Multiple shifts between violet and ultraviolet vision in a family of passerine birds with associated changes in plumage coloration

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Colour vision in diurnal birds falls into two discrete classes, signified by the spectral sensitivity of the violet- (VS) or ultraviolet-sensitive (UVS) short wavelength-sensitive type 1 (SWS1) single cone. Shifts between sensitivity classes are rare; three or four are believed to have happened in the course of avian evolution, one forming UVS higher passerines. Such shifts probably affect the expression of shortwave-dominated plumage signals. We have used genomic DNA sequencing to determine VS or UVS affinity in fairy-wrens and allies, Maluridae, a large passerine family basal to the known UVS taxa. We have also spectrophotometrically analysed male plumage coloration as perceived by the VS and UVS vision systems. Contrary to any other investigated avian genus, Malurus (fairy-wrens) contains species with amino acid residues typical of either VS or UVS cone opsins. Three bowerbird species (Ptilonorhynchidae) sequenced for outgroup comparison carry VS opsin genes. Phylogenetic reconstructions render one UVS gain followed by one or more losses as the most plausible evolutionary scenario. The evolution of avian ultraviolet sensitivity is hence more complex, as a single shift no longer explains its distribution in Passeriformes. Character correlation analysis proposes that UVS vision is associated with shortwave-reflecting plumage, which is widespread in Maluridae.

Keywords: UV vision; SWS1 opsin sequencing; plumage spectrophotometry; Maluridae; Malurus; fairy-wren

1. INTRODUCTION

The eyes of diurnal birds are highly adapted to detect colour contrasts in the natural environment. The avian retina has possibly the most complex structure found in vertebrates, containing seven different classes of photoreceptors [1]. Of these, the four single-cone classes appear to be involved in colour vision [2–4]. Although birds live in a wide variety of habitats, avian colour vision is remarkably invariant. Significant variation has, however, been found in the ultraviolet-/violet-sensitive (short wavelength-sensitive type 1) single cone (‘SWS1’ or ‘UVS/VS’). Its wavelengths of maximum sensitivity ($\lambda_{\text{max}}$) fall into two discrete classes [5]: UVS ($\lambda_{\text{max}}$ 355–380 nm) and VS ($\lambda_{\text{max}}$ 402–426 nm; reviewed by Odeen et al. [6]).

The ancestral and most common sensitivity class in birds appears to be VS [7,8], which is found in all examined groups except shorebirds and Passerida passerines, parrots and the rhea, with the possible addition of trogons ([8] and references therein) [9–12]. Since Psittaciformes and Passeriformes may be sister orders [13,14], the shift from VS to UVS has thus happened independently at least three times [15]. The UVS trait is common to all 22 Passerida species investigated with microspectrophotometry (MSP) and molecular sequencing [8,9,16–23], whereas other passerine species, including other oscines, share the trait (two species of Tyrannidae, five species of Meliphagidae, four of Corvidae and three of Thamnophilidae [8,9,22,24], GenBank accession number DQ451006). The simplest explanation to the presently understood distribution of SWS1 classes in Passeriformes is a single VS to UVS opsin shift in an ancestor of Passerida, but the patchy taxon sampling outside Passerida may easily mask any additional shifts.

Ultraviolet vision is associated with several costs. One is that short wavelength radiation is absorbed by, and destroys, biological tissue through photo-oxidation [25]. Most VS birds have ocular media that long-pass filter ultraviolet radiation [26], thereby avoiding the hazards of photo-oxidation. Assuming that photodamage accumulates over time, ultraviolet vision will be more costly in long-lived animals (cf. [11]). The observation that long-lived birds possess physiological mechanisms to counter...
oxidative damage confirms the reality of this cost [27]. Another cost comes from the fact that an ultraviolet shift in spectral sensitivity will reduce visual acuity and contrast for the SWS1 cone by chromatic aberration and Rayleigh scattering, especially in large eyes [28]. These problems may be partly mitigated through improved optics, but the remedies will carry a cost all the same. Nevertheless, several groups of birds have acquired the UVS trait, indicating that it carries significant advantage under some circumstances. The shortwave shift in SWS1 single-cone spectral sensitivity increases the realized volume of colourspace and consequently the ability to discriminate between colours, especially in the short wavelength end of the spectrum (see [9,29]). It will probably increase the importance, hence the level of expression, of social colour signals in general and shortwave-dominated signals in particular (see [30]).

Consequently, shifts from VS to UVS should most probably occur in groups that consist of small and relatively short-lived birds, which communicate extensively with colour signals—blues and ultraviolets in particular. A family of basal oscines that fits these criteria well is the Maluridae, the 14 species of fairy-wrens, grass-wrens and emu-wrens, endemic to Australia and New Guinea.

In this study, we have used genomic DNA sequencing on a dense sample of Maluridae taxa to estimate the $\lambda_{\text{max}}$ of the SWS1 cone opsin and determine VS or UVS affinity. We have also analysed colour variation of male breeding plumage—the presence of short wavelength reflectance—in relation to any changes between spectral sensitivity classes.

2. MATERIAL AND METHODS

(a) Opsin gene analysis

To estimate approximate spectral sensitivity, i.e. UVS or VS affinity, we chose to perform molecular sequencing of the SWS1 single-cone opsin gene, targeting the second alpha-helical transmembrane region. The sheer number of birds rendered any other established method, i.e. ERG (electroretinography), MSP, in vitro regeneration of photopigments or behavioural testing, prohibitively time-consuming. DNA extractions from tissue samples, PCR and sequencing, as well as $\lambda_{\text{max}}$ calculation from the key amino acid (aa) residues 86, 90 and 93, followed the protocols outlined in Ödeen & Hästad [8] and Ödeen et al. [6]. We used the forward primers SU200Ca, 5'-AYTACATCYTGGTGAAACATCTCC-3', SU200a in (sequencing only), 5'-AYTACATCYTGGTGAA CACTCCS-3' [6], SU193a, 5'-CCSCTYAAYTACATCC TGTT-3', SU161a, 5'-KGCTACCCRTYMRKTAACAA-3' and SU149a, 5'-CCRTSCTSDRSG7TCAC-3' [8], and the reverse primers SU306b, 5'-SCITTSCGAAGAYR AAAGT-3' [6] (in sequencing only) and SU306b, 5'-SYBCT TSCCGAAGAYRAAGTF-3' [8]. Our sample comprised 32 Maluridae individuals from 16 species and a total of 21 subspecies, plus three species of bowerbirds (Ptilonorhynchidae), one individual each, as outgroups (table 1 and figure 1).

(b) Spectrometry

Measurements from 320 to 700 nm of plumage colour were taken on specimens from museum collections in Australia and the USA, typically with an Ocean Optics USB2000 spectroradiometer and a PX-2 xenon light source (Ocean Optics, Dunedin, FL, USA), and calibrated against a WS-1 white standard, which reflects greater than 98 per cent from 250 to 1500 nm wavelengths. Measurements were taken at 90° angle to the feathers of the crown, back, tail, throat, belly and wing coverts of three breeding-coloured males from each taxon, with the exception of Malurus cyanopeplus cyanopeplus. M. c. myorensis, M. leucipterus edouardi and Cbytonyas insignis (one male each). An Ocean Optics S2000 spectroradiometer, Top Sensor Systems DH2000 deuterium–halogen light source and Avantes WS-2 white standard were used on the latter two taxa. More details of our spectrometrical measurements are presented in Armenta et al. [33]. Each patch was measured five times, and an average spectrum was calculated for each patch of every individual. Readings with a maximum reflectance of less than 5 per cent were excluded to minimize measurement noise. If all readings from a single patch were excluded, the average spectrum was replaced by achromatic grey.

The taxa for which spectrometer readings were obtained were emperor fairy-wrens M. c. cyanopeplus and M. c. myorensis, lovely fairy-wren M. amabilis, variegated fairy-wrens M. lamberti lamberti and M. assimilis, blue-breasted fairy-wren M. pulcherimus, red-winged fairy-wren M. elegans, superb fairy-wren M. cyanus cyanus, splendid fairy-wrens M. splendens splendens and M. i. melanotus, purple-crowned fairy-wren M. coronatus macgillivrayi, white-shouldered fairy-wren M. albocapillus kutubu, red-backed fairy-wren M. melanopeplus melanopeplus, white-winged fairy-wrens M. leucipterus edouardi and M. i. leuconotus, orange-crowned fairy-wren Cbytonyas insignis oorti and striated grass-wren Amytornis striatus. The majority of specimens were collected in the 1990’s and only the C. insignis, M. c. cyanopeplus and M. c. myorensis more than 50 years ago (1958, 1931 and 1896). Plumage samples less than 50 years old should not have changed considerably in plumage reflectance compared with live birds [34].

(c) Colour space plots

We modelled spectral sensitivities for the Maluridae species using SWS1 $\lambda_{\text{max}}$ estimated from the aa sequences and data from other species for all missing parameters, following the studies of Hart & Vorobyev [26] and Govardovskii et al. [35]. Judged by the MSP data available to date, spectral sensitivities of VS cones seem to form two clusters, which one may call ‘Pigeon VS’ ($\lambda_{\text{max}}$ 404–406 nm) and ‘Chicken VS’ ($\lambda_{\text{max}}$ 415–426 nm) [5,36,37]. The opsin genetics defining these types is currently unknown. We chose to model spectral sensitivities for the VS Maluridae species using pigeon sensitivities [26] owing to the closer evolutionary affinities between passerines and Pigeon VS species. We, however, assumed an ultraviolet transparent ocular media owing to the small eye size and to reduce the number of assumed physiological differences between closely related VS and UVS species. Spectral sensitivities of UVS species were modelled with data for the bluetit [26]. An average patch value for each species was calculated and plotted in VS and UVS quantum catch tetrahedra (figure 2).

(d) Character correlation analysis

We tested independence in the evolution of spectral sensitivity and plumage coloration treated as binary states: UVS or VS single cones and presence or absence of shortwave-dominated (blue to humans) patches in breeding male plumage. The Pagel [38] correlation method was used as implemented
Table 1. SWS1 opsin genes fragments and the type of colour vision, violet- or ultraviolet-sensitive (VS or UVS), predicted from the spectral tuning amino acid (aa) sites 86, 90 and 93, marked in bold (see §4). (Further detail on sampling location is available at ENA http://www.ebi.ac.uk/ena/data/view/)<ACCESSION NUMBERS HE588090-HE588124>.)

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\(^a\)Academy of Natural Sciences, Philadelphia (ANSP), Australian Museum (O), Australian National Wildlife Collection (ANWC), Museum of Victoria, Melbourne (MVM), University Kansas Museum of Natural History (Kam, KUNHM), University of Washington, Burke Museum (UWBM), Western Australian Museum (WAM).

\(^b\)Odeen & Hästad [9], GenBank accession number: GQ305959.
bird species, *Ailuroedus crassirostris* (Meliphagidae: see [9]) by the apparent aa substitution base-pair identical, differing from other Meliphagoidea Malurus transition in Val94. The remaining six Met85Leu and a synonymous T to C third position trans-

M. amabilis
M. splendens melanotus
M. amabilis 1
M. pulcherrimus 1
M. coronatus 2
M. cyanocephalus 1
M. amabilis 1
M. pulcherrimus 1
M. cyanocephalus 1
M. amabilis 1
M. coronatus 1
M. splendens 1
M. melanocephalus 3
M. grayi 1
Clytomyias insignis 1
Stipiturus mallee 1
Amytornis striatus 1
Amytornis barbatus 1
Ptilonorhynchidae 3

Figure 1. Type of colour vision in the family Maluridae and closely related taxa (UVS, black and VS, white) predicted from SWS1 opsin gene fragments and mapped onto two different molecular gene tree reconstructions. Phylogenies have been reproduced from (a) Driskell et al. [31] and (b) Gardner et al. [32]. *Acanthorhynchus tenuirostris* in (a) and Ptilonorhynchidae in (b) correspond to outgroups used in the phylogenetic reconstructions. Taxa with shortwave-dominated plumage patches are shown in bold font. Number of individuals for which the opsin gene has been sequenced is listed after each taxon.

in Mesquite [39] with the following parameters: estimating p-values, 20 iterations, all branch lengths set to 1 and 200 replicates. We defined shortwave-dominated patches as those where more than 60 per cent of the total visible reflectance (320–700 nm) falls inside the spectral sensitivity ranges of the SWS1 and SWS2 cone classes (320–510 nm). Two recent gene-based phylogenetic reconstructions (figure 1) were tested using the same character matrix.

3. RESULTS

(a) Opsin gene analysis

PCR amplified fragments of the SWS1 opsin gene were 64, 74, 107 or 119 bp long, with primer SU200Ca, SU193a, SU161a or SU149a, respectively, paired with SU306b. Overlapping strands of identifiable nucleotides spanned, at the least, the aa sites 84–94. aa translation showed that the opsin of *Malurus grayi*, *M. cyanochlamys*, *M. coronatus*, *M. alboscopatus*, *M. melanophaeus*, *M. leucophaeus* and the non-*Malurus* Maluridae species contains the residue Ser90, followed by Val91 (table 1 and figure 1). The gene sequences of these taxa are base-pair identical, differing from other Meliphagoidea (Meliphagidae: see [9]) by the apparent aa substitution Met85Leu and a synonymous T to C third position transition in Val94. The remaining six *Malurus* species, *M. amabilis*, *M. lamberti*, *M. pulcherrimus*, *M. elegans*, *M. cyaneus* and *M. splendens* as well as two of the bowerbird species, *Ailuroedus crassirostris* and *Chlamydera nuchalis*, are base-pair identical to the other Maluridae, except for the aa residues Cys90 (codon AGC replaced by GTC) and Ile91 (GTC to ATC; table 1 and figure 1).

The remaining bowerbird, *Sericulus chrysocephalus*, shows a synonymous T to C third position transition in Thr93. Ser90 is common to all VS birds, identified with MSP: see [6]. Furthermore, site-directed mutagenesis has demonstrated that Ser90Cys and its reverse substitution Cys90Ser, shift avian SWS1 pigment $\lambda_{\text{max}}$ by $-29$ to $-46$ nm and 35–60 nm, respectively [10,40–42]. The taxa with Ser90 should hence be VS and those with Cys90 be UVS.

The DNA sequences presented in this study have been deposited in the European Nucleotide Archive (ENA) under accession numbers HE588090-HE588124.

(b) Colour space plots

As can be seen in figure 2, all significant ultraviolet reflection in the plumage patches was associated with a strong reflectance in the human visible range (i.e. strong SWS1 cone stimulation was always associated with SWS2 stimulation). Two main spectral shapes were found, SWS1–SWS2 or long wavelength-sensitive (LWS) stimulation, plus the combination of both (in *M. coronatus*).

(c) Character correlation analysis

The following taxa were classified as carrying shortwave-dominated (blue) patches according to the 60 per cent criterion: *M. cyanochlamys*, *M. amabilis*, *M. lamberti*, *M. pulcherrimus*, *M. elegans*, *M. cyaneus*, *M. splendens*, *M. coronatus* and *M. l. leuconotus* (bold in figure 1). Although we did not have access to skins from *M. grayi* or *Stipiturus mallee*, available images clearly show that these species carry large patches of blue plumage. These
two VS species were, therefore, coded as shortwave-dominated. If erroneous, this tentative classification would have a conservative effect on our test results, biasing them against a correlation between presence of UVS cones and shortwave plumage.

Tested according to the phylogeny in figure 1a [31], the log likelihood for the four parameter model (independent evolution between VS–UVS shifts and shortwave plumage) was −219.5 and for the eight parameter model (dependent changes) was −214.3 with a simulated $p$-value of 0. According to the alternative phylogeny (figure 1b) [32], log likelihoods were −15.5 and −10.1, respectively, with $p = 0$.

4. DISCUSSION

(a) Spectral sensitivity

Contrary to what has been found in any other investigated avian genus (cf. [12]), Malurus contains both species with aa residues typical of VS and of UVS birds. The evolution of UVS vision in Passeriformes is hence more complex than previously indicated. Evidently, a single shift from VS colour vision in an ancestor of Passerida does not suffice to explain the phylogenetic distribution of ultraviolet sensitivity in Passeriformes.

The molecular evidence mapped onto two recent gene-based phylogenies (figure 1) suggests that Maluridae is ancestrally a VS family, similar to other non-passerida passerines [8,9,22,24] (GenBank accession number DQ451006), such as bowerbirds (Ptilonorhynchidae: this study). The three bowerbird species from different genera have VS-type sequences identical to the VS Maluridae bar-ring one synonymous substitution. Within Maluridae, there are two clades of fairy-wrens with UVS-type opsins resulting from the aa substitution Ser90Cys combined with Val91Ile. These two UVS clades are not resolved as sisters in either phylogeny, and a hypothesis of two independent gains of UVS opsins is the most parsimonious reconstruction of character evolution for one tree [31] (figure 1a), which suggests the possibility of parallel
evolution of UVS from VS opsin involving double substitutions (at aa sites 90 and 91), in the Maluridae. A scenario of one gain of UVS opsin and a subsequent loss is the most parsimonious reconstruction for the other phylogenetic hypothesis [32] (figure 1b). However, the phylogeny in Driskell et al. [31] is based on extensive sampling of fairy-wrens, while a number of Malurus species are missing from the analysis of Gardner et al. [32]. If added they may change the topology of the tree in this region. Support for some nodes of both phylogenies is only moderately strong, but two or more need to collapse to yield a one gain, one loss reconstruction on the tree from the study of Driskell et al. [31] (figure 1a).

The aa residue combinations Ser90 + Val91 and Cys90 + Ile91 are common to all birds that have been demonstrated by retinal MSP to be VS and UVS, respectively (see [6]). Only some terns (Sternaeidae) combine Ser90 with Ile91, among the bird species that have been sequenced so far [12]. Most published SWS1 opsin sequences are as short as ours, which highlights the possibility of other associations with site 90 falling outside the sequenced gene fragments. We find it unlikely that Val91Ile would counteract the VS shift expected from Cys90Ser because Ile91 is not UVS-specific in any other MSP-investigated vertebrates than birds [43]. Neither do we expect the Thr93 residue common to Maluridae to influence spectral tuning to a large degree, since it is present in VS and UVS species alike (see review in Ödeen et al. [6]) and since mutagenesis at the site has produced only a 3 nm shift in $\lambda_{\text{max}}$ [40]. The implications of Ser90 being combined with Cys86, which is a pattern that has been demonstrated in other passerines too [8,9,22], are less clear, as Ser90 is always paired with Ser86 in MSP-investigated birds. As demonstrated by Carvalho et al. [10] however, the pigment of the common cormorant Phalacrocorax carbo, which naturally combines Cys86 and Ser90, maximally absorbs at 405 nm (VS) when regenerated and expressed in vitro. Furthermore, site-directed mutagenesis substituting Ser86 for Cys86 has failed to produce a shortwave shift in the Ser90 pigment of the VS pigeon Columba livia [10].

(b) Plummage colour
The fact that shifts between the UVS and VS type of SWS1 opsin gene have simple molecular mechanisms, but are rare among higher order avian taxa [8] suggests generally strong selection against the resulting shifts in colour vision. Selection on VS colour vision in Maluridae should thus have been relaxed or reversed for some reason in the past. New and different habitats might have changed the visual environment to that effect. Ambient light, reflectance of the background and spectral sensitivity are critical determinants in colour perception of items in the physical world [44], such as potential mates or food. It is likely that shifts to the shorter wavelength sensitivity of UVS single cones are selected for in relative to ultraviolet-rich, open habitats. Passerines, such as redwing Turdus iliacus [45] and bluetit Cyanistes caeruleus [46] have been shown to use ultraviolet contrast in foraging. Furthermore, ultraviolet has a naturally low intensity compared with longer wavelengths even in unobstructed daylight, which renders retinae with UVS single cones inherently noisier than VS retinae [30]. Although the fairy-wren species with UVS vision (bold) do not occupy a unique habitat relative to other species in the family (table 2), in general, they occur in habitats characterized by a mixture of open, sparsely vegetated areas and open or closed forests. This pattern of distribution contrasts with the VS species, which occupy primarily open habitats (Malurus alboscapulatus, M. melanocephalus, M. leucopterus, Stipiturus spp., Amytornis spp.) and those VS species that occur primarily in forested habitats (M. gravi, M. cyanocephalus, M. coronatus, C. insignis).

Instead, sexual selection and perception of male breeding plumage may offer plausible explanations to why UVS colour vision has been favoured in Maluridae species. A shift from VS to UVS colour vision has the effect of differentiating visibility of social signals to conspecifics and predators [48]. An obvious feature of the mixed habitats typically occupied by UVS fairy-wrens is the much higher number of avian predators one finds there compared with other Maluridae habitats (S. Pruett-Jones, 1998–2010 personal observation). Raptors, which are important predators on small passerines, have the VS type of SWS1 opsin gene [8,22]. By exploiting differences in spectral sensitivity, the UVS fairy-wrens may maximize the visibility of their plumage to other UVS birds, while limiting the correlated increase in conspicuousness to VS birds, such as raptors (cf. [48]).

The character correlation analyses significantly reject evolutionary independence between spectral sensitivity

### Table 2. Natural habitats of the Maluridae species included in this study. (Species with UVS single cones, as predicted from a Cys90 aa residue in the SWS1 opsin gene, are bold. Habitat classification is from Rowley & Russell [47].)

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<tr>
<td>Malurus cyanopeplus</td>
<td>tropical lowland forests</td>
</tr>
<tr>
<td>Malurus ambilis</td>
<td>tropical grasslands, open tropical forest, second growth areas</td>
</tr>
<tr>
<td>Malurus lamberti, Malurus splendens</td>
<td>scrub, mixed forest and grassland, savannah, scattered trees</td>
</tr>
<tr>
<td>Malurus cyaneus</td>
<td>parkland habitat, mixed forest and open areas, not dense forest</td>
</tr>
<tr>
<td>Malurus coronatus</td>
<td>dense riparian vegetation, scattered trees, subtropical areas</td>
</tr>
<tr>
<td>Malurus albescapulatus</td>
<td>tropical grasslands, few and scattered trees</td>
</tr>
<tr>
<td>Malurus melanocephalus</td>
<td>subtropical savannah, open forests, second growth areas, edge habitat</td>
</tr>
<tr>
<td>Malurus leucopterus</td>
<td>desert grasslands, scrub, few or scattered trees</td>
</tr>
<tr>
<td>Malurus alboscapulatus</td>
<td>tropical forests, edge habitat, second growth areas</td>
</tr>
<tr>
<td>Malurus pulcherrimus, Malurus elegans</td>
<td>desert grasslands, coastal heath, few if any trees</td>
</tr>
<tr>
<td>Malurus splendens, Malurus lamberti</td>
<td>swamp floodplains, dense scrub habitat, few canopy trees</td>
</tr>
<tr>
<td>Amytornis barbatus</td>
<td>desert grasslands, scrub, few or scattered trees</td>
</tr>
<tr>
<td>Amytornis striatus</td>
<td>tropical lowland forests</td>
</tr>
</tbody>
</table>


and plumage coloration, despite small sample sizes. The UVS clades *M. cyaneus* plus *M. splendens* and the chestnut-shouldered fairy-wrens *M. lamberti*, *M. pulcherrimus*, *M. amabilis* plus *M. elegans* bear shortwave plumage without exception and evidence of strong species and subspecies differentiation in coloration [47]. From the presence of shortwave-dominated plumage patches in *M. cyanocephalus* (figure 1a) and *M. leucopeterus* (figure 1b), we may deduce that acquisition of shortwave-dominated plumage patches preceded changes to UVS cones. Perhaps, an associated improvement in shortwave colour discriminability favoured further elaboration of plumage signals in this part of the spectrum, which in turn selected against shifting back to VS cones.

Shifts from violet to ultraviolet spectral sensitivity are simple in molecular terms, yet phylogenetically rare. It is likely that the new spectral sensitivity rarely reaches fixation. Our results support a link between shortwave signalling and ultraviolet sensitivity, perhaps surprisingly, suggesting that shortwave plumage signalling facilitates fixation of the UVS genotype, rather than UVS vision pushing colour signals towards the shortwave end of the spectrum directly.

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### References


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