

Evolutionary dynamics of Rh2 opsins in birds demonstrate an episode of accelerated evolution in the New World warblers (*Setophaga*)

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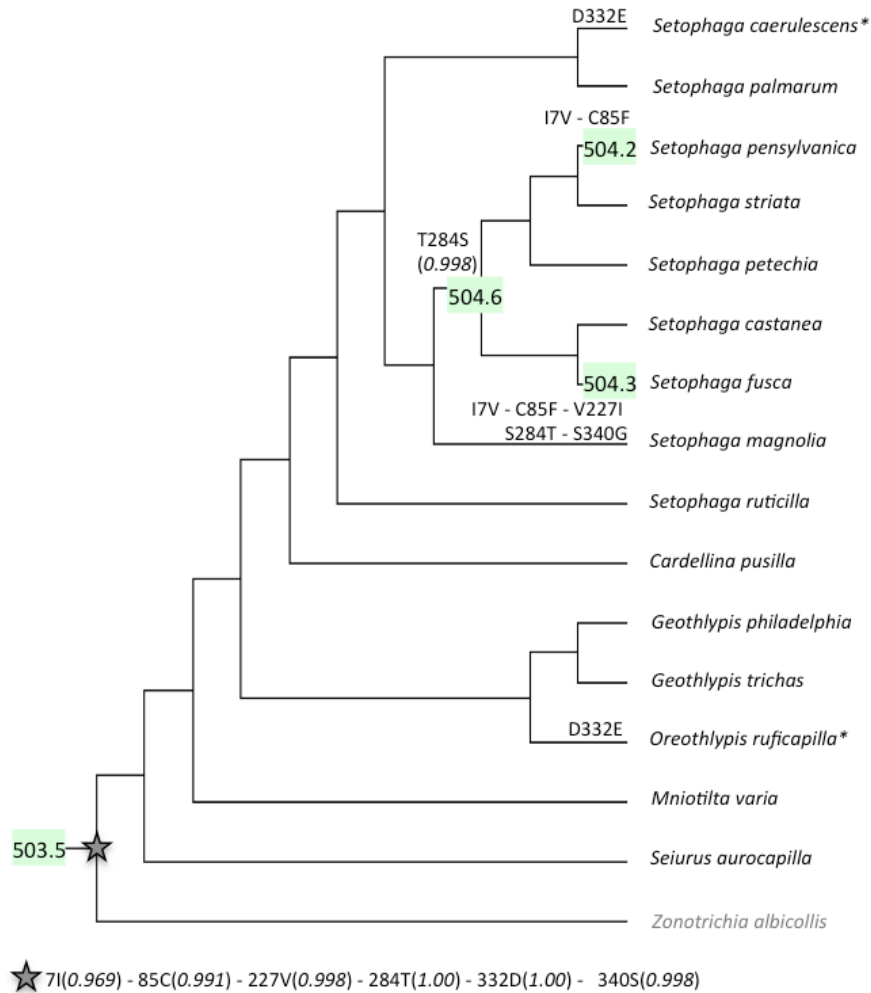
Numbering corresponds to Bovine rhodopsin (GenBank M21606). Grey boxes indicate the seven transmembrane helices (H-I through H-VII) (Palczewski et al. 2000). The variable sites across warbler clades are highlighted indicating their position relative to the transmembrane domains and colored according to amino acid identity. Species names are abbreviated as the first letter of the genus and the three first letters of the species. Full names can be found in Figs. 1 & 2.

Figure S2: Rh2 variable site summary based on consensus of all clades.

	1111111111122222222222333333*
	3558003555567901112334488033344
	79265489014859882787130849423607
New World warblers	ScaeE.....
	ScasS.....
	Sfus V...F.....I.....G.
	SmagS.....
	SpalS.....
	Spen V...F.....S.....
	SpetS.....
	SstrS.....
	GtriS.....
	GphiS.....
	MvarS.....
	SaurS.....
	SrutS.....
	OrufE.....
CpusS.....	
Old World warblers	ZalbE.....
	PcyaS.....
	ScanV.....S...P.....E....
	TgutL.....E.....
	GforCA.....
	FalbS.....
	PhumS.....
	PmacS.....
	PoccS.....
	PchlS.....
PpulS.....	
PregS.....	
SwisS.....	
Bowerbirds	ChypIV.....I.....E....
	RregL.....S.....
	PshuA.....
	Aino ---.MV...V...I.....----
	Amac ---.V...V...I.....----
	Cnuc ---.V...V...I.....----
	Pvio ---.V...I.....----
	Cgut ---.V...I.....----
	Cmac ---.V...I.....----
	Apap ---.V...I.....----
	Acra ---.V...VV...I.....----
	Asub ---.V...V...I.....----
	Pnew ---.V...V...I.....----
	Mund VLLF.VS...FMY...VI...RSSV..A.I
Ggal ..LLF.VV.T.FMY...VI.....V...	
CONS ILFFCVIIASMAIIFFFVIVRASKTAIDTTSV	

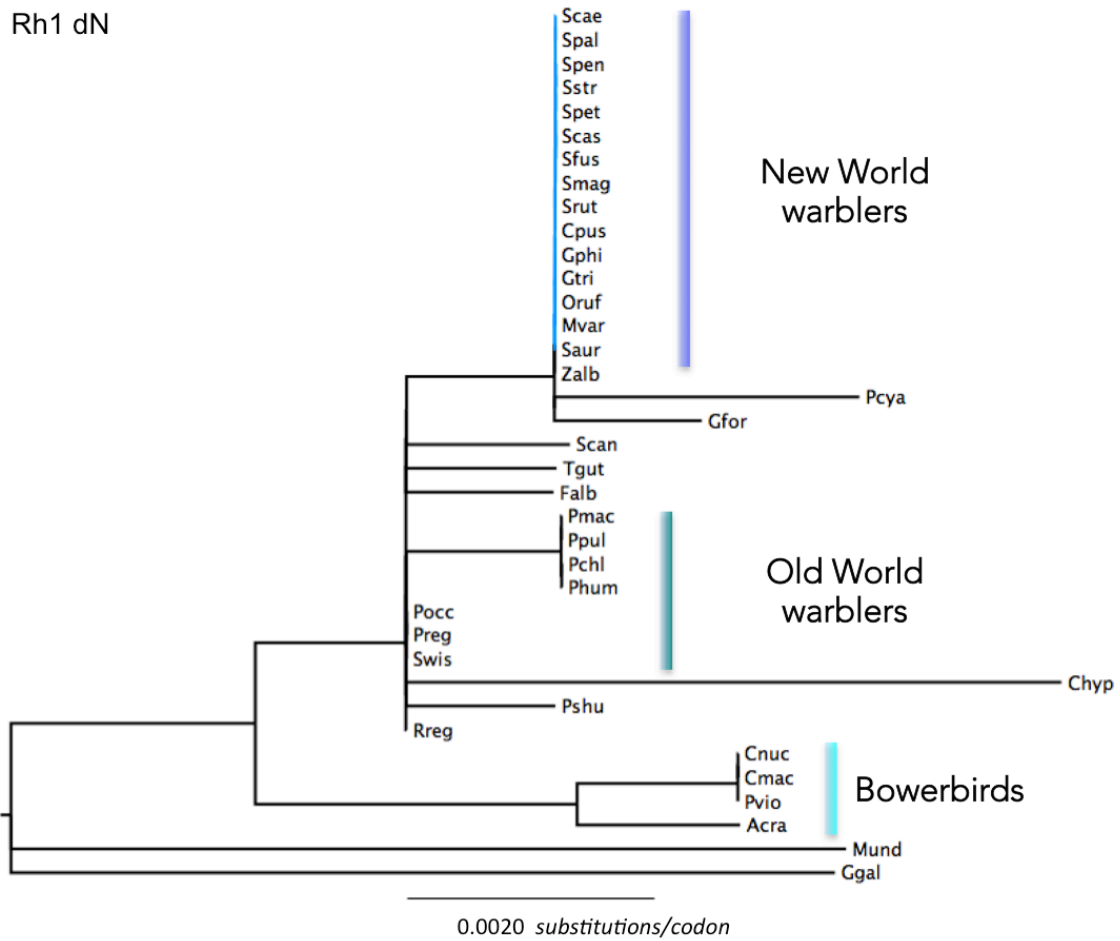
Numbers correspond to amino acid positions standardized by the bovine Rhodopsin (GenBank M21606). Here complete amino acid sequences were used. The last line (CONS) corresponds to the consensus sequence. Dots indicate the identity of the amino acids with the consensus sequences at each site, thus species that only have dots match the consensus amino acid sequence at all sites. Dashes (-) indicate positions for which we do not have sequence information in bowerbirds (Sites missing in the limited sequence dataset because Bowerbird sequences from Coyle et al. (2012) cover amino acids residues 55 to 314). *Indicates the site that was inferred to be evolving under positive selection in models using the limited sequence dataset. Species names are abbreviated as the first letter of the genus and the three first letters of the species see Fig. 1 & 2 for full species names). Abbreviations of bowerbird species correspond to *Ailuroedus crassirostris*, *Archboldia papuensis*, *Amblyornis macgregoriae*, *Amblyornis subalaris*, *Amblyornis inornatus*, *Chlamydera nuchalis*, *Chlamydera maculata*, *Chlamydera guttata*, *Prionodura newtoniana*, *Ptilonorhynchus violaceus*.

Figure S3: Cladogram of the New World warbler species tracing the accumulation of Rh2 substitutions across New World warblers.



Substitutions are shown next to the branch where they occur. The phylogeny for New World warblers and *Z. albicollis* is based on Lovette et al. (2010). Posterior probabilities for variable amino acid sites at every node from Empirical Bayes ancestral reconstructions are shown in parentheses. All ancestral reconstruction models agreed on the identity of amino acids at all nodes. We show posterior probabilities for nucleotide EB models, which were the most conservative compared to codon and amino acid models. Values in green boxes indicate the λ_{\max} values of the RH2 pigment for the ancestor of New World warblers and each species or node where a substitution occurred. Unless otherwise indicated the RH2 of all species following a node have the same λ_{\max} .

Figure S4: Variation in Rh1 non-synonymous rates of evolution (d_N) across Passerines.



Branch lengths are scaled by d_N rates. Branch lengths were estimated using a free ratio model as implemented in PAML, that estimates independent d_N rates for each branch in the tree. Branches in blue highlight New World warblers. Scale bar indicates value of d_N as in the Rh2 trees in Fig. 4.

2. Supplementary tables

Table S1: Species with available Rh2 and Rh1 sequences used in evolutionary analyses

Order	Family	Species	Common name	Rh2 Accession #	Rh1 Accession #	Reference
Passeriformes	Estrildidae	<i>Taeniopygia guttata</i>	Zebrafinch	AF222332	AF222329	(Yokoyama <i>et al.</i> 2000)
Passeriformes	Thraupidae	<i>Geospiza fortis</i>	Medium ground-finch	XM_005428907	NA	Derived from genome by computational analysis
Passeriformes	Muscicapidae	<i>Ficedula albicollis</i>	Collared flycatcher	XM_005059479	NA	Derived from genome by computational analysis
Passeriformes	Fringillidae	<i>Serinus canaria</i>	Canary	AJ277923	AJ277926	(Das <i>et al.</i> 1999)
Passeriformes	Paridae	<i>Pseudopodoces humilis</i>	Hume's ground-tit	XM_005529357	NA	Derived from genome by computational analysis
Psittaciformes	Psittacidae	<i>Melopsittacus undulatus</i>	Budgerigar	AF021241	AJ277926	(Heath <i>et al.</i> 1997)
Galliformes	Phasianidae	<i>Gallus gallus</i>	Chicken	NM205517	NM_001030606	(Okano <i>et al.</i> 1992)
Passeriformes	Ptilonorhynchidae	<i>Ailuroedus crassirostris</i>	Green catbird	JQ034374	JQ034381	(Coyle <i>et al.</i> 2012)
Passeriformes	Ptilonorhynchidae	<i>Archboldia papuensis</i>	Archbold bowerbird	JQ034403	NA	(Coyle <i>et al.</i> 2012)
Passeriformes	Ptilonorhynchidae	<i>Amblyornis macgregoriae</i>	Macgregor bowerbird	JQ034402	NA	(Coyle <i>et al.</i> 2012)
Passeriformes	Ptilonorhynchidae	<i>Amblyornis subalaris</i>	Streaked bowerbird	JQ034400	NA	(Coyle <i>et al.</i> 2012)
Passeriformes	Ptilonorhynchidae	<i>Amblyornis inornatus</i>	Vogelkop bowerbird	JQ034401	NA	(Coyle <i>et al.</i> 2012)
Passeriformes	Ptilonorhynchidae	<i>Chlamydera nuchalis</i>	Great bowerbird	JQ034395	JQ034384	(Coyle <i>et al.</i> 2012)
Passeriformes	Ptilonorhynchidae	<i>Chlamydera maculata</i>	Spotted bowerbird	JQ034394	JQ034385	(Coyle <i>et al.</i> 2012)
Passeriformes	Ptilonorhynchidae	<i>Chlamydera guttata</i>	Western bowerbird	JQ034393	NA	(Coyle <i>et al.</i> 2012)
Passeriformes	Ptilonorhynchidae	<i>Chlamydera cerviniventris</i>	Fawn-breasted bowerbird	JQ034396	NA	(Coyle <i>et al.</i> 2012)
Passeriformes	Ptilonorhynchidae	<i>Prionodura newtoniana</i>	Golden bowerbird	JQ034399	NA	(Coyle <i>et al.</i> 2012)
Passeriformes	Ptilonorhynchidae	<i>Ptilonorhynchus violaceus</i>	Satin bowerbird	JQ034392	JQ034383	(Coyle <i>et al.</i> 2012)

Table S2: Primers used to amplify full opsin coding sequences

A		Rh2	
		Outer	Nested
Initial Degenerate Primers	for rev	GGG ACG GAR GGS RTC AAT TTT TA ACT TGG CTG GAG GAR AYR GA	TTT ACG TGC CTA TGT CCA ACA AGA C GAC CTC RGT CTT GCT CTG GGA TA
RACE primers <i>(used in combination with universal primers (UOP & NUP))</i>	5' 3'	AAA GCA GGC CAT GCA CA TTC GGC TGG TCC AGG TAC	GCT TCT TGT GCT TGA AGG TG TTC GGC TGG TCC AGG TAC
Primers for coding sequences amplification	for rev	CAG GGC AGC ATG AAC GGG ACG G GCT CGG CAG GTC ACC CCA GTT C	ATG AAC GGG ACG GAG GGG AT ACC CCA GTT CAA ACT GTC CAT GC

B		Rh1	
		Outer	Nested
Initial Degenerate Primers	for rev	ATG AAC GGG ACM GAA GGC CAA GAG ACC TGG CTG GTG GAR ACG GA	GAC TTC TAY GTG CCC ATG TCC AA CTC TGT CTT GCC MGC AGA YGT GT
RACE primers <i>(used in combination with universal primers (UOP & NUP))</i>	5' 3'	TGT ACA TGG TGG TTG TGA AGC C TGG TTC ACT TCA TGA TCC CGC T	TTG TGC TGG ATG GTG ACG TA TTC TGC TAT GGG AAC CTG GTC T
Primers for coding sequences amplification	for rev	TGG GGT GAC AGT GAA GGC TGA TTT CAG CCT TGT TCC CAG GGT	CAT GAA CGG GAC AGA AGG CCA CCT ATG CAG GAG AGA CCT GGC TG

Primers used for amplification of full coding sequences for A) Rh2 and B) Rh1 opsin genes.

UOP: Universal outer primer. NUP: Nested universal primer.

**These primers are the same. "for" stands for forward primer, "rev" stands for reverse primers.

Table S3: Sequences used in the construction of the time-calibrated phylogeny of species used in this study.

SPECIES	SWS1	RAG1	Myoglobin	ODC	GAPDH	Cytb	ND2
<i>Mniotilta varia</i>	KM516261	KC007795.1				AF383006.1	
<i>Setophaga ruticilla</i>	KM516263	KC007796.1	GU932298.1	EU325831.1		AF383008.1	AF383124.1
<i>Setophaga palmarum</i>	KM516255					EU815685.1	JN568665.1
<i>Setophaga fusca</i>	KM516253		GU932300.1			AY340208.1	
<i>Setophaga castanea</i>	KM516252					EU815675.1	
<i>Setophaga striata</i>	KM516258		GU932313.1			EU815688.1	EU815772.1
<i>Setophaga petechia</i>	KM516257		GU932308.1			AF382996.1	JQ218962.1
<i>Setophaga pensylvanica</i>	KM516256		GU932307.1			AY216828.1	
<i>Setophaga magnolia</i>	KM516254					EU815682.1	
<i>Setophaga caerulescens</i>	KM516251		GU932289.1			EU815674.1	JN568626.1
<i>Oreothlypis ruficapilla</i>	KM516264		GU932348.1			GU932421.1	
<i>Seiurus aurocapilla</i>	KM516262		GU932257.1			AF383007.1	AF383123.1
<i>Geothlypis philadelphia</i>	KM516260					GU932399.1	
<i>Geothlypis trichas</i>	KM516259					AF383003.1	AF383119.1
<i>Cardellina pusilla</i>	KM516265					GU932416.1	
<i>Setophaga coronata</i>		KC007793.1	GU932292.1			EU815677.1	AY030146.1
<i>Cardellina canadensis</i>		KC007805.1	GU932342.1			AF383016.1	
<i>Phylloscopus pulcher</i>	KM516270		KJ454867.1	KJ455871.1	KJ455124.1	KJ456396.1	KJ455565.1
<i>Phylloscopus maculipennis</i>	KM516267		AY887701.1	KJ455868.1	KJ455121.1	KJ456395.1	KJ455562.1
<i>Phylloscopus reguloides</i>	KM516271		AY887709.1	KJ455872.1	KJ455125.1	KJ456397.1	KJ455566.1
<i>Phylloscopus chloronotus</i>	KM516269		DQ008556.1	KJ455864.1		KJ456391.1	KJ455558.1
<i>Phylloscopus humei</i>	KM516266		KJ454866.1	KJ455867.1	KJ455120.1	KJ456394.1	KJ455561.1
<i>Phylloscopus occipitalis</i>	KM516268		AY887703.1	KJ455870.1	KJ455123.1	AY635057.1	KJ455564.1
<i>Seicercus whistleri</i>	KM516272	KJ456144	AY887726.1	KJ455876	KJ455174.1	AY635065.1	KJ455643.1

New World warblers

Old World warblers

<i>Regulus regulus</i>	N. Bloch, unpublished data	JX236451.1					
<i>Chelidorhynch hypoxantha</i>	N. Bloch, unpublished data			GQ369664.1		EF081357.1	GQ145403.1
<i>Zonotrichia albicollis</i>	N. Bloch, unpublished data	XM_005480147.1	XM_005488552.1		XM_005486061.1	EF529931.1	FJ547327.1
<i>Passerina cyanea</i>	N. Bloch, unpublished data		EU191798.1			AF089047.1	AF447296.1
<i>Serinus canaria</i>	AJ277922.1		JN715301.1	JN715393.1	JN715213.1	AY914116.1	JN715484.1
<i>Taeniopygia guttata</i>	NM_001076704.1	XM_002199682.1	XM_002199380.2		NM_001198610.1	AY491525.1	AY323596.2
<i>Geospiza fortis</i>	XM_005430818.1	XM_005419485.1	XM_005421806.1		XM_005420667.1	AF447369.1	AF447282.1
<i>Ficedula albicollis</i>	XM_005061513.1	XM_005046928.1	XM_005039827.1		XM_005038447.1	DQ674491.1	DQ674464.1
<i>Pseudopodoces humilis</i>	XM_005533413.1	XM_005522398.1	XM_005527553.1	KF183744.1	XM_005523849.1	EU382201.1	KF183833.1
<i>Melopsittacus undulatus</i>	Y11787.1	XM_005150647.1	XM_005150402.1		XM_005146692.1	DQ143295.1	EU327633.1
<i>Gallus gallus</i>	NM_205438.1	NM_001031188.1	NM_001167752.2		NM_204305.1	L08376.1	KF792740.1

Table S4: Time calibrations used for date estimates in the phylogeny construction

CALIBRATION TAXON	SISTER TAXA IN TREE	CALIBRATION SOURCE	DATE RANGE (UPPER TO LOWER)	LOWER BOUND	CALIBRATION TYPE	ORIGINAL REFERENCES
<i>Chlorospingus ophthalmicus</i>	<i>Chlorospingus pileatus</i>	Biogeographic (age of Talamanca uplift)	4.5 to 0.0	NA	Uniform	(Gräfe <i>et al.</i> 2002; Weir & Schluter 2008)
<i>Tangara dowii</i>	<i>Tangara nigroviridis</i>	Biogeographic (formation of isthmus of Panama)	3.5 to 0.0	NA	Uniform	(Coates & Obando 1996; Burns & Naoki 2004; Weir & Schluter 2008)
<i>Sylvia melanothorax</i>	<i>Sylvia rueppelli</i>	Biogeographic (reformation of Cyprus)	5.3 to 0.0	NA	Uniform	(Krijgsman <i>et al.</i> 1999; Böhning Gaese <i>et al.</i> 2006; Weir & Schluter 2008)
<i>Cyanocorax yncæ</i>	Old World crows and jays	Fossil <i>Miocitta galbreathi</i> (earliest New World jay)	80.0 to 16.3-13.6	14.95	Uniform	(Brodkorb 1972; Becker 1987)
<i>Oreothlypis celata</i>	<i>Oreothlypis ruficapilla</i>	Fossil <i>Oreothlypis celata</i>	80.0 to 2.0	2.0	Uniform	(Emslie 1998; Lovette & Hochachka 2006; Weir & Schluter 2008)
<i>Psittaculirostris edwardsii</i>	<i>Melopsittacus undulatus</i>	Fossil <i>Melopsittacus</i>	80.0 to 5.5	5.5	Uniform	(Boles 1998)
<i>Acanthisitta chloris</i>	All Eupasserres	Fossil <i>Wieslochia weissi</i> (Oldest Stem Eupasserres)	80.0 to 34.0-30.0	32.0	Uniform	(Mayr & Manegold 2004; 2006)
<i>Passeriformes</i>	Psittaciformes	Fossil <i>Cyrilavis</i> (Halcyornithidae) member of Pan-Psittaciformes (oldest stem parrot lineage)	135.0 to 51.81	51.81	Uniform	(Smith 2010; Ksepka <i>et al.</i> 2011; Ksepka & Clarke 2012)
<i>Coraciidae</i>	Alcedinidae	Fossil <i>Primobucco mcgrewi</i> (oldest stem Coraciidae)	135.0 to 51.81	51.81	Uniform	(Clarke <i>et al.</i> 2009; Ksepka & Clarke 2010)
<i>Eudyptula minor</i>	<i>Puffinus griseus</i>	Fossil <i>Waimanu manningi</i> (oldest stem penguin)	135.0 to 61.0	61.0	Uniform	(Slack <i>et al.</i> 2006)

All time-calibration taxa are a subset of those used in (Price *et al.* 2014).

Table S5: Parameters used to choose the most appropriate models for Empirical Bayes ancestral reconstructions

GENE	TREE	MODEL	lnL	LRT	
Rh2	NEW WORLD WARBLERS	CODON		F61 + Γ	
		F1/61 + Γ	-1920.22	382.56**(60)	
		F1X4 + Γ	-1891.15	324.45**(57)	
		F3X4 + Γ	-1783.62	109.37**(51)	
		F61	-1728.94	NA	
		NUCLEOTIDE		HKY85 + Γ	GRT + Γ
		HKY85+G	-1889.43	NA	53.44** (4)
		JC69+G	-1946.54	114.20** (4)	
		F81+G	-1916.15	53.44 ** (1)	
		K80+G	-1921.22	63.58 ** (3)	
		GTR+G	-1916.15	-	
		AMINO ACID			
		Poisson + F + Γ	-1150.21		
		Jones + F + Γ	-1095.48		
		Dayhoff78 + F + Γ	-1100.69		
		MTMAM + F + Γ	-1097.83		
			PASSERINES	CODON	
F1/61 + Γ	-3591.51			206.07**(60)	
F1X4 + Γ	-3558.90			140.85**(57)	
F3X4 + Γ	-3494.61			12.26 ^{NS} (51)	
F61	-3488.48			NA	
NUCLEOTIDE				HKY85 + Γ	GRT + Γ
HKY85+G	-3629.34			NA	26.77**(4)
JC69+G	-3765.51			272.34**(4)	
F81+G	-3729.57			200.47**(1)	
K80+G	-3670.89			83.11**(3)	
GTR+G	-3615.95			-	
AMINO ACID					
Poisson + F + Γ	-1485.42				
Jones + F + Γ	-1360.03				
Dayhoff78 + F + Γ	-1369.71				
MTMAM + F + Γ	-1364.34				
Rh1	PASSERINES			CODON	
		F1/61 + Γ	-3144.81	323.04**(60)	
		F1X4 + Γ	-3132.38	298.18**(57)	
		F3X4 + Γ	-3045.48	124.39**(51)	
		F61	-2983.29	NA	

Summary of likelihood ratio test performed to chose best Codon, Nucleotide and Amino Acid models for ancestral reconstruction and positive selection tests (codon models). LRT = 2(lnL1- lnL2) and its significance is approximated to the χ^2 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))

Table S6: Parameter estimates for passerine Rh2 clade model D

RH2		Purifying selection site class			Neutral site class		Divergent selection site class			<i>lnL</i>	np	<i>p-value</i> LRT vs M3 (k=2) (df)	<i>p-value</i> LRT vs M3 (k=3) (df)
Model: Foreground clade	Tree length	κ	ω_0	p_0	ω_1	p_1	ω_2 ω_3 ω_4	p_2					
CmD: Setophaga warblers	1.73	6.02	0.000	0.754	0.031	0.225	0.28 2.26	0.021	-2716.29	90	0.029 (3)	0.009 (1)	
CmD: New World warblers	1.73	6.01	0.000	0.758	0.314	0.221	0.303 1.265	0.021	-2718.09	90	0.15 (3)	0.07 (1)	
CmD: Old World warblers	1.72	6.00	0.000	0.804	0.529	0.014	0.051 0.000	0.182	-2716.83	90	0.048 (3)	0.016 (1)	
CmD: Bowerbirds*	1.71	5.98	0.003	0.906	0.080	0.080	0.443 0.753	0.014	-2719.63	90	0.51 (3)	0.64 (1)	
CmD: Setophaga* + Old World warblers	1.74	6.01	0.000	0.747	0.029	0.231	0.354 2.170 0.000	0.022	-2714.24	91	0.011(4)	0.004 (2)	
M3 (k=3)	1.72	6.00	0.000	0.782	0.037	0.199	0.441	0.019	-2719.74	89	0.35 (2)	-	
M3 (k=2)	1.70	5.98	0.004	0.951	0.213	0.049			-2720.79	87	-	-	

CmD: Clade model D with $k = 3$, a model that allows for different ω between a clade set as foreground and the rest of the tree. The framework is similar to Clade model C (CmC), but CmD places no constraint on the estimates of ω for any of the site classes. In each case the clade associated with each test was set as foreground clade. ω_3 , ω_4 are the ratios for the “foreground” clades. *Based on “limited sequence dataset”. Tree length is defined as the sum of the branch lengths along the tree in substitutions per codon.

κ (kappa) transition/transversion rate

p_i refers to the proportion of sites inferred to be in each selection category.

Table S7: Parameter estimates for passerines Rh1 clade models (CmC)

RH1	Model: Foreground clade	Tree length	κ	PURIFYING SELECTION SITE CLASS		NEUTRAL SITE CLASS		DIVERGENT SELECTION SITE CLASS		<i>lnL</i>	np	<i>p-value</i> LRT vs M2a_rel (df)
				ω_0	p_0	ω_1	p_1	ω_2 ω_3	p_2			
	CmC: Setophaga warblers	1.52	3.56	0.000	0.605	1.0	0.003	0.032 0.000	0.391	-3437.96	78	0.009 (1)
	CmC: New World warblers	1.54	3.62	0.011	0.997	1.0	0.00	0.031 0.000	0.387	-3438.38	78	0.014 (1)
	CmC: Old World warblers	1.52	3.57	0.055	0.000	1.0	0.003	0.115 0.000	0.997	-3440.29	78	0.14
	CmC: Bowerbirds	1.52	3.58	0.004	0.807	1.0	0.003	0.031 0.151	0.190	-3440.06	78	0.1
	M2a_rel	1.52	3.57	0.000	0.600	1.0	0.003	0.263	0.396	-3441.4	77	-

CmC: Clade model C, a model that allows for different ω between a clade set as foreground and the rest of the tree. In each case the clade associated with each test was set as foreground clade. ω_3 is the ratio for the “foreground” clade. M2a_rel is the improved test for use as a null model in the LRT by (Weadick & Chang 2012a).

Tree length is defined as the sum of the branch lengths along the tree in substitutions per codon.

κ (kappa) transition/transversion rate

p_i refers to the proportion of sites inferred to be in each selection category.

Table S8: Synonymous and non-synonymous rates of evolution estimated for Rh2 and Rh1

Rh2							
Tree/species considered	model	dN Tree length	dS Tree length	κ	l	np	ω
Main dataset	Free ratio	0.062	3.668	4.711	-3420.87	131	Variable
Limited sequence including bowerbirds	Free ratio	0.057	4.985	5.517	-2713.76	167	Variable
Rh1							
Tree/species considered	model	dN Tree length	dS Tree length	κ	l	np	ω
Main dataset	Free ratio	0.039	2.390	3.584	-3454.60	143	Variable

Estimates obtained from a free-ratio model in PAML. Graphical summaries of each branch dN rates can be found in Fig. 4 & S4.

3. Supplementary references

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