biology
letters
Molecular evolution

Biol. Lett. (2010) **6**, 370–374
doi:10.1098/rsbl.2009.0877

Evolution of ultraviolet vision in shorebirds (Charadriiformes)

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Diurnal birds belong to one of two classes of colour vision. These are distinguished by the maximum absorbance wavelengths of the SWS1 visual pigment sensitive to violet (VS) and ultraviolet (UVS). Shifts between the classes have been rare events during avian evolution. Gulls (Laridae) are the only shorebirds (Charadriiformes) previously reported to have the UVS type of opsin, but too few species have been sampled to infer that gulls are unique among shorebirds or that Laridae is monomorphic for this trait. We have sequenced the SWS1 opsin gene in a broader sample of species. We confirm that cysteine in the key amino acid position 90, characteristic of the UVS class, has been conserved throughout gull evolution but also that the terns *Anous minutus, A. tenuirostris* and *Gygis alba*, and the skimmer *Rynchops niger* carry this trait. Terns, excluding *Anous* and *Gygis*, share the VS conferring serine in position 90 with other shorebirds but it is translated from a codon more similar to that found in UVS shorebirds. The most parsimonious interpretation of these findings, based on a molecular gene tree, is a single VS to UVS shift and a subsequent reversal in one lineage.

Keywords: gulls; UV visual pigment; opsin; phylogeny

1. INTRODUCTION

There is a categorical, physiological difference in colour vision between groups of diurnal birds (Cuthill *et al.* 2000). These have short-wavelength sensitive type 1 (SWS1) cone-opsin-based visual pigments of either an ultraviolet sensitive (UVS) type with maximum absorbance wavelengths ($\lambda_{\max}$) between 355 and 380 nm or a violet sensitive (VS) type with $\lambda_{\max}$ between 402 and 426 nm (reviewed by Ödeen *et al.* 2009). The VS type appears to be ancestral (Yokoyama 2002) and phylogenetically most widespread (Ödeen & Håstad 2003) in birds. Independent shifts to UVS may have occurred only three or four times (Håstad *et al.* 2009): in shorebirds (Charadriiformes), in a common ancestor of passerines and psittaciforms, in *Rhea americana* and possibly in trogons (Carvalho *et al.* 2007). Gulls are the only shorebirds reported to be UVS—demonstrated through the presence of cysteine in amino acid (aa) position 90 (bovine rod opsin numbering) in their SWS1 opsin gene and ocular media with spectral transmission similar to that of other UVS birds (Ödeen & Håstad 2003; Håstad *et al.* 2005, 2009).

There is an increased cost of UV transmittance owing to photo-oxidation of the retina (e.g. Boulton *et al.* 2001), making the presence of UV tuning in gulls rather puzzling. Unlike other UVS birds, no selective advantage of UV vision has been demonstrated in gulls, whether for detecting sexual signals (e.g. Bennett *et al.* 1997) or foraging (e.g. Church *et al.* 1998). It has been suggested to allow eavesdropping on UV communication in schools of fish swimming close to the surface (Ödeen & Håstad 2003) but evidence seems weak. All auks, waders and terns investigated so far have SWS1 opsins of the VS type (Håstad *et al.* 2005), and some, especially terns, are ecologically similar to gulls. However, too few species have been sampled to safely infer that no other charadriiforms have UV-tuned visual pigments, or that this character state does not vary within Laridae.

The SWS1 $\lambda_{\max}$ pigments in avian retinae closely follows aa sequence variation in the SWS1 opsin gene. *In vitro* studies have demonstrated that $\lambda_{\max}$ can be predicted on the basis of the opsin's aa sequence (Wilkie *et al.* 2000; Yokoyama *et al.* 2000; Carvalho *et al.* 2007). The cone sensitivity can then be estimated from the opsin sequence, owing to covariance between the visual pigment's $\lambda_{\max}$ and the spectral absorptance properties of the cone oil droplets (Hart & Vorobyev 2005). As spectral tuning of the SWS1 single cone is under direct genetic control, it becomes possible to identify the type of short-wavelength sensitivity in a bird from a sample of genomic DNA. This method was conceived by Ödeen & Håstad (2003) and validated by Ödeen *et al.* (2009). We have used it here to search for gross differences in colour vision in a significantly widened sample of gulls and other shorebirds. The aim has been to assess how stable the SWS1 pigment sensitivity has been during the evolution of shorebirds in general and gulls in particular. As the basis for this analysis, we have inferred a phylogeny, mainly using GenBank sequence data.

2. MATERIAL AND METHODS

We studied a broad selection of species in the order Charadriiformes (table 1) with special emphasis on including a representative sample of various Laridae taxa. We sequenced a fragment of the SWS1 opsin gene containing the aa residues of positions 84–94 in up to six species each of seven shorebird families (table 1). We estimated $\lambda_{\max}$ from three key positions, as outlined by Ödeen *et al.* (2009).

For the phylogenetic analyses, we used GenBank sequences of three mitochondrial and three nuclear loci, plus own β fibrinogen (FGB7) and myoglobin sequences of *Anous tenuirostris* and *Gygis alba*. Phylogenetic trees were constructed by maximum likelihood for one locus at a time and by Bayesian inference in the following combinations: (i) mitochondrial CO1, ND2 and cytochrome *b*, (ii) nuclear RAG-1, FGB7 and myoglobin intron 2 (myoglobin), and (iii) all loci together. For details, see the electronic supplementary material.

3. RESULTS

(a) *Opsin gene analysis*

All gull species investigated in this and previous studies (table 1) carry the UVS-pigment-conferring Ser to Cys

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2009.0877 or via http://rsbl.royalsocietypublishing.org.

Received 23 October 2009
Accepted 27 November 2009

Table 1. SWS1 opsin gene fragments from shorebirds. The spectral tuning amino acid (aa) sites 86, 90 and 93 are marked in bold. Type of SWS1 opsin is either VS (violet sensitive) or UVS (UV-sensitive), as predicted from the gene fragment aa sequences. NRM, Swedish Museum of Natural History; LSUMZ, LSU Museum of Natural Science; UWBM, Burke Museum, University of Washington; ZMUC, Zoological Museum, University of Copenhagen.

family	species	common name	aa seq. 84–94	aa90 codon	type	unknown effect	origin/voucher no.	tissue no.	GenBank acc. no.	reference
Scolopacidae	*Tringa glareola*	Wood Sandpiper	FI**A**CIF**S**VF**T**V	agc	VS		Department of Animal Ecology, U. U.	031218-2	GU129664	this study
	Actitis hypoleucos	Common Sandpiper	FI**A**CIF**S**VF**T**V	agc	VS				AY960716	Håstad *et al.* (2005)
	Phalaropus fulicarius	Red Phalarope	FI**A**CIF**S**VF**T**V	agc	VS				AY960718	Håstad *et al.* (2005)
	Gallinago gallinago	Common Snipe	FI**A**CIF**S**VF**T**V	agc	VS				AY960717	Håstad *et al.* (2005)
	Philomachus pugnax	Ruff	FI**A**CIF**S**VF**T**V	agc	VS		F. Widemo	11 individuals	GU129665	this study
Thinocoridae	*Thinocorus orbignyianus*	Grey-breasted Seedsnipe	FI**A**CIF**S**VF**T**V	agc	VS		UWBM 54407	DAB 808	GU129666	this study
Laridae	*Larus atlanticus*	Olrog's Gull	FI**I**CVF**C**IS**I**V	tgc	UVS	Ile86, 93	P. Yorio, J.-M. Pons		GU129667	this study
	Larus marinus	Great Black-backed Gull	FI**I**CVF**C**IS**I**V	tgc	UVS	Ile86, 93			AY227162	Ödeen & Håstad (2003)
	Larus argentatus	European Herring Gull	FI**I**CVF**C**IS**I**V	tgc	UVS	Ile86, 93			AY227160	Ödeen & Håstad (2003)
	Larus michahellis	Yellow-legged Gull	FI**I**CVF**C**IS**I**V	tgc	UVS	Ile86, 93			FJ790325	Håstad *et al.* (2009)
	Larus fuscus	Lesser Black-backed Gull	FI**I**CVF**C**ISIV	tgc	UVS	Ile86, 93			AY227161	Ödeen & Håstad (2003)
	Ichthyaetus hemprichii	Sooty Gull	FI**I**CVF**C**IS**I**V	tgc	UVS	Ile86, 93			AY960710	Håstad *et al.* (2005)
	Leucophaeus pipixcan	Franklin's Gull	FI**I**CVF**C**IS**I**V	tgc	UVS	Ile86, 93	UWBM 80595	CSW 6795	GU129668	this study
	Chroicocephalus hartlaubii	Hartlaub's Gull	FI**I**CVL**C**IS**I**V	tgc	UVS	Ile86, 93			AY960709	Håstad *et al.* (2005)
	Chroicocephalus ridibundus	Black-headed Gull	FI**I**CVL**C**IS**I**V	tgc	UVS	Ile86, 93			AY960711	Håstad *et al.* (2005)
	Rhodostethia rosea	Ross's Gull	FV**I**CVF**C**IS**L**V	tgc	UVS	Ile86, Leu93	ZMUC 139640		GU129669	this study
	Rissa tridactyla	Black-legged Kittiwake	FI**I**CVF**C**IS**I**V	tgc	UVS	Ile86, 93			AY960712	Håstad *et al.* (2005)
	Pagophila eburnea	Ivory Gull	FI**I**CVF**C**IS**I**V	tgc	UVS	Ile86, 93	ZMUC 132130		GU129670	this study
	Xema sabini	Sabine's Gull	FI**I**CVF**C**IS**I**V	tgc	UVS	Ile86, 93	LSUMZ B-4066		GU129671	this study
	Creagrus furcatus	Shallow-tailed Gull	FI**I**CVF**C**IS**I**V	tgc	UVS	Ile86, 93	LSUMZ B-15450		GU129672	this study
Rynchopidae	*Rynchops niger*	Black Skimmer	FV**A**CVF**C**IF**T**V	tgc	UVS		LSUMZ B-2457		GU129673	this study
Sternidae	*Anous minutus*	Black Noddy	FI**A**CIF**C**IF**T**V	tgc	UVS		ZMUC 137802		GU129674	this study
	Anous tenuirostris	Lesser Noddy	FI**A**CIF**C**IF**T**V	tgc	UVS		ZMUC 113339		GU129675	this study
	Onychoprion anaethetus	Bridled Tern	FV**T**CVF**S**IF**T**V	tcc	VS	Thr86	ZMUC 112708		GU129676	this study

(*Continued.*)

Table 1. (*Continued.*)

family	species	common name	aa seq. 84–94	aa90 codon	type	unknown effect	origin/voucher no.	tissue no.	GenBank acc. no.	reference
	Onychoprion fuscatus	Sooty Tern	FV**T**CVF**S**IF**T**V	tcc	VS	Thr86	ZMUC 113320		GU129677	this study
	Gygis alba	White Tern	FI**A**CIF**C**IF**T**V	tgc	UVS		ZMUC 113340		GU129678	this study
	Sternula albifrons	Little Tern	FV**T**CIF**S**IF**T**V	tcc	VS	Thr86	NRM 20076398		GU129679	this study
	Chlidonias niger	Black Tern	FV**T**CIF**S**IF**T**V	tcc	VS	Thr86	NRM 20066936		GU129680	this study
	Chlidonias hybrida	Whiskered Tern	FV**T**CIF**S**IF**T**V	tcc	VS	Thr86	ZMUC 131916		GU129681	this study
	Sterna hirundo	Common Tern	FV**T**CIF**S**IF**T**V	tcc	VS	Thr86	Department of Animal Ecology, U. U.	031218-9	GU129682	this study
	Thalasseus sandvicensis	Sandwich Tern	FV**T**CIF**S**IF**T**V	tcc	VS	Thr86			AY960720	Håstad *et al.* (2005)
	Sterna paradisaea	Arctic Tern	FV**T**CIF**S**IF**T**V	tcc	VS	Thr86			AY960719	Håstad *et al.* (2005)
Alcidae	*Uria aalge*	Common Murre	FL**A**CIF**S**VF**T**V	agc	VS				AY227163	Ödeen & Håstad (2003)
	Alca torda	Razorbill	FV**A**CIF**S**VF**T**V	agc	VS				AY227159	Ödeen & Håstad (2003)
Stercorariidae	*Stercorarius longicaudus*	Long-tailed Jaeger	FV**A**CIF**S**VF**T**V	agc	VS		NRM 976537		GU129683	this study
	Stercorarius parasiticus	Parasitic Jaeger	FV**A**CIF**S**VF**T**V	agc	VS		NRM 20066101		GU129684	this study
	Stercorarius maccormicki	South Polar Skua	FV**A**CIF**S**VF**T**V	agc	VS		ZMUC 131807		GU129685	this study
Glareolidae	*Glareola pratincola*	Collared Pratincole	FL**A**CVF**S**VF**T**V	agc	VS		M. Wilson, A.Ö.	Ug35	GU129686	this study
	Glareola nuchalis	Rock Pratincole	FL**A**CIF**S**VF**T**V	agc	VS		ZMUC 113386		GU129687	this study
	Rhinoptilus chalcopterus	Bronze-winged Courser	CL**A**CLF**S**VF**T**V	agc	VS		ZMUC 131583		GU129688	this study
Recurvirostridae	*Himantopus himantopus*	Black-winged Stilt	FV**A**CIF**S**VF**T**V	agc	VS				AY227156	Ödeen & Håstad (2003)
Haematopodidae	*Haematopus ostralegus*	Eurasian Oystercatcher	FI**A**CIF**S**VF**T**V	agc	VS				AY227155	Ödeen & Håstad (2003)
Charadriidae	*Pluvialis apricaria*	Eurasian Golden Plover	FI**A**CIF**S**VF**T**V	agc	VS		Department of Animal Ecology, U. U.	Ljungpipare98	GU129689	this study
	Charadrius dubius	Little Ringed Plover	FI**A**CIF**S**VF**T**V	agc	VS				AY227154	Ödeen & Håstad (2003)

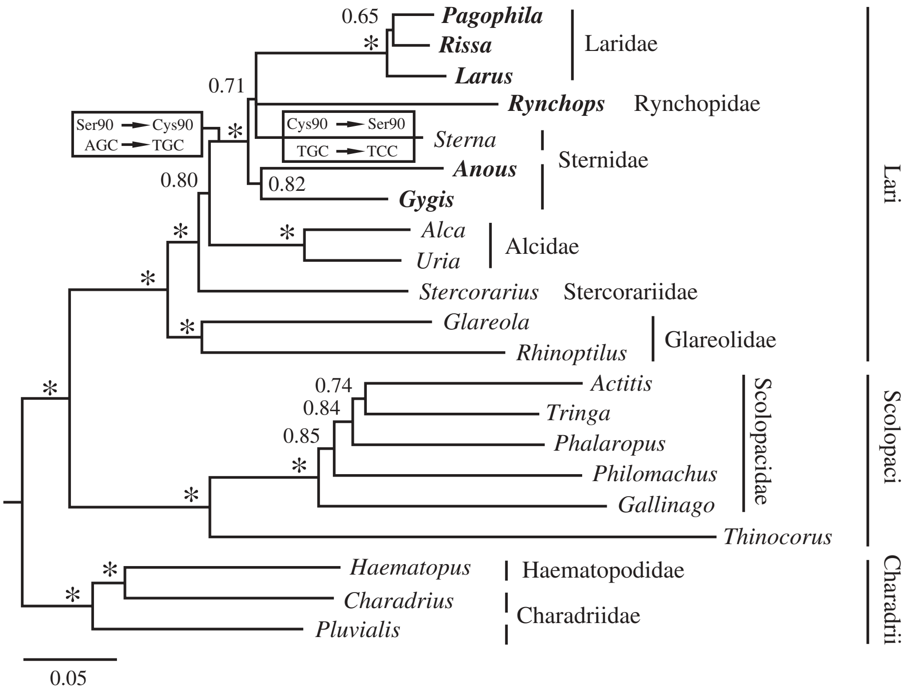

Figure 1. Bayesian inference tree of shorebirds based on mitochondrial ND2, cytochrome *b* and CO1, and nuclear RAG-1, FGB7 and myoglobin (approx. 7.4 kbp). The species having the UVS-pigment-conferring Ser90Cys substitution in their SWS1 opsin are in bold. The most parsimonious reconstruction of the sequence of mutations in the corresponding codon is shown in boxes. Numbers at nodes are posterior probabilities (values of 1.00 indicated by *). The name of the family Thinocoridae (comprising *Thinocorus*) is not marked owing to lack of space.

substitution at aa position 90 (Ser90Cys: Wilkie *et al.* 2000; Yokoyama *et al.* 2000), as specified by the codon TGC in the SWS1 opsin gene. We found this trait in four other shorebirds, namely *A. minutus, A. tenuirostris*, *G. alba* (Sternidae) and *Rynchops niger* (Rynchopidae). All other species examined carry Ser90, which is ancestral to Charadriiformes (Ödeen & Håstad 2003; Håstad *et al.* 2005), but translated from different synonymous codons. In general, VS shorebirds carry AGC; the exception is Sternidae (excluding *Anous* and *Gygis*), where all sequences read TCC instead. The distribution of these traits on the phylogeny is shown in figure 1 (and the electronic supplementary material, figures S1 and S2).

(b) *Phylogeny*

All analyses produced results largely in agreement with previous studies (Ericson *et al.* 2003; Paton *et al.* 2003; Paton & Baker 2006; Baker *et al.* 2007; Fain & Houde 2007), and identify three main clades corresponding to Lari, Scolopaci and Charadrii. Only the tree based on all loci (figure 1) is shown (combined nuclear and mitochondrial gene trees in the electronic supplementary material, figures S1 and S2). All trees recover a strongly supported clade comprising Laridae, Sternidae and *Rynchops*. Within this clade, in the tree based on all loci, Laridae, *Rynchops* and *Sterna* form a poorly supported clade, which is sister to an insufficiently supported clade comprising *Anous* and *Gygis*. The nuclear tree has the same topology, but even weaker support. In contrast, in the mitochondrial tree, Laridae, *Rynchops* and *Anous* form a moderately well-supported trichotomy that is sister to a strongly supported clade containing *Gygis* as sister to the rest of Sternidae (excluding *Anous*).

4. DISCUSSION

Our results agree with previous studies in showing that gulls have Cys90 in their SWS1 opsins, characteristic of the UVS class of colour vision. This trait has been conserved during gull evolution, as revealed by the denser taxon sampling of this study compared with previous ones (Håstad *et al.* 2005). Another new finding is that gulls are not unique among shorebirds in being UVS, as the terns *A. minutus*, *A. tenuirostris* and *G. alba* and the skimmer *R. niger* share the UVS opsin forming Ser90Cys substitution.

The evolutionary interpretation of our results hinges on the correct inference of the phylogeny. As the relationships in the Laridae/Sternidae/Rynchopidae clade are somewhat uncertain, different interpretations are possible. However, the most parsimonious reconstruction suggests a Ser90Cys substitution in the common ancestor to Laridae/Sternidae/Rynchopidae, and a subsequent reversal to Ser in the Sternidae lineage (except *Anous* and *Gygis*), in all formed by two consecutive point mutations in the codon at aa position 90, from AGC to TGC to TCC. This is true even if the uncertainty in the gene tree is taken into account by considering the Sternidae branch (except *Anous* and *Gygis*) in alternative positions. For example, the posterior probability that *Sterna* is outside the clade comprising Laridae, *Rynchops*, *Anous* and *Gygis*—which would support a single Ser90Cys substitution but no subsequent reversal—is lesser than 0.06. Accordingly, our favoured hypothesis based on the available data is a single shift from VS to UVS in the common ancestor to Laridae/Sternidae/Rynchopidae and a subsequent reversal in the Sternidae lineage (except *Anous* and *Gygis*).

All key aas we report in positions 86, 90 and 93 have already been described (Wilkie *et al.* 2000; Yokoyama *et al.* 2000; Ödeen & Håstad 2003), but the spectral tuning effects of some of them are still unknown. The effect of Cys86 in birds is probably negligible (Ödeen *et al.* 2009), but neither the effects of Thr86 or Ile86 nor Ile93 has been investigated *in vitro*. However, the λ_{max} of SWS1 pigment of *Anas platyrhynchos*, which carries Ile93, is not significantly different from that of species with aas of known effect in this position (Ödeen *et al.* 2009).

In one aspect, avian colour vision is under simple genetic control: the VS/UVS pigment opsin character depends on the Ser90Cys substitution, which can be caused by a single nucleotide mutation. Still, this character is surprisingly conserved among birds, indicating strong stabilizing selection. This is a rare example of a system where two very similar and well-understood variants of the same gene, which have a significantly different effect on the phenotype and ecology of the carrier, have been equally successful in a large taxon.

Tissue and DNA samples were kindly provided by Burke Museum, University of Washington, Seattle; Zoological Museum, University of Copenhagen, Copenhagen; LSU Museum of Natural Science, Baton Rouge; Swedish Museum of Natural History, Stockholm; Department of Animal Ecology, Uppsala University; Fredrik Widemo, Jean-Marie Pons and Malcom Wilson. This study was funded by the Swedish Research Council Formas (A.Ö.). Two anonymous reviewers provided constructive criticism, which has improved this paper.

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