangered taxa. UV-sensitivity can further be mediated by the level of ocular media transparency to UV wavelengths as well as by higher-level neural processes (see Machovsky Capuska et al. 2011), as well as the relative abundance/density of SWS1 cones in the retina, making behavioral studies a primary goal for assessing the degree of UV-sensitivity in any species.

Phylogenetics and character state reconstruction

SWS1 opsin DNA sequence has been used to produce a reliable phylogeny among most vertebrate classes consistent with both morphological and molecular phylogenies most likely due to its relatively homogeneous substitution rates (van Hazel et al. 2006). Our phylogenetic analysis of the SWS1 nucleotide sequences showed three distinct, basally resolved clades of Passeriformes, Psittaciformes, and paleognaths (Fig. 1). The lack of high resolution at the apices is likely due to the relatively short lengths of our sequences as well as the inclusion of only one gene, yet the overall intra-order affinities of our samples ensure the quality/validity of our sequence data. It is indeed compelling that the Passeriformes clade appears to be split functionally as VS SWS1-assigned (S90) taxa resolved outside the UVS SWS1-assigned (C90) taxa, whereas the ubiquitously UVS SWS1 Psittaciformes and paleognaths appear to better retain their phylogenetic history within their respective clades (Fig. 1).

The close phylogenetic relationship between Passeriformes and Psittaciformes is increasingly well established (Hackett et al. 2008; Suh et al. 2011), our phylogenetic analysis also places Psittaciformes with Passeriformes. Future work including multiple gene sequences and/or a longer SWS1 sequence is needed both to produce a phylogeny in better consensus with the established literature, and increase resolution within the phylogeny. Nonetheless, the phylogeny reported here allowed for informative ancestral character state reconstruction of key spectral tuning sites along the SWS1 opsin within each separate clade (Fig. 2).

The ancestral state of the vertebrate SWS1 opsin is believed to be UVS (Yokoyama and Shi 2000; Shi et al. 2001), with the avian lineage having a VS SWS1 ancestral state, evolving a UVS SWS1 opsin multiple times (Ödeen and Håstad 2003; Carvalho et al. 2007; Hunt et al. 2009; Ödeen et al. 2011). Here we show that an ancient lineage of birds, the paleognaths, likely possess UVS SWS1 opsins due to the presence of C90 and F86 (Fig. 2), the latter of which is highly conserved in UV-sensitive mammals (Hunt et al. 2009). A F86S substitution has been shown to be the integral substitution involved in the evolution of a VS SWS1 opsin in avian ancestors (Carvalho et al. 2007). The ubiquitous presence of F86 in the ancient paleognath lineage is particularly exciting because it was present in the ancestral UVS SWS1 opsin of all vertebrates (Yokoyama and Shi 2000; Shi et al. 2001; Hunt et al. 2009), especially because the paleognaths were the earliest clade to diverge within the avian lineage (e.g., Hackett et al. 2008).

If UV-sensitivity was lost among avian ancestors and subsequently reacquired in the avian lineage, our results suggest that this may have occurred earlier than has been previously suggested. Recent phylogenetic work placed the extinct moa as closely related to the cassowary, emu, kiwi, and tinamous with the rhea and ostrich having diverged earlier (Phillips et al. 2010). If all paleognath species have UVS SWS1 pigments as our results suggest, a UVS state is likely to have evolved in a species ancestral to all the paleognaths rather than only among the rheas, as has been previously suggested (e.g., Ödeen and Håstad 2003).

A single amino acid substitution (C90S) was responsible for the evolution of UV SWS1 sensitivity in avian species

(Yokoyama et al. 2000), though no paleognath species were included in that study. To date, however, no avian species have been identified that do not possess either S90 or C90, suggesting site 90 is a highly conserved, functionally relevant site among all avian lineages. Our reconstruction analyses did not support F86 or C90 as the root ancestral states (Fig. 2), though this is probably due to the choice of outgroup (Galliformes), the relatively short sequence used in the phylogenetic analysis, and the lack of diverse taxon sampling throughout the avian (and vertebrate) lineage.

Functional relevance of UV-sensitivity among paleognaths

Our results suggest that all paleognath birds have the molecular pigments for perceiving UV light, though the function of this perception is not clear. There is no evidence that paleognath plumage reflects UV light, so it is not clear whether UV signals play a major role in mate choice (Mullen and Pohland 2008). However, moa species with predominantly paternal care (Huynen et al. 2010) may have used UV-reflectance spectra in egg identification, which is potentially important in numerous other bird species (e.g., Cherry and Bennett 2001; Polačiková et al. 2007; Honza et al. 2007), particularly those that nest in enclosed, dark cavities (Avilés et al. 2006). Further, extant tinamous lay conspicuously colored eggs despite being ground nesting species which typically lay camouflaged eggs (Kilner 2006). These non-cryptic eggs may serve as an intraspecific signal of female quality to incubating males and/or a signal of nest location for laying females (Brennan 2010).

For example, males may attend to UV-reflectance of eggs as a predictor of female quality, as has been suggested in spotless starlings and garnering moderate, if correlative, empirical support (López-Rull et al. 2007). Eggshells of extinct moa species are often found in caves, suggesting that UV egg reflectance may have facilitated egg location and recognition in these species. Ostrich, emu, and extinct stout-legged moa eggshells are known to reflect UV light (Igic et al. 2010a) which may play a functional role in signaling quality of both mother and chicks in other species (Moreno et al. 2005; López-Rull et al. 2007, 2008).

Fadzly et al. (2009) has examined the spectral reflectance curves of lancewood tree (*Pseudopanax crassifolius*) leaves once exploited by moa species as a food source. The leaves showed some, albeit minimal, reflectance in the UV portion of the light spectrum and it is possible that moa could have used this UV-reflectance to locate suitable saplings. However, the relative densities of UV-sensitive cones within their retinas are unknown, making this claim purely speculative.

Functional relevance of UV-sensitivity among parrots

All parrot species sampled in this study possess a C at amino acid site 90, with very little variation along the entire length of the sequence and are identical in all parrot lineages sampled (Table 2). Although the behavioral relevance of UV-spectra among parrot species is fairly well documented (see Berg and Bennett 2010), little is known about its role in the kakapo, an endangered, nocturnal parrot of New Zealand. Evolving nocturnality tends to favor either an increased or decreased reliance on visual stimuli; nocturnal species relying heavily on visual stimuli tend to possess large eyes with poor acuity (Hall and Ross 2007), whereas those that rely on senses other than vision often possess smaller eyes (e.g., Martin et al. 2007). A recent study by Corfield et al. (2011) showed the kakapo has evolved a visual system not typically observed in nocturnal animals as it possesses visual system traits characteristic of both nocturnal and diurnal birds. This could be because the kakapo evolved from a diurnal parrot ancestor (Corfield et al. 2011). Given that our data suggest that the kakapo has pigments sensitive to UV-spectra, future studies are necessary to establish its role in the kakapo's behavioral repertoire and communication behavior.

Functional relevance of UV-sensitivity among passerines

Each of the North American species from the present study has been reported as a host of the brown-headed cowbird, allowing us to assess whether or not UV-sensitivity is related to parasitic egg rejection rates. According to Peer and Sealy (2004), rejecter hosts (those that typically remove parasitic cowbird eggs) include the eastern kingbird, American robin, and gray catbird. The northern mockingbird is classified as an intermediate rejecter (with some foreign eggs rejected from its nests), and the eastern phoebe, wood thrush, yellow warbler, song sparrow, red-winged blackbird, and common grackle are classified as accepter species. Our results suggest that the tyrant flycatchers (eastern kingbird and eastern phoebe) possess a VS visual system, as has been previously shown among the Tyrannidae (Ödeen and Håstad 2003). In keeping with Underwood and Sealy's (2008) conclusions, we found no obvious relationship between hosts' predicted UV-sensitivity and their rejecter status and thereby do not find support for the UV-matching hypothesis (Cherry and Bennett 2001) in North American hosts of the brown-headed cowbird.

The New Zealand endemic whitehead is a non-ejector host of the long-tailed cuckoo (*Eudynamis taitensis*) (McLean and Waas 1987; Briskie 2003), so it is unlikely

that UV-sensitivity plays a functional role in egg discrimination. However, it may be involved in detecting the sexual dichromatism of whitehead feathers (Igic et al. 2010b). The gray warbler is a non-ejector host of the shining bronze cuckoo (*Chrysococcyx lucidus*) (Briskie 2003) but unlike the whitehead, it is predicted to be a VS species. However, because these were the only two Australasian brood parasite hosts sampled, support for the UV-matching hypothesis in this system remains equivocal.

Although we did not find overwhelming support for the UV-matching hypothesis between egg accepters and rejecters (Cherry and Bennett 2001; Underwood and Sealy 2008) in any of our sampled songbirds, alternative roles of UV-reflectance in behavioral repertoires in songbirds remain unclear. Our results support ubiquitous UVS pigments in the true sparrows and a VS state in suboscines (Tyrannidae), as has been previously shown (Ödeen and Håstad 2003). By including previously unsequenced representative songbird (oscine) Passeriformes from the New World sparrows, New World blackbirds, mimids, thrushes, whistlers, Australasian robins and Australasian warblers, we show that all appear to be UVS, except the New Zealand *G. igata* which is predicted to be VS.

Our results suggest a ubiquitous UVS SWS1 opsin for Psittaciformes and paleognath species based on the presence of SWS1 C90 in all samples tested, and is the first study to investigate SWS1 pigments in extinct paleognaths. We also predict variable SWS1 opsin sensitivity among the Passeriformes based on the presence of S90 or C90. Although further confirmation (microspectrophotometry, site-directed mutagenesis, ocular media transparency and behavioral studies) would be ideal, genetic sequencing of the SWS1 photoreceptor gene is the only method currently available for studying extinct taxa. Given that paleognath eggshells reflect a large proportion of UV light suggests that UV-sensitivity may be behaviorally relevant for these species. Our results also expand and support previous suggestions of ubiquitous UVS pigments for the entire parrot order (Psittaciformes). Our passerine samples yielded the only taxonomic group to demonstrate any variability in predicted UV-sensitivity throughout the order, though we did not observe any intra-family variability. Where possible, behavioral, microspectrophotometric, and ocular media transparency studies should follow-up on the results presented here in order to better describe the function of UV signals in these species.

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