

Supplementary Material

Visual pigments in a palaeognath bird, the emu *Dromaius novaehollandiae*: implications for spectral sensitivity and the origin of ultraviolet vision

Nathan S. Hart^{1,2*}, Jessica K. Mountford^{2,3,4}, Wayne I. L. Davies^{2,3,4}, Shaun P. Collin^{2,3,4} and David M. Hunt^{2,4}

¹Department of Biological Sciences, Macquarie University, North Ryde, NSW 2109, Australia

²School of Animal Biology, University of Western Australia, Crawley, WA 6009, Australia

³Oceans Institute, University of Western Australia, Crawley, WA 6009, Australia

⁴Lions Eye Institute, University of Western Australia, Nedlands, WA 6009, Australia

*Corresponding author: nathan.hart@mq.edu.au

Running title: Emu photoreceptors and visual pigments

Key words: Spectral tuning, opsin evolution, microspectrophotometry, oil droplets, Casuariiformes

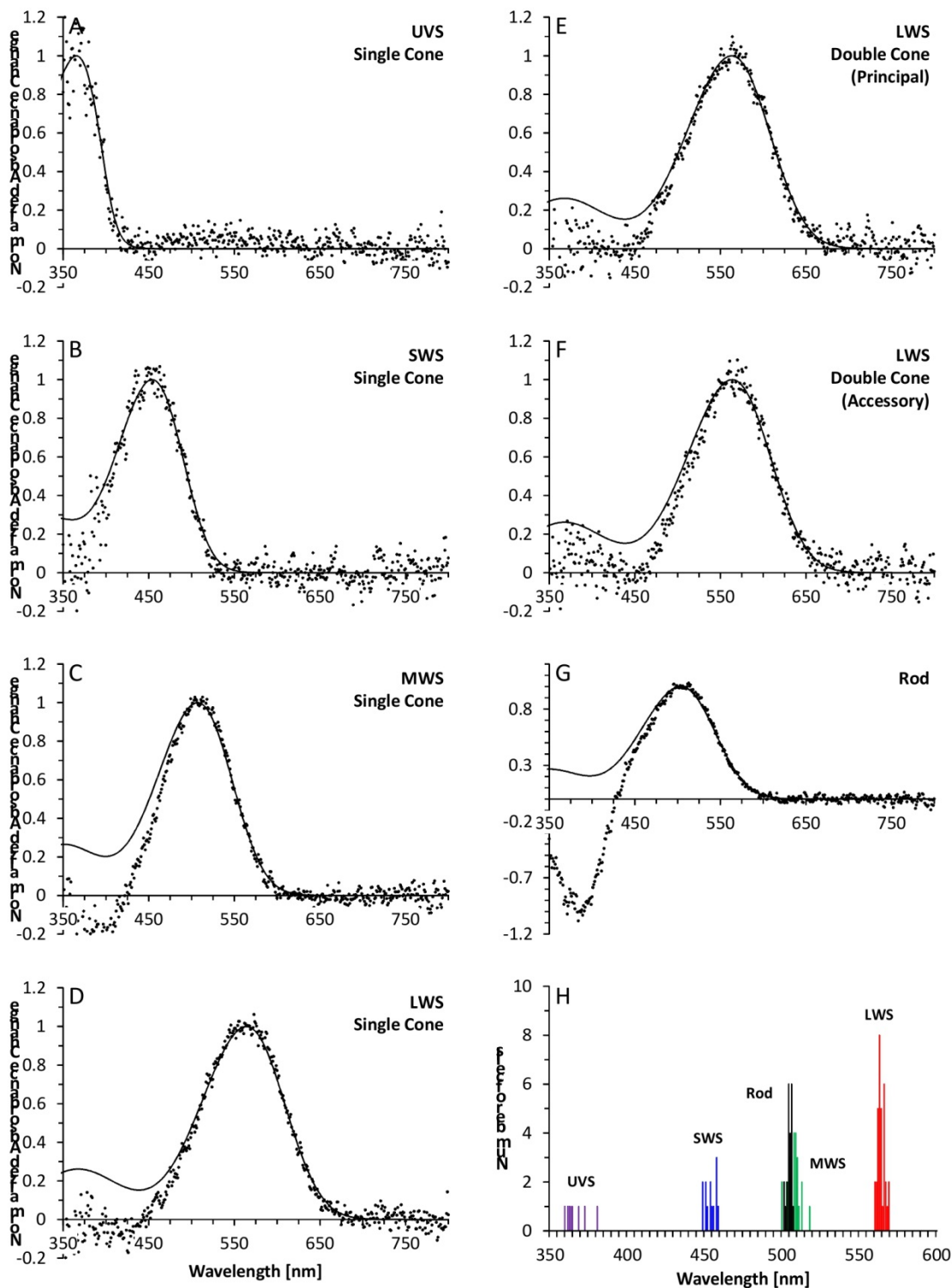


Figure S1. (a–g) Normalised mean bleaching difference spectra (black circles) of visual pigments measured using microspectrophotometry from emu photoreceptor outer segments. Difference spectra are overlaid with best-fit rhodopsin (vitamin A1) templates (black line). (h) Histogram shows the spectral distribution of the wavelength of maximum absorbance change (λ_{max}) values for individual photoreceptor cell outer segments that were used to generate the mean spectra. The λ_{max} value distribution of long-wavelength-sensitive (LWS) pigments includes measurements from LWS single cones as well as both the principal and accessory members of double cones. UVS, ultraviolet-sensitive; SWS, short-wavelength-sensitive; MWS, medium-wavelength-sensitive.

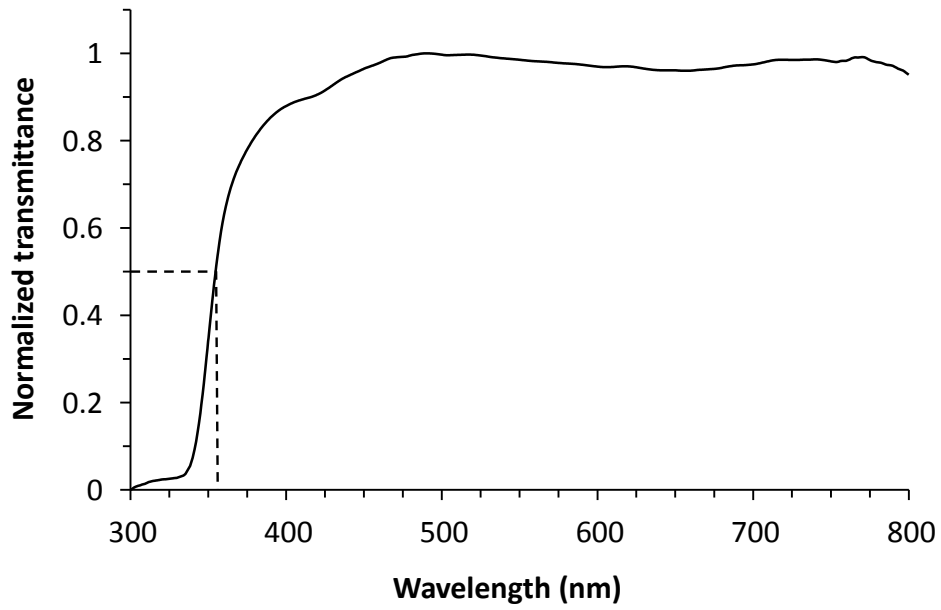


Figure S2. Normalised mean spectral transmittance of the combined ocular media (cornea, aqueous humour and lens) of the emu ($n = 2$ eyes from different birds). The wavelength at 0.5 normalised transmittance ($\lambda_{T0.5}$) is 355 nm and is indicated by the dashed line.

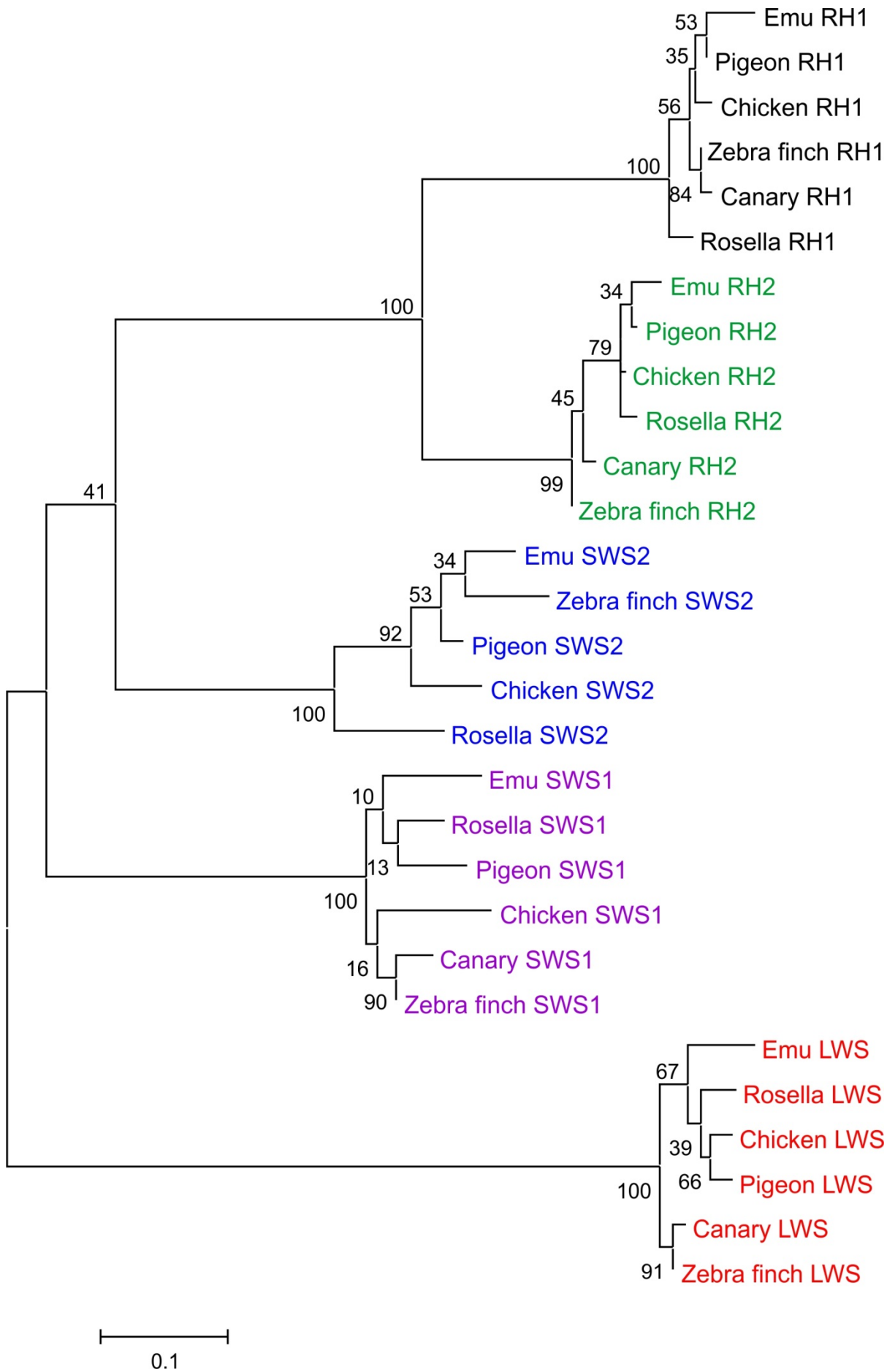


Figure S3. Maximum Likelihood phylogenetic analysis of emu (*Dromaius novaehollandiae*) LWS, SWS1, SWS2, RH2 and RH1 visual opsins compared to orthologues present in other representative birds with *Drosophila* RH1 opsin (GenBank accession no. X65877) as an outgroup (not shown). The degree of support for internal branching is expressed as a percentage with the scale bar indicating the number of amino acid substitutions per site. The

sequences used for generating the tree are as follows: (1) RH1 opsin class: emu (*Dromaius novaehollandiae*), KU568456; pigeon (*Columba livia*), AH007730; chicken (*Gallus gallus*), NM001030606; zebra finch (*Taeniopygia guttata*), NM001076695; canary (*Serinus canaria*), AJ277926; crimson rosella (*Platycercus elegans*), KF134487; (2) RH2 opsin class: emu (*Dromaius novaehollandiae*), KU568455; pigeon (*Columba livia*), AH007731; chicken (*Gallus gallus*), M92038; crimson rosella (*Platycercus elegans*), KF134489; canary (*Serinus canaria*), AJ277924; zebra finch (*Taeniopygia guttata*), NM001076696; (3) SWS2 opsin class: emu (*Dromaius novaehollandiae*), KU568454; zebra finch (*Taeniopygia guttata*), NM001076697; pigeon (*Columba livia*), AH007799; chicken (*Gallus gallus*), NM205517; crimson rosella (*Platycercus elegans*), KF134491; (4) SWS1 opsin class: emu (*Dromaius novaehollandiae*), KU568453; crimson rosella (*Platycercus elegans*), KF134492; pigeon (*Columba livia*), AH007798; chicken (*Gallus gallus*), NM205438; canary (*Serinus canaria*), AJ277922; zebra finch (*Taeniopygia guttata*), NM001076704; and (5) LWS opsin class: emu (*Dromaius novaehollandiae*), KU568452; crimson rosella (*Platycercus elegans*), KF134493; chicken (*Gallus gallus*), NM205440; pigeon (*Columba livia*), AH007800; canary (*Serinus canaria*), AJ277925; zebra finch (*Taeniopygia guttata*), NM001076702.

SWS1 sequences

Zebra finch M-DEEEFYLFKNGSSVGPWDGQYHIAPMWAFYLTQIFMGLVFAVGTPLNAIVLIVTIKY
 Canary M-DEEEFYLFKNGSSVGPWDGQYHIAPMWAFYLTQIFMGLVFAVGTPLNAIVLIVTIKY
 Chicken MSSDDDFYLFPTNGSSVGPWDGQYHIAPMWAFYLTQIFMGLVFAVGTPLNAIVLIVTIKY
 Pigeon MSGDEEFYLFKNGSSVGPWDGQYHIAPMWAFYLTQIFMGLVFAVGTPLNAIVLIVTIKY
 Rosella MSGDEEFYLFKNGSSVGPWDGQYHIAPMWAFYLTQIFMGLVFAVGTPLNAIVLIVTIKY
 Emu -----SSVGPWDGQYHIAPMWAFYLTQIFMGLVFAVGTPLNAIVLIVTIKY

86 93

Zebra finch KKLRLQPLNLYLVNLSVSGIMCCVFCIPTVFVASSQGYFVFGHMCFAFBGAGATGGVLTG
 Canary KKLRLQPLNLYLVNLSVSGIMCCVFCIPTVFVASSQGYFVFGHMCFAFBGAGATGGVLTG
 Chicken KKLRLQPLNLYLVNLSVSGIMCCVFCIPTVFVASSQGYFVFGHMCFAFBGAGATGGVLTG
 Pigeon KKLRLQPLNLYLVNLSVSGIMCCVFCIPTVFVASSQGYFVFGHMCFAFBGAGATGGVLTG
 Rosella KKLRLQPLNLYLVNLSVSGIMCCVFCIPTVFVASSQGYFVFGHMCFAFBGAGATGGVLTG
 Emu -----KKLRLQPLNLYLVNLSVSGIMCCVFCIPTVFVASSQGYFVFGHMCFAFBGAGATGGVLTG

Zebra finch WSLAFLAFERYIVICKPFGNFRFNSRHALLVVAATWIIIGVGVATIPFFGWSRYIEEGLQC
 Canary WSLAFLAFERYIVICKPFGNFRFNSRHALLVVAATWIIIGVGVATIPFFGWSRYIEEGLQC
 Chicken WSLAFLAFERYIVICKPFGNFRFNSRHALLVVAATWIIIGVGVATIPFFGWSRYIEEGLQC
 Pigeon WSLAFLAFERYIVICKPFGNFRFNSRHALLVVAATWIIIGVGVATIPFFGWSRYIEEGLQC
 Rosella WSLAFLAFERYIVICKPFGNFRFNSRHALLVVAATWIIIGVGVATIPFFGWSRYIEEGLQC
 Emu -----WSLAFLAFERYIVICKPFGNFRFNSRHALLVVAATWIIIGVGVATIPFFGWSRYIEEGLQC

Zebra finch SOGPDWYTVGTYKYS EYTWLFLIFCFIIVPLLSLII FSYQLLSALRAVAQQQSATTQK
 Canary SOGPDWYTVGTYKYS EYTWLFLIFCFIIVPLLSLII FSYQLLSALRAVAQQQSATTQK
 Chicken SOGPDWYTVGTYKYS EYTWLFLIFCFIIVPLLSLII FSYQLLSALRAVAQQQSATTQK
 Pigeon SOGPDWYTVGTYKYS EYTWLFLIFCFIIVPLLSLII FSYQLLSALRAVAQQQSATTQK
 Rosella SOGPDWYTVGTYKYS EYTWLFLIFCFIIVPLLSLII FSYQLLSALRAVAQQQSATTQK
 Emu -----SOGPDWYTVGTYKYS EYTWLFLIFCFIIVPLLSLII FSYQLLSALRAVAQQQSATTQK

Zebra finch AEREVSRLVVMVGSFCMCFVYAAALAMVNNRHGLDLRLVITPAF FSKSSCVYNIIP
 Canary AEREVSRLVVMVGSFCMCFVYAAALAMVNNRHGLDLRLVITPAF FSKSSCVYNIIP
 Chicken AEREVSRLVVMVGSFCMCFVYAAALAMVNNRHGLDLRLVITPAF FSKSSCVYNIIP
 Pigeon AEREVSRLVVMVGSFCMCFVYAAALAMVNNRHGLDLRLVITPAF FSKSSCVYNIIP
 Rosella AEREVSRLVVMVGSFCMCFVYAAALAMVNNRHGLDLRLVITPAF FSKSSCVYNIIP
 Emu -----A

Zebra finch YCFMKNQFRACIMETVCGRPMTDDSDVSSAQRTEVSSVSSSQVGPS----
 Canary YCFMKNQFRACIMETVCGRPMTDDSDVSSAQRTEVSSVSSSQVGPS----
 Chicken YCFMKNQFRACIMETVCGRPMTDDSDVSSAQRTEVSSVSSSQVGPS----
 Pigeon YCFMKNQFRACIMETVCGRPMTDDSDVSSAQRTEVSSVSSSQVGPS----
 Rosella YCFMKNQFRACIMETVCGRPMTDDSDVSSAQRTEVSSVSSSQVGPS----
 Emu -----YCFMKNQFRACIMETVCGRPMTDDSDVSSAQRTEVSSVSSSQVGPS----

RH2 sequences

Zebra finch MNGTBEINFEVPMNKTGVVRSPEEYPCYLAEPWKYRACVCIIFFLISGTFINFLITLL
 Canary MNGTBEINFEVPMNKTGVVRSPEEYPCYLAEPWKYRACVCIIFFLISGTFINFLITLL
 Chicken MNGTBEINFEVPMNKTGVVRSPEEYPCYLAEPWKYRACVCIIFFLISGTFINFLITLL
 Pigeon MNGTBEINFEVPMNKTGVVRSPEEYPCYLAEPWKYRACVCIIFFLISGTFINFLITLL
 Rosella MNGTBEINFEVPMNKTGVVRSPEEYPCYLAEPWKYRACVCIIFFLISGTFINFLITLL
 Emu -----MNGTBEINFEVPMNKTGVVRSPEEYPCYLAEPWKYRACVCIIFFLISGTFINFLITLL

83 86 97

Zebra finch VTEKHKIKRQPLNLYLVNLAADLMAFCFIVTFTYANNGYFVFGVPGVCAVBGFPAATLG
 Canary VTEKHKIKRQPLNLYLVNLAADLMAFCFIVTFTYANNGYFVFGVPGVCAVBGFPAATLG
 Chicken VTEKHKIKRQPLNLYLVNLAADLMAFCFIVTFTYANNGYFVFGVPGVCAVBGFPAATLG
 Pigeon VTEKHKIKRQPLNLYLVNLAADLMAFCFIVTFTYANNGYFVFGVPGVCAVBGFPAATLG
 Rosella VTEKHKIKRQPLNLYLVNLAADLMAFCFIVTFTYANNGYFVFGVPGVCAVBGFPAATLG
 Emu -----VTEKHKIKRQPLNLYLVNLAADLMAFCFIVTFTYANNGYFVFGVPGVCAVBGFPAATLG

Zebra finch GQVALWSLVVLAIERYIVICKPFGNFRFNSRHALLVVAATWIIIGVGVATIPFFGWSRYIEEGLQC
 Canary GQVALWSLVVLAIERYIVICKPFGNFRFNSRHALLVVAATWIIIGVGVATIPFFGWSRYIEEGLQC
 Chicken GQVALWSLVVLAIERYIVICKPFGNFRFNSRHALLVVAATWIIIGVGVATIPFFGWSRYIEEGLQC
 Pigeon GQVALWSLVVLAIERYIVICKPFGNFRFNSRHALLVVAATWIIIGVGVATIPFFGWSRYIEEGLQC
 Rosella GQVALWSLVVLAIERYIVICKPFGNFRFNSRHALLVVAATWIIIGVGVATIPFFGWSRYIEEGLQC
 Emu -----GQVALWSLVVLAIERYIVICKPFGNFRFNSRHALLVVAATWIIIGVGVATIPFFGWSRYIEEGLQC

Zebra finch EGMQCS CGE DYYTHNDPHNESVYLYMFI IHF IIPVWVIFFS YGRLIKVR BAAQQQES
 Canary EGMQCS CGE DYYTHNDPHNESVYLYMFI IHF IIPVWVIFFS YGRLIKVR BAAQQQES
 Chicken EGMQCS CGE DYYTHNDPHNESVYLYMFI IHF IIPVWVIFFS YGRLIKVR BAAQQQES
 Pigeon EGMQCS CGE DYYTHNDPHNESVYLYMFI IHF IIPVWVIFFS YGRLIKVR BAAQQQES
 Rosella EGMQCS CGE DYYTHNDPHNESVYLYMFI IHF IIPVWVIFFS YGRLIKVR BAAQQQES
 Emu -----EGMQCS CGE DYYTHNDPHNESVYLYMFI IHF IIPVWVIFFS YGRLIKVR BAAQQQES

Zebra finch ATTQKAKEVTRMVLMLVGLFPLAWTPYVAWAIPFTNKGDFTATLMSVAFPSKSSSL
 Canary ATTQKAKEVTRMVLMLVGLFPLAWTPYVAWAIPFTNKGDFTATLMSVAFPSKSSSL
 Chicken ATTQKAKEVTRMVLMLVGLFPLAWTPYVAWAIPFTNKGDFTATLMSVAFPSKSSSL
 Pigeon ATTQKAKEVTRMVLMLVGLFPLAWTPYVAWAIPFTNKGDFTATLMSVAFPSKSSSL
 Rosella ATTQKAKEVTRMVLMLVGLFPLAWTPYVAWAIPFTNKGDFTATLMSVAFPSKSSSL
 Emu -----ATTQKAKEVTRMVLMLVGLFPLAWTPYVAWAIPFTNKGDFTATLMSVAFPSKSSSL

Zebra finch YNPIIYVIMNKGFRNCMITITICOGKNPFGDEETSSTVSQSKTEVSSVSSSQVSPA
 Canary YNPIIYVIMNKGFRNCMITITIT-----
 Chicken YNPIIYVIMNKGFRNCMITITICOGKNPFGDEDSSTVSQSKTEVSSVSSSQVSPA
 Pigeon YNPIIYVIMNKGFRNCMITITICOGKNPFGDEDSSTVSQSKTEVSSVSSSQVSPA
 Rosella YNPIIYVIMNKGFRNCMITITICOGKNPFGDEETSSTVSQSKTEVSSVSSSQVSPA
 Emu -----YNPIIYVIMNKGFRNCMITITICOGKNPFGDEETSSTVSQSKTEVSSVSSSQVSPA

SWS2 sequences

Zebra finch MPKP REMDELEDFYI PMSLETPLNTALSPFLVQTHLGSFGPKAMAAFMFLVLLGV
 Chicken MHPF RPTD-LP EDFYI PMALDAPNITALSPLVQTHLGSFGPKAMAAFMFLVLLGV
 Pigeon MQRAREARDELDFYI PMALDAPNITALSPLVQTHLGSFGPKAMAAFMFLVLLGV
 Rosella -----PQTHLGSAGLFAAMAAFMFLVLLGV
 Emu -----

Zebra finch PINALTICTAKYKLRSHLNYLVNLAANLVLCVGS TTAAYFSQMYFALGPTACKI
 Chicken PINALTICTARFKLRSHLNYLVNLAANLVLCVGS TTAAYFSQMYFALGPTACKI
 Pigeon PINALTICTARFKLRSHLNYLVNLAANLVLCVGS TTAAYFSQMYFALGPTACKI
 Rosella PINALTICTARFKLRSHLNYLVNLAANLVLCVGS TTAAYFSQMYFALGPTACKI
 Emu -----PINALTICTARFKLRSHLNYLVNLAANLVLCVGS TTAAYFSQMYFALGPTACKI

Zebra finch EGFATLGGMVSLSLAWAFERFLVICKPLGNFTFRGSHAVLGCATWIFGLIASLPL
 Chicken EGFATLGGMVSLSLAWAFERFLVICKPLGNFTFRGSHAVLGCATWIFGLIASLPL
 Pigeon EGFATLGGMVSLSLAWAFERFLVICKPLGNFTFRGSHAVLGCATWIFGLIASLPL
 Rosella EGFATLGGMVSLSLAWAFERFLVICKPLGNFTFRGSHAVLGCATWIFGLIASLPL
 Emu -----EGMVSLSLAWAFERFLVICKPLGNFTFRGSHAVLGCATWIFGLIASLPL

Zebra finch FGWSRYIIFBGLQCS CGPDWYTVNNHWNNE SVVIFLFCFCGPGVPLTIVFSGYRLLITLRA
 Chicken FGWSRYIIFBGLQCS CGPDWYTVNNHWNNE SVVIFLFCFCGPGVPLTIVFSGYRLLITLRA
 Pigeon FGWSRYIIFBGLQCS CGPDWYTVNNHWNNE SVVIFLFCFCGPGVPLTIVFSGYRLLITLRA
 Rosella FGWSRYIIFBGLQCS CGPDWYTVNNHWNNE SVVIFLFCFCGPGVPLTIVFSGYRLLITLRA
 Emu -----FGWSRYIIFBGLQCS CGPDWYTVNNHWNNE SVVIFLFCFCGPGVPLTIVFSGYRLLITLRA

269

Zebra finch VARQQEQSASTQKAREVTRMVMVVMVGLFVLCWAPYAFALWVTHRGPFVGLIASIPS
 Chicken VARQQEQSASTQKAREVTRMVMVVMVGLFVLCWAPYAFALWVTHRGPFVGLIASIPS
 Pigeon VARQQEQSASTQKAREVTRMVMVVMVGLFVLCWAPYAFALWVTHRGPFVGLIASIPS
 Rosella VARQQEQSASTQKAREVTRMVMVVMVGLFVLCWAPYAFALWVTHRGPFVGLIASIPS
 Emu_SWS2 VARQQEQSASTQKAREVTRMVMVVMVGLFVLCWAPYAFALWVTHRGPFVGLIASIPS

Zebra finch VFSKAS TVNPIIYVFMNKGFRSCMLKLPFCGRS PFGDEEDVSSGSGATQVSSV-SS-
 Chicken VFSKAS TVNPIIYVFMNKGFRSCMLKLPFCGRS PFGDEEDVSSGSGATQVSSV-SS-
 Pigeon VFSKAS TVNPIIYVFMNKGFRSCMLKLPFCGRS PFGDEEDVSSGSGATQVSSV-SS-
 Rosella VFSKAS TVNPIIYVFMNKGFRSCMLKLPFCGRS PFGDEEDVSSGSGATQVSSV-SS-
 Emu -----VFSKAS TVNPIIYVFMNKGFRS-----

Zebra finch QVSPA
 Chicken QVSPA
 Pigeon QVSPA
 Rosella -----
 Emu -----

LWS sequences

Zebra finch MATGWDGAVFAARRRHEDEDTT RDSIFTYTNNT RGFEGFNHYIAPRWVYNTLSW
 Canary MATGWDGAVFAARRRHEDEDTT RDSIFTYTNNT RGFEGFNHYIAPRWVYNTLSW
 Chicken MAAWB-AAFAARRRHEDEDTT RDSVFTYTNNT RGFEGFNHYIAPRWVYNTLSW
 Pigeon MDGFAAARRRHEDEDTT RDSVFTYTNNT RGFEGFNHYIAPRWVYNTLSW
 Rosella MAAWB-AVMAARRRHEDEDTT RDSVFTYTNNT RGFEGFNHYIAPRWVYNTLSW
 Emu -----MAAWB-AVMAARRRHEDEDTT RDSVFTYTNNT RGFEGFNHYIAPRWVYNTLSW

Zebra finch IFVVAASVFTINGLVAVATAKFKLRHPLNWI LVNLAADLGETVIASITSVVQIFGYEYI
 Canary IFVVAASVFTINGLVAVATAKFKLRHPLNWI LVNLAADLGETVIASITSVVQIFGYEYI
 Chicken IFVVAASVFTINGLVAVATAKFKLRHPLNWI LVNLAADLGETVIASITSVVQIFGYEYI
 Pigeon IFVVAASVFTINGLVAVATAKFKLRHPLNWI LVNLAADLGETVIASITSVVQIFGYEYI
 Rosella IFVVAASVFTINGLVAVATAKFKLRHPLNWI LVNLAADLGETVIASITSVVQIFGYEYI
 Emu -----IFVVAASVFTINGLVAVATAKFKLRHPLNWI LVNLAADLGETVIASITSVVQIFGYEYI

164

Zebra finch LGHPMVCVIEGYTVSACGI TALWS LAI ISWERWF WCKPFGNIFKFDGLAVAGVLESWWS
 Canary LGHPMVCVIEGYTVSACGI TALWS LAI ISWERWF WCKPFGNIFKFDGLAVAGVLESWWS
 Chicken LGHPMVCVIEGYTVSACGI TALWS LAI ISWERWF WCKPFGNIFKFDGLAVAGVLESWWS
 Pigeon LGHPMVCVIEGYTVSACGI TALWS LAI ISWERWF WCKPFGNIFKFDGLAVAGVLESWWS
 Rosella LGHPMVCVIEGYTVSACGI TALWS LAI ISWERWF WCKPFGNIFKFDGLAVAGVLESWWS
 Emu -----LGHPMVCVIEGYTVSACGI TALWS LAI ISWERWF WCKPFGNIFKFDGLAVAGVLESWWS

181

Zebra finch CMTAPPIFGWSRYWPHGLKTS CGPDVFGSS SDPGVQSVMMVLMVTCFFPLAVIIFCYL
 Canary CMTAPPIFGWSRYWPHGLKTS CGPDVFGSS SDPGVQSVMMVLMVTCFFPLAVIIFCYL
 Chicken CMTAPPIFGWSRYWPHGLKTS CGPDVFGSS SDPGVQSVMMVLMVTCFFPLAVIIFCYL
 Pigeon CMTAPPIFGWSRYWPHGLKTS CGPDVFGSS SDPGVQSVMMVLMVTCFFPLAVIIFCYL
 Rosella CMTAPPIFGWSRYWPHGLKTS CGPDVFGSS SDPGVQSVMMVLMVTCFFPLAVIIFCYL
 Emu -----CMTAPPIFGWSRYWPHGLKTS CGPDVFGSS SDPGVQSVMMVLMVTCFFPLAVIIFCYL

261 269

Zebra finch QWLAI RAVAAQKES ESTQKAEKVS RMVVMVILAYCFWGEYTFACFAAANFGYAFH
 Canary QWLAI RAVAAQKES ESTQKAEKVS RMVVMVILAYCFWGEYTFACFAAANFGYAFH
 Chicken QWLAI RAVAAQKES ESTQKAEKVS RMVVMVILAYCFWGEYTFACFAAANFGYAFH
 Pigeon QWLAI RAVAAQKES ESTQKAEKVS RMVVMVILAYCFWGEYTFACFAAANFGYAFH
 Rosella QWLAI RAVAAQKES ESTQKAEKVS RMVVMVILAYCFWGEYTFACFAAANFGYAFH
 Emu -----QWLAI RAVAAQKES ESTQKAEKVS RMVVMVILAYCFWGEYTFACFAAANFGYAFH

292

Zebra finch PLTAAALPAFFAKSATIYNPIIYVFMNKGFRNICLQLFGKVVDDGSEVSTRTVESSVNS
 Canary PLTAAALPAFFAKSATIYNPIIYVFMNKGFRNICLQLFGKVVDDGSEVSTRTVESSVNS
 Chicken PLTAAALPAFFAKSATIYNPIIYVFMNKGFRNICLQLFGKVVDDGSEVSTRTVESSVNS
 Pigeon PLTAAALPAFFAKSATIYNPIIYVFMNKGFRNICLQLFGKVVDDGSEVSTRTVESSVNS
 Rosella PLTAAALPAFFAKSATIYNPIIYVFMNKGFRNICLQLFGKVVDDGSEVSTRTVESSVNS
 Emu -----PLTAAALPAFFAKSATIYNPIIYVFMNKGFRNICLQLFGKVVDDGSEVSTRTVESSVNS

Zebra finch SVS PA
 Canary SVS PA
 Chicken SVS PA
 Pigeon SVS PA
 Rosella SVS PA
 Emu -----

Figure S4. Alignment of the amino acid sequences of four cone opsins (SWS1, SWS2, RH2 and LWS) expressed in the emu (*Dromaius novaehollandiae*) compared to orthologues found in other bird species, including zebra finch (*Taeniopygia guttata*), canary (*Serinus canaria*), chicken (*Gallus gallus*), pigeon (*Columba livia*), and the crimson rosella (*Platycercus elegans*). Gaps inserted to maintain a high degree of sequence identity and unsequenced regions are indicated by dashes (-). Spectral tuning sites mentioned in the text are highlighted in red.

Zebra finch MNGTEGQDFYVPM SNKTGVVRS PF EY P Q Y Y L A E P W K F S A L A A Y M F M L I L L G F P I N F L T L Y
Canary -----PFEY P Q Y Y L A E P W K F S A L A A Y M F M L I L L G F P I N F L T L Y
Chicken MNGTEGQDFYVPM SNKTGVVRS PF EY P Q Y Y L A E P W K F S A L A A Y M F M L I L L G F P V N F L T L Y
Pigeon MNGTEGQDFYVPM SNKTGVVRS PF EY P Q Y Y L A E P W K F S A L A A Y M F M L I L L G F P V N F L T L Y
Rosella -----T L Y
Emu -----P I S N K T G V V R S P F E Y P Q Y Y L A E P W K F S A L A A Y M F M L I L L G F P I N F L T L Y
Bovine MNGTEGPNFYV P F S N K T G V V R S P F E A P Q Y Y L A E P W Q F S M L A A Y M F L L I M L G F P I N F L T L Y

Zebra finch V T I Q H K K L R T P L N Y I L L N L A V A D L F M V F G G F T T T M Y T S M N G Y F V F G V T G C Y I E G F F A T L G
Canary V T I Q H K K L R T P L N Y I L L N L A V A D L F M V F G G F T T T M Y T S M N G Y F V F G V T G C Y I E G F F A T L G
Chicken V T I Q H K K L R T P L N Y I L L N L V A D L F M V F G G F T T T M Y T S M N G Y F V F G V T G C Y I E G F F A T L G
Pigeon V T I Q H K K L R T P L N Y I L L N L A I A D L F M V F G G F T T T M Y T S M N G Y F V F G V T G C Y I E G F F A T L G
Rosella V T I Q H K K L R T P L N Y I L L N L A V A D L F M V F G G F T T T M Y T S M N G Y F V F G V T G C Y I E G F F A T L G
Emu V T I Q H K K L R T P L N Y I L L N L A V A N L F M V F G G F T T T L Y T S M H G Y F V F G V T G C Y I E G F F A T L G
Bovine V T V Q H K K L R T P L N Y I L L N L A V A D L F M V F G G F T T T L Y T S L H G Y F V F G P T G C N L E G F F A T L G

Zebra finch G E I A L W S L V V L A I E R Y V V V C K P M S N F R F G E N H A I M G V A F S W I M A L A C A A P P L F G W S R Y I P
Canary G E I A L W S L V V L A I E R Y V V V C K P M S N F R F G E N H A I M G V A F S W I M A L A C A A P P L F G W S R Y I P
Chicken G E I A L W S L V V L A E R Y V V V C K P M S N F R F G E N H A I M G V A F S W I M A M A C A A P P L F G W S R Y I P
Pigeon G E I A L W S L V V L A I E R Y V V V C K P M S N F R F G E N H A I M G V A F S W M A L A C A A P P L F G W S R Y I P
Rosella G E I A L W S L V V L A I E R Y V V V C K P M S N F R F G E N H A I M G V A F S W I M A L A C A A P P L F G W S R Y I P
Emu G E I A L W S L V V L A I E R Y V V V C K P V S N F R F G E N H A I M G L A L T W M A L A C A A P P L F G W S R Y I P
Bovine G E I A L W S L V V L A I E R Y V V V C K P M S N F R F G E N H A I M G V A F T W M A L A C A A P P L V G W S R Y I P

Zebra finch E G M Q C S C G I D Y Y T L K P E V N N E S F V I Y M F V V H F M I P L S I I F F C Y G N L V C T V K E A A A Q Q Q E S
Canary E G M Q C S C G I D Y Y T L K P E V N N E S F V I Y M F V V H F M I P L L I I F F C Y G N L V C T V K E A A A Q Q Q E S
Chicken E G M Q C S C G I D Y Y T L K P E I N N E S F V I Y M F V V H F M I P L A V I F F C Y G N L V C T V K E A A A Q Q Q E S
Pigeon E G M Q C S C G I D Y Y T L K P E I N N E S F V I Y M F V V H F M I P L M V I F F C Y G N L V C T V K E A A A Q Q Q E S
Rosella E G M Q C S C G I D Y Y T L K P E I N N E S F V I Y M F V V H F M I P L M I I F F C Y G N L V C T V K E A A A Q Q Q E S
Emu E G M Q C S C G I D Y Y T L K P E V N N E S F V I Y M F V V H F T I P L M V I F F C Y G N L V C T V K E A A A Q Q Q E S
Bovine E G M Q C S C G I D Y Y T P H E E T N N E S F V I Y M F V V H F I I P L I V I F F C Y G Q L V F T V K E A A A Q Q Q E S

Zebra finch A T T Q K A E K E V T R M V I I M V I A F L I C W V P Y A S V A F Y I F T N Q G S D F G P I F M T I P A F F A K S S A I
Canary A T T Q K A E K E V T R M V I I M V I S F L I C W V P Y A S V A F Y I F T N Q G S D F G P I F M T I P A F F A K S S A I
Chicken A T T Q K A E K E V T R M V I I M V I A F L I C W V P Y A S V A F Y I F T N Q G S D F G P I F M T I P A F F A K S S A I
Pigeon A T T Q K A E K E V T R M V I I M V I A F L I C W L P Y A S V A F Y I F T N Q G S D F G P I F M T I P A F F A K S S A I
Rosella A T T Q K A E K E V T R M V I I M V I A F L I C W V P Y A S V A F Y I F T N Q G S D F G P I F M T I P A F F A K S S S I
Emu A T T Q K A E K E V T R M V I M V I A F L I C W L P Y A S V A F Y I F T N Q G S D F G P I F M T I P A F F A K S S A I
Bovine A T T Q K A E K E V T R M V I I M V I A F L I C W L P Y A G V A F Y I F T H Q G S D F G P I F M T I P A F F A K T S A V

Zebra finch Y N P V I Y I V M N K Q F R N C M I T T L C C G K N P L G D E D T S A G K T E T S S V S T S Q V S P A
Canary Y N P V I Y I V M N K Q F R N C M I T T L C C G K N P L G D E D T S A G K T E T S S -----
Chicken Y N P V I Y I V M N K Q F R N C M I T T L C C G K N P L G D E D T S A G K T E T S S V S T S Q V S P A
Pigeon Y N P V I Y I V M N K Q F R N C M I T T L C C G K N P L G D E D T S A G K T E T S S V S T S Q V S P A
Rosella F N P L I Y V F R D K Q F R N C M I T T L C C G K N P L G D E D T S A G K T E T S S V S T S Q V S P A
Emu Y N P V I Y I V M N K Q F R N C M I -----
Bovine Y N P V I Y I M M N K O F R N C M V T T L C C G K N P L G D E A S T --- T V S K T E T S O V A P A

Figure S5. Alignment of the amino acid sequences of the emu (*Dromaius novaehollandiae*) rod opsin (RH1) compared to orthologues found in other bird species, including zebra finch (*Taeniopygia guttata*), canary (*Serinus canaria*), chicken (*Gallus gallus*), pigeon (*Columba livia*), and the crimson rosella (*Platycercus elegans*), as well as the common cow (*Bos taurus*). Gaps inserted to maintain a high degree of sequence identity and unsequenced regions are indicated by dashes (–). Spectral tuning sites mentioned in the text are highlighted in red.

Supplementary Tables:**Table S1.** Primers used to isolate and amplify emu opsin gene sequences from retinal cDNA.

Primer	Sequence
LWS_F1	AAGCGTATTYAYTTAYACCRACASCAACAA
LWS_R1	CATCCTBGACACYTCCYTCTCVGCCTTCTG
LWS_F2	AGTGTCATCAACCAGWTCTYBGGSTAYTTC
LWS_R2	CATCATCCACYTTYTTSCCRAASAGCTGCA
SWS1_F1	TCCCATGTCCGGAGAVGAVGABTTYTACCT
SWS1_R1	CACCACSACCATSCGVGASACCTCCCGCTC
SWS1_F2	GGCCTTCGARGHTACATYGYATCTGCAA
SWS1_R2	TTAGCTGGGGCYGACYTGRCTGGAGGACAC
SWS2_F1	CAACATCACRRCSTSAGCCCBTTCTGGT
SWS2_R1	CAGGAAGCCCADSACCATSACYACYACCAT
SWS2_F2	CTGCAAGATAGAGGGNTTYDCBGCMACGCT
SWS2_R2	AAGAATTTTABGCBGGGGMSACBTGGCTGG
RH2_F1	ATCAACATCCTCACCYTVYTKGTSACCTTC
RH2_R1	CAAGGAGGAATCCMADCACCATSARRATCA
RH2_F2	CTTCTCTGCCACTCAYGCCWTRWTRGGCAT
RH2_R2	CACTTGGCTGGAAGARAYRGAVGAKACCTC
RH1_F1	GTCAAAAATTTCTAYRTBCCCWTKCCAACA
RH1_R1	ACAGTGCAGACAAGRYKYCCRTAGCAGAAG
RH1_F2	AATAGGATGCWRCWTYGARGGCTTCTTTGC
RH1_R2	ATTCTTTCCACARCARAGRGTBRTGATCAT

Table S2. Spectral absorption characteristics of retinal photoreceptors in the emu measured using MSP. λ_{\max} , wavelength of maximum absorbance/absorbance change; λ_{cut} , cut-off wavelength; λ_{mid} , wavelength of half maximum absorbance. Values are ± 1 standard deviation. Avian rods do not contain oil droplets and no oil droplets were observed in the accessory members of double cones in the emu (although a diffuse pigmentation was present). T-, C-, Y-, R- and P-type oil droplets are located in the UVS, SWS, MWS and LWS single cones, and the principal members of the LWS double cones, respectively. D, dorsal retina; V, ventral retina; N, number of cells used in the analysis. Subscripts 1 and 2 identify two different spectral types of P-type oil droplet located in the dorsal retina.

Visual Pigments	Single Cones				Double Cones				Rods	
	UVS	SWS	MWS	LWS	Principal	Accessory				
Mean λ_{\max} of prebleach spectra (nm)	367.0 \pm 2.5	453.2 \pm 2.3	502.3 \pm 2.8	562.0 \pm 2.0	562.8 \pm 2.6	563.4 \pm 2.0	501.0 \pm 1.0			
λ_{\max} of mean prebleach spectrum (nm)	366.8	453.1	501.2	562.0	562.9	562.1	500.7			
Mean transverse absorbance at λ_{\max}	0.018 \pm 0.008	0.012 \pm 0.003	0.016 \pm 0.005	0.018 \pm 0.004	0.013 \pm 0.004	0.012 \pm 0.003	0.023 \pm 0.004			
Mean λ_{\max} of difference spectra (nm)	366.6 \pm 6.8	453.7 \pm 3.5	507.2 \pm 4.0	564.2 \pm 1.9	562.8 \pm 2.8	563.5 \pm 2.3	504.0 \pm 1.7			
λ_{\max} of mean difference spectrum (nm)	366.5	454.0	507.0	564.0	563.0	563.7	503.5			
N prebleach (difference)	9 (8)	9 (13)	21 (25)	10 (14)	12 (12)	7 (8)	21 (22)			

Oil Droplets	T-type		C-type		Y-type		R-type		P-type			A-type		
	D	V	D	V	D	V	D	V	D ₁	D ₂	V	D	V	
Mean λ_{cut} of absorbance spectra (nm)	-	-	408.4 \pm 4.3	408.5 \pm 3.5	508.1 \pm 1.9	507.0 \pm 4.0	558.8 \pm 3.8	559.9 \pm 4.2	404.0 \pm 2.0	475.9 \pm 11.8	491.1 \pm 3.8	480.2 \pm 3.9	479.5 \pm 2.0	-
λ_{cut} of mean absorbance spectrum (nm)	-	-	409.3	407.5	507.6	506.8	559.2	560.6	403.5	479.2	492.3	479.2	480.1	-
Mean λ_{mid} of absorbance spectra (nm)	-	-	426.0 \pm 1.9	432.0 \pm 4.9	524.6 \pm 3.4	526.8 \pm 4.2	580.9 \pm 3.8	582.2 \pm 4.2	433.5 \pm 5.9	499.1 \pm 6.2	507.4 \pm 2.3	494.4 \pm 3.5	491.9 \pm 0.6	-
λ_{mid} of mean absorbance spectrum (nm)	-	-	426.2	430.9	524.3	527.3	581.4	582.8	432.9	500.6	508.1	494.6	492.1	-
Mean maximum absorbance	0.04 \pm 0.02	0.05 \pm 0.03	0.54 \pm 0.09	0.47 \pm 0.11	0.84 \pm 0.08	0.84 \pm 0.06	0.85 \pm 0.08	0.83 \pm 0.08	0.46 \pm 0.10	0.66 \pm 0.14	0.58 \pm 0.10	0.21 \pm 0.13	0.20 \pm 0.08	-
Mean diameter (μm)	2.4 \pm 0.2	3.0 \pm 0.4	3.3 \pm 0.8	3.7 \pm 0.6	3.3 \pm 0.6	3.6 \pm 0.4	3.2 \pm 0.3	3.5 \pm 0.5	2.8 \pm 0.3	3.1 \pm 0.3	3.1 \pm 0.4	-	-	-
N	5	4	12	11	18	22	13	19	13	11	20	9	7	-

Table S3. Amino acid differences between ultraviolet-sensitive (UVS) and violet-sensitive (VS) short-wavelength-sensitive-1 (SWS1) visual pigments at known or potential tuning sites, including residues 46, 49, 52, 114 and 118 [1]; 261, 269 and 292 [2]; 86 and 90 [3, 4]; and 93 [5]. Sequence data for avian SWS1 pigments are derived from this study and published articles [6-13], and are compared to residues found in the green anole (*Anolis carolinensis*) SWS1 pigment (GenBank Accession Number AH007736).

Pigment	Tuning sites											
	46	49	52	86	90	93	114	118	164	261	269	292
Avian UVS	Phe Leu Val	Leu Met Val	Thr	Ala Cys	Cys	Thr	Gly	Ala	Gly	Phe	Ala	Ala
Avian VS	Phe Ile	Ala Leu	Thr	Cys Ser	Ser	Thr Val	Ala Gly	Ala Thr	Gly	Phe	Ala	Ala
Anole UVS	Phe	Phe	Thr	Phe	Ser	Thr	Ala	Ser	Gly	Phe	Ala	Ala
Emu UVS	Phe	Phe	Thr	Phe	Cys	Met	Gly	Ser	Gly	Phe	Ala	Ala

Supplementary References (for Table S3):

- [1] Shi, Y., Radlwimmer, F.B. & Yokoyama, S. 2001 Molecular genetics and the evolution of ultraviolet vision in vertebrates. *Proc. Natl. Acad. Sci. U S A* **98**, 11731-11736. (doi:10.1073/pnas.201257398).
- [2] Yokoyama, S. & Radlwimmer, F.B. 1998 The "five-sites" rule and the evolution of red and green color vision in mammals. *Mol. Biol. Evol.* **15**, 560-567.
- [3] Cowing, J.A., Poopalasundaram, S., Wilkie, S.E., Robinson, P.R., Bowmaker, J.K. & Hunt, D.M. 2002 The molecular mechanism for the spectral shifts between vertebrate ultraviolet- and violet-sensitive cone visual pigments. *Biochem. J.* **367**, 129-135. (doi:10.1042/BJ20020483).
- [4] Wilkie, S.E., Robinson, P.R., Cronin, T.W., Poopalasundaram, S., Bowmaker, J.K. & Hunt, D.M. 2000 Spectral tuning of avian violet- and ultraviolet-sensitive visual pigments. *Biochemistry* **39**, 7895-7901. (doi:10.1021/bi992776m).
- [5] Carvalho, L.S., Davies, W.L., Robinson, P.R. & Hunt, D.M. 2012 Spectral tuning and evolution of primate short-wavelength-sensitive visual pigments. *Proc. R. Soc. Lond. B* **279**, 387-393. (doi:10.1098/rspb.2011.0782).
- [6] Aidala, Z., Huynen, L., Brennan, P.L., Musser, J., Fidler, A., Chong, N., Machovsky Capuska, G.E., Anderson, M.G., Talaba, A., Lambert, D., et al. 2012 Ultraviolet visual sensitivity in three avian lineages: paleognaths, parrots, and passerines. *J. Comp. Physiol. A* **198**, 495-510. (doi:10.1007/s00359-012-0724-3).
- [7] Carvalho, L.S., Cowing, J.A., Wilkie, S.E., Bowmaker, J.K. & Hunt, D.M. 2007 The molecular evolution of avian ultraviolet- and violet-sensitive visual pigments. *Mol. Biol. Evol.* **24**, 1843-1852. (doi:10.1093/molbev/msm109).

- [8] Knott, B., Davies, W.I., Carvalho, L.S., Berg, M.L., Buchanan, K.L., Bowmaker, J.K., Bennett, A.T. & Hunt, D.M. 2013 How parrots see their colours: novelty in the visual pigments of *Platyercus elegans*. *J. Exp. Biol.* **216**, 4454-4461. (doi:10.1242/jeb.094136).
- [9] Odeen, A. & Hastad, O. 2010 Pollinating birds differ in spectral sensitivity. *J. Comp. Physiol. A* **196**, 91-96. (doi:10.1007/s00359-009-0474-z).
- [10] Odeen, A. & Hastad, O. 2013 The phylogenetic distribution of ultraviolet sensitivity in birds. *BMC Evol. Biol.* **13**, 36. (doi:10.1186/1471-2148-13-36).
- [11] Ödeen, A. & Håstad, O. 2003 Complex distribution of avian color vision systems revealed by sequencing the SWS1 opsin from total DNA. *Mol. Biol. Evol.* **20**, 855-861. (doi:10.1093/molbev/msg108).
- [12] Odeen, A., Hastad, O. & Alstrom, P. 2010 Evolution of ultraviolet vision in shorebirds (Charadriiformes). *Biol. Lett.* **6**, 370-374. (doi:10.1098/rsbl.2009.0877).
- [13] Odeen, A., Hastad, O. & Alstrom, P. 2011 Evolution of ultraviolet vision in the largest avian radiation - the passerines. *BMC Evol. Biol.* **11**, 313. (doi:10.1186/1471-2148-11-313).