Supplementary Material

# SWS2 visual pigment evolution as a test of historically contingent patterns of plumage color evolution in warblers.

Natasha I. Bloch, James M. Morrow, Belinda S.W. Chang, and Trevor D. Price.

## TABLE OF CONTENTS

# Supplementary figures:

### Figure S1: Amino acid sequence alignment of SWS2 opsins of New World and Old World warblers.

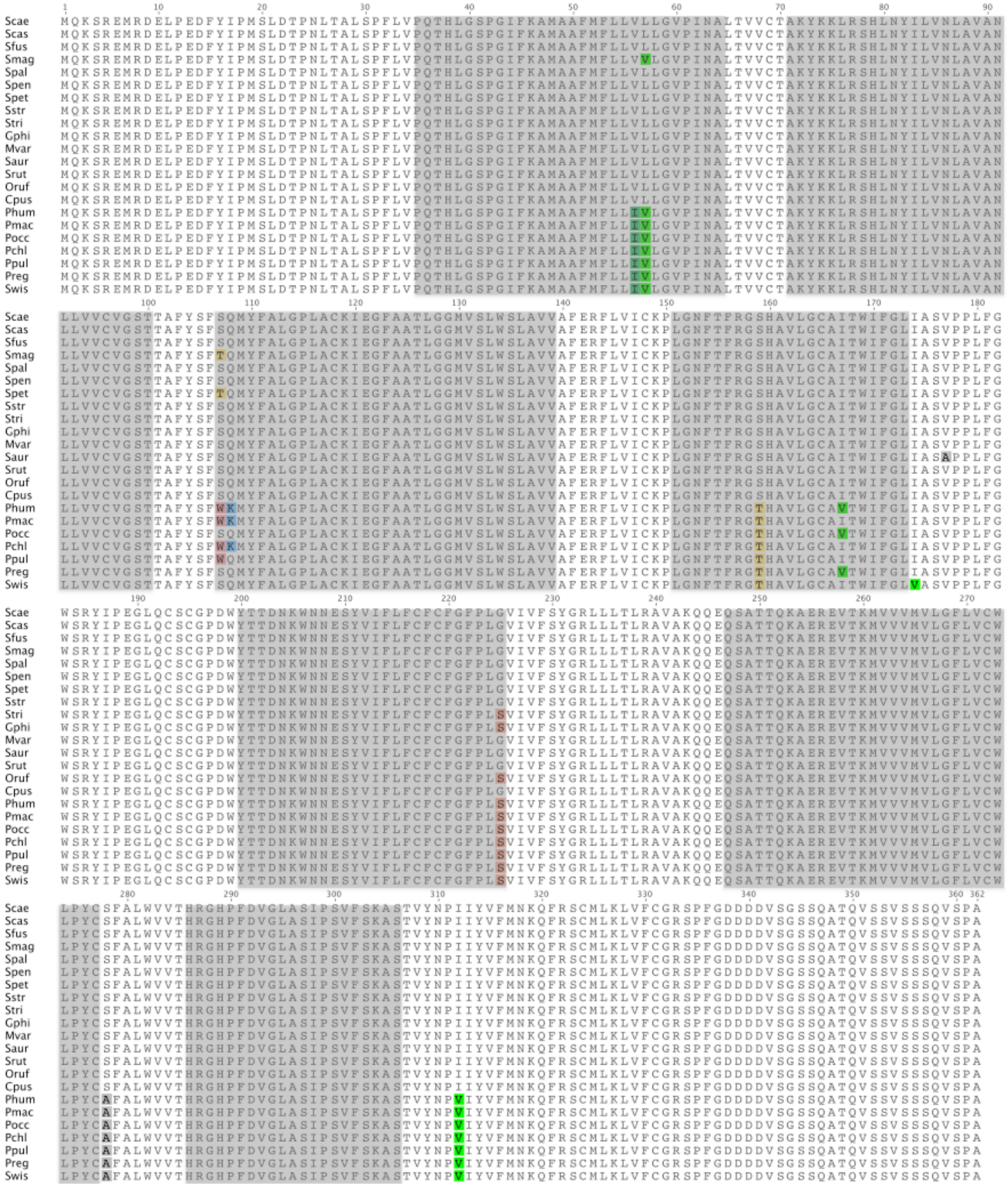

**Figure S1.** Numbering corresponds to Bovine rhodopsin (RH1). Grey boxes indicate the seven transmembrane helices (H-I through H-VII) (Palczewski et al. 2000). The 11 variables sites across warbler clades are highlighted indicating their position relative to the transmembrane domains and colored according to amino acid identity. Species names are abbreviated following Fig. 3 and full names can be found in Fig. 1.

## Figure S2: Cladogram mapping SWS2 substitutions for all species used in ancestral reconstructions.

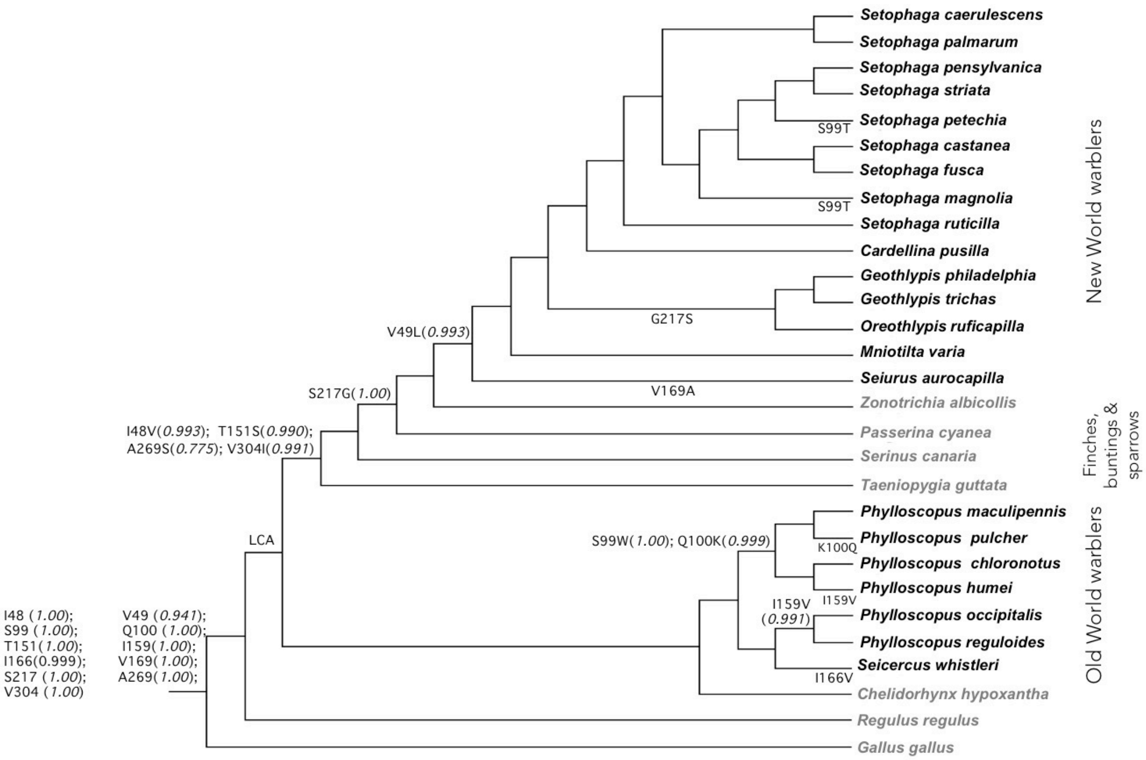

**Figure S2.** We used our own outgroup species as well as SWS2 sequences for species available on GenBank for ancestral reconstructions (accession numbers AF222332, NM205517 and AJ277923). Substitutions are shown next to the edge where they occur with their corresponding posterior probabilities from Empirical Bayes models in parentheses and italics. We show ancestral reconstruction results for codon models, which always had the highest posterior probabilities, but all models yielded identical results for all substitutions except a minor difference for S217G on the branch leading to the New World ancestor (in nucleotide models it occurs on the same branch as V49L). LCA corresponds to the last common ancestor between New and Old World warblers. The phylogeny is based on Lovette et al. (2010) and Price et al. (2014).

## Figure S3: Parsimony ancestral reconstructions.

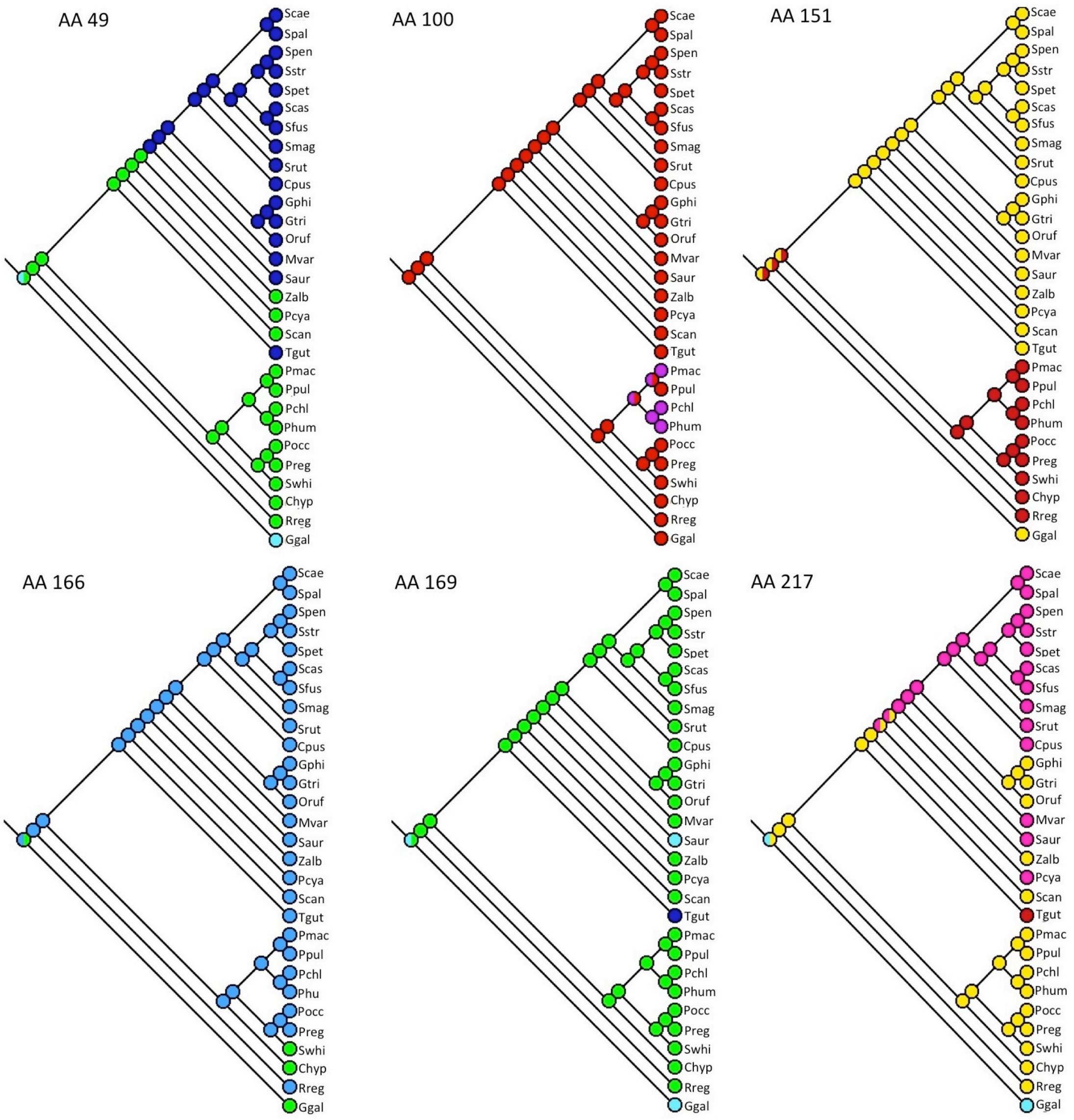

**Figure S3.** Cladograms indicating the amino acid identity at variables sites for SWS2 according to parsimony reconstruction (branch lengths are not proportional to time). Nodes with two colors indicate two equally parsimonious states. Species abbreviations correspond to the first letter of the genus and the three first letters of the species name. See Fig. 1 for full species names.

## Figure S4: Cladogram illustrating correlated evolution of SWS2 spectral sensitivity and foraging habitat in songbirds.

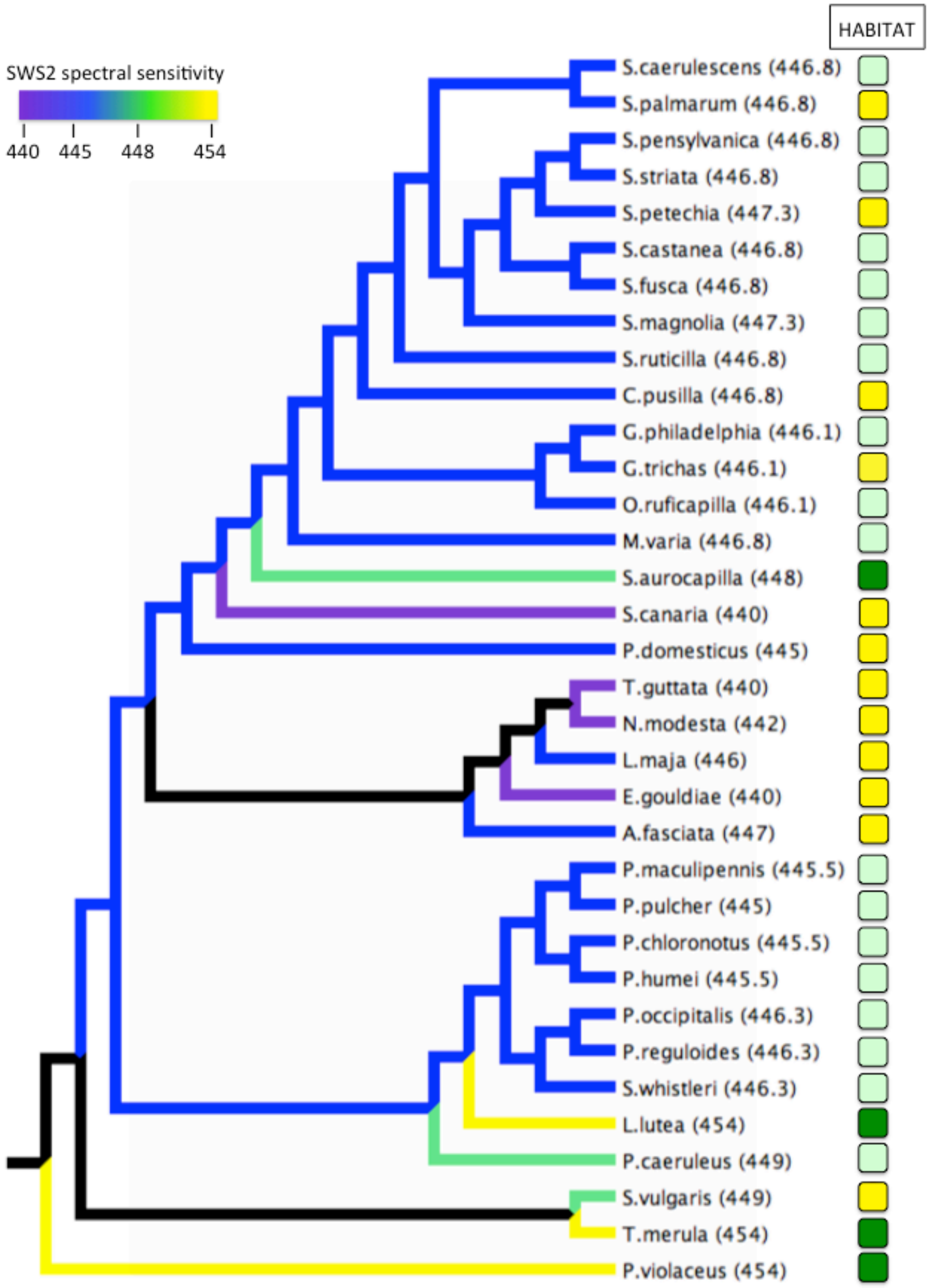

**Figure S4.** This figure is complementary to Figure 7 in the main text, which shows a scatter plot of the relationship between SWS2 $\lambda_{max}$ against foraging height in these species. Branches are colored according to each species or the inferred ancestors' $\lambda_{max}$ (when data is available; black branches correspond to ancestor for which inferred $\lambda_{max}$ is not known). Habitat categories are: Forest understory (dark green), arboreal (light green) and open (yellow).

## Figure S5: Scatter plot of SWS2 λmax against foraging height in passerines and non-passerines.

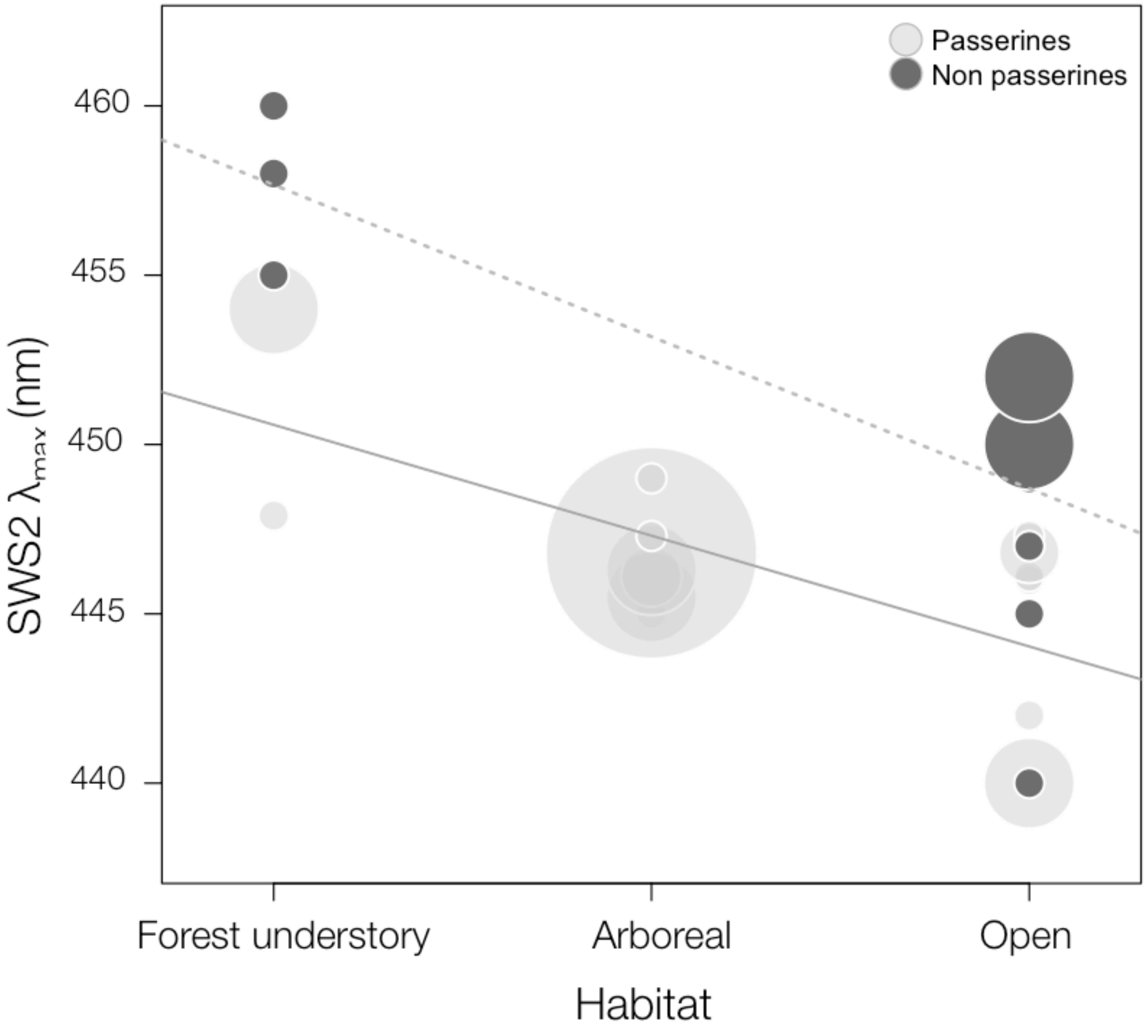

**Figure S5.** Scatter plot of SWS2 $\lambda_{max}$ against foraging height in passerines (light grey; as in Figure 7) and non-passerines (dark grey). Circle size corresponds to number of species. Breeding season foraging habitat categories correspond to (1) species foraging in the forest understory, on or near the ground, (2) all the remaining strata of the forest and (3) outside forests in the open. The regression line for passerines is y= 450.6-3.28x and for non-passerines is y= 457.7-4.48x. Data used are in table S2. The relationship was found to be significant in both clades (see text).

# Supplementary tables

## Table S1: Primers used to amplify full coding sequences for SWS2 opsin genes

| | | SWS2 | |
|---|---|---|---|
| | | **Outer** | **Nested** |
| **Initial Degenerate Primers** | for | TKACGGCGCTSAGCCCSTTCCTGGT | CCSCARACSCACCTGGGCAGCCC |
| | rev | ATGCSGMCGCGGAACTGCTTGTTCATGA | TTGTAGACGGTGGAGGMCTTGGAGAAGA |
| **RACE primers** *(used in combination with universal primers (UOP & NUP)* | 5’ | TGTCCGTCGTGTACCAGTCCG | CTCGGGGATGTACCTGCTCCA |
| | 3’ | TGGAGCAGGTACATCCCCGAG | CGGACTGGTACACGACGGACA |
| **Primers for coding sequence amplification** | for | CAGAAGTCGCGGGAGATGCGGGAC | CGGGACGAGCTCCCCGAGGATTT |
| | rev | CTACGCCGGGGACACCTGGCT | ACCTGGCTGGAGGACACCGAGG |

## Table S2: Data used for evolutionary correlations of SWS2 $\lambda_{max}$ against foraging habitat.

| **Clade** | **Species** | SWS2 $\lambda_{max}$ (nm) | SWS1 $\lambda_{max}$ (nm) | Foraging Height | Habitat information reference | Spectral tuning reference |
|---|---|---|---|---|---|---|
| *New World warblers* | *Setophaga caerulescens* | 446.8± 0.1 | 365.7± 0.1 | Arboreal | (Dunn and Garrett 1997; Poole 2005) | *Present study* |
| | *Setophaga palmarum* | 446.8± 0.1 | 365.7± 0.1 | Open | (Dunn and Garrett 1997; Poole 2005) | *Present study* |
| | *Setophaga pensylvanica* | 446.8± 0.1 | 365.7± 0.1 | Arboreal | (Dunn and Garrett 1997; Poole 2005) | *Present study* |
| | *Setophaga striata* | 446.8± 0.1 | 365.7± 0.1 | Arboreal | (Dunn and Garrett 1997; Poole 2005) | *Present study* |
| | *Setophaga petechia* | 447.3± 0.1 | 365.7± 0.1 | Open | (Dunn and Garrett 1997; Poole 2005) | *Present study* |
| | *Setophaga castanea* | 446.8± 0.1 | 365.7± 0.1 | Arboreal | (Dunn and Garrett 1997; Poole 2005) | *Present study* |
| | *Setophaga fusca* | 446.8± 0.1 | 365.7± 0.1 | Arboreal | (Dunn and Garrett 1997; Poole 2005) | *Present study* |
| | *Setophaga magnolia* | 447.3± 0.1 | 365.7± 0.1 | Arboreal | (Dunn and Garrett 1997; Poole 2005) | *Present study* |
| | *Setophaga ruticilla* | 446.8± 0.1 | 365.7± 0.1 | Arboreal | (Dunn and Garrett 1997; Poole 2005) | *Present study* |
| | *Cardellina. pusilla* | 446.8± 0.1 | 365.7± 0.1 | Open | (Dunn and Garrett 1997; Poole 2005) | *Present study* |
| | *Geothlypis philadelphia* | 446.1± 0.1 | 365.7± 0.1 | Arboreal | (Dunn and Garrett 1997; Poole 2005) | *Present study* |
| | *Geothlypis trichas* | 446.1± 0.1 | 365.7± 0.1 | Open | (Dunn and Garrett 1997; Poole 2005) | *Present study* |
| | *Oreothlypis ruficapilla* | 446.1± 0.1 | 365.7± 0.1 | Arboreal | (Dunn and Garrett 1997; Poole 2005) | *Present study* |
| | *Mniotilta varia* | 446.8± 0.1 | 365.7± 0.1 | Arboreal | (Dunn and Garrett 1997; Poole 2005) | *Present study* |
| | *Seiurus aurocapilla* | 448± 0.3 | 364.8± 0.2 | Forest understory | (Dunn and Garrett 1997; Poole 2005) | *Present study* |
| *Old World warblers* | *Phylloscopus maculipennis* | 445.5± 0.2 | 365.7± 0.1 | Arboreal | (Price and Gross 2005) | *Present study* |
| | *Phylloscopus pulcher* | 445.0± 0.0 | 365.7± 0.1 | Arboreal | (Price and Gross 2005) | *Present study* |
| | *Phylloscopus chloronotus* | 445.5± 0.2 | 365.7± 0.1 | Arboreal | (Price and Gross 2005) | *Present study* |
| | *Phylloscopus humei* | 445.5± 0.2 | 365.7± 0.1 | Arboreal | (Price and Gross 2005) | *Present study* |
| | *Phylloscopus occipitalis* | 446.3± 0.2 | 365.7± 0.1 | Arboreal | (Price and Gross 2005) | *Present study* |
| | *Phylloscopus reguloides* | 446.3± 0.2 | 365.7± 0.1 | Arboreal | (Price and Gross 2005) | *Present study* |
| | *Seicercus whistleri* | 446.3± 0.2 | 365.7± 0.1 | Arboreal | (Price and Gross 2005) | *Present study* |
| *Other passerines* | *Serinus canaria* | 440±2.2 | 363±4.7 | Open | (Snow et al. 1998) | (Das et al. 1999) |
| | *Taeniopygia guttata* | 440±1 | 359±1 | Open | (Hart et al. 2000a) | (Yokoyama et al. 2000) |
| | *Amadina fasciata* | 447±1.6 | 370±2.4 | Open | (Hart et al. 2000a) | (Hart et al. 2000a) |
| | *Erythrura gouldiae* | 440±2.7 | 370±0.8 | Open | (Hart et al. 2000a) | (Hart et al. 2000a) |
| | *Leiothrix lutea* | 454±5.5 | 355† | Forest understory | (Amano et al. 2002) | (Maier and Bowmaker 1993) |
| | *Lonchura maja* | 445.5±1.5 | 373±1.9 | Open | (Hart et al. 2000a) | (Hart et al. 2000a) |
| | *Neochmia modesta* | 442±2.9 | 373±3.3 | Open | (Hart et al. 2000a) | (Hart et al. 2000a) |
| | *Parus caeruleus* | 449±2.5 | 372±3.8 | Arboreal | (Blondel et al. 1993) | (Hart et al. 2000b) |
| | *Passer domesticus* | 445 † | NA Predicted UV type | Open | (Romero et al. 2006) | (Hart and Hunt 2007) |
| | *Sturnus vulgaris* | 449±5.4 | 362±2.8 | Open | (Feare 1985) | (Hart et al. 1998) |
| | *Turdus merula* | 454±2.2 | 373±5.0 | Forest understory | (Hoyo et al. 1994) | (Hart et al. 2000b) |
| | *Ptilonorhynchus violaceus* | 454±1.4 | 410±2.8 | Forest understory | (Coyle et al. 2012) | (Coyle et al. 2012) |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Non-passerines* | *Anas platyrhynchos* | 452±5.0 | 415±9.5 | Open | (Hoyo et al. 1994) | (Jane and Bowmaker 1988) |
| | *Puffinus pacificus* | 450±3.9 | 406±4.2 | Open | (Hoyo et al. 1994) | (Hart 2004) |
| | *Puffinus puffinus* | 452† | 402† | Open | (Hoyo et al. 1994) | (Bowmaker et al. 1997) |
| | *Spheniscus humboldti* | 450† | 403±5.1 | Open | (Hoyo et al. 1994) | (Bowmaker and Martin 1985) |
| | *Columba livia* | 452±5.6 | 404±10.2 | Open | (Hoyo et al. 1994) | (Bowmaker et al. 1997) |
| | *Coturnix japonica* | 450±3.0 | 418±5.0 | Open | (Hoyo et al. 1994) | (Bowmaker et al. 1993) |
| | *Gallus gallus* | 453±3.8 | 418±5.1 | Forest understory | (Hoyo et al. 1994) | (Bowmaker et al. 1997) |
| | *Meleagris gallopavo* | 460±1.9 | 420±1.3 | Forest understory | (Hoyo et al. 1994) | (Hart et al. 1999) |
| | *Pavo cristatus* | 458±2.0 | 424±2.3 | Forest understory | (Hoyo et al. 1994) | (Hart 2002) |
| | *Melopsittacus undulatus* | 440±8.0 [a] | 365±3[b] | Open | (Hoyo et al. 1994) | [a](Bowmaker et al. 1997) [b](Wilkie et al. 1998) |
| | *Struthio camelus* | 445±2.2 | 405±6.5 | Open | (Hoyo et al. 1994) | (Wright and Bowmaker 2001) |

† Standard deviation or error not available

§ Not included in models correcting for SWS1 $\lambda_{max}$ as this value is not available.

Except for warblers, all errors correspond to stand deviation (SD). For species that have become human commensals, we considered habitats for ancestral wild populations.

## Table S3: Site models for positive selection

| Gene | Clade | Model | lnL | np | Parameter Estimates | Positively selected (BEB) | p-value (LRT) |
|---|---|---|---|---|---|---|---|
| **SWS2** | NWW | M0 | -1771.79 | 31 | $\omega_0$=0.0527 | - | |
| | | M1a | -1763.22 | 32 | $p_0$= 0.9607, $\omega_o$=0.039 | - | NS (0.32) |
| | | M2a | -1762.09 | 34 | $p_0$= 0.979, $\omega_o$=0.025, $p_2$= 0.0207, $\omega_2$=2.717 | 99 217 | |
| | | M7 | -1765.05 | 32 | p= 0.0098, q= 0.174 | - | 0.051 |
| | | M8 | -1762.09 | 34 | $p_0$= 0.979 ($p_1$=0.021), $\omega$=2.717 | 99 217 | |
| **SWS2** | OWW | M0 | -1699.91 | 17 | $\omega_0$=0.072 | - | |
| | | M1a | -1691.78 | 18 | $p_0$= 0.0.951, $\omega_o$=0. 0000 | - | NS (0.11) |
| | | M2a | -1689.57 | 20 | $p_0$= 0.981, $\omega_o$=0.0000, $p_2$= 0.0191, $\omega_2$=4.327 | 99 159 | |
| | | M7 | -1693.04 | 18 | p= 0.009, q= 0.139 | - | 0.031 |
| | | M8 | -1689.57 | 20 | $p_0$=0.981 ($p_1$=0.019), $\omega$=4.327 | 99 159 | |

PAML results M0: One-ratio model; M1a neutral model, M2a: selection model; M7: β; M8:β and ω $(= d_N/d_S)$ (Yang et al. 2000). lnL stands for the log value of the model's likelihood score given the data and provided phylogeny. Likelihood ratio tests (LRT) were performed to test whether the two more parameter rich models, M2a and M8, that allow for a category of positively selected sites, fit the data better than their nested, simpler counterparts, M1a and M7 respectively. The resulting test statistic was compared to a $\chi^2_2$ distribution (Yang et al. 2000). Positively selected sites identified by Bayes Empirical Bayes factors (BEB) are shown. NS = non-significant; np = number of parameters.

## Table S4: Model choice for Empirical Bayes ancestral reconstructions

| GENE | CLADE | MODEL | lnL | LRT | |
|---|---|---|---|---|---|
| **SWS2** | ALL | **CODON** | | **F61 + Γ** | |
| | | F1/61 + Γ | -3285.52 | 670.66**(60) | |
| | | F1X4 + Γ | -3198.05 | 495.71**(57) | |
| | | F3X4 + Γ | -3043.92 | 187.45**(51) | |
| | | **F61 + Γ** | **-2950.19** | NA | |
| | | **NUCLEOTIDE** | | **HKY85 + Γ** | **GRT + Γ** |
| | | HKY85+G | -3234.56 | NA | 83.62** (4) |
| | | JC69+G | -3343.54 | 217.97** (4) | |
| | | F81+G | -3240.25 | 11.39**(1) | |
| | | K80+G | -3341.33 | 213.54**(3) | |
| | | **GTR+G** | **-3192.75** | - | |
| | | **AMINO ACID** | | | |
| | | Poisson + F + Γ | -1744.37 | | |
| | | **Jones + F + Γ** | **-1590.60** | | |
| | | Dayhoff78 + F + Γ | -1600.50 | | |
| | | MTMAM + F + Γ | -1605.46 | | |

Summary of likelihood ratio tests performed to chose best Codon, Nucleotide and Amino Acid models for ancestral reconstruction and positive selection tests (codon models). LRT = $2(lnL_1\text{-}lnL_2)$ and its significance is approximated to the $\chi^2_{df}$ distribution with degrees of freedom indicated in parentheses. ** LRT p-values <0.05. Best fitting models are highlighted in **bold**. For the explanation of the notation see Yang (2007).

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