

**Dark nests and egg colour in birds: a possible functional role  
of ultraviolet reflectance in egg detectability**

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Due to the conspicuousness of ultraviolet colour in dark environments, natural selection might have selected for ultraviolet egg coloration because it would enhance egg detectability by parents in murky nests. Here we tested this hypothesis by using comparative and experimental approaches. First we studied variation in egg coloration of 98 species of European passerines measured using UV-visible reflectance spectrometry (300-700 nm) in relation to nesting habits. Analyses based on raw data and controlling for phylogenetic distances both at the species and the family level revealed that hole-nester species showed eggs with higher ultraviolet reflectance than those nesting in open habitats. The experimental approach consisted on the manipulation of ultraviolet reflectance of experimental eggs introduced outside the nest-cup of the hole-nester spotless starling *Sturnus unicolor* and the study of retrieval of these eggs. Ultraviolet-reflecting eggs (“controls”) were more frequently retrieved to the nest cup than non-reflecting (“-UV”) eggs. These results were not due to “-UV” eggs being recognized by starlings as parasitic because when a parasitic egg is detected, starling removed it from the nest-box. Therefore, these results are consistent with the hypothesis that ultraviolet egg colours are designed to provide highly detectable targets for parent birds in dark nest environments.

**Keywords:** egg colour, dark nest, detectability, ultraviolet reflectance

## 1. INTRODUCTION

Egg coloration in birds exhibits an extraordinary variation among and within species, yet what role, if any, bird egg coloration plays and why it varies among species remains controversial (Underwood & Sealy 2002). Cryptic eggs may remain unnoticed to predators for species that lay their eggs directly on the ground (e.g. Solis & de Lope 1995). The functional role of cryptic eggs, however, is less obvious for shrub and canopy nester species (Gotmark 1992; Weidinger 2001). A second functional explanation is provided by brood parasitism studies. Host discrimination of parasitic eggs appears to have been the main selective pressure for the evolution of egg mimicry in the European cuckoo *Cuculus canorus* (Davies 2000). Also, it has been suggested that a high colour similarity among the eggs of a clutch may enhance recognition of cuckoo eggs by hosts (e.g. Soler & Møller 1996; Stokke *et al.* 2002), or individual clutch recognition in colonial birds nesting at high densities (Birkhead 1978). More recently, it has been proposed that blue and green egg colours may function as a post-mating sexually selected signal of female's phenotypic quality to their mates in order to induce a higher allocation of paternal care (Moreno & Osorno 2003). Finally, Gosler *et al.* (2005) have proposed that egg speckling caused by protoporphyrin pigments might compensate for reduced eggshell-thickness due to calcium deficiency.

The interaction between the spectral properties of ambient light and the reflectance spectra of the objects affects the conspicuousness of coloured elements and, thus, their detectability by conspecific and/or predators (e.g. Marchetti 1993; Endler & Théry 1996). Nests of birds exhibit a wide range of spectral properties that arise from their variable geometry and exposure to solar radiation (Hunt *et al.* 2003). Thus nest light properties may have influenced the evolution of egg and nestling traits used in visual detection by parents (Heeb *et al.* 2003, Hunt *et al.* 2003). In this vein, it has been

recently proposed that the importance of ultraviolet radiation for conspicuousness is particularly highlighted in dark environments such as hole-nests because the radiance from the background (nest material and wooden cavity) is considerably lower for the ultraviolet light than in open nests (Hunt *et al.* 2003; Veiga & Polo 2005). Indeed, females of spotless starling *Sturnus unicolor* regularly carry feathers to their hole nest which are arranged to maximize their conspicuousness in the ultraviolet (Veiga & Polo 2005). Also, recent comparative evidence suggests that conspicuousness of nestlings' mouths is maximal in the ultraviolet (Hunt *et al.* 2003), and, it may be important for parental decisions about food allocation in the nest (Jourdie *et al.* 2004).

Here we propose and provide comparative and experimental evidence for the hypothesis that ultraviolet egg colours in birds may enhance egg detectability in dark nesting environments. Due to a retinal cone type that is tuned to UV wavelengths (Chen *et al.* 1984) passerines can perceive the reflectance of ultraviolet light, whose importance has been demonstrated in a wide number of species and situations (review in Cuthill *et al.* 2000; Hausmann *et al.* 2003). In addition, UV-VIS reflectance spectrophotometry has revealed that ultraviolet reflectance of eggs is a variable trait among the European passerines (UV chroma of eggs ranges between 0.13 and 0.22; table 1, Electronic Appendix). The idea that certain colours of eggs tend to be associated with certain type of nesting sites is not new. Early comparative studies reported that conspicuous white egg colours are most often found in species that nest in cavities or crevices than in open nesting species (Lack 1958; von Haartmann 1957; Oniki 1985). However, these studies scored egg coloration based on human vision which does not consider ultraviolet reflectance, and did not use the comparative method to account for species similarity due to common descent. In a first step we aimed to report whether egg coloration measured with UV-VIS spectrophotometry is related to nesting habits among

the European passerines after considering their phylogenetic relationships into account. We predicted that (i) eggs of species nesting in dark environments showed stronger UV reflectance than those of open nesting species.

Conspicuous egg coloration in dark environment has been typically interpreted as a consequence of the lack of selection for cryptic coloration in species that nest in cavities or enclosures (Lack 1958; Underwood & Sealy 2002). A recent comparative study, however, suggests no support for the nest predation hypothesis since the selection pressure exerted by nest predation failed to explain egg colour among the European passerines (Soler *et al.* 2005). Alternatively, particular egg coloration may have a positive selection value enabling the parents to see them in a dark nest (von Haartmann 1957; Lack 1958). Here we aimed to explore the functionality of ultraviolet reflectance of spotless starling eggs in determining egg retrieval of experimental eggs in a field experiment. Starlings are strict hole-nesting species, and microspectrophotometry suggests that they can detect UV light due to a vision cone with peak absorption in the UV (362 nm, Hart *et al.* 1998). Indeed, experimental evidence supports that mate assessment depends on ultraviolet vision in the relative European starling *Sturnus vulgaris* (Bennett *et al.* 1997). Here we coated experimental starling eggs with a ultraviolet-light blocker (“-UV”) and with horse fat as a control treatment which provides eggs with a similar touching that the “-UV” eggs. We artificially placed a “-UV” and a “control” egg in alternate days within nest-boxes with incubated and completed clutches. Eggs were situated outside the nest-cup and starling retrieval responses recorded. Since eggs with reduced reflectance in the ultraviolet region show a poorer contrast with the nest background we predicted ii) that “-UV eggs” were less frequently retrieved within the nest-cup, thus being more frequently adjudged as “odd-eggs”, than “control” eggs in which reflectance was not modified.

## **2. MATERIAL AND METHODS**

### **(A) COMPARATIVE ANALYSIS**

#### **(a) Species data**

##### *(i) Egg colour*

A total of 5598 eggs belonging to 89 species and 16 families were sampled in museum collections (Median = 26 eggs per species, range = 2-1807 eggs, Table 1 in Appendix). This sample comprised the 66.4 % of the species and the 100 % of the families breeding in the Western Palaearctic (i.e. 134 species belonging to 16 families, according to Sibley & Ahlquist 1990). Egg coloration was estimated by using UV-VIS spectrophotometry, which is a technique providing highly repeatable measures of egg colour even for species with spotted eggs (Cherry & Bennet 2001; Langmore *et al.* 2003; Aviles *et al.* 2004; Soler *et al.* 2005), and allowing the record of ultraviolet information to which humans are blind (Endler 1990). Reflectance spectra in the range 300-700 nm were obtained from all eggs using a spectroradiometer with a deuterium and a halogen light source (DH 2000, Ocean Optics Europe). A fiber-optic probe provided illumination at a 45° angle and transferred reflected light to the spectrometer (S2000, Ocean Optics). Data from the spectrometer were converted into digital information by a DAQ Card 700 and passed into a computer, where a software package (Spectrawin 4.1) calculated reflectance spectra relative to a standard white reference (WS-2). Total reflectance was obtained for the ultraviolet (300-400 nm), blue (400-475 nm), green (475-550 nm), yellow (550-625 nm), and red (625-700 nm) intervals. We performed all our measurements under standardized light condition to avoid an effect of ambient light.

(ii) *Nesting site*

The hypothesis of some avian egg colour being more perceptible under particular microhabitat conditions predicts for among species variation in egg colours related to nesting sites. Each species was classified either as strict hole nester (a score of 1) or open nester (a score of 3) based on information provided by Harrison (1975). Most sampled species only used a single nesting site, however 12 species were more variable (e.g. hole nesting species in a area that also nested in open nests in other area). These species were treated as non-strict hole nesters (a score of 2)(table 1, Electronic Appendix). We assume throughout the paper that non-strict hole-nesters are an intermediate category between open and strict hole nesters.

(iii) *Confounding variables*

Based on information from Cramp (1998), we quantified several factors that may affect the predicted link between egg coloration and nesting habits. Different habitats are likely to differ in ambient light as a consequence of their geometry and weather (Endler 1993; Marchetti 1993). To control for this potential confounding effect we classified species as inhabiting open (score of 1) or forested habitats (score of 3). Species of open habitats include species nesting in open lands, old fields and riparian habitats. The forest species are mostly strictly forest breeders (score of 1). Birds inhabiting both kinds of habitats, or preferring scrubs, clearing or forest edges were categorized as living in mixed habitats (score of 2).

It has been recently suggested that blue-green egg coloration in birds may function as a post-mating sexual signals of female quality to males (Moreno & Osorno 2003). Indeed, supporting the sexual selection hypothesis, blue-green egg-colour intensity is associated with parental effort as estimated from the duration of the nestling period among the European passerines (Soler *et al.* 2005). To control for the possibility

that the strength of sexual selection on egg coloration varied among nesting sites we entered the length of the nestling period as an additional predictor in our comparative framework. Also, if producing pigments colouring the eggs are costly to produce (see Moreno & Osorno 2003), egg colour, such as other egg traits, may trade with other life-history traits as part of an overall life-history strategy (Martin *et al.* 2006). We included information on clutch size and body mass in our comparative framework to control for this possibility. All raw data used for the analyses are summarized in the Table 1 of the Appendix.

## **(b) Statistical methods**

### *(i) Egg colour analyses*

Colour variables are typically interrelated (Endler 1990) so we performed principal component analysis (PCA) on reflectance data to reduce the number of correlated variables into a few orthogonal variables summarizing colour variation (Cuthill *et al.* 1999). PCA allowed us to distinguish between achromatic “brightness” variation represented by the first principal component (PC1) and chromatic variation represented by PC2 and PC3 (Endler & Théry 1996). All together, these three first components explained 99.7 % of the total variance of sampled eggs (Table 1). PC1 had high and negative loadings for the five colour regions; hence, it described achromatic variation explaining 95.0 % of the overall variation in coloration (Table 1). PC2 had high and positive loadings at the blue wavelengths and high negative ones at red wavelengths and it explained 62 % of the chromatic variance (i.e. remaining variance after excluding the achromatic variance, Table 1). PC3, however, had high positive loadings at the ultraviolet waveband and explained 34 % of chromatic variance (Table 1). Therefore, we used the scores from those axes in subsequent analyses since they expressed brightness (PC1) and chroma (PC2 and PC3) for sampled eggs respectively.



Table 1 about here

(ii) *Exploratory analyses*

We used a variance component analysis (Harvey & Pagel 1991) to examine how variation in egg colour traits and nesting habits is partitioned among the different taxonomic levels (species, genus and family, following Sibley & Ahlquist 1990). Most of the variation in egg coloration and nesting habit was distributed at the species level (table 2, Electronic Appendix). Thus, it is relevant to consider how variation in egg colour traits correlates to nesting habits at lower taxonomic levels, such as among species, but taking into account the phylogenetic relationships among species because a considerable amount of variance is also explained by higher taxonomical levels (table 2, Electronic Appendix).

(iii) *Species-level analyses*

The prediction that egg coloration varies with nesting habits was tested with a General Linear Model (GLM) with scores defining egg coloration (i.e. PC1 “brightness”, PC2 “blue-red” and PC3 “ultraviolet”) as dependent variables and nesting habit as independent variable. Brightness and blue-red were normally distributed and ultraviolet was log transformed before this analysis. To adjust for the potentially confounding effects of habitat type, body mass, clutch size and nestling period on our prediction we performed a second GLM entering these variables together with the traits of interest.

Taxonomic groups such as species cannot be considered statistically independent observations due to the confounding effects of common ancestry (Felsenstein 1985). Thus we reassessed the association between egg colour traits and nesting habits using Felsenstein’s (1985) independent contrasts method as implemented in the computer program PDAP (Vers. 6.0, module PDTREE) by Garland *et al.* (1999) and Garland & Ives (2000). Our phylogenetic hypothesis was based on the molecular phylogeny of

Sibley & Ahlquist (1990), completed with recently published information (Sheldon & Winkler 1993; Blondel *et al.* 1996; Cibois & Pasquet 1999) (figure 1, Electronic Appendix). We assume all polytomies to be unresolved, and branch lengths were assigned following three different methodologies: (i) all were set equal to one; (ii) by arbitrarily assigning all inter-node branch segments to one, but constraining tips to be contemporaneous (Pagel 1992); and (iii), by tips being contemporaneous, the depth of each node being arbitrarily set to one less than the number of tip species that descend from it (Grafen 1989). We checked whether the contrasts were adequately standardized by plotting absolute values of standardized contrasts versus their standard deviations (square roots of sums of corrected branch lengths) (see Garland *et al.* 1991; Garland 1992; Pagel 1992). Only scores for PC1 correlated with SD ( $r = 0.23$ ,  $P = 0.03$ ,  $N = 89$ ) when branch length was assigned following the Pagel (1992) method, although this relationship was non-significant under Bonferroni standards. However, in no case did we find a significant correlation ( $P > 0.1$ ) when branch lengths was set equal to one or assigned following the Grafen (1989) method. The resulting contrasts for each variable were then used to perform general linear models (GLM) through the origin. Results from phylogenetically independent contrasts provided consistent results regardless of the method used to estimate branch length. We, therefore, only report results based on branch length estimated according to Grafen (1989) method.

#### (iv) *Family-level analyses*

Although most of the variance in egg colour traits and nesting habit is at the species taxonomic level (Table 2, Appendix), analysis based on species could represent a significant problem because there is no single objective measurement that allows nesting habit to be examined in a continuous fashion such as is desirable for the chosen

phylogenetic approach (Harvey & Pagel 1991). Therefore, in a second set of analyses we estimated the magnitude of family-level differences in nesting habits by using the percentage of strict hole nesting species (arcsine square-root transformed) in a family. These percentages provide a reliable measure of the occurrence of hole nesting habits within a given family and were related with the average egg colour traits (i.e. PC1 “brightness”, PC2 “blue-red” and PC3 “ultraviolet” scores) of each family using least-squares regressions (for a similar approach see Sol *et al.* 2005). We repeated the analyses using the method of independent contrasts to deal with phylogenetic effects (Felsenstein 1985). The phylogenetic hypothesis for our 16 families was based on DNA hybridization (Sibley & Ahlquist 1990), using the genetic distance to estimate branch lengths. Phylogenetically independent contrasts for the percentage of strict hole nesting species and the average value for the three egg colour traits within each family were calculated with the software PDAP (Garland *et al.* 1999) and related with ordinary regressions forced through the origin.

## **B) FIELD STUDY**

### **(a) *Study area***

The field study was carried out in Guadix (37°18'N, 3°11'W), southern-east Spain, during the breeding season of 2005, in nest-boxes recently (2005) installed close or within colonies of spotless-starlings already established in old buildings of the area. The species is polygynous (Veiga *et al.* 2001), nestlings usually hatch asynchronously (Cramp 1998) and are mainly fed with insects (Motis *et al.* 1997) by females and, sometimes, also by males (Veiga *et al.* 2002).

### **(b) *Experimental design***

2-4 days after clutch completion we placed one experimental egg (“-UV” or “control”) outside the nest-cup close to the internal side of the nest-box in 25 randomly selected

nests. All tested pairs were incubating and females returned to the nests only a few minutes after our visit. Two hours after the female returned to the nest we went back and recorded the position of the experimental egg before definitively removing them as: egg retrieved if the egg was found within the nest-cup, or egg adjudged as “odd” if the experimental egg remained in the same position as placed or was ejected out of the nest-box. The following day the same nest was tested with the other treatment. The order of exposure to the “-UV” or control treatments was randomly assigned to each nest. Therefore for all 25 tested nests we gathered paired information on retrieval of “-UV” and control experimental eggs.

Our main focus is interpreting differences in egg retrieving by starlings in terms of detectability linked to UV reflectance. However, the spotless starling is a species in which intraspecific nest parasitism occurs (Calvo *et al.* 2000), and this breeding strategy has selected for recognition and ejection of odd eggs in this species (Eens & Pinxten 1999). Therefore, because the “-UV” eggs differ from eggs of the nest owner more than control eggs, a low retrieving of “-UV” eggs could also be interpreted in the light of recognition of parasitic eggs (i.e. “-UV” eggs may be identified as parasitic eggs and then do not retrieved). To control for this possibility we introduced a second egg within the nest-cup with the same treatment than that situated outside the nest-cup. If differences in egg retrieving were due to brood parasitism (i.e. egg recognition) we would expect that pairs that recognize as parasitic (i.e. do not retrieve) the egg situated outside the nest-cup would also recognize as parasitic (i.e. eject from the nest cup) the second experimental egg of the same treatment introduced in the nest-cup. In other words, if starlings do not retrieve the first experimental egg but eject the second experimental egg from the nest cup, the non-retrieve behaviour should be interpreted in

the light of brood parasitism selection pressure. Otherwise, results should be interpreted as UV-colouration affecting egg detectability by starling adults.

Experimental eggs were collected from 2005 abandoned-starling nests and used fresh after collection. Further, experimental eggs were kept into closed boxes to diminish possible fading and re-utilized twice with the same experimental treatment. The “-UV” treatment consisted of coating real starling eggs with an ultraviolet-light blocker (50/50 w/w blend of Parsol 1789 and MCX, Roche). The UV-block effect persisted at 24h after coating, and it did not alter spectral shape of starling eggs at the experimental nests after 48 h. suggesting that the treatment was not transferred to adjacent eggs. Control starling eggs were coated with horse fat that provides similar touching to eggs than the ultraviolet-light blocker (Aviles *et al.* 2006). Changes in spectral shape of starling eggs for the two treatments are shown in the figure 2 in the Electronic Appendix. The -UV and control treatments minimally affected reflectance of starling eggs in the human visible spectrum, while the -UV treatment markedly reduced starling egg reflectance in the range below 400 nm.

### **(c) *Statistical methods***

Determinants of egg retrieval were studied by using generalized linear models for binary dependent variables (SAS macro GLIMMIX; SAS Institute 1999) involving experimental treatment (UV-reduced versus control), clutch size (< 4 eggs versus => 4 eggs) and their interaction as independent fixed factors and nest as a random factor. Probability of retrieval of each experimental egg was modelled as a binomial response variable (1 = rejection, 0 = acceptance) using a logistic link function.

## **3. RESULTS**

**(a) Does ultraviolet egg coloration in birds vary with nesting habits?**

*(i) Species-level analyses*

Analyses based on raw data revealed that egg coloration differed significantly among nesting habits ( $F_{3,85} = 18.21$ ,  $P < 0.00001$ ). Hole nesting species have more brilliant (i.e. lower PC1 scores;  $F_{1,87} = 33.73$ ,  $P < 0.00001$ ), bluer and more ultraviolet (i.e. higher PC2 and PC3 scores;  $F_{1,87} = 5.64$ ,  $P < 0.019$  and  $F_{1,87} = 4.45$ ,  $P < 0.037$ , respectively) eggs than open and semihole nesting species (figure 1). Differences still remained significant when the potential confounding effects of habitat type, body mass, clutch size and nestling period were considered in our GLM (table 2). Also, differences in egg coloration between hole nesting and the rest of species still held when applying phylogenetically controlled GLM in which we adjusted for potential confounding factors (table 2). These analyses revealed that species nesting in dark environments showed eggs with higher brightness and ultraviolet reflectance than those nesting in open habitats (table 2).

**Figure 1 and Table 2 about here**

*(ii) Family-level analyses*

The above results were partially supported by our analyses at the family level. At this taxonomic level, the relationship between average brightness and percentage of strict hole-nesting species does not reach significance ( $R^2 = 0.19$ ,  $F_{1,14} = 3.39$ ,  $p = 0.09$ ).

However, families with a higher tendency to nest in holes tended to have species with more coloured blue and ultraviolet eggs (blue-red:  $R^2 = 0.28$ ;  $F_{1,14} = 5.62$ ,  $p = 0.03$ ; ultraviolet:  $R^2 = 0.45$ ,  $F_{1,14} = 11.43$ ,  $p = 0.004$ , figure 2), which was qualitatively confirmed when phylogenetic independent contrasts were used (blue-red:  $R^2 = 0.40$ ;  $F_{1,14} = 9.63$ ,  $p = 0.007$ ; ultraviolet:  $R^2 = 0.37$ ,  $F_{1,14} = 8.25$ ,  $p = 0.01$ , figure 2)

**Figure 2 about here**

**(b) Does ultraviolet colour affect egg detection in dark nests?**

Spotless starlings retrieved 44.0% of the eggs situated outside their nest-cups (22 out of 50 experimental added eggs), but not all the tested pairs exhibited this behaviour (random effect of nest identity,  $Z = 1.74$ ,  $P = 0.04$ ). Probability of egg retrieval was influenced by original clutch size of the tested pair (fixed effect of clutch size,  $F_{1,23} = 6.75$ ,  $P = 0.016$ ). Pairs with two or three eggs were more prone to put the experimental eggs within the nest-cup than pairs with four or more eggs (15 out of 22 pairs with 2-3 eggs *versus* 7 out of 28 pairs with four or more eggs). Interestingly, modifying ultraviolet reflectance of eggs led to significant differences in probability of egg retrieval (fixed effect of treatment,  $F_{1,23} = 5.40$ ,  $P = 0.029$ ). Ultraviolet-reflecting eggs (“controls”) were more retrieved than non-reflecting (“-UV”) eggs (figure 3). Higher retrieval of control as compared to UV-reduced eggs was unaffected by the clutch size of the tested pairs (treatment \* clutch size effect,  $F_{1,23} = 2.16$ ,  $P = 0.15$ ).

**figure 3 about here**

Only 3 out (6 %) of 50 experimental eggs introduced within the nest cup were ejected and, in all three cases, eggs were ejected from the nest box. Therefore, because the low percentage of rejection, we used Fisher exact tests for the analyses. Although all rejected eggs were “-UV” the effect of experimental treatment did not reach statistical significance (3 out of 25 “-UV” eggs *versus* 0 out of 25 control eggs ejected outside the nest; Fisher exact test,  $P = 0.23$ ). Further, variation in clutch size did not explain ejection of experimental eggs within the nest cup (21 out of 22 pairs with 2-3 eggs *versus* 26 out of 28 pairs with four or more eggs; Fisher exact test,  $P = 1.00$ ), and the two only pairs that ejected the eggs situated outside the nest-cup also ejected the eggs situated within the nest-cup. Therefore, these results suggest that most of the responses

against experimental eggs outside the nest cup recorded in this study are not due to UV-blocked eggs being detected as parasitic egg by adult starlings.

#### **4. DISCUSSION**

##### ***(a) Does ultraviolet egg coloration in birds vary with nesting habits?***

We have found that intensity of ultraviolet colour of the eggs is associated with certain types of nesting sites among the European passerines. More precisely, hole nesting species have more ultraviolet eggs than open nesting species (figure 1), a pattern that held significant when we controlled for phylogenetic effects. This relationship was not due to the confounding effect of habitat type, body mass, clutch size and nestling period since intensity of ultraviolet coloration remained significantly associated with nesting habits when all the confounding effects were included in the same model (Table 2). Moreover, those families with a higher tendency to nest in holes tended to have species with more ultraviolet eggs (figure 2). These results are therefore consistent with the hypothesis that ultraviolet colours in the eggs may have been selected to provide detectable cues in poorly lit environments.

Our results extend previous findings suggesting that nest light properties may have influenced the evolution of coloration of different traits that are used in visual detection within nests. This hypothesis has received strong support for nestling traits. Actually, comparative studies have shown that the mouth of hole-nestlings is brighter than that of open-nestlings (Ficken 1965; Kilner & Davies 1998). Experimental evidence has confirmed a functional role of such nest-light variable mouth coloration in nestling's detectability by their parents (Heeb *et al.* 2003). The use of non-anthropocentric UV-VIS spectrometry to quantify mouths colour has allowed to confirm these findings based on the human vision and emphasized the importance of



ultraviolet radiation for mouth conspicuousness in hole-nesters (Hunt *et al.* 2003; Jourdie *et al.* 2004). Conspicuousness of reflective in the ultraviolet traits in hole-nests is based on the particularly low levels of radiance in the ultraviolet of the nest background that increases the detectability of the traits in those environments (Hunt *et al.* 2003).

Particular egg colour traits may also enhance egg detectability by parents in dark nests and thus may have been favoured by natural selection. Indeed, early comparative studies that did not control for common phylogenetic descent had shown that highly conspicuous (i.e. more brilliant) white eggs were most often found in species that nest in cavities or enclosures than in open nests (von Haartmann 1957; Lack 1958; Oniki 1985; Underwood & Sealy 2002). Our comparative analysis of egg colour across European passerines by using UV-VIS spectrometry confirms previous findings based on human vision since eggs of hole nesters are globally brighter than those of open nesters (figure 1, Table 2). However, beyond global brightness, we have also reported that hole-nesting European passerines have more intensely coloured ultraviolet eggs than open nesting species. That is, selection may have favoured highly reflective (i.e. more brilliant) eggs, but also eggs coloured in a way that enhanced conspicuousness in the ultraviolet to enhance egg detectability in the poor lit conditions of a hole.

Other plausible alternative hypotheses to that of the egg detectability may explain why ultraviolet egg colours are associated with dark nesting habits in our sample. The first hypothesis is that hole-nesting birds may have more reflective eggs in the ultraviolet because ultraviolet colour eggs were less detected by predators in holes. However, in a recent work we have found no support for the predation hypothesis since egg coloration remained unrelated to nest predation among the European passerines (Soler *et al.* 2005). Nests predators may rely on other more obvious cues denoting nest

presence than egg color as for instance parental activity (Martin *et al.* 2000), or the nest itself (Gotmark 1992). However, we could not discard that predators on holes and open nests had differently tuned colour sensory systems (e.g. Hastad *et al.* 2005). The second hypothesis is that selection for camouflage was lower in hole nesters than in open nesters and, thus, that the higher relative influence of ultraviolet in hole nesting species was a by-product of positive selection on brown and/or red colours at higher wavelengths (Underwood & Sealy 2002). We have found that eggs of open nesting passerines were redder and less blue (Figure 1) than those of the open nesting species. However, when tested in a multivariable model the association between ultraviolet colour and nesting habits controlling for PC2 (i.e. “blue-red”) and all the confounding variables our results remain unchanged (Table 3, Appendix). Then, selection for camouflage in open nests does not explain the resulting differences in UV-reflectance of eggs between hole and open nesting species.

**(b) Does ultraviolet colour affect egg detectability in dark nests?**

von Haartmann (1957) hypothesized that brilliant egg colour may have an adaptive value enabling the incubating bird to see its eggs more easily in hole-nests (see also Lack 1958). The egg detectability hypothesis may potentially explain why hole-nester species have brighter and more ultraviolet coloured eggs (this study). This hypothesis would clearly predict changes in egg detectability by incubating birds as colour of eggs is manipulated in a way perceptible to birds. To date, only the study by Holyoak (1969) has provided some support for this possibility. Holyoak (1969) compared the survival of jackdaw *Corvus monedula* eggs blackened with ink and of the white naturally coloured eggs and found that the former disappeared significantly more than the white eggs. He concluded that egg detectability may play a main role either in nest location within a hole and/or the movement associated with egg-turning or settling onto the eggs in the jackdaw.

To test the detectability hypothesis in relation to the UV-colour intensity of eggs we also performed experiments in the hole-nester spotless starling. We manipulated starling eggs by reducing reflectance at wavelengths below 400 nm, and with a control treatment that minimally affected original reflectance. Ultraviolet-reflecting eggs (“controls”) placed outside the nest-cup were more commonly retrieved by incubating starlings than non-reflecting (“-UV”) eggs (figure 3). Therefore, our result suggests that the ultraviolet reduction treatment affected egg retrieval by starlings, and thus that ultraviolet reflectance of eggs may favour egg detectability in this particular system.

An alternative explanation for our experimental result is related to intraspecific brood-parasitism selection pressure that selects for parasitic-egg recognition and rejection (Petrie & Møller 2001). Because “-UV” eggs would differ more than control eggs from eggs of the nest owner, an alternative explanation of our results would be that

“-UV” eggs are more easily detected as parasitic eggs and, thus, are rejected (non-retrieved to the nest cup) more often than control eggs. To rule out this possibility, we introduced within the nest-cup a second experimental egg with the same treatment than that placed outside the nest-cup. However, starlings never moved experimental eggs from inside to outside nest-cup, and, when they recognize a parasitic egg, they ejected the egg from the nest box (three out of 50 experiments). More importantly, when one of the experimental eggs was removed from the nest box, the second experimental was also ejected from the nest boxes. Thus, our experimental results cannot be explained in a brood-parasitic scenario where retrieving a foreign egg to the nest-cup would not be of selective advantage.

Interestingly we also found that egg retrieval behaviour was affected by original clutch size of the tested pairs that, although remain to be further studied, may reveal a role of clutch size on male investment. Accordingly, because clutch size reflects phenotypic quality females that obtain an extra egg in the nest-cup could signal their male a high quality in order to obtain a higher male attendance.

In conclusion, relying on a wide range of passerine species in the Palaearctic, we found comparative support for an association between ultraviolet egg coloration and nesting habits in birds. In addition, we found experimental support for the role of ultraviolet egg coloration in egg retrieval in the spotless starling. These findings agree with expectations for the egg detectability hypothesis suggesting that ultraviolet egg coloration enhance egg detectability by parents in dark nests.

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Table 1. Factor loadings of axes from a principal component analysis on reflectance data. Percent of variance explained by each axis is also shown.

	PC1	PC2	PC3
UV	-0.967	0.049	0.248
Blue	-0.978	0.198	-0.026
Green	-0.978	0.150	-0.138
Yellow	-0.987	-0.140	-0.058
Red	-0.965	-0.259	-0.022
% of variance	95.00	3.01	1.69

### Legend to figures

Figure 1. Differences (mean  $\pm$  SE) in egg colouration ((A) brightness; (B) blue-red and (C) ultraviolet) in relation to nesting habits in Western Palaearctic passerine birds. The number of species appears on error bars.

Figure 2. Relationship between mean blue-red and ultraviolet colour and proportion of hole nesting species per family in Western Palaearctic passerine birds: A-C) non-controlling and B-D) controlling for the phylogenetic effects by using independent contrast analysis.

Figure 3. Starling pairs retrieving eggs within the nest-cup in relation to a treatment affecting UV reflectance. Starlings can retrieve the control, the “-UV” or the two kinds of experimental eggs. (N = 25 tested pairs with the two egg treatments).

Figure 1. Avilés et al.

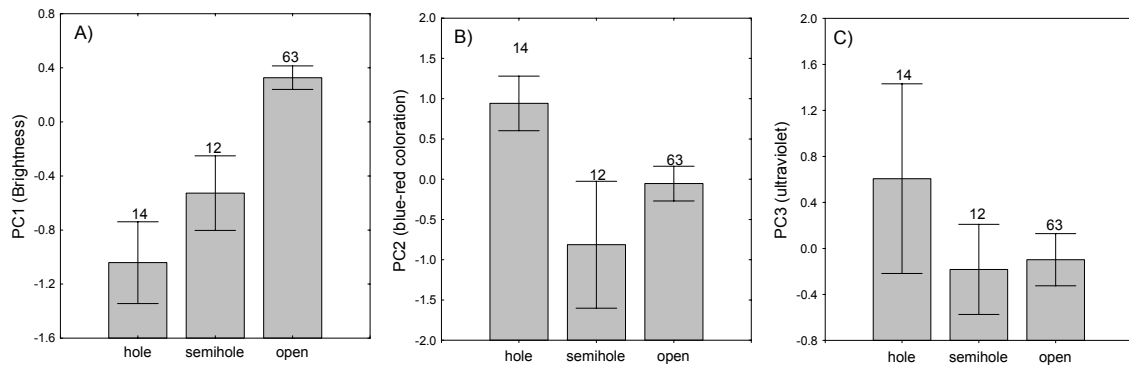


Figure 2. Avilés et al.

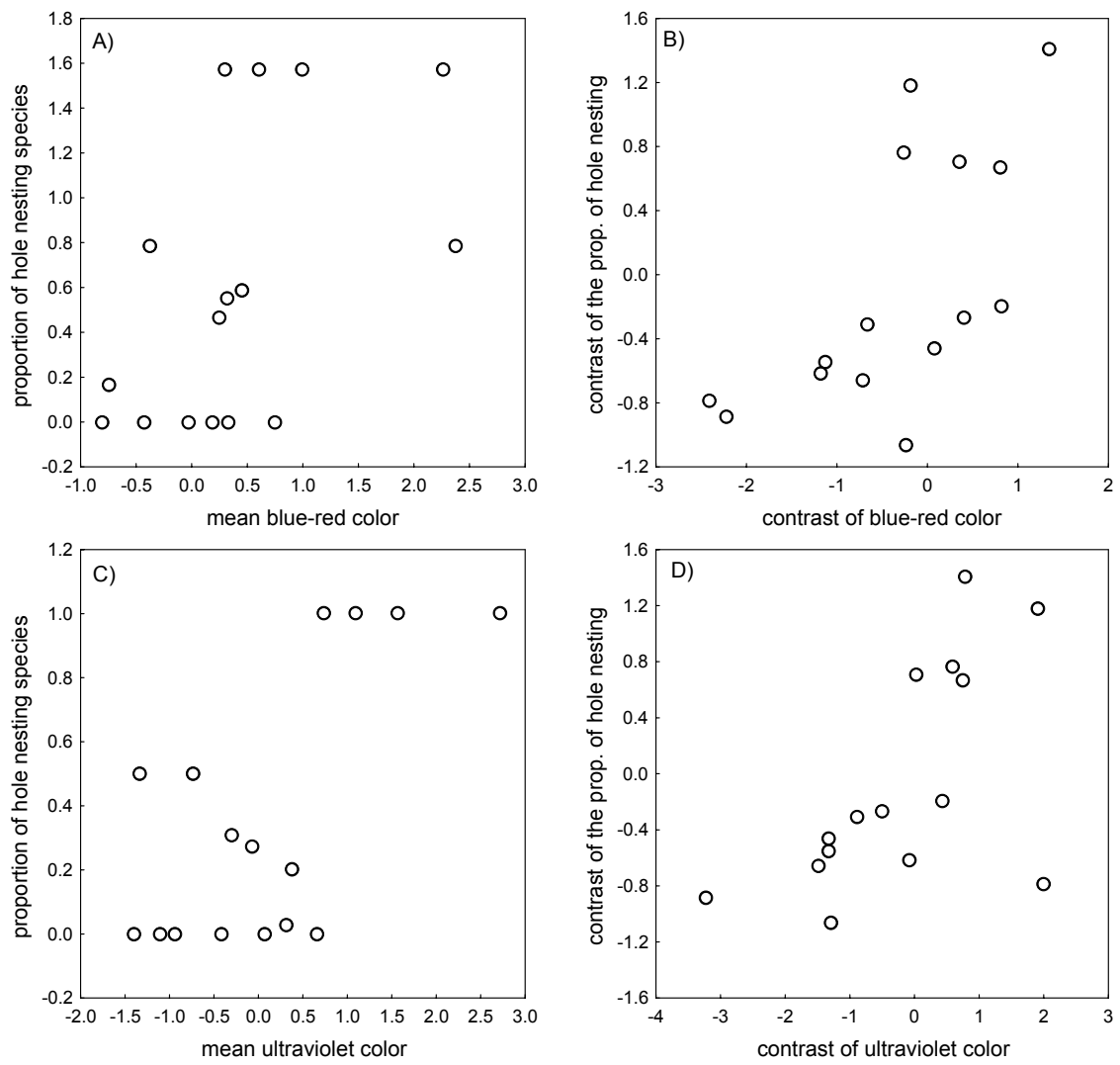
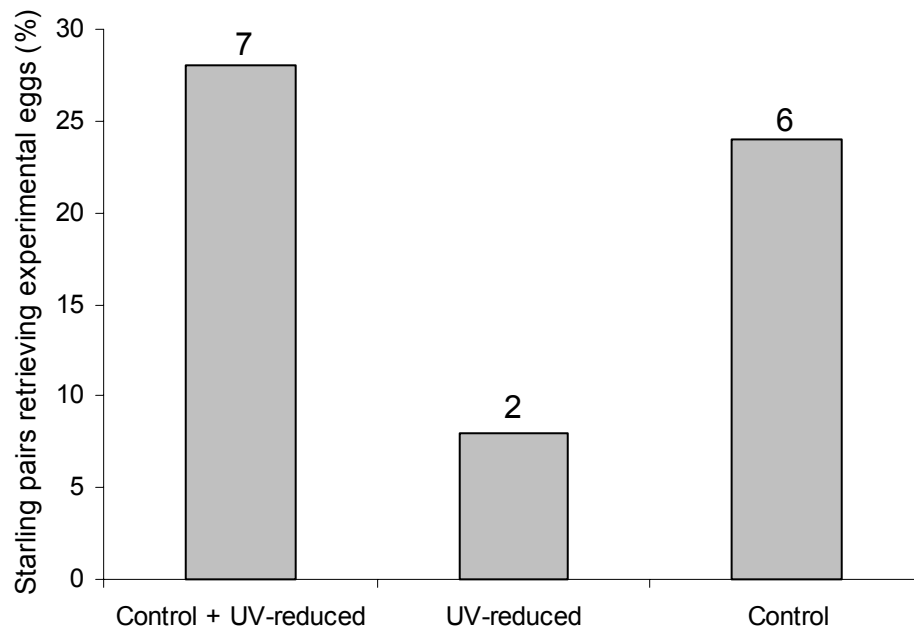


Figure 3. Avilés et al.







**Table 2.** Results of general linear models including variables defining egg colour as dependent variables and nesting site, habitat type, body mass, clutch size and duration of the nestling period as independent variables. Analyses were performed on raw data (i.e. species as independent data points) and on phylogenetically independent contrasts. In the last case the regression line was forced through the origin and degrees of freedom were corrected by subtracting the number of polytomies in the phylogenetic tree.

	Variables in the model	<i>F</i>	df	<i>P</i>	PC1 (brightness)				PC2 (blue versus red)				PC3 (UV)			
					Beta	(SE)	<i>t</i>	<i>P</i>	Beta	(SE)	<i>t</i>	<i>P</i>	Beta	(SE)	<i>T</i>	<i>P</i>
Raw data	Intercept	2.04	3,81	0.15			-0.10	0.92			1.08	0.285			2.05	0.04
	nesting site	14.66	3,81	< 0.0001	0.41	0.10	4.15	< 0.001	-0.37	0.11	-3.44	0.001	-0.27	0.12	-2.36	0.02
	habitat type	1.64	3,81	0.19	0.05	0.09	0.57	0.57	-0.18	0.10	-1.83	0.071	-0.08	0.11	-0.71	0.48
	body mass	5.09	3,81	< 0.01	0.35	0.13	2.67	0.01	0.45	0.14	3.16	< 0.001	-0.04	0.15	-0.26	0.79
	clutch size	1.69	3,81	0.18	0.08	0.11	0.72	0.47	0.00	0.11	0.01	0.992	-0.25	0.12	-2.00	0.05
	nestling period	1.83	3,81	0.15	-0.29	0.13	-2.20	0.03	-0.14	0.14	-1.02	0.311	0.02	0.15	0.13	0.90
Contrast	nesting site	9.20	3,52	< 0.0001	0.40	0.10	3.86	0.0003	-0.13	0.11	-1.20	0.23	-0.31	0.11	-2.91	0.004
	habitat type	1.18	3,52	0.32	0.14	0.10	1.46	0.15	-0.09	0.10	-0.86	0.39	-0.05	0.10	-0.50	0.62
	body mass	2.30	3,52	0.09	0.13	0.12	1.11	0.27	0.25	0.13	1.96	0.05	0.18	0.13	1.44	0.15
	clutch size	0.45	3,52	0.72	0.05	0.11	0.51	0.61	0.10	0.11	0.91	0.37	-0.07	0.11	-0.66	0.51
	nestling period	0.78	3,52	0.51	-0.14	0.12	-1.23	0.22	0.07	0.13	0.59	0.56	0.06	0.12	0.47	0.64

