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

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Owing to the conspicuousness of ultraviolet (UV) colour in dark environments, natural selection might have selected UV 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 levels revealed that hole-nester species produced eggs with higher UV reflectance than those nesting in open habitats. The experimental approach consisted of the manipulation of UV reflectance of the experimental eggs introduced outside the nest-cup of the hole-nester spotless starling *Sturnus unicolor* and the study of the 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, starlings removed it from the nest-box. Therefore, these results are consistent with the hypothesis that UV 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 by 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 the parasitic eggs appears to have been the main selective pressure for the evolution of egg mimicry in the European cuckoo, *Cuculus canorus* (Davies 2000). In addition, 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). Recently, it has been proposed that blue and green egg colours may function as a post-mating sexually selected signal of female 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 objects affects the conspicuousness of coloured elements, and thus, their detectability by conspecifics and/or predators (e.g. Marchetti 1993; Endler & Théry 1996). Birds' nests 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 recently been proposed that the importance of ultraviolet (UV) 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 UV 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 nests, which are arranged to maximize their conspicuousness in the UV (Veiga & Polo 2005). In addition, recent comparative evidence suggests that conspicuousness of nestlings' mouths is maximal in the UV (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 UV egg colours in birds may enhance egg detectability in dark nesting environments. Owing to a retinal cone type that is tuned to UV wavelengths (Chen *et al.* 1984), passerines can perceive the reflectance of UV 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–visible (UV–vis) reflectance spectrophotometry has revealed that UV reflectance of eggs is a variable trait among the European passerines (UV chroma of eggs ranges between 0.13 and 0.22; table 3, electronic supplementary material). 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 more often found in species that nest in cavities or crevices than in open-nesting species (von Haartmann 1957; Lack 1958; Oniki 1985). However, these studies scored egg coloration based on human vision, which does not detect UV reflectance, and did not use the comparative method to account for species similarity due to common descent. In the first step, we aimed to discover whether egg coloration measured with UV–vis spectrophotometry is related to nesting habits among the European passerines after taking their phylogenetic relationships into account. We predicted that eggs of species nesting in dark environments showed stronger UV reflectance than those of open-nesting species.

Conspicuous egg coloration in dark environments has typically been 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). However, a recent comparative study provides 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 that enables the parents to see eggs in a dark nest (von Haartmann 1957; Lack 1958). Here, we aimed to explore the functionality of UV reflectance of spotless starling eggs by determining 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 retinal cone with peak absorption in the UV (362 nm; Hart *et al.* 1998). Indeed, experimental evidence demonstrates that mate assessment depends on UV vision in the relative European starling *Sturnus vulgaris* (Bennett *et al.* 1997). Here, we coated experimental starling eggs with a UV-light blocker (–UV) and with horse fat as a control treatment, which provides eggs with a similar texture to the '–UV' eggs. We artificially placed a '–UV' and a 'control' egg in alternate days within nest-boxes in which laying had finished and incubation had already started. The eggs were situated outside the nest-cup and starling retrieval responses were recorded. Since eggs with reduced reflectance in the UV region show a poorer contrast with the nest background, we predicted that '–UV eggs' would be 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*
(i) *Species data*
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 3, electronic supplementary

material). This sample comprised 66.4% of species and 100% of families breeding in the Western Palaearctic (i.e. 134 species belonging to 16 families, according to Sibley & Ahlquist (1990)). The 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 & Bennett 2001; Langmore *et al.* 2003; Avilés *et al.* 2004; Soler *et al.* 2005), and allowing the recording of UV information to which humans are blind (Endler 1990). The 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 fibre-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 v. 4.1) calculated reflectance spectra relative to a standard white reference (WS-2). The total reflectance was obtained for the UV (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.

Nesting site
The hypothesis that some avian egg colour is more perceptible under particular microhabitat conditions predicts that among-species variation in egg colours is 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 the 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 an area that also nested in open nests in other area). These species were treated as non-strict hole-nesters (a score of 2; table 3, electronic supplementary material). We assume throughout the paper that non-strict hole-nesters are an intermediate category between open- and strict hole-nesters.

Confounding variables
Based on the 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 this potential confounding effect, we classified species as inhabiting open (score of 1) or forested habitats (score of 3). The species of open habitats include those nesting in open lands, old fields and riparian habitats. Mostly, the forest species are strictly forest breeders (score of 1). Birds inhabiting both kinds of habitats or preferring scrubs, clearings 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 signal 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 the nesting sites, we entered the length of nestling period as an additional predictor in our comparative framework. In addition, if pigments colouring the eggs are costly to produce (see Moreno & Osorno 2003),

Table 1. Factor loadings of axes from a principal component analysis on reflectance data. (Percentage 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
percentage of variance	95.00	3.01	1.69

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 the raw data used for analyses are summarized in the table 3 in the electronic supplementary material.

(ii) *Statistical methods*
Egg colour analyses
Since colour variables are typically interrelated (Endler 1990), 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 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 UV wavelength 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.

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 4, electronic supplementary material). 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 4, electronic supplementary material).

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 'UV') as dependent variable and nesting habit as independent variable. Brightness and blue–red were normally distributed and UV 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 contrast methods as implemented in the computer program PDAP (v. 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 4 in the electronic supplementary material). We assume all polytomies to be unresolved, and branch lengths were assigned following three different methodologies: (i) by setting all equal to 1; (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 1 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 s.d. ($r = 0.23$, $p = 0.03$, $N = 89$) when branch length was assigned following the Pagel (1992) method, although this relationship was non-significant under Bonferroni standards. However, in no cases did we find a significant correlation ($p > 0.1$) when branch lengths was set equal to 1 or assigned following the Grafen (1989) method. The resulting contrasts for each variable were then used to perform GLMs through the origin. The 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.

Family-level analyses
Although most of the variance in egg-colour traits and nesting habit is at the species taxonomic level (table 4, electronic supplementary material), 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 'UV' 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
(i) 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 to or within colonies of spotless starlings already established in old buildings in 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 also sometimes by males (Veiga *et al.* 2002).

(ii) Experimental design
After 2–4 days of 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 the tested pairs were incubated and females returned to the nests only a few minutes after our visit. Two hours after the female returned to the nest, we recorded the position of the experimental egg as 'egg retrieved' if the egg was found within the nest-cup, or 'odd' if the egg was judged still to be in its original position, or if it had been ejected out of the nest-box. The experimental egg was then removed. On 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 the 25 tested nests, we gathered paired information on the retrieval of '–UV' and control experimental eggs.

Our main focus is interpreting differences in egg retrieval 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 it is for this reason that this breeding strategy has been selected for the 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 retrieval 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 not retrieved). To control for this possibility, we introduced a second egg within the nest-cup with the same treatment as that situated outside the nest-cup. If differences in egg retrieval were due to brood parasitism (i.e. egg recognition), we would expect that pairs that recognize the egg situated outside the nest-cup as parasitic (i.e. do not retrieve it) would also recognize the second experimental egg of the same treatment introduced to the nest-cup as parasitic (i.e. eject it from 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-retrieval behaviour should be interpreted in the light of brood parasitism selection pressure. Otherwise, results should be interpreted as UV-coloration affecting starling adults' ability to detect eggs.

The experimental eggs were collected from abandoned-starling nests in 2005 and used fresh after collection. Further, experimental eggs were kept in 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 a UV-light blocker (50/50 w/w blend of Parsol 1789 and MCX, Roche). The UV-block effect persisted at 24 h after coating, and it did not alter the 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, which provides similar touching to eggs to the UV-light blocker (Avilés *et al.* 2006). Changes in the spectral shape of starling eggs for the two treatments are shown in figure 5 in the electronic supplementary material. 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.

(iii) Statistical methods
The 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 (less than four eggs versus four or more eggs) and their interaction as independent fixed factors and nest as a random factor. The probability of retrieval of each experimental egg was modelled as a binomial response variable (1 = rejection and 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 UV eggs (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) than open- and semi-hole-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). In addition, 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 UV reflectance than those nesting in open habitats (table 2).

(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 blue-coloured and UV eggs (blue–red: $R^2 = 0.28$; $F_{1,14} = 5.62$, $p = 0.03$; UV: $R^2 = 0.45$, $F_{1,14} = 11.43$, $p = 0.004$; figure 2), which was qualitatively confirmed when phylogenetic independent contrasts were used (blue–red: $R^2 = 0.40$; $F_{1,14} = 9.63$, $p = 0.007$; UV: $R^2 = 0.37$, $F_{1,14} = 8.25$, $p = 0.01$; figure 2).
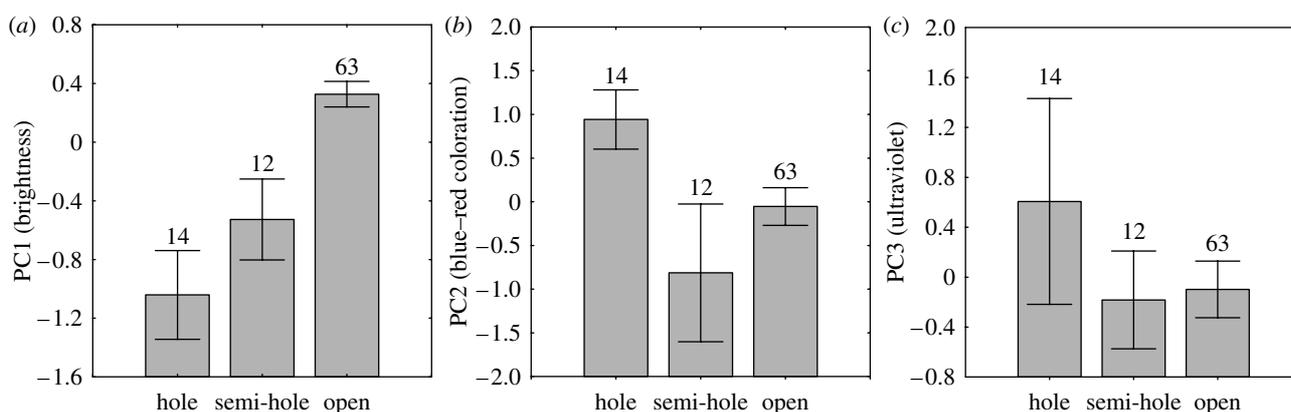
Figure 1. Differences (mean ± s.e.) in egg colouration ((a) brightness; (b) blue–red and (c) ultraviolet) in relation to nesting habits in western Palaearctic passerine birds. The numbers of species appear on error bars.

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

The spotless starlings retrieved 44.0% of the eggs situated outside their nest-cups (22 out of 50 experimentally 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 the 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 the 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 UV 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 frequently 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, $F_{1,23} = 2.16$, $p = 0.15$).

Only 3 out of 50 (6%) experimental eggs introduced within the nest-cup were ejected, and in all 3 cases, eggs were ejected from the nest-box. Therefore, owing to the low percentage of rejection, we used Fisher's exact tests for the analyses. Although all the 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's exact test, $p = 0.23$). Further, variation in clutch size did not explain the ejection of experimental eggs within the nest-cup (21 out of 22 pairs with 2–3 eggs versus 26 out of 28 pairs with 4 or more eggs; Fisher's exact test, $p = 1.00$), and the only two 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 eggs by adult starlings.

4. DISCUSSION

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

We have found that intensity of egg UV colour is associated with certain types of nesting sites among the European passerines. More precisely, the hole-nesting species have more UV eggs than open-nesting species

(figure 1), a pattern that held significant when we controlled for phylogenetic effects. This relationship was not due to a confounding effect of habitat type, body mass, clutch size or nestling period since intensity of UV 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 UV eggs (figure 2). These results are therefore consistent with the hypothesis that UV colours in the eggs may have been selected to provide detectable cues in poorly lit environments.

Our results extend the 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 nestlings' months in hole-nesting species are brighter than in open-nesting species (Ficken 1965; Kilner & Davies 1998). Experimental evidence has confirmed a functional role of such variablility of mouth coloration with nest light in nestling's detectability by their parents (Heeb *et al.* 2003). The use of non-anthropocentric UV–vis spectrometry to quantify mouth colour has allowed us to confirm these findings based on human vision and emphasized the importance of UV radiation for mouth conspicuousness in hole-nesters (Hunt *et al.* 2003; Jourdie *et al.* 2004). Conspicuousness of reflective eggs in the UV traits in hole nests is based on the particularly low levels of radiance in the UV of the nest background, which 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 common phylogenetic descent had shown that highly conspicuous (i.e. more brilliant) white eggs were more 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 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 UV

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 nesting period as independent variables. (Analyses were performed on raw data (i.e. species as independent data points) and on phylogenetically independent contrasts. In the latter 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	PC1 (brightness) F	d.f.	p	β	(s.e.)	t	p	PC2 (blue versus red) β	(s.e.)	t	p	PC3 (UV) β	(s.e.)	T	p
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
nesting 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
nesting 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

eggs than open-nesting species. In other words, selection may have favoured not only highly reflective (i.e. more brilliant) eggs, but also eggs coloured in a way that enhanced conspicuousness in the UV to enhance egg detectability in the poorly lit conditions of a hole.

Other plausible alternative hypotheses to that of the egg detectability may explain why UV 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 UV because UV coloured eggs were less detectable 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). The nests predators may rely on other more obvious cues denoting nest presence than egg colour, such as, for instance, parental activity (Martin *et al.* 2000), or the nest itself (Gotmark 1992). However, we could not dismiss the possibility that predators on hole 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 UV 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 UV colour and nesting habits controlling for PC2 (i.e. 'blue–red') and all the confounding variables, our results remain unchanged (table 5, electronic supplementary material). Therefore, 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 UV-coloured eggs (this study). This hypothesis would clearly predict changes in egg detectability by incubating birds as the 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 white naturally coloured eggs and found that the former disappeared significantly more often 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
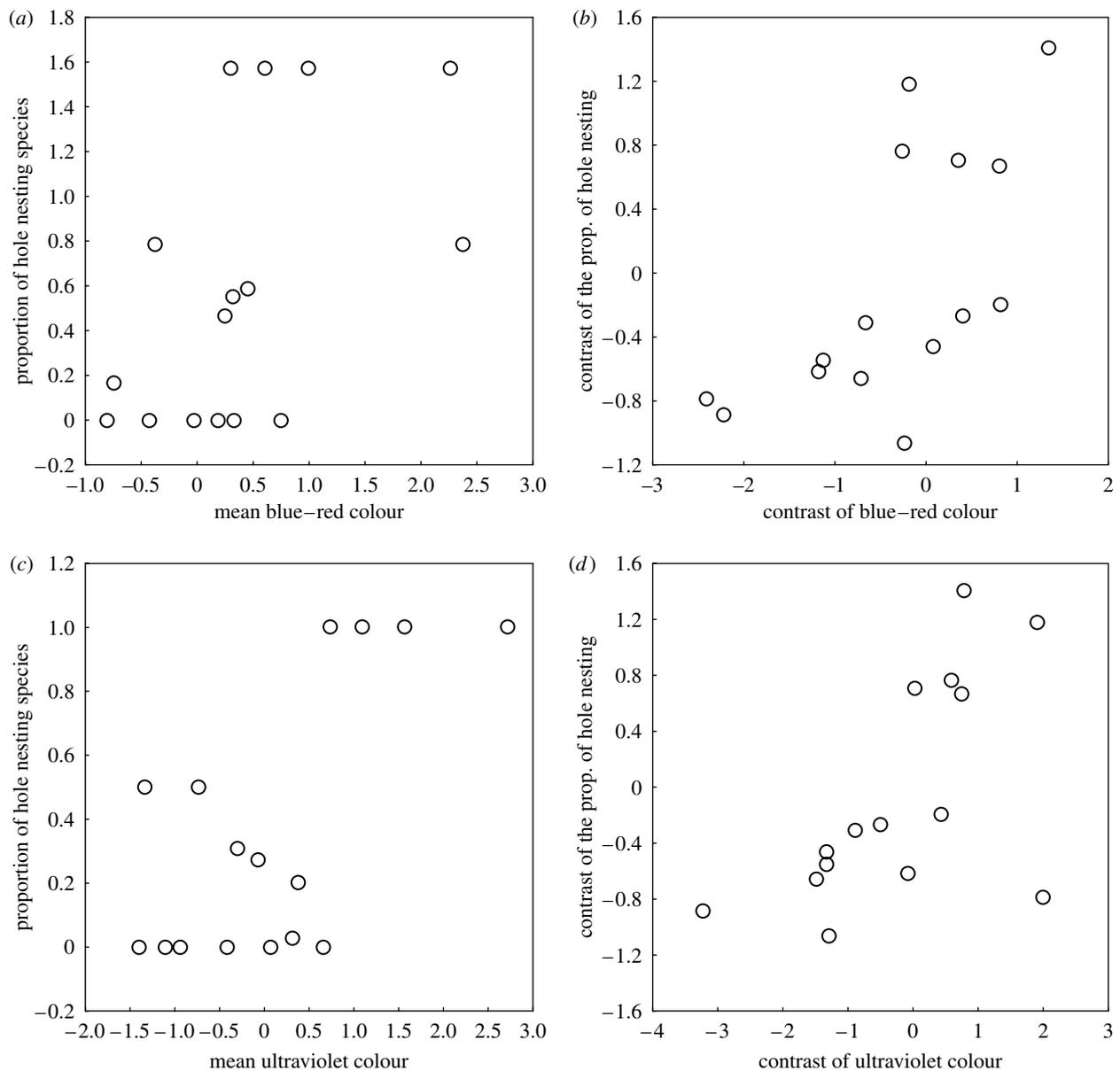
Figure 2. Relationship between mean blue–red and UV colour and proportion of hole-nesting species per family in Western Palaearctic passerine birds: (*a,c*) non-controlling and (*b,d*) controlling for the phylogenetic effects using independent contrast analysis.

commonly retrieved by incubating starlings than non-reflecting (–UV) eggs (figure 3). Therefore, our result suggests that the UV reduction treatment affected egg retrieval by starlings, and thus, that UV 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 1991). Since '–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 (not 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 as the egg placed outside the nest-cup. However, starlings never moved experimental eggs from inside to outside nest-cup, and when they recognized a parasitic egg, they ejected the egg from the
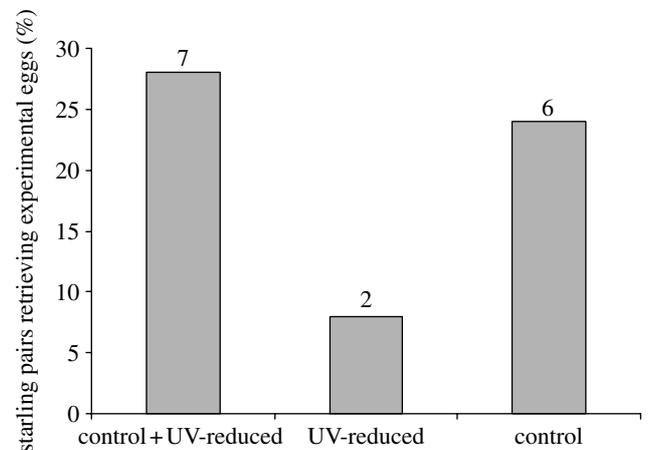

Figure 3. Starling pairs retrieving eggs within the nest-cup in relation to a treatment affecting UV reflectance. Starlings can retrieve the control, the '–UV' or the two kinds of experimental eggs (*N*=25 tested pairs with the two egg treatments).

nest-box (3 out of 50 experiments). More importantly, when one of the experimental eggs was removed from the nest-box, the second experimental egg 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. Although this remains to be further studied, it 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 a higher quality to their male 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 UV egg coloration and nesting habits in birds. In addition, we found experimental support for the role of UV egg coloration in egg retrieval in the spotless starling. These findings agree with expectations for the egg detectability hypothesis, suggesting that UV egg coloration enhances egg detectability by parents in dark nests.

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