

SHORT COMMUNICATION

Nestling coloration is adjusted to parent visual performance in altricial birds irrespective of assumptions on vision system for *Laniidae* and owls, a reply to Renoult *et al.*

J. M. AVILÉS & J. J. SOLER

Departamento de Ecología Funcional y Evolutiva, Estación Experimental de Zonas Áridas (C.S.I.C.), Almería, Spain

Keywords:

Laniidae;
owls;
PGLS models;
visual system assumptions.

Abstract

We have recently published support to the hypothesis that visual systems of parents could affect nestling detectability and, consequently, influences the evolution of nestling colour designs in altricial birds. We provided comparative evidence of an adjustment of nestling colour designs to the visual system of parents that we have found in a comparative study on 22 altricial bird species. In this issue, however, Renoult *et al.* (*J. Evol. Biol.*, 2009) question some of the assumptions and statistical approaches in our study. Their argumentation relied on two major points: (1) an incorrect assignment of vision system to four out of 22 sampled species in our study; and (2) the use of an incorrect approach for phylogenetic correction of the predicted associations. Here, we discuss in detail re-assignment of vision systems in that study and propose alternative interpretation for current knowledge on spectrophotometric data of avian pigments. We reanalysed the data by using phylogenetic generalized least squares analyses that account for the alluded limitations of phylogenetically independent contrasts and, in accordance with the hypothesis, confirmed a significant influence of parental visual system on gape coloration. Our results proved to be robust to the assumptions on visual system evolution for *Laniidae* and nocturnal owls that Renoult *et al.* (*J. Evol. Biol.*, 2009) study suggested may have flawed our early findings. Thus, the hypothesis that selection has resulted in increased detectability of nestling by adjusting gape coloration to parental visual systems is currently supported by our comparative data.

Introduction

Nestlings of altricial birds must be fed by adults promptly after hatching. A large body of functional hypotheses suggests that, throughout their coloration, nestlings may signal to their parents some individual characteristics that may help them to decide how to allocate their feeding effort (reviewed in Kilner, 2006). Parental preference for

a coloration reflecting a particular characteristic would lead to selection on variable nestling coloration. A necessary corollary of the signalling hypothesis is that parents are able to discriminate the coloured signals emitted by nestlings. Gape and body skin colours are therefore viewed as an adaptation to increase nestling detectability to feeding parents (Heeb *et al.*, 2003; Kilner, 2006). Nowadays, most of the effort in the understanding of perceptual efficacy of begging signals has been devoted to study the adjustment of nestling coloration to the luminal characteristics in the nests (Ficken, 1965; Kilner & Davies, 1998; Hunt *et al.*, 2003; Avilés *et al.*, 2008), but recently we proposed that visual systems of parents could also affect nestling detectability by parents and,

Correspondence: Jesús M. Avilés, Departamento de Ecología Funcional y Evolutiva, Estación Experimental de Zonas Áridas (C.S.I.C.), General Segura 1, E-04001 Almería, Spain.
Tel.: +34 950 281 045; fax: +34 950 277 110;
e-mail: javiles@eeza.csic.es

consequently influences the evolution of nestling colour designs (Avilés & Soler, 2009).

Visual sensitivity of birds varies across species in the short-wavelength sensitive (SWS1) ultraviolet/violet visual pigment λ_{\max} (Hart & Vorobyev, 2005; Hart & Hunt, 2007), leading to differences among species in how they perceive ultraviolet and violet colours, but also of all non-spectral colours in which SWS1 were involved (Cuthill *et al.*, 2000). It follows that nestling colour designs were adjusted to the visual system of their parents as an adaptation to enhance nestling detectability (Avilés & Soler, 2009). An obvious prerequisite for the proposed hypothesis is that the two different avian visual systems performed differently at detecting nestling coloration. In our article we first used a colour discrimination model that allowed us to estimate nestling conspicuousness of 22 altricial bird species from the perspective of UV-tuned and violet-tuned receivers, and, found that the UV eye enhanced the performance of the violet eye when looking at the nestling traits under the light regimes prevailing at their nests (Avilés & Soler, 2009). In a second step we explored variation in achromatic and chromatic components of nestling coloration in relation to parental visual systems. Analyses performed on species as independent data points revealed that nestlings of UVS species displayed more yellow and less pure ultraviolet mouths than nestlings of VS species (Avilés & Soler, 2009). This pattern was confirmed when analyses were performed on phylogenetic independent contrasts, and when we used phylogenetically controlled ANOVAS (Avilés & Soler, 2009). Our results agreed with the prediction for the hypothesized adaptive parent–offspring communication scenario where nestlings' colours tuned the perception capacities of their parents.

In this journal volume, however, Renoult *et al.* (2009) questioned some of the assumptions and statistical approaches in our study and, based on new analyses on our data, claimed that their study refuted the association of gape coloration with parental visual sensitivity that we had previously reported. Their argumentation relied on two major points: (1) an incorrect assignment of vision system to four out of 22 sampled species in our study; and (2) the use of an incorrect approach for phylogenetic correction of the predicted associations. Briefly, they reported that the association between nestling gape coloration and parental visual system vanished when relying on phylogenetically independent standardized linear contrasts once the two nocturnal birds were removed and the *Lanius* species were reassigned as VS. We appreciate Renoult *et al.*'s effort for testing the robustness of our previous results, and for driving our attention to possible weakness in the assumptions and analytical approach of our work. It gives us the opportunity of further explaining and discussing reasons under our assumptions and analysing our prediction with

a more suitable analytical approach for phylogenetic corrections.

Here, therefore, we discuss in detail Renoult *et al.* (2009) re-assignment of vision systems, propose alternative interpretation for current knowledge on spectrophotometric data of avian pigments, and provide new phylogenetic analyses that provided results consistent with our previous findings that supported the hypothesis of an adjustment of nestling coloration to parental vision system in altricial birds.

On the assignment of species to visual systems

We concur with and thank Renoult *et al.*'s (2009) suggestions of considering the two sampled shrike species as violet sensitive following the assumption of strong phylogenetic inertia of avian visual systems. We think, however, that their suggestion of excluding the two owl species, viz. Scops owl *Otus scops* and Little owl *Athene noctua*, from our analyses is not justified based on current knowledge.

Renoult *et al.* (2009) suggest that the inclusion of two owl species as violet sensitive was unjustified given the assumed principle of strong phylogenetic inertia for bird visual systems. They base their argumentation in the fact that an early work by Bowmaker & Martin (1978) based on microspectrophotometric measures taken on Tawny owl *Strix aluco* retinas failed to report the presence of a fourth pigment, although in the same work the authors explicitly admitted the existence of four visual cones in the retina of tawny owls. They argue that an ulterior failure to amplify a fragment of the SWS1 gene in this species (Ödeen & Håstad, 2003) is consistent with their argumentation of owl trichromacy. We do not agree with that conclusion and will, therefore, first discuss evidence in support of owl trichromacy and, later on, the importance of not detecting SWS1 pigment by Ödeen & Håstad (2003).

Bowmaker & Martin (1978) finished their article saying, together with other considerations, that the Tawny owl has a trichromatic photopic system. However, they also stated, even in the abstract, that 'microspectrophotometer measurements of the oil droplets and visual pigments in the receptors of the tawny owl ... have demonstrated the presence of at least four types of oil droplets and three visual pigments'. Thus, although they found evidence of ('at least') three different pigments, they also found four different cones and oil droplet types, which is evidence of having four different types of cone opsins. In fact, they described in a figure (Fig. 5 in Bowmaker & Martin, 1978) the absorbance spectra for the 'pigment–oil droplet combinations in the four recognised cone types'. In agreement, Vorobyev *et al.* (1998, p. 621), even after explicitly mentioning that only three type of cone pigment were detected in the Tawny owl and in one species of penguin (*Spheniscus humboldti*),

concluded that possibly ALL birds have retained four cone opsins.

As additional evidence supporting their idea of owl trichromacy, Renoult *et al.* (2009), by citing Bennett & Cuthill (1994) and Cuthill *et al.* (2000), argued that the absence of SWS1 pigment in the tawny owl is probably linked to its nocturnal habit. They seemed to ignore recent behavioural and molecular evidence supporting the existence of SWS1 opsin types that have recently been published. On the one hand behavioural studies have demonstrated that the Eagle owl *Bubo bubo* and the Scops owl *Otus scops* (two species of the *Strigidae* family) responded to manipulations of visual signals in the wavelengths that would primarily be perceived by SWS1 pigments (Penteriani *et al.*, 2007a;b; Parejo *et al.*, 2009). On the other hand, Ödeen & Håstad (2003) reported that the European nightjar *Caprimulgus europaeus*, a species that as all members of its family shares nocturnal habits with owls, has a SWS1 opsin protein biased toward violet (see also Hart & Hunt, 2007). Interestingly, DNA–DNA hybridization data (Sibley & Ahlquist, 1990; Espinosa de los Monteros, 2000) suggested a sister group relationship between Strigiformes and Caprimulgiformes. Other molecular phylogenies have joined *Strigidae* to *Falconidae* (Mindell *et al.*, 1997; Harrison *et al.*, 2004) or *Accipitridae* (Hackett *et al.*, 2008). Evidence suggests that all these possible sister families of owls are tetrachromatic (Vorobyev *et al.*, 1998) and possess a violet sensitive vision system (Ödeen & Håstad, 2003; Hart & Hunt, 2007). Thus, neither nocturnal habits, nor phylogenetic inertia would support the hypothesized owl trichromacy.

The second source of evidence supporting owl trichromacy reported by Renoult *et al.* (2009) is that Ödeen & Håstad (2003) did not find evidence of the presence of SWS1 opsin in the Tawny owl. They, however, explicitly stated that, 'for unknown reasons', neither they were able to amplify the SWS1 opsin sequence for this and another species including Brant *Branta bernicla*, Green-winged teal *Anas crecca*, Common swift *Apus apus*, Golden eagle *Aquila chrysaetos*, and the Great crested grebe *Podiceps cristatus* (Ödeen & Håstad, 2003, p. 858). Some of these species have close relatives (even belonging to the same genus) that were amplified for that SWS1 opsin (see Table 1 in Ödeen & Håstad, 2003), and/or have exhibited a behavioural response to experimental manipulation of visual signals that are captured by SWS1 pigments (e.g. Bize *et al.*, 2006). Therefore, it is possible that the non-amplification of SWS1 opsin sequence in Tawny owl was in fact the result of methodological problems rather than a lack of the SWS1 pigment. Inconsistencies when studying the existence of cone pigments are in fact no rare in the literature. For instance, by sequencing part of the gene coding for the ultraviolet and violet absorbing opsin in the avian retina, Ödeen & Håstad (2003) revealed the existence of a SWS1 opsin in two species of the genus *Corvus*, a group of birds where early

microspectrophotometric analyses had failed to reveal the presence of a SWS1 pigment (Bowmaker, 1979).

Microspectrophotometry was the standard method used to examine the chromatic ocular disposition of animals in the last few decades. Due to the complexity of the method (Hart *et al.*, 1999), however, the absorbance of visual pigments has only been examined in a limited number of species, being relatively frequent obtaining partial results for location of pigments in some bird species (see Table 2 in Hart & Hunt, 2007). Thus, the non-detection of a fourth pigment in a bird species should cautiously be interpreted and does not necessarily constitute a proof of visual trichromatism. Summarising, we think that methodological problems during early microspectrophotometric analyses rather than a lack of SWS1 pigment may explain the alluded owl trichromacy defended by Renoult *et al.* (2009).

Based on these considerations, and pending new data for the considered owl species, we believe that the most parsimonious classification of owls regarding their vision system is violet sensitive.

On the phylogenetic approach

Renoult *et al.* (2009) questioned the use of phylogenetically independent contrasts (PICs hereafter, sensu Felsenstein, 1985) to control for the possible effects of common ancestry on the relationship between nestling coloration and parental visual system. They, however, built up their conclusion of a lack of relationship between nestling colour and parental vision system using PICs (i.e. the same phylogenetic approach that we had used), which is surprising given that they are aware of the weakness and limitations of this method.

We appreciate, however, Renoult *et al.*'s (2009) considerations on the possible weakness of our analytical approach and, accordingly, reassessed the predicted association between nestling colour traits and visual system using phylogenetic generalized least squares analyses (PGLS; Martins & Hansen, 1997); a phylogenetic approach that account for the alluded limitations. The main advantage of PGLS is that it is not constrained to a single evolutionary model, as are PICs. Brownian motion or other models of evolution can be incorporated as long as they allow for a variance-covariance matrix to be constructed (Martins & Hansen, 1997; Rohlf, 2006). Thus, PGLS methods should be preferred when the data show departure from Brownian motion, which is likely to be the case if branch lengths are set to equal values as in our study (see Avilés & Soler, 2009). A simulation study by Martins *et al.* (2002) showed that performance of PICs is compromised when the model of evolution shows marked departure from Brownian motion. Furthermore, PICs rely on reconstruction of ancestral states, which can be difficult for labile traits as it seems to be the case for gape coloration (see results below). Another advantage of using PGLS is that the value of the

Table 1 Relationships between gape colour (PC1 and PC2 for flanges and mouth) and nesting site and parental visual system as independent variables.

	Dependent variable	Lambda	P-value (=1)	P-value (=0)	F (Parental visual system)	P-value	F (Nesting site)	P-value
PGLS with Avilés & Soler classification	PC1 (flange)	0.00008	< 0.0001	1.00	16.45	0.0006	13.12	0.002
	PC2 (flange)	0.47	0.009	0.20	0.02	0.87	0.29	0.59
	PC1 (mouth)	0.56	0.053	0.11	0.73	0.40	13.91	0.001
	PC2 (mouth)	0.80	0.006	0.06	10.86	0.003	3.42	0.08
PGLS with Lanius as VS and including owls	PC1 (flange)	0.00008	< 0.0001	1.00	20.21	0.0002	14.51	0.001
	PC2 (flange)	0.48	0.0009	0.14	0.17	0.68	0.29	0.59
	PC1 (mouth)	0.52	0.03	0.11	1.37	0.25	14.55	0.001
	PC2 (mouth)	0.84	0.02	0.09	4.83	0.04	3.06	0.09
PGLS with Lanius as VS and excluding owls	PC1 (flange)	0.00007	< 0.0001	1.00	15.45	0.001	22.06	0.0002
	PC2 (flange)	0.50	0.002	0.14	0.08	0.77	0.19	0.67
	PC1 (mouth)	0.53	0.05	0.13	0.93	0.35	12.86	0.002
	PC2 (mouth)	0.85	0.03	0.12	4.39	0.051	2.81	0.111

Results are from phylogenetic generalized least squares analyses (PGLS).

additional parameter estimated during the analysis (either lambda or alpha depending on the model of evolution) also provides information on how the traits have been evolving.

Therefore, to control for such phylogenetic relationship we used phylogenetic generalized least square regression (PGLS) models (Pagel, 1997, 1999) as implemented in *R* statistical environment with additional unpublished function by R. Freckleton (*pglm3.3.r*; available on request), which allow the inclusion of discrete variables as independent factors. The PGLS approach characterizes evolutionary changes along each branch of a phylogeny through the variance components of traits and controls for the non-independence among species by incorporating a matrix of the covariances among species based on their phylogenetic relationships (Martins & Hansen, 1997; Pagel, 1997, 1999). The method applies likelihood ratio statistics to test hypotheses of correlated trait evolution and also to estimate the importance of phylogenetic corrections in the models (Freckleton *et al.*, 2002). We conducted all analyses setting the degree of phylogenetic dependence (λ) to the most appropriate degree evaluated for each model. Values of $\lambda < 1$ would correspond to traits being less similar amongst species than expected from their phylogenetic relationship, while $\lambda = 1$ suggests the reverse. Models were built with PC1 and PC2 colour scores for flanges and mouth as separate dependent variables and parental visual system and nesting site as independent variables.

Using the original classification provided in our work and PGLS to control for the phylogenetic relationships among the sampled species we found the same significant influence of parental visual system on PC1 of flanges and on PC2 of mouths that reported in Avilés & Soler (2009) (Table 1). We obtained qualitatively identical results when we repeated the analysis including the two *Lanius* species as violet sensitive and included the two owl

species as tetrachromats with violet sensitive vision (Table 1). Finally, although we consider it unlikely (see above), we contrasted the possible influence of the alluded by Renoult *et al.* (2009) owl trichromatism on our results. Interestingly, and demonstrating the robustness of our previous results, we found that PC1 of flanges and PC2 of mouths differed between ultraviolet and violet sensitive species once we removed the two owl species from the analysis (Table 1).

Interestingly, the PGLS also revealed a low degree of phylogenetic dependence for gape coloration irrespective of the assumptions made for the evolution of vision system in *Laniidae* and owls (Table 1). Thus, phylogenetic approaches based on PICs clearly overestimated phylogenetic dependence in previous analyses, which may have resulted in the lack of adjustment reported by Renoult *et al.* (2009) for their most parsimonious scenario.

Conclusion

Our results are consistent with our previous findings (Avilés & Soler, 2009) and have proved to be robust to the assumptions on visual system evolution for *Laniidae* and nocturnal owls that Renoult *et al.* (2009) suggested may have flawed our early findings. Thus, the hypothesis that selection has resulted in increased detectability of nestling by adjusting gape coloration to parental visual systems is currently supported by our comparative data.

References

- Avilés, J.M. & Soler, J.J. 2009. Nestling colouration is adjusted to parent visual performance in altricial birds. *J. Evol. Biol.* **22**: 376–386.
- Avilés, J.M., Soler, J.J., Navarro, C. & Pérez-Contreras, T. 2008. Dark nests and nestling conspicuousness in color patterns of altricial birds. *Am. Nat.* **171**: 327–338.

- Bennett, A.T.D. & Cuthill, I.C. 1994. Ultraviolet vision in birds – what is its function. *Vision Res.* **34**: 1471–1478.
- Bize, P., Piau, R., Moureau, B. & Heeb, P. 2006. A UV signal of offspring condition mediates context-dependent parental favouritism. *Proc. R. Soc. Lond. B* **273**: 2063–2068.
- Bowmaker, J.K. 1979. Visual pigments and oil droplets in the pigeon retina, as measured by microspectrophotometry, and their relation to spectral sensitivity. In: *Neural Mechanisms of Behavior in the Pigeon* (A.M. Granda & J.H. Maxwell, eds), pp. 287–305. Plenum, New York.
- Bowmaker, J.K. & Martin, G.R. 1978. Visual pigments and color-vision in a nocturnal bird, *Strix aluco* (Tawny Owl). *Vision Res.* **18**: 1125–1130.
- Cuthill, I.C., Partridge, J.C., Bennett, A.T.D., Church, S.C., Hart, N.S. & Hunt, S. 2000. Ultraviolet vision in birds. *Adv. Study Behav.* **29**: 159–214.
- Espinosa de los Monteros, A. 2000. Higher-level phylogeny of Trogoniformes. *Mol. Phyl. Evol.* **14**: 20–34.
- Felsenstein, J. 1985. Phylogenies and the comparative method. *Am. Nat.* **125**: 1–15.
- Ficken, M.S. 1965. Mouth color of nestling passerines and its use in taxonomy. *Wilson Bull.* **77**: 71–75.
- Freckleton, R.P., Harvey, P.H. & Pagel, M. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.* **160**: 712–726.
- Hackett, S.J., Kimball, R.T., Reddy, S., Bowie, R.C.K., Braun, E.L., Braun, M.J., Chojnowski, J.L., Cox, W.A., Han, K.L., Harshman, J., Huddleston, C.J., Marks, B.D., Miglia, K.J., Moore, W.S., Sheldon, F.H., Steadman, D.W., Witt, C.C. & Yuri, T. 2008. A phylogenomic study of birds reveals their evolutionary history. *Science* **320**: 1763–1768.
- Harrison, G.L., McLenachan, P.A., Phillips, M.J., Slack, K.E., Cooper, A. & Penny, D. 2004. Four new avian mitochondrial genomes help get to basic evolutionary questions in the late Cretaceous. *Mol. Biol. Evol.* **21**: 974–983.
- Hart, N.S. & Hunt, D.M. 2007. Avian visual pigments: characteristics, spectral tuning, and evolution. *Am. Nat.* **169**: S7–S26.
- Hart, N.S. & Vorobyev, M. 2005. Modelling oil droplet absorption spectra and spectral sensitivities of bird cone photoreceptors. *J. Comp. Physiol. A* **191**: 381–392.
- Hart, N.S., Partridge, J.C. & Cuthill, I.C. 1999. Visual pigments, cone oil droplets, ocular and predicted spectral sensitivity in the domestic (*Meleagris gallopavo*). *Vision Res.* **39**: 3321–3328.
- Heeb, P., Schwander, T. & Faoro, S. 2003. Nestling detectability affects parental feeding preferences in a cavity-nesting bird. *Anim. Behav.* **66**: 637–642.
- Hunt, S., Kilner, R.M., Langmore, N.E. & Bennett, A.T.D. 2003. Conspicuous, ultraviolet-rich mouth colours in begging chicks. *Proc. R. Soc. Lond. B* **270**: S25–S28.
- Kilner, R.M. 2006. Function and evolution of color in young birds. In: *Bird Coloration: Function and Evolution* (G.E. Hill & K.J. McGraw, eds), pp. 201–232. Vol. 2. Harvard University Press, Cambridge.
- Kilner, R. & Davies, N.B. 1998. Nestling mouth colour: ecological correlates of a begging signal. *Anim. Behav.* **56**: 705–712.
- Martins, E.P. & Hansen, T.F. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am. Nat.* **149**: 646–667.
- Martins, E.P., Diniz-Filho, J.A.F. & Housworth, E.A. 2002. Adaptive constraints and the phylogenetic comparative method: a computer simulation test. *Evolution* **56**: 1–13.
- Mindell, D.P., Sorenson, M.D., Huddleston, C.J., Miranda, H.C. Jr, Knight, A., Swachuk, S.J. & Yuri, T. 1997. Phylogenetic relationships among and within select avian orders based on mitochondrial DNA. In: *Avian Molecular Evolution and Systematics* (D.P. Mindell, ed), pp. 213–247. Academic Press, San Diego, CA.
- 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.
- Pagel, M. 1997. Inferring evolutionary processes from phylogenies. *Zool. Scripta* **26**: 331–348.
- Pagel, M. 1999. Inferring the historical patterns of biological evolution. *Nature* **401**: 877–884.
- Parejo, D., Avilés, J.M. & Rodríguez, J. 2009. Visual cues and parental favouritism in a nocturnal bird. *Biol. Lett.* doi: 10.1098/rsbl.2009.0769.
- Penteriani, V., Delgado, M.M., Alonso-Alvárez, C. & Sergio, F. 2007a. The importance of visual cues for nocturnal species: eagle owl signal by badge brightness. *Behav. Ecol.* **18**: 143–147.
- Penteriani, V., Delgado, M.M., Alonso-Alvárez, C., Viqueira Pina, N., Sergio, F., Bartolommei, P. & Thompson, L.J. 2007b. The importance of visual cues for nocturnal species: eagle owl fledglings signal with white mouth feathers. *Ethology* **113**: 934–943.
- Renoult, J.P., Courtiol, A. & Kjellberg, F. 2009. When assumptions on visual system evolution matter: nesting colouration and parental visual performance in birds. *J. Evol. Biol.* (in press).
- Rohlf, F.J. 2006. A comment on phylogenetic correction. *Evolution* **60**: 1509–1515.
- Sibley, C.G. & Ahlquist, J.E. 1990. *Phylogeny and Classification of Birds: A Study in Molecular Evolution*. Yale University Press, New Haven and London.
- Vorobyev, M., Osorio, D., Bennett, A.T.D., Marshall, N.J. & Cuthill, I.C. 1998. Tetrachromacy, oil droplets and bird plumage colours. *J. Comp. Physiol. A* **183**: 621–633.

Received 1 October 2009; accepted 9 October 2009