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BLUE AND GREEN EGG-COLOR INTENSITY IS ASSOCIATED WITH PARENTAL EFFORT AND MATING SYSTEM IN PASSERINES: SUPPORT FOR THE SEXUAL SELECTION HYPOTHESIS

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Abstract.—Among several adaptive explanations proposed to account for variation in avian egg color, that related to sexual selection is of particular interest because of its possible generality. Briefly, it proposes that because biliverdin (the pigment responsible for blue-green eggshell coloration) is an antioxidant, deposition in the eggshell by laying females may signal the capacity of females to control free radicals, despite the handicap of removing this antioxidant from their body. If males adjust parental effort in response to the intensity of the blue coloration of eggs, thereby investing more in the offspring of high-quality mates, blue eggs may represent a postmating sexually selected signal in females. Here, by image and spectrophotometric analyses of the eggs of European passerines, we tested two different predictions of the hypothesis. First, variables related to intraspecific variation in parental effort (i.e., the duration of the nestling period controlled for body mass) should be positively related to the intensity of blue-green color of the eggshell across species. Second, there should be a positive relationship between intensity of blue-green color of eggs and degree of polygyny. These predictions were supported: intensity of blue-green coloration (i.e., chroma) was significantly related to the duration of the nestling period and to degree of polygyny after controlling for possible confounding variables (i.e., body mass, incubation period, and nest type) and similarity due to common descent. Nest type (hole or nonhole) also explained a significant proportion of variation in egg chroma, perhaps reflecting different selection pressures (i.e., light conditions, risk of parasitism) affecting species with the two types of nests.

Key words.—Egg color evolution, hole nesters, mating systems, postmating sexual selection, predation, sexual selection.

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Egg coloration in birds varies greatly among species from completely pale to beautifully decorated eggs. Adaptive explanations for such variation are of great interest for evolutionary biologists, and several hypotheses have been proposed. However, there is no general consensus on the importance of different factors for explaining variation in egg coloration of birds. Possible functions of egg coloration vary from those related to physical properties of pigments (i.e., filtering solar radiation or strengthening the eggshell) to those related to the adaptive function of color, with the latter having received most attention (see review in Underwood and Sealy 2002). For instance, egg color may cause clutches to mimic the environment, making it more difficult for predators to find eggs (e.g., Solis and de Lope 1995). This would mainly apply to species building inconspicuous nests (Götmark 1992, 1993) because otherwise predators may detect clutches by directly searching for nests (e.g., Møller 1990) or parental activity (e.g., Martin et al. 2000). However, results from recently published experimental tests in species with low nest conspicuousness do not support the hypothesis that egg camouflage reduces the risk of predation (Weidinger 2001).

Another functional hypothesis of avian egg coloration is related to the probability of detecting parasitic eggs in a clutch. Brood parasites cause a dramatic reduction in breeding success of their hosts (e.g., Davies 2000), and hosts, by laying eggs of a homogeneous color pattern (i.e., low intraclutch variation), would be able to more easily distinguish foreign eggs in their clutches (Petrie and Møller 1991; Soler and Møller 1996; Stokke et al. 2002). Experimental and comparative support for this functional hypothesis (Soler and Møller 1996; Stokke et al. 1999, 2002; Soler et al. 2000) indicate that brood parasitism is a powerful selective force in the evolution of egg coloration in birds. However, because variation in egg coloration persists among species that are not exploited by brood parasites with mimetic eggs, even among passerines (e.g., Stokke et al. 2002) the generality of this functional explanation is limited.

A new hypothesis attempting to explain avian egg coloration suggests that egg color is a sexually selected signal of females displaying their phenotypic quality or physical condition to males (Moreno and Osorno 2003). Briefly, biliverdin and protoporphyrines are two kinds of pigments responsible for egg coloration and, they are likely costly to produce and maintain (Moreno and Osorno 2003). The first is responsible for blue and green colors, while the latter determines reddish and brown egg colors (Kennedy and Vevers 1976; Miksik et al. 1996). Biliverdin is a strong antioxidant (Neuzil and Stocker 1993; McDonagh 2001; Kaur et al. 2003). Accordingly, deposition in the eggshell by laying females may signal their capacity to control free radicals, despite the handicap

of removing this antioxidant from their body by deposition in eggs (Moreno and Osorno 2003). Therefore, if male investment in reproduction depends on the amount of biliverdin included in the eggshell, as reflected by the intensity of blue-green coloration of eggs, egg color would be a postmating sexually selected signal explaining the adaptive significance of blue eggs (Moreno and Osorno 2003), which for most species until recently was considered a mystery (Götmark 1992; Underwood and Sealy 2002). Moreno et al. (2004) found a positive relationship between blue color intensity of pied flycatcher (*Ficedula hypoleuca*) eggs and feeding rate by the attending male, a result consistent with this functional hypothesis.

Here we test the importance of egg coloration as a postmating sexually selected signal by comparing two different predictions of the hypothesis. First, we study the relationship between egg color of European passerines and a variable related to level of parental effort (i.e., relative duration of the nestling period after controlling for body mass of different species). The assumption is that in species with protracted nestling care and male help, paternal contribution may be more important and variable than in species with short nestling periods, and consequently selection for signaling of female quality may be stronger (Moreno and Osorno 2003). Therefore, although Moreno and Osorno (2003) predicted a positive relationship between intraspecific variation in paternal care and egg-color intensity across avian species, duration of the nestling period is an appropriate, although imperfect, variable for testing the prediction given its potential association with intraspecific variability and consequent opportunities for selection. Second, we study the relationship between egg coloration and mating system (i.e., monogamy, monogamy-polygyny, polygyny, and polygynandry). The rationale is that, because male contribution to parental care is traded against mate attraction activities (Smith 1995; Moreno et al. 1999), females of polygynous species experience stronger competition for male contribution to parental care than those of monogamous species. Thus, females in polygynous systems may benefit more from inducing a higher level of paternal care through egg coloration than females of monogamous species (Moreno and Osorno 2003). Therefore, a positive relationship between egg-color intensity and degree of polygyny is predicted at the interspecific level.

Finally, we analyzed the relationship between egg color and incidence of nest predation in different species (Martin and Clobert 1996) to test if selection pressure exerted by nest predators affects egg coloration of passerines.

Material and Methods

The dataset includes information on egg-color and life-history variables of 152 species. Females incubate alone in 70.1% of the species, and males and females contribute to feed the offspring in 98.7% of the species (see Appendix 1, available online only at http://dx.doi.org/10.1554/04-159.1.s1).

Predictions derived by Moreno and Osorno (2003) from their hypothesis of certain avian egg colors being sexually selected signals by females that induce a higher allocation of paternal care refer more to intraspecific variation than to absolute levels of male contribution to feeding of offspring. However, we were unable to find data in the literature on intraspecific variation in male provisioning of nestlings for a sufficient number of species that would allow testing the prediction. Instead, we used the duration of the nestling period (as reported primarily by Perrins 1987), while for species with no information we used that in Cramp (1998), as a measure of parental effort (see Soler et al. 1998). Nestling care is the most important component of parental effort (Winkler and Wilkinson 1988), and the period when male help is most important for females in many species (Breitwisch 1989). Thus, the relative duration of the nestling period has previously been used as a measure of parental effort and potential male contribution (Soler et al. 1998). Moreover, this variable is likely related to variation in male contribution to feeding the offspring simply because sexual conflict would increase as specific requirements of parental effort for the offspring increases. Males are normally the sex with less reproductive investment and, thus, the sex more likely to vary in its contribution to feeding offspring (Andersson 1994). Therefore, we believe that the relative duration of the nestling period is an appropriate variable to test the predictions derived from Moreno and Osorno (2003).

The duration of the nestling period is negatively related to the risk of nest predation (Lack 1968; Bosque and Bosque 1995), which may interact with egg-color intensity (see introduction). Therefore, the hypothetical relationship between egg-color intensity and relative duration of the nestling period could be mediated by differences in risk of predation suffered by different species if, for instance, eggs of hole-nesting species (i.e., low risk of predation) differ in color from those of nonhole nesters. We partially controlled for this potential problem by including information on nest site as a dummy variable (holes = 2, nonholes = 1) in the analyses (Harrison 1975). We also included in the analyses the duration of the incubation period (from Perrins 1987; Cramp 1998), a variable related to the duration of the nestling period and to the risk of predation, but not to paternal investment in nestling care (Bennett and Owens 2002).

Body mass for each species was estimated as the average values reported by Perrins (1987) for males and females, which was logarithmically transformed before analyses. Information on mating systems was extracted from Cramp (1998) and used as an ordinal variable with the following values: monogamy = 0, monogamy-polygyny (i.e., some males being polygynous) = 1, polygyny (i.e., most males being polygynous) = 2, and polygynandry = 3. We assigned the larger value to the polygynandrous species because in this system females are competing with other females for the paternal care of more than one male. Incidence of nest predation in different species was from Martin and Clobert (1996).

Egg-Color Estimation by Computer Analysis

Egg-color intensity was estimated following two different methods and sources. First, we used photographs published in the electronic version of Cramp's collection (1998) and estimated background color of eggs using the computer program Photoshop (ver. 4.0; Adobe Systems Inc., San Jose,

CA) following a method similar to that described in Villafuerte and Negro (1998). Briefly, for each egg, we used the magic-wand option to select the background part of the eggs exhibiting a similar color and, later expanding the resulting selection up to the edges of the egg pictures by excluding spots and light-reflecting areas. Once background color of an egg was selected, the program calculated median values of pixels within the selected area for luminosity, red, green, and blue channels (range = 0–255). This procedure was repeated twice by applying the magic wand to different randomly selected areas of the egg background to estimate repeatability of egg-color estimations. For nonspotted eggs with uniform color, we took a single measure because the selected area was always the whole egg. Because repeatability of color values was highly significant for all three channels and luminosity (Pearson correlation coefficient, $R > 0.98$, $P < 0.0001$), we used mean values from the two measurements in the analysis. For each species, Cramp (1998) showed a variety of eggs from different clutches including the extremes in coloration for each species. However, when possible we avoided extreme colored eggs (i.e., nonspotted eggs and those almost completely covered with spots from the same species) and analyzed four randomly chosen eggs except for those species with fewer than four eggs on the plates (see sample sizes in Appendix 1, available online). One-way analyses of variance revealed consistently greater variances among than within species for all three standardized color values ($F_{153, 413} > 3.3$, $P < 0.0001$), and, therefore, background egg coloration estimated here is a species-specific character.

Egg-Color Estimation by Spectroradiometer

The second estimation of egg color was obtained using a spectroradiometer on eggs in museum collections. Because most eggs were collected more than 50 years ago and from different time periods, eggs may have faded in color, and those from different time periods may have faded to different degrees. However, that bias may be negligible: the analysis of 342 reed warbler clutches collected in the island of Zealand (Denmark) between 1922 and 1962, and saved at the Zoological Museum in Copenhagen (Denmark) and at the Zoological Museum of the University of Lund (Sweden) revealed no significant relationships between year of collection and reflectance at the ultraviolet, blue, green, yellow, and red wavelengths ($R < -0.04$, $P > 0.3$, J. M. Avilés and A. P. Møller, unpubl. data).

A total of 5878 eggs belonging to 98 species were sampled (median = 26 eggs per species, range = 2–1807). However, information for other variables in the analyses was available only for 88 of those species. We obtained reflectance spectra in the range 300–700 nm from all clutches using a spectroradiometer (Ocean Optics Europe, Duiven, The Netherlands). We measured color twice in two randomly selected areas of the surface of the eggs, each covering about 1 mm^2. The illuminant was a deuterium and halogen light source (DH 2000, Ocean Optics). The light was transferred to the eggs through a quartz optic fiber (Ocean Optics) and reached the eggs at 45°. The sampling optic was placed in the same cable as the optic fiber and, thus, at 45° to the surface of the sample. It was connected to a spectrometer (S2000, Ocean Optics) by a second quartz fiber-optic cable. Data from the spectroradiometer were converted into digital information by DAQ Card 700 (Ocean Optics) and passed into a computer with appropriate software (Spectrawin 4.1, Ocean Optics). The measurements were relative and referred to the dark (by applying the extreme of the probe to a black card with the lamps switched off) and to a standard white reference (WS-2). We made a reference and dark calibration before measurement of each clutch. 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.

Spectrophotometric techniques may have flaws when quantifying the background color of a spotted egg because the diameter of the optic fiber may exceed for some species the surface between two spots. Therefore, we did not avoid spots and used randomly selected areas of the entire eggs. However, repeatability analyses of different measures from different randomly selected areas allowed us to quantify color variability within and among eggs (Avilés et al. 2004). We performed the analysis on 144 eggs of the great reed warbler (*Acrocephalus arundinaceus*), a species with heavily spotted eggs, and on 190 eggs of the redstart (*Phoenicurus phoenicurus*), with pure blue eggs. First, we measured twice in the same randomly selected area of a single egg and found a high repeatability (Pearson correlation coefficients, $R > 0.94$, $P < 0.0001$ for pure blue eggs, and $R > 0.79$, $P < 0.0001$ for spotted eggs). Secondly, we measured each egg in two randomly selected areas and found a significant repeatability (Pearson correlation coefficients, $R > 0.46$, $P < 0.0001$ for pure blue eggs, and $R > 0.54$, $P < 0.0001$ for spotted eggs). Consequently, all our measurements were reliable irrespectively of spottiness and, thus, we used mean values for each egg for each established interval. Moreover, one-way analyses of variance revealed consistently greater variance among than within species for all colors ($F_{97,3958} > 96.15$, $P < 0.00001$), which is an important prerequisite for comparative analyses. Therefore, we assumed that despite considerable intraspecific variation, the background coloration of eggs is as a species-specific characteristic that can be reliably estimated. Sample sizes and species used in the analyses are listed in Appendix 1 (available online).

Statistical and Comparative Analyses

Because color variables from either image or spectroradiometer analyses were interrelated, we performed principal component analyses (PCA), which have the advantage of reducing the number of variables to a few orthogonal and statistically independent variables that summarize most of the variation. The first principal component (PC1) for reflectance spectra from natural objects typically describes achromatic variation, essentially brightness (Endler 1990; Endler and Théry 1996), and often explains more than 90% of the spectral variation. PC2 and PC3 then represent variation in spectral shape and are related to chromatic variation (Hurlbert 1986; Cherry and Bennett 2001) and, together with PC1, often constitute close to 100% of the total variance. In accordance, PCA on reflectance variables generated three orthogonal axes explaining 99.8% of total variance. The first one (PC1r) was strongly negatively related to all five different color variables

TABLE 1. Factor loading of axes from principal component analysis on reflectance (PC1r, PC2r, PC3r) and image (PC1i, PC2i, PC3i) variables. Percent of variance explained by each axis is also shown.

	Spectroradiometry analysis			Image analysis	
	PC1r	PC2r	PC3r	PC1i	PC2i
UV	−0.967	0.056	0.249		
Blue	−0.978	0.200	−0.032	−0.962	−0.265
Green	−0.978	0.145	−0.141	−0.995	−0.008
Yellow	−0.987	−0.144	−0.054		
Red	−0.964	−0.260	−0.020	−0.968	0.240
Luminosity				−0.999	0.031
% of variance	95.0	3.1	1.7	96.3	3.2

(UV, blue, green, yellow, and red), therefore reflecting achromatic brightness. The second one (PC2r) was positively related to blue and green reflectance and negatively to yellow and red reflectance. Finally, the third component (PC3r) was positively related to UV reflectance (Table 1). In addition, results from the PCA performed on variables from image analysis were quite similar. It produced two axes explaining 99.5% of total variance. The first axis (PC1i) was negatively related to all color variables (luminosity, blue, green, and red), therefore reflecting achromatic brightness. The second one (PC2i) was negatively related to blue but positively related to red color values. Therefore, we used scores from those axes in our analyses because they reflected brightness (PC1r and PC1i) and chroma for which it was possible to distinguish the importance of different colors (UV by PC3r, blue by +PC2r and −PC2i, green by +PC2r, yellow by −PC2r, and red by −PC2r and + PC2i).

Phylogenetic relationships among species were estimated following Sibley and Alhquist (1990) and more recently published papers (Sheldon and Winkler 1993; Blondel et al. 1996; Cibois and Pasquet 1999; see Appendix 2, available online only at http://dx.doi.org/10.1554/04-159.1.s1). We assume all polytomies to be unresolved, and branch lengths were assigned following three different methods: (1) all were set equal to one; (2) arbitrarily assigning all internode branch segments to one, but constraining tips to be contemporaneous (Pagel 1992); and (3) 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). Lacking information on actual branch length, we used this approach to test the robustness of our results to varying branch-length assumptions.

To control for the possible effect of common phylogenetic descent, we used Felsenstein's (1985) independent contrasts method as implemented in the computer program PDAP (ver. 6.0, module PDTREE) by Garland et al. (1999) and Garland and Ives (2000). This method finds a set of independent pairwise differences or contrasts, assuming that changes along the branches of the phylogeny can be modeled by a Brownian motion process (successive changes are independent of one another) and that the expected total change summed over many independent changes is zero (Harvey and Pagel 1991). The advantage of the independent contrasts approach is that, by partitioning the variation appropriately, all contrasts can be used to assess a hypothetical comparative relationship (Harvey and Pagel 1991). These contrasts were estimated for each variable using the three different methods of assigning branch length. Moreover, to check whether the contrasts were adequately standardized, we plotted 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). In no case did we find a significant correlation ($P > 0.1$). The resulting contrasts for each variable were then used to perform general linear model (GLM) analyses through the origin. We also analyzed data without phylogenetic correction. GLMs were performed with egg-color variables as dependent variables and mating system and the duration of the nestling period as independent variables. Body mass, which is positively related to the duration of the nestling period (Peters 1983), nest site, and the incubation period (both related to risk of predation, which could affect egg colors, but also to nestling period) were also included in the model to control for possible confounding factors (i.e., predation pressure and allometric effects). Because we were interested in partial correlation coefficients, Type II decomposition of variance was used (StatSoft, Inc. 2001). Moreover, although sample sizes were quite small for a few species, results did not change when excluding species with fewer than three eggs sampled. The analysis of incidences of nest predation and egg color do not include other variables due to the lack of data for some species. Finally, although predictions were clearly directional, we conservatively used two tailed *P*-values.

RESULTS

Scores for PC1i and PC2i estimated for each species from the image analysis of Cramp's (1998) plates were significantly related to scores for PC1r and PC2r estimated from the spectroradiometer ($N = 88$ species; PC1i vs. PC1r: $R = 0.602$, $P < 0.0001$; PC2i vs. PC2r: $R = -0.63$, $P < 0.0001$). These significant correlations validate the computer image analyses. Moreover, reflectance was estimated for the entire eggs without avoiding spotted areas and, therefore, values of egg color for different species should vary depending on methodology (i.e., image analyses or spectroradiometer) used for that estimation, explaining the relatively low correlations found. Finally, PC1i was significantly positively related to PC2r ($R = 0.22$, $P = 0.037$), suggesting that PC1i not only indicates brightness but also chroma to a lesser degree.

After controlling for possible confounding variables, mating system and the duration of the nestling period explained egg coloration of passerines (Table 2). However, the duration of the nestling period was the main predictor when using data

TABLE 2. Results of general linear models including variables defining egg color as dependent variables and log body mass, mating system (monogamous = 0, monogamous-polygynous = 1, polygynous = 2, polygynandrous = 3), duration of the nestling and incubation period, and nest site (hole = 1, nonhole = 2) as independent variables. The analyses were performed for principal component analysis scores from image (PC1i, PC2i) and spectroradiometer (PC1r, PC2r, and PC3r) analyses, and considering species as independent datapoints (scores) but also taking phylogenetic relationships into account (contrasts) with all branch lengths equal to one, or arbitrarily assigned following the methods of Pagel (1992) or Grafen (1989). Because results from phylogenetic independent contrasts are similar regardless of the assigned branch length, we report for each variable the smallest resulting values for the statistics and the largest estimated *P*-values. Degrees of freedom were corrected by subtracting the number of polytomies in the phylogenetic trees.

Branch length	Variables in the model	*F*	df	*P*	PC1 (brightness)				PC2 (chroma)				PC3 (UV)			
					Beta (SE)	*t*	df	*P*	Beta (SE)	*t*	df	*P*	Beta (SE)	*t*	df	*P*
Image analysis																
Scores	body mass	45.70	2,145	<0.0001	0.65 (0.09)	7.27	146	<0.0001	−0.50 (0.09)	5.33	146	<0.0001				
	mating systems	6.95	2,145	0.001	0.08 (0.07)	1.14	146	0.26	−0.24 (0.07)	3.40	146	0.001				
	nestling period	17.80	2,145	<0.0001	−0.65 (0.11)	5.96	146	<0.0001	−0.14 (0.11)	1.30	146	0.19				
	incubation period	1.72	2,145	0.18	0.05 (0.11)	0.45	146	0.65	0.20 (0.11)	1.85	146	0.066				
	nest site	3.34	2,145	0.038	−0.03 (0.07)	0.42	146	0.68	−0.20 (0.08)	2.59	146	0.01				
Contrasts	body mass	10.26	2,81	0.0001	0.22 (0.08)	2.70	84	0.008	−0.30 (0.08)	3.67	84	0.0004				
	mating systems	2.76	2,81	0.069	0.05 (0.08)	0.65	84	0.51	−0.16 (0.08)	2.28	84	0.025				
	nestling period	3.46	2,81	0.036	−0.24 (0.09)	2.55	84	0.013	−0.06 (0.09)	0.64	84	0.52				
	incubation period	1.51	2,81	0.23	−0.03 (0.09)	0.40	84	0.69	0.15 (0.09)	1.71	84	0.09				
	nest site	2.24	2,81	0.11	−0.16 (0.08)	1.18	84	0.07	−0.08 (0.08)	0.96	84	0.34				
Reflectance analysis																
Scores	body mass	9.18	3,80	<0.0001	0.44 (0.12)	3.55	83	0.001	0.47 (0.11)	4.28	83	<0.0001	0.02 (0.14)	0.12	83	0.91
	mating systems	6.33	3,80	0.001	0.02 (0.10)	0.21	83	0.84	0.39 (0.09)	4.40	83	<0.0001	−0.04 (0.11)	0.40	83	0.69
	nestling period	2.60	3,80	0.06	−0.38 (0.17)	2.23	83	0.023	0.13 (0.15)	0.83	83	0.41	0.19 (0.19)	0.99	83	0.32
	incubation period	2.04	3,80	0.12	0.05 (0.16)	0.29	83	0.77	−0.26 (0.15)	1.75	83	0.08	−0.27 (0.18)	1.45	83	0.15
	nest site	12.75	3,80	<0.0001	−0.33 (0.10)	3.37	83	0.001	0.38 (0.09)	4.29	83	<0.0001	0.17 (0.11)	1.56	83	0.12
Contrast	body mass	2.51	3,51	0.069	0.17 (0.13)	1.34	54	0.19	0.24 (0.11)	2.07	54	0.043	0.14 (0.12)	1.18	54	0.24
	mating systems	5.33	3,51	0.003	−0.03 (0.10)	0.24	54	0.81	0.35 (0.09)	3.73	54	0.0005	−0.16 (0.10)	1.58	54	0.12
	nestling period	2.55	3,51	0.066	−0.18 (0.15)	1.24	54	0.22	0.28 (0.13)	2.15	54	0.036	0.09 (0.14)	0.62	54	0.54
	incubation period	2.30	3,51	0.088	−0.01 (0.14)	0.11	54	0.91	−0.31 (0.12)	2.57	54	0.013	−0.04 (0.13)	0.27	54	0.79
	nest site	6.65	3,51	0.0007	−0.26 (0.11)	2.33	54	0.024	0.21 (0.10)	2.16	54	0.035	0.25 (0.11)	2.41	54	0.019

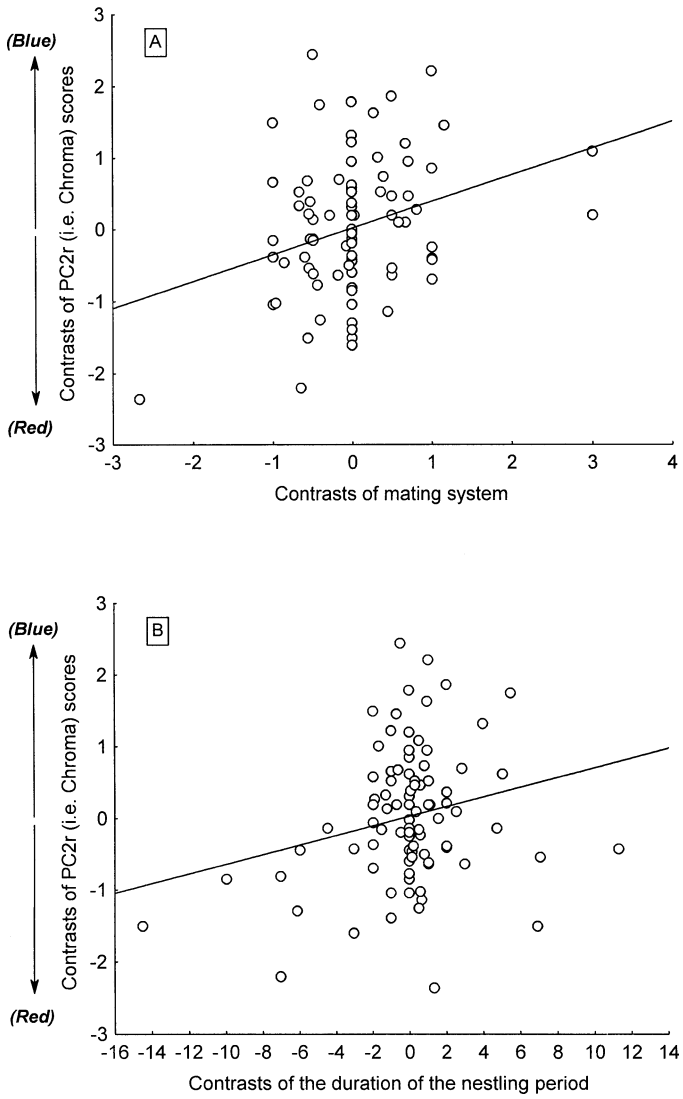

FIG. 1. Relationships between background color intensity (chroma, PC2r) estimated from spectrophotometric analysis and (A) mating system (monogamous = 0, monogamous-polygynous = 1, polygynous = 2, and polygynandrous = 3) and (B) the duration of the nestling period. Data are phylogenetically independent contrasts, which were estimated based on phylogenetic trees with branch lengths estimated following Pagel's (1992) method.

from image analysis, while mating system were the main predictor when using reflectance data (Table 2).

When looking at the relationships between dependent and independent variables separately in our GLM models, results were quite similar regardless of the methodology used to estimate egg color. In the image analysis, PC1i was significantly related to length of nestling period, whereas PC2i was related to mating system. Similarly, in the reflectance analysis, PC1r was related to nestling period but only in the nonphylogenetic analysis, whereas PC2r was strongly related to mating system in both analyses. A weaker relationship between PC2r and nestling period also was found in the phylogenetically controlled analysis (Fig. 1, Table 2).

Some of the additional independent variables included in the model were significantly related to PC axes reflecting brightness (PC1i and PC1r) but also to those reflecting chroma (PC2i, PC2r, and PC3r). For instance, when using reflectance data, nest site and incubation period predicted egg coloration. While incubation period was negatively related to blue-green color intensity, eggs of hole-nester species were brighter, more blue, and reflecting more intensely at UV wavelengths than those of nonhole nesting species. Although these results could suggest a role for risk of predation on the evolution of egg color in passerines, percentage of nest predation suffered by different species did not explain egg-color variables. That was the case regardless of the method used to estimate egg color and whether phylogenetically independent contrasts were used ($N = 30$, strongest relationships found: PC1i: $F_{1,28} < 1.24$, $P > 0.25$; PC2i: $F_{1,28} < 0.84$, $P > 0.36$; PC1r: $F_{1,22} < 0.58$, $P > 0.4$; PC2r: $F_{1,22} < 1.32$, $P > 0.26$; PC3r: $F_{1,22} < 0.55$, $P > 0.4$). Therefore, at least for the species with information on risk of predation, the prediction from the hypothesis of egg coloration being the consequence of selection due to nest predation is not fulfilled.

Finally, body mass was negatively related to brightness and positively to blue color intensity of eggs (Table 2).

DISCUSSION

Certain life-history traits, such as mating system and the duration of the nestling period, explained a significant proportion of variance of passerine egg coloration. More precisely, when using reflectance data, we found that species with longer nestling periods laid eggs with a greater intensity of blue-green coloration. In addition, females of species more likely to compete for paternal investment because of their polygynous mating system laid eggs with a greater intensity of blue-green color. This is consistent with the hypothesis that certain avian egg colors are sexually selected signals produced by females to induce a higher allocation of paternal care (Moreno and Osorno 2003).

We used the duration of the nestling period as a variable related to parental effort and, thereby, possibly reflecting intraspecific variation in paternal care (see Material and Methods for justification). Variation in paternal investment after fledging may also influence selection on egg color, but this stage was not considered in our analyses. In addition, the duration of the nestling period is related to body size (Peters 1983) and to risk of nest predation (Bosque and Bosque 1995; Remes and Martin 2002), which may also affect egg color (Underwood and Sealy 2002). In our analysis, we controlled for body size and indirectly for nest predation by including nest site and duration of incubation, variables correlated to the risk of predation (Martin 1995; Bennett and Owens 2002). We also tested directly for the hypothetical association between risk of predation (i.e., predation rate) and egg color, both including (reflectance analysis) and excluding (image analysis) spots, but these effects were far from significant. Thus, our results are consistent with the hypothesis of blue-egg color intensity being a female signal used in postmating sexual selection (Moreno and Osorno 2003).

Another prediction of this hypothesis is that, because females would gain more relative parental investment from their males in polygynous than in monogamous species, mating systems adopted by different species should predict blue-

green color intensity of their eggs. In agreement with that prediction, we found that intensity of blue-green egg coloration (i.e., PCA axes reflecting chromatic but not brightness aspects of egg color) was positively related to the presumable amount of competition among females for male help (i.e., mating system). Eggs of monogamous species were lower in blue-green coloration, while eggs of polygynous and polygynandrous species had greater blue-green color intensity. We are aware of no other hypothesis that would predict a relationship between the intensity of blue-green egg coloration and mating system. Therefore, our results are consistent with the sexual selection hypothesis.

The results presented here are stronger when using a spectroradiometer to estimate egg color, although image analyses gave qualitatively similar results. Color vision of birds differs from that of humans because birds can detect UV radiation (< 400 nm) due to cones in their retinas being sensitive to UV light (Chen et al. 1984; Bowmaker et al. 1997). Moreover, Cherry and Bennet (2001) suggested a role for UV reflectance of eggs in the context of avian brood parasitism, and, therefore, we used the reflectance spectrum including UV wavelengths (300–400 nm). However, neither the duration of the nestling period nor mating system explained a significant amount of variation in UV color intensity (i.e., PC3r).

We found a significant association between nest site and egg coloration. Eggs of hole-nesting species are brighter, more blue, and reflect more at UV wavelengths than those of nonhole nesting species. Because hole-nesting species experience a lower probability of predation (Martin 1995; Bennett and Owens 2002), this result suggests a role of predation risk in the evolution of avian egg coloration. Although we did not find a significant relationship between probability of predation and egg coloration (see Results), sample sizes for these analyses were small. Thus, it is at least possible that the darker, less colorful eggs of nonhole nesting species allows a higher degree of crypsis with the environment (see Underwood and Sealy 2002). Since risk of predation may constrain the exaggeration of sexually selected characters in general (see examples in Andersson 1994), and egg coloration in particular (e.g., Solis and de Lope 1995), hole-nesting species should particularly show an exaggerated intensity of egg coloration. In agreement with this scenario, hole nesters tended to lay eggs with a greater intensity of blue-green but also UV coloration.

An alternative and nonexclusive explanation is related to egg coloration being favored because of light properties in holes. Brighter colors may for example allow proper detection of position of eggs in the nest, permitting proper management of eggs when incubating individuals change position. It is known that some colors are more easily detected in dark environments such as holes. Accordingly, it has been suggested that yellow but not other colors of nestling gapes are more easily detectable by parents of hole-nesting species (Heeb et al. 2003; Hunt et al. 2003). Moreover, UV color has recently been demonstrated to be important for nestlings in hole nests in the context of parental decisions about food allocation (Jourdie et al. 2004), which could explain the higher intensity of UV chroma (i.e., PC3r) of eggs of hole nesters. However, this hypothesis cannot explain the more intense blue-green egg coloration of hole nesters (Underwood and Sealy 2002), which could be accounted for by the exaggeration of blue-green coloration (sexually selected signals) being less constrained in species that nest in holes (see above).

Finally, a second alternative but nonexclusive explanation for differences in blue color intensity of eggs of hole and nonhole nesters is that antioxidants are more important for hole nesters than for open nesters. It is known that holenesters suffer from more and/or more virulent parasites, resulting in the evolution of a stronger immune system in hole-nesting birds (Møller and Erritzøe 1996). Activation of the immune system results in the production of free radicals due to cell proliferation, and such free radicals must be neutralized to prevent damage to DNA and other molecules (Ames 1983; Krinsky 1989, 1998; Chew 1996; Edge et al. 1997; Møller et al. 2000). This scenario implies that antioxidants such as biliverdin should be more important for hole nesters than for open nesters, providing a particularly strong selection pressure for reliable signaling of antioxidant properties by female hole nesters.

Surprisingly, we found that eggs of species with large body mass were darker and bluer than those of smaller species. Although it is difficult to interpret this result, a possible explanation is that, for species of large body size, paternal contribution to feeding the offspring is relatively more important than for species with smaller body size. Thus, according to the hypothesis of blue-green coloration being a sexually selected signal of females (Moreno and Osorno 2003), exaggeration of such signal should be greater in species of large body mass. A second possibility is that metabolic rate has negative allometry with an exponent less than one. This means that large species have relatively fewer free radicals, leaving more antioxidants for coloring the eggs. Another possibility is related to the relationship between egg size and signal detection by males. If, for instance, males were able to more easily detect small differences in color with eggs with large volume, sexual selection may differentially facilitate the exaggeration of the signal in such species. Another nonfunctional possibility is that species of small body mass produced relatively large amounts of protoporphyrin, which is natural metabolite and is responsible for yellow-red egg colors (Moreno and Osorno 2003). Because our second PCA is related not only to blue, but also to yellow-red coloration (with opposite sign), differential production of protoporphyrin by smaller species would explain our finding. All the possibilities explaining the detected relationship between egg coloration and body mass are merely speculations, although they may encourage further research to understand why body size and eggs color are significantly correlated.

Taking into account all the results presented here, we conclude that a significant proportion of variance in egg coloration of European passerines is explained by two different life-history variables, mating system and duration of nestling care. This is the first comparative evidence consistent with the hypothesis that blue-green coloration of eggs in passerines is a postmating sexually selected signal (Moreno and Osorno 2003). However, because we used an indirect measure of potential intraspecific variation in paternal care, experimental manipulations of egg coloration in different species varying in life-history traits are needed before accepting the

generality of the hypothesis explaining variation in blue-green coloration of avian eggs. Moreover, the results presented here are correlational and other alternative explanations, including nonadaptive ones, may also explain blue-green egg coloration. However, explanations other than that of signaling (Moreno and Osorno 2003), including the possibility that pigment in eggshells can be used directly by embryos, have antibacterial properties, or are a by-product of metabolism reflecting female stress during the laying period, require clear predictions with respect to the association between egg coloration and life-history traits before they can be tested properly.

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