

Attractive blue-green egg coloration and cuckoo–host coevolution

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Recent evidence suggests that blue-green coloration of bird eggshells may be related to female and/or egg phenotypic quality, and that such colour may affect parental effort and therefore the nutritional environment of developing nestlings. Here we suggest that these relationships and the signal function of eggshell coloration would affect the outcome of coevolution between avian brood parasites and their hosts in at least three different non-exclusive evolutionary pathways. First, by laying blue-green coloured eggs, cuckoo females may exploit possible sensory biases of their hosts, constraining the evolution of parasitic egg recognition, and thus avoid rejection. Second, because of the relatively high costs of laying blue eggs, cuckoo females may be limited in their ability to mimic costly blue-green eggs of their hosts because cuckoo females lay many more eggs than their hosts. Furthermore, costs associated with foreign egg recognition errors would be relatively higher for hosts laying blue eggs. Third, cuckoos may use coloration of host eggs for selecting individuals or specific hosts of appropriate phenotypic quality (i.e. parental abilities). We here explored some predictions emerging from the above scenarios and found partial support for two of them by studying egg coloration of European cuckoos (*Cuculus canorus*) and that of their 25 main hosts, as well as parasitism and rejection rate of hosts. Cuckoo hosts parasitized with more blue, green, and ultraviolet cuckoo eggs, or those laying more blue-green eggs, were more prone to accept experimental parasitism with artificial cuckoo eggs. In addition, coloration of cuckoo eggs is more variable when parasitizing hosts laying bluer-greener eggs, even after controlling for the effect of host egg coloration (i.e. degree of egg matching). Globally, our results are consistent with the proposed hypothesis that host egg traits that are related to phenotypic quality of hosts, such as egg coloration, may have important implications for the coevolutionary interaction between hosts and brood parasites. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **106**, 154–168.

ADDITIONAL KEYWORDS: blue-green egg colour – cuckoo parasitism – host egg discrimination – pigment limitation – sensory biases – sexual signals.

INTRODUCTION

Avian brood parasites exploit the parental care of other species of birds (hosts) by laying their eggs in nests of hosts that incubate and raise parasite offspring. Avian brood parasitism reduces breeding success of parasitized hosts and, therefore, selects for

defensive mechanisms such as parasite egg recognition and rejection. This in turn selects for counter-defences in brood parasites, such as laying eggs that mimic those of their hosts, giving rise to coevolutionary arms races (e.g. Davies, 2000). The European cuckoo, *Cuculus canorus*, and its coevolutionary relationship with some of its hosts constitutes one of the best examples of parasitic eggs mimicking those of their hosts as a consequence of selection created by

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hosts rejecting eggs that do not match the appearance of their own eggs (Brooke & Davies, 1988). Matching of host eggs by cuckoos even occurs in species that parasitize multiple host species because different individual cuckoos specialize in parasitizing a single host species for which their eggs are mimetic, and egg colour patterns are apparently inherited from parents to offspring leading to the evolution of genetically differentiated 'gentes' (host races) within the same parasite species (see Gibbs, Brooke & Davies, 1996; Gibbs *et al.*, 2000; Fossøy *et al.*, 2010).

Extensive surveys on more than 100 bird species have revealed the simultaneous presence of two main pigments in the eggshell of birds, namely biliverdins and protoporphyrines that are responsible for blue-green and brown-red egg colorations, respectively (Kennedy & Vevers, 1976; Miksik, Holan & Deyl, 1996). Empirical data have shown that not only derived (Miksik *et al.*, 1996), but also basal avian lineages including their extinct close relatives used the same pigments to colour their eggs (Igic *et al.*, 2010). Thus, it is likely that cuckoos share with their hosts the chemical basis to colour their eggs and therefore have the potential to evolve eggs that mimic those of their hosts (Igic *et al.*, 2011). Biliverdins possess strong antioxidant activity (McDonagh, 2001; Kaur *et al.*, 2003), which was the basic argument supporting the hypothesis of blue-green egg coloration functioning as a postmating sexually selected signal of female birds (Moreno & Osorno, 2003). This hypothesis first assumes that biliverdin-based egg colours reveal the physiological condition of females during breeding (see review in Reynolds, Martin & Cassey, 2009), and, secondly, suggests that the use of colour intensity to differentially allocate parental effort would be beneficial for fathers (Moreno & Osorno, 2003). Whereas the use of eggshell coloration for adjusting paternal breeding effort has received mixed support (see below), there is widespread support for a relationship between eggshell colour and physiological condition (Riehl, 2011). Indeed, it has been shown that blue-green egg-colour intensity is related to body condition, immunocompetence, and oxidative status of females at breeding onset (Moreno *et al.*, 2005; Siefferman, Navara & Hill, 2006; Krist & Grim, 2007; Hanley, Heiber & Dearborn, 2008; Morales, Velando & Moreno, 2008; Soler *et al.*, 2008). In addition, it has been shown that blue-green coloration of eggs is heritable (Morales *et al.*, 2010) and that it may also signal the physiological condition of eggs and developing embryos as shown by positive correlations between blue-green colour intensity of eggshells and immunoglobulin (Morales, Sanz & Moreno, 2006), testosterone (Lopez-Rull, Miksik & Gil, 2008), and antioxidant concentrations in the eggs (Hargitai *et al.*, 2008, 2010; Navarro *et al.*, 2011; but

see Cassey *et al.*, 2008a). Interestingly, these associations were also detected for European cuckoo eggs in nests of the great reed warbler, *Acrocephalus arundinaceus* (Hargitai *et al.*, 2010).

Laying blue-green eggs would therefore be relatively costly for both hosts and parasites, and we here hypothesize that costs of producing biliverdin-pigmented eggs may affect the coevolutionary relationships between hosts and parasites. Indeed, there is correlative evidence suggesting limitation of pigments to colour eggs (Underwood & Sealy, 2002) including those responsible for blue-green coloration (Moreno *et al.*, 2005). Moreover, it is known that experimentally handicapped females (with some primary feathers clipped) of spotless starlings (*Sturnus unicolor*) laid paler blue eggs than control females, which demonstrated the existence of such costs (Soler *et al.*, 2008). In addition, food-supplemented female pied flycatchers laid bluer eggs than controls (Moreno *et al.*, 2006). Thus, on the one hand, laying exaggerated blue-green coloured eggs is costly and might reduce clutch size or the ability to mimic blue-green host eggs, mainly for brood parasites that lay many more eggs than their hosts (Payne, 1973, 1974, 1977). On the other hand, costs incurred by hosts because of breakage or erroneous rejection of their own eggs when rejecting and detecting parasitic eggs would be relatively higher for hosts laying more intensely blue-green than for hosts laying paler eggs. These two hypothetical scenarios would then affect the evolution of egg recognition by hosts and, consequently, the degree of mimicry of parasitic eggs.

The sexual selection hypothesis driving the evolution of blue-green coloration of avian eggs posits that colour intensity predicts the nutritional environment that offspring will experience in the nests (Moreno & Osorno, 2003). In this scenario, at the intraspecific level cuckoos may select good quality nutritional environments for their offspring by selecting host nests with intense blue-green coloration. The hypothetical relationship between blue-green egg colour intensity and variables related to nutritional environments experienced by offspring has received mixed support. A relationship between egg coloration and paternal investment (Moreno *et al.*, 2004; Hanley *et al.*, 2008; Soler *et al.*, 2008; English & Montgomerie, 2011) or nutritional condition of nestlings (Soler *et al.*, 2008) has been detected under natural conditions in some study systems, although it is not always the case (Krist & Grim, 2007; Honza *et al.*, 2011). At the inter-specific level, we have previously found a positive relationship between blue-green colour intensity and duration of the nestling period, which was interpreted as evidence of egg coloration predicting parental investment (Soler *et al.*, 2005). Moreover, there exist intraspecific correlative data suggesting nonrandom

cuckoo parasitism in relation to host egg coloration (Avilés *et al.*, 2006; Cherry, Bennett & Moskat, 2007) and phenotypic quality of female hosts (Polacikova *et al.*, 2011). If that was the case, brood parasites could use host sexual signals (i.e. blue-green coloration) revealing parental abilities for host selection. The use of sexual signals by avian brood parasites has been described in several systems (Soler *et al.*, 1995; Clotfelter, 1998; Moskat & Honza, 2000; Garamszegi & Avilés, 2005; Parejo & Avilés, 2007). This preferential selection might constrain the exaggeration of eavesdropped sexual signals (i.e. intensity of blue-green eggshell colour) such as has been suggested for sexually selected nest traits of magpies (*Pica pica*) selected by great spotted cuckoos (*Clamator glandarius*) (Soler *et al.*, 1999).

Cuckoos may also exploit sensory biases in hosts (see Alvarez, 1999) by exaggerating the amount of biliverdin-related pigments in the eggshell if hosts showed an innate preference for biliverdin-based colorations. At the intraspecific level, we know that a single experimental egg of deep-blue coloration acts as a super-normal stimulus for pied flycatchers, *Ficedula hypoleuca*, resulting in improved physical condition of nestlings (Moreno *et al.*, 2008). If parasitic blue-green eggs were attractive for hosts, this may reduce the probability of egg rejection at the intraspecific level. At the interspecific level, blue-green parasitic eggs could affect the evolution of egg rejection, if hosts differ in sensory bias toward blue-green eggs, and/or if the degree of blue-green colour intensity of cuckoo eggs differs when parasitizing different host species. Therefore, the use of sexually selected signals by cuckoos, either for host selection or as a super-normal stimulus would affect the evolution of host defences (i.e. cuckoo egg recognition and rejection) and, therefore, the coevolutionary process between brood parasites and their hosts (Dawkins & Krebs, 1979).

Here, by studying egg coloration of the European cuckoo and its hosts, as well as parasitism rate and foreign-egg rejection rates of potential hosts, we develop and test interspecific predictions of the above non-exclusive scenarios suggesting new hypotheses whereby eggshell coloration of cuckoos and hosts would influence the coevolutionary process. (1) If parasitic blue-green coloured cuckoo eggs are attractive to hosts, blue-green colour intensity of cuckoo eggs should be negatively related to level of host defences (i.e. parasitic egg recognition) after controlling for intensity of selection owing to parasitism. Otherwise, hosts laying costly blue eggs may be those suffering larger costs associated with foreign egg recognition, with lower rejection rates allowing effective cuckoo parasitism through eggs that do not resemble those of the hosts. In addition (2) if cuckoos eavesdrop

on sexual signals of their hosts (i.e. intensity of blue-green eggshell colour) reflecting parental investment (Soler *et al.*, 2005), at the interspecific level, cuckoos should prefer potential host species with such signals. Finally (3) cuckoos may exploit possible sensory biases of some hosts toward blue-green eggs (e.g. at the cost of for instance reducing clutch size) and lay bluer-greener eggs than their hosts, or lay nonmimetic (i.e. pale) eggs if recognition would rarely evolve in hosts laying blue-green eggs (see above). These two possibilities predict that colour variability of cuckoo eggs would increase as intensity of blue-green coloration of host eggs increases.

MATERIAL AND METHODS

DATA SET FROM THE LITERATURE

We gathered exhaustive information on egg-colour and brood parasitism of 25 different European passerines commonly parasitized by the European cuckoo (Appendix 1). We did not use any selection criteria, but simply used all species for which information on brood parasitism related variables were available in the literature described below and for which clutches for measuring coloration were available in the visited museums. Species-specific information on degree of recognition of cuckoo parasitism and on level of parasitism relied on rates of rejection of nonmimetic model cuckoo eggs (i.e. model eggs that looked markedly different from the host eggs to the human eye) reported by Moksnes *et al.* (1991) and Davies & Brooke (1989), and percentage of cuckoo parasitized nests reported in Soler (1999) for European passerines. Previous work has shown that rejection rate is a highly repeatable defence of host species across different populations (Soler & Møller, 1996; Garamszegi & Avilés, 2005), and thus that level of recognition of cuckoo eggs can be considered as a species-specific attribute in comparative analyses. Information on cuckoo parasitism for more than a single host population is absent for most European passerines, which precluded a sound analysis of reliability for this trait (Soler, 1999). This information is however available for many North American passerines, and Garamszegi & Avilés (2005) found consistently greater variance amongst than within species in parasitism rate by the brown headed cowbird, *Molothrus ater*. Thus, it is also likely that cuckoo parasitism suffered by different host species was consistent in different host populations.

The suitability of species as cuckoo hosts is related to foreign egg rejection rates because suitable host species are more likely to have experienced a long-term relationship with the parasite (Brooke & Davies, 1988; Moksnes *et al.*, 1991). Thus, we divided host

species into two groups with respect to their suitability according to Moksnes & Røskaft (1995), who classified as unsuitable species those that build their nests in holes or concealed places, those that feed their young with food unsuitable for the cuckoo chick, or those having nests/eggs that are too big to permit successful ejection by the young cuckoo. Moreover, the sex responsible for rejecting cuckoo eggs varies amongst host species and apparently egg rejection by males occurs in species in which males incubate (e.g. Soler, Martín-Vivaldi & Pérez-Contreras, 2002). Therefore, information on the sex involved in incubation was simultaneously considered with the traits of interest in our comparative framework. We classified species using a two-point scale as 0 (strict female incubators) when no evidence of males incubating existed, and 1 (both female and male incubators) when evidence was reported for males incubating according to information in Cramp (1998). Information on nestling and incubation periods, nesting habits, and degree of polygyny were obtained from the appendices in Soler *et al.* (2005).

EGG COLOUR ESTIMATION FROM MUSEUM COLLECTIONS

Cuckoo and host egg coloration and degree of matching of host eggs by cuckoos were estimated by using a spectroradiometer on 494 clutches belonging to 25 hosts species parasitized by the European cuckoo and conserved at the Zoological Museums of Helsinki and Copenhagen (median = seven clutches per species, range = one to 259 clutches per species; Appendix 1). Each of the measured clutches included a single cuckoo egg. The results presented here come from information on the 25 species, but identical statistical significance was reached when analyses were restricted to species with more than three available clutches. Eggshell coloration may degrade with time (Starling *et al.*, 2006; Moreno, Lobato & Morales, 2011) and thus the use of eggshells conserved in museums may be problematic. However, this effect is most likely to influence components of between-clutch variation (within species), and it can be considered negligible when exploring interspecific variation (Cassey *et al.*, 2010) as is here the case.

Reflectance spectra in the range 300–700 nm were obtained from all clutches using a spectrophotometer (S2000, Ocean Optics) with a deuterium and a halogen light source (DH 2000, Ocean Optics Europe). Colour was measured in two randomly selected areas of the surface of the eggs, each of *c.* 1 mm². A fibre-optic probe provided illumination at a 45° angle and transferred reflected light to the spectrophotometer. Data from the spectrophotometer 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 each nm waveband from 300 to 700 nm.

Accuracy of spectrophotometric measures may be reduced when quantifying the background colour of a spotted egg because the diameter of the optic fibre sometimes exceeds the surface between two spots. However, repeatability analyses of different measures from the same and from different randomly selected areas allowed us to quantify colour variability within and amongst heavily spotted eggs of the great reed warbler *Acrocephalus arundinaceus* (144 eggs) and of 190 pure immaculate blue eggs of the redstart, *Phoenicurus phoenicurus* (see Avilés & Møller, 2004). Measurements of the same randomly selected area of a single egg resulted in highly repeatable reflectance at the ultraviolet (300–400 nm), blue (400–475 nm), green (475–550 nm), yellow (550–625 nm), and red (625–700 nm) wavebands (Pearson correlation coefficients, $R \geq 0.94$, $P \leq 0.0001$ for immaculate eggs, and $R \geq 0.79$, $P \leq 0.0001$ for spotted eggs). When comparing measurements in two randomly selected areas of the same egg, we found a moderate repeatability in reflectance at the five colour regions as shown by the highly significant correlation between the two measurements ($R \geq 0.46$, $P \leq 0.0001$ for immaculate eggs, and $R \geq 0.54$, $P \leq 0.0001$ for spotted eggs). Therefore, we used mean values for each egg. In addition, amongst-clutch variation was larger than within-clutch variation. Thus, a mean host spectrum for each clutch was calculated. By using these mean values we do not consider colour pattern (e.g. spot size, etc.), but a general value of egg coloration that is related to pigment concentration in the eggshell. The following analyses were therefore based on 494 pairs of spectra (one mean host and cuckoo spectra for each clutch).

Before proceeding with comparative analyses, we tested whether mean coloration values for host and cuckoo eggs saved at the museums and calculated on mean host and cuckoo clutch spectra could be treated as species-specific features. Analyses of the clutches from the 25 host species allowed us to quantify colour variability within and amongst host species, and within and amongst cuckoo eggs laid in the nests of different host species. We found a moderate repeatability of egg coloration for all five spectra intervals (i.e. colours) of host eggs ($R \geq 0.612$, $F_{24,469} \geq 23.97$, $P \leq 0.00001$), which is an important prerequisite for comparative analyses. Likewise, although repeatability was low ($R \geq 0.178$), variation in coloration of cuckoo eggs laid in different host nests was higher than variation amongst eggs found in a single host species ($F_{24,469} \geq 4.14$, $P \leq 0.0001$). Therefore, coloration

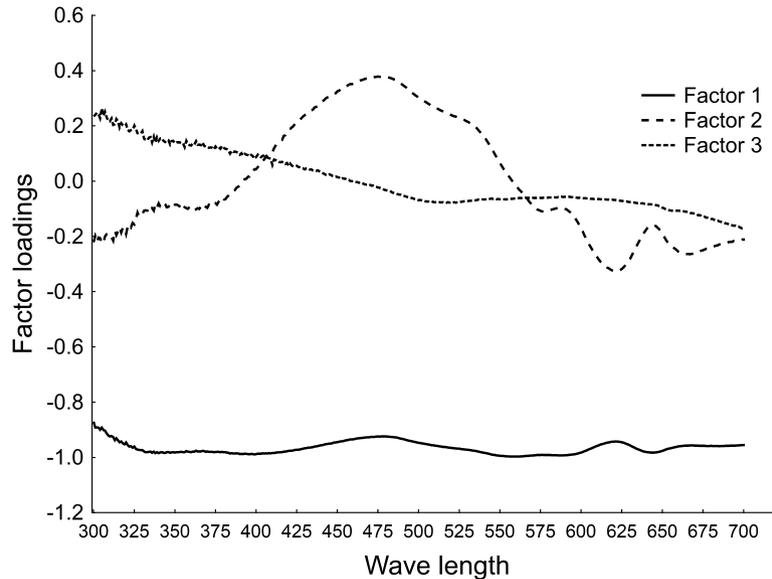


Figure 1. Factor loadings of the three first principal components from a principal components analysis as a function of wavelength, derived from reflectance spectra from European cuckoo and host eggs.

tion of host and cuckoo eggs laid in nests of different hosts can be considered species and cuckoo host race-specific attributes, respectively.

STATISTICAL AND COMPARATIVE ANALYSES

Reflectance spectra of natural colours are smooth and, therefore, the reflectance of adjacent wavelengths is typically highly autocorrelated (Endler, 1990). Thus, we performed principal component analyses (PCAs) on reflectance data to reduce the number of correlated variables into a few orthogonal variables summarizing colour variation (Cuthill *et al.*, 1999; Cherry & Bennett, 2001; Soler *et al.*, 2005; Avilés *et al.*, 2006). 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). Combined, these three first components explained 99.1% of the total variance of sampled eggs. PC1 had high and negative loadings across the spectra (Fig. 1) and hence, it described achromatic variation explaining 93.2% of the overall variation in coloration. PC2 had high and positive loadings at the blue and green regions and high negative ones at red wavelengths, and it explained 70.6% of the chromatic variance (i.e. 4.8% of total variance). PC3 had positive loadings at ultraviolet wavelengths and explained 16.1% of chromatic variance (i.e. 2.0% of total variance) (see Fig. 1).

As we found that host egg coloration explained variance in cuckoo egg coloration (see Results), variability in PCA scores describing coloration of cuckoo eggs found in different species of hosts was estimated

as the absolute residual value of the phylogenetically controlled regression between cuckoo and host PC scores.

However, values describing coloration of eggshells of different species cannot be considered statistically independent data observations because of common ancestry, and phylogenetic relationships between species should be taken into account in comparative analyses (Harvey & Pagel, 1991). We incorporated phylogenetic relationships (Appendix 2) based on Jönsson & Fjeldså (2006), and polytomies of basal nodes were solved following Sibley & Ahlquist (1990). Branch lengths were arbitrarily assigned to one, but constraining tips to be contemporaneous (Pagel, 1992).

To control for possible effects of a common ancestor we performed phylogenetic generalized least square regression (PGLS) analyses (Pagel, 1997, 1999) as implemented in the R statistical computing environment (R Development Core Team, 2010) using 'MASS' (Venables & Ripley, 2002), 'ape' (Paradis, Claude & Strimmer, 2004), and 'mvtnorm' (Genz & Bretz, 2011) libraries) with an additional unpublished function by R. Freckleton (University of Sheffield, pglm3.3.r available upon request). The PGLS model is the same as the usual least-squares regression model except for the structure of the error term that includes a variance-covariance matrix of the phylogenetic relationship amongst species that takes into consideration the expected lack of independence of the observations owing to the phylogenetic history of the species (Martins & Hansen, 1997; Pagel, 1997, 1999; Rohlf, 2001). We used the principle of maximum likelihood

to fit models and likelihood ratio tests for testing evolutionary hypotheses and also to estimate the importance of phylogenetic corrections in the models (λ) (Freckleton, Harvey & Pagel, 2002), which vary between 0 (phylogenetic independence) and 1 (species traits covary in direct proportion to their shared evolutionary history) (Pagel, 1997, 1999). We simultaneously estimated the parameters of regression models and the degree of phylogenetic dependence (λ), which was included within the residual error term.

Comparative analyses were corrected for heterogeneity in data quality because of the large variation in sample sizes (i.e. number of hosts with estimates of egg coloration) amongst species by using weights (Garamszegi & Møller, 2010). Briefly, following Garamszegi & Møller (2007), we combined variance factors owing to phylogenetic and weight effects as error terms in the form of a matrix using the $Q = V + cW$ equation, where V is the phylogeny matrix; W is the diagonal matrix of 1/weights; and c is a constant (Martins & Hansen, 1997). By varying the c -constant, we calculated the maximum likelihood of different combinations of the phylogeny and weight matrices. At the combination that resulted in the highest maximum likelihood, we determined the slope of the effect in focus. This additional PGLS exercise was also performed in the R statistical computing environment. However, λ and c cannot be simultaneously estimated in the same model, and we set the degree of phylogenetic dependence (λ) to the most appropriate degree evaluated for each unweighted model.

Before the analyses, rejection rates were arcsine square-root transformed whereas parasitism rates were transformed by the formula: $\log(100 \times (\text{arcsine square-root}(\text{rate of parasitism}) + 0.01))$. After these transformations, frequency distributions of both rejection and parasitism rates did not differ from normality (Kolmogorov–Smirnov test for continuous variables, $P > 0.2$). The frequency distributions of all variables quantifying eggshell coloration did not differ from normality (Kolmogorov–Smirnov test for continuous variables, $P > 0.15$). The tested set of predictions required holding constant the effect of a third variable when evaluating the degree of association between brood parasitic and egg colour variables. Therefore, we used partial correlation coefficients and type III decomposition of variance in our PGLS models. Some of the predictions tested assume that interspecific variation in blue-green colour intensity of host eggs is associated with variation in intensity of sexual selection (i.e. polygyny) and parental investment (duration of nestling period); trends that were found in a larger data set by Soler *et al.* (2005). These associations were reassessed with the subsample of potential host species of the European cuckoo used here.

RESULTS

HOST EGG COLORATION INDICATES INTERSPECIFIC VARIATION IN DEGREE OF POLYGyny AND PARENTAL INVESTMENT

Supporting the assumption that interspecific variation in blue-green colour intensity of host eggs is associated with variation in intensity of variables reflecting sexual selection and parental investment, we found that scores of this colour component (i.e. PC2) were positively related to degree of polygyny and duration of the nestling period (Table 1). This was the case after controlling for the significant positive association with body mass and nesting habits (i.e. larger values of blue-green colour intensity for hole nesters) (Table 1). However, these are correlations and other variables may contribute to these relationships.

HOST RESISTANCE AND COLORATION OF CUCKOO AND HOST EGGS

Rejection rates of artificial nonmimetic eggs were lower in potential host species parasitized with more blue-green (i.e. PC2 colour scores) (Fig. 2) and ultraviolet cuckoo eggs after accounting for parasitism rate, and the sex implicated in incubation (Table 2). Similar results were found when only suitable host species were considered in the analyses (Table 2). This negative association also appeared when considering host rather than cuckoo eggs in the statistical models. However, in this case the model explained half the variance explained by cuckoo eggs and the effect of PC2 colour scores of host eggs did not reach statistical significance when only suitable hosts were considered (Table 2).

CUCKOO PARASITISM AND HOST EGG COLORATION

None of the variables describing host egg coloration (PC1, PC2, PC3) were significantly associated with rate of cuckoo parasitism once we controlled for rejection rate and the sex implicated in incubation (Table 3). Similar results were found when only suitable host species were considered in the analyses (Table 3).

VARIATION IN CUCKOO EGG COLORATION IN RELATION TO HOST EGG COLORATION

As cuckoos may exploit sensory biases (by exaggerating the blue-green coloration of eggs) or enjoy possible low rejection rates of hosts laying blue eggs, we predicted that variation in cuckoo eggs should be greater when parasitizing hosts with blue-green eggs. When analysing the relationship between colour components of cuckoo and host eggs, brightness of cuckoo

Table 1. Relationship between variables defining host eggs coloration [principal component 1 (PC1), PC2, and PC3] and log body mass, mating system (monogamous = 0, monogamous-polygynous = 1, polygynous = 3; polygynandrous = 3), duration of nestling and incubation periods, and nest site (hole = 1, nonhole = 2). Analyses are phylogenetic generalized linear models weighted by sample size

Overall models	PC1			PC2			PC3		
	Beta (SE)	t_{20}	P	Beta (SE)	t_{20}	P	Beta (SE)	t_{20}	P
	$F_{5,19} = 2.55, P = 0.06, R^2_{\text{adj.}} = 0.24$			$F_{5,19} = 10.3, P = 0.001, R^2_{\text{adj.}} = 0.66$			$F_{5,19} = 0.60, P = 0.70, R^2_{\text{adj.}} = -0.09$		
Independent variables	Beta (SE)	t_{20}	P	Beta (SE)	t_{20}	P	Beta (SE)	t_{20}	P
Nest site	0.02 (0.73)	0.03	0.973	1.07 (0.46)	2.32	0.032	0.48 (0.70)	0.69	0.498
Body mass	0.92 (0.51)	1.82	0.085	1.19 (0.39)	3.09	0.006	-0.09 (0.72)	-0.12	0.905
Mating system	-0.01 (0.37)	-0.03	0.979	1.13 (0.22)	5.05	0.000	0.34 (0.32)	1.03	0.314
Incubation period	0.24 (0.34)	0.71	0.484	-0.45 (0.22)	-2.08	0.051	0.06 (0.30)	0.22	0.830
Nestling period	-0.53 (0.25)	-2.15	0.045	0.44 (0.17)	2.60	0.018	0.05 (0.24)	0.19	0.851

eggs was positively related to brightness of host eggs [PGLS; Beta (SE) = 0.23 (0.06), $t = 3.81$, $N = 25$, $P = 0.0001$; Fig. 3]. However, chromatic values of cuckoo and host eggs were not statistically significantly associated [PGLS; PC2: Beta (SE) = 0.24 (0.13), $t = 1.87$, $N = 25$, $P = 0.074$; PC3: Beta (SE) = 0.18 (0.09), $t = 1.88$, $N = 25$, $P = 0.07$] (Fig. 2). Thus, we used absolute residuals of those relationships as indicating variance in cuckoo egg coloration that was not explained by colour of host eggs.

As predicted, variation in cuckoo eggs along a blue-red axis increased as the intensity of blue-green coloration of host eggs increased [Fig. 2; PGLS; all species: Beta (SE) = 0.18 (0.06), $t = 2.92$, $N = 25$, $P = 0.008$; suitable species: Beta (SE) = 0.17 (0.07), $t = 2.35$, $N = 20$, $P = 0.03$]. This association did not appear for other colour components [PGLS, brightness: all species, Beta (SE) = -0.04 (0.04), $t = 1.04$, $N = 25$, $P = 0.31$; suitable species: Beta (SE) = -0.002 (0.04), $t = 0.06$, $N = 20$, $P = 0.95$; UV: all species, Beta (SE) = 0.03 (0.07), $t = 0.49$, $N = 25$, $P = 0.63$, suitable species Beta (SE) = -0.04 (0.07), $t = 0.52$, $N = 20$, $P = 0.61$].

DISCUSSION

We found that (1) host species parasitized with bluer-greener cuckoo eggs were more prone to accept experimental parasitism with nonmimetic model eggs (Fig. 2); and that (2) variation in cuckoo egg coloration was more pronounced when parasitizing hosts that laid blue-green eggs; however (3) host egg coloration did not predict the frequency of parasitism by the European cuckoo. In addition, for the pool of host species considered, previously detected associations between interspecific variation in blue-green colour intensity of eggs and amongst-species variation in mating system and duration of nestling period were corroborated. These comparative results provide support for two of the three proposed scenarios, suggesting a role of blue-green coloration of host and cuckoo eggs in the coevolutionary interaction between brood parasites and their hosts. Below we discuss these results and their possible implications for the coevolution of hosts and parasites using egg signals that are attractive for hosts.

The interspecific relationship between cuckoo egg coloration and probability of detecting and rejecting nonmimetic model eggs was only observed for the chromatic component of coloration, and this appeared to be independent of possible confounding factors such as level of parasitism and the role of sexes in incubation. Recognition and rejection of parasitic eggs is the most common and effective host defence against interspecific brood parasitism in birds (Rothstein, 1990), probably reflecting intensity of selection from

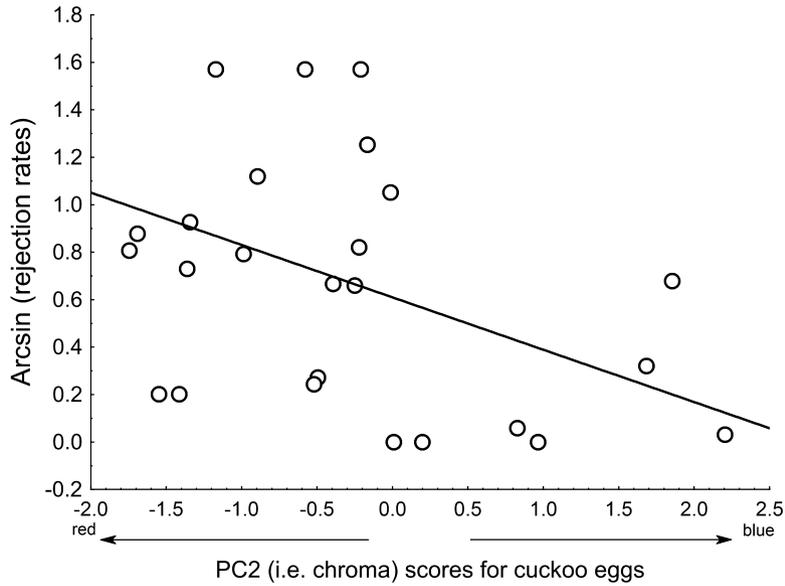


Figure 2. Relationships between rejection rate of artificial nonmimetic cuckoo eggs and coloration [i.e. principal component 2 (PC2) scores] of cuckoo eggs. The slope in the figure was estimated by means of phylogenetic generalized linear models.

Table 2. Rejection rate of artificial cuckoo eggs in relation to egg coloration of cuckoos (C) and hosts (H) as estimated by the scores of a principal component analysis on spectrophotometric values (PC1, PC2, and PC3), the sex implicated in incubation and level of parasitism. Analyses are phylogenetic generalized linear models

Overall models	All potential hosts ($N = 25$)			Suitable host species ($N = 20$)		
	$F_{5,19} = 4.16, P = 0.01, R^2_{adj} = 0.40$			$F_{5,14} = 5.46, P = 0.005, R^2_{adj} = 0.54$		
Independent variables	Beta (SE)	t_{20}	P	Beta (SE)	t_{15}	P
Parasitism	-0.14 (0.04)	3.33	0.004	-0.17 (0.04)	4.18	0.001
PC1 C	0.32 (0.25)	1.28	0.216	0.43 (0.25)	1.76	0.101
PC2 C	-0.22 (0.08)	2.74	0.013	-0.18 (0.08)	2.30	0.037
PC3 C	-0.34 (0.15)	2.26	0.036	-0.45 (0.15)	3.06	0.009
Incubating sex	0.25 (0.18)	1.39	0.180	0.27 (0.17)	1.60	0.132
Overall models	$F_{5,19} = 2.17, P = 0.10, R^2_{adj} = 0.20$			$F_{5,14} = 1.79, P = 0.18, R^2_{adj} = 0.17$		
Parasitism	-0.04 (0.05)	0.87	0.400	-0.05 (0.05)	0.97	0.351
PC1 H	-0.12 (0.11)	1.14	0.270	-0.14 (0.12)	1.22	0.242
PC2 H	-0.18 (0.07)	2.47	0.023	-0.16 (0.08)	2.01	0.064
PC3 H	0.12 (0.10)	1.13	0.270	0.10 (0.11)	0.85	0.410
Incubating sex	0.25 (0.20)	1.26	0.224	0.26 (0.21)	1.23	0.238

brood parasitism (Davies & Brooke, 1989). Here we used rejection rates of experimental nonmimetic eggs as a proxy for egg recognition and rejection ability of potential hosts. Thus, our results are in agreement with our expectation that for a similar intensity of selection owing to parasitism, the evolution of recognition and rejection ability is relatively more delayed in hosts that are parasitized with ‘attractive’ eggs than in other hosts.

The existence of sensory biases towards blue-green egg coloration by hosts at the intraspecific level would predict that relatively more blue-green model cuckoo eggs were accepted more frequently than less blue-green cuckoo models. Available information from such experiments in the literature, however, does not unequivocally support this prediction. Davies & Brooke (1989) experimentally parasitized the nests of 22 host species of the European cuckoo with four

Table 3. Level of cuckoo parasitism in relation to host egg coloration as estimated by the scores of a principal component analysis on spectrophotometric values (PC1r, PC2r, and PC3r), the sex implicated in incubation and rejection rate of artificial cuckoo eggs. Analyses are phylogenetic generalized linear models

Overall models	All potential host species ($N = 25$)			Suitable host species ($N = 20$)		
	$F_{5,19} = 3.39, P = 0.023, R^2_{\text{adj}} = 0.33$			$F_{5,14} = 2.97, P = 0.049, R^2_{\text{adj}} = 0.34$		
Independent variables	Beta (SE)	t_{20}	P	Beta (SE)	t_{15}	P
Rejection	-1.32 (0.66)	-1.99	0.061	-1.58 (0.72)	2.21	0.045
PC1	0.48 (0.27)	1.77	0.092	0.43 (0.29)	1.50	0.156
PC2	-0.28 (0.33)	-0.84	0.412	0.12 (0.40)	0.29	0.777
PC3	0.49 (0.27)	1.80	0.088	0.27 (0.33)	0.82	0.424
Incubating sex	1.26 (0.79)	1.60	0.125	1.34 (0.78)	1.73	0.106

different model cuckoo eggs in Britain. Aside testing rejection with model eggs resembling in appearance the host ones (i.e. mimetic models), each host species in that study except the wren (*Troglodytes troglodytes*) was tested against at least two nonmimetic models. Models painted with acrylic paints matched the colour of four gentes of cuckoo females, namely pied wagtail (*Motacilla alba*), meadow pipit (*Anthus pratensis*), reed warbler (*Acrocephalus scirpaceus*), and redstart (*Phoenicurus phoenicurus*). The background coloration of the four cuckoo models ranged from immaculate pale blue eggs in the redstart to the brownish-grey eggs of the meadow pipit type (Davies & Brooke, 1989), therefore providing for those host species parasitized with blue model eggs and laying nonblue eggs ($N = 15$ host species) a test of the value of blue egg coloration in recognition of nonmimetic eggs. They failed to find biases toward acceptance of blue models amongst the tested host species (table 3 in Davies & Brooke, 1989). Similarly, Peer, Robinson & Herkert (2000) performed egg recognition experiments in nests of seven hosts of brown-headed cowbird with mimetic eggs (white with brown spots) and nonmimetic blue eggs. One host did not reject any eggs, but five hosts rejected the nonmimetic blue eggs more often than the mimetic white eggs. It is apparent in these studies that it is the degree of similarity that affects host rejection. More recently, however, in a population of song thrushes, *Turdus philomelos*, Honza, Polacikova & Prochazka (2007) performed egg recognition experiments with a battery of mimetic and nonmimetic model eggs varying in blue-green colour intensity. They found that two of the mimetic model blue eggs were rejected at a high rate and that nonmimetic green eggs were more frequently accepted than mimetic eggs. However, these mimetic model eggs for human vision were highly contrasted against host eggs for avian visual perception (Cassey *et al.*, 2008b). These results taken together at least suggest that the importance of

blue-green colorations of brood parasitic eggs as a host supernormal stimulus varied amongst host species.

The above scenario suggests that cuckoos laying exaggerated blue-green parasitic eggs may gain advantages in terms of reducing host rejection of eggs. However, blue-green pigment used to colour eggs is presumably limited (Moreno *et al.*, 2005; Soler *et al.*, 2008), and is correlated with concentrations of antioxidants and other costly substances in the egg yolks (see Introduction). Thus, exaggeration of blue-green egg coloration will not be easy for cuckoos because they may lay more than 20 eggs per season (Payne, 1973, 1974). Cuckoos lay small eggs relative to their body mass and could in any case reduce clutch size and lay a smaller number of eggs, at least when exploiting hosts with a sensory bias towards blue-green eggs. Information for testing the association between clutch size and colour of cuckoo eggs is currently unavailable and, therefore, this possibility cannot be further explored. By contrast, hosts laying costly blue-green eggs would experience higher costs associated with recognition errors, which therefore may delay the evolution of foreign egg recognition. We found partial support for this scenario as blue-green colour intensity of host eggs predicted rejection rates of their foreign eggs when considering all potential hosts species, although this relationship disappeared when only considering suitable hosts.

The two scenarios listed above (exaggeration of blue-green coloration by cuckoos or the low rejection rates of hosts that lay blue eggs) suggest that cuckoos may exploit sensory biases of some hosts towards blue-green eggs, or may lay nonmimetic eggs as rejection rate is negatively related to intensity of blue-green coloration of host eggs. These two possibilities would mainly occur in host species laying blue-green eggs because a sensory bias toward exaggerated blue-green eggs is more likely, and recognition errors are

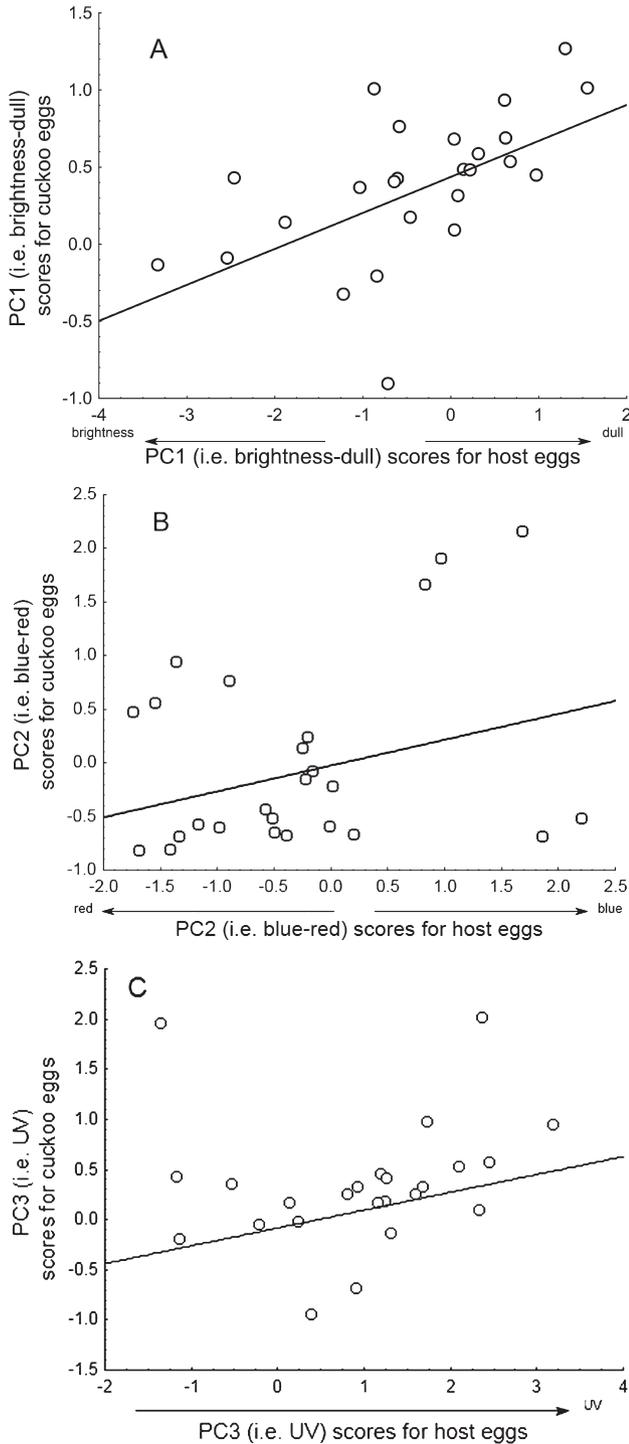


Figure 3. Relationships between (A) brightness [principal component 1 (PC1)] and (B) chroma [PC2: blue-red (B); PC3: UV (C)] of cuckoo and host eggs. The slope in the figure was estimated by means of phylogenetic generalized linear models.

relatively costlier for them. However, our data do not allow the relative importance of these two possibilities explaining colour variation in cuckoo eggs to be tested. However, together these possibilities predicted a positive association between colour variability of cuckoo eggs and intensity of blue-green coloration of host eggs (see Introduction). In accordance with this prediction we found that residuals of cuckoo egg coloration after controlling for coloration of host eggs were larger when parasitizing species that lay more blue-green eggs (Fig. 3). Cuckoos parasitizing the five host species with more blue-green eggs lay intensely coloured blue eggs in nests of *Oenanthe oenanthe*, *Ficedula hypoleuca*, and *Phoenicurus phoenicurus*, but not in nests of *Prunella modularis* (three cuckoo eggs in dunnock nests in Denmark were as blue as the eggs of the host, A. P. Møller, unpubl. data) or *Turdus philomelos*. When parasitizing the first three species, cuckoos may be exploiting a sensory bias of hosts toward intensely coloured blue-green eggs or simply mimicking host eggs. When parasitizing the other two host species, they could exploit the poor rejection rates of foreign eggs of hosts laying costly blue-green eggs, which may be the case for the dunnock and the song thrush (see rejection rates of nonmimetic model eggs in Appendix 2). In any case, mechanisms underlying the detected relationships should be tested interspecifically to reach firm conclusions. Independently of the mechanisms acting on different host-parasite systems, apart from the hypothesis of blue-green eggshell colour being a costly signal that may be attractive and/or costly for hosts (at least for some species), we are unaware of alternative hypotheses able to explain the detected trends between cuckoo egg variation (i.e. residual colour) and host eggs.

The last proposed evolutionary scenario is related to the possibility that cuckoos eavesdrop on host egg coloration for host selection (Parejo & Avilés, 2007). We however did not find evidence of such relationships in our data set. It is possible that, at the intraspecific level, cuckoos select individual hosts of good parental ability as shown by the blue-green colour intensity of their eggs and that, at the interspecific level, other host characteristics were more important in determining prevalence of brood parasitism.

To summarize, we proposed different non-exclusive evolutionary scenarios where attractiveness or costliness of blue-green pigmented eggs could potentially affect the coevolutionary relationships between brood parasites and their hosts. We found comparative evidence consistent with two out of three scenarios, but intraspecific experimental approaches are necessary to evaluate the importance of this hypothesis for different host species of brood parasites.

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APPENDIX 1

Potential cuckoo host species considered in the analyses with information on role of sexes in incubation [Incubating sex, only female incubates (F) and both female and male incubate (FM)], host suitability (HS; S, suitable; U, unsuitable), parasitism rate (PR, %), rejection rate (RR, %), nest site (Open, domed (Semi-) and Hole nests), mating system (polyg, polygynous), incubation and nestling periods, and values of principal component analysis (PCA) scores of host and cuckoo eggs. Achromatic (ΔQ) and chromatic (ΔS) visual contrasts between cuckoo and hosts eggs are also shown. Sample sizes (N) refer to number of clutches on which spectrophotometry analyses were based.

Species (N)	Incubating sex	HS	PR (%)	RR (%)	Nest site	Mating system	Incubation period (days)	Nestling period (days)	PCA 1	PCA 2	PCA 3	PCA 1	PCA 2	PCA 3	ΔS	ΔQ
<i>Acrocephalus palustris</i> (14)	FM	S	1.88	86.84	Open	Mono-polyg	12	12	-0.608	-0.013	1.672	0.427	-0.585	0.338	4.546	4.524
<i>Acrocephalus schoenobaenus</i> (13)	FM	S	0.33	20	Open	Mono-polyg	14	13	0.972	-1.414	1.722	0.449	-0.803	0.978	4.772	3.106
<i>Acrocephalus scirpaceus</i> (259)	FM	S	5.54	61.82	Open	Mono-polyg	12	12	0.676	-0.394	1.307	0.536	-0.675	-0.128	1.393	1.404
<i>Anthus pratensis</i> (14)	F	S	2.66	26.83	Open	Mono-polyg	14	14	1.552	-0.495	3.177	1.015	-0.642	0.955	2.002	5.528
<i>Carduelis cannabina</i> (1)	F	U	0.11	0	Open	Mono-polyg	13	12	-0.873	0.008	-1.367	1.009	-0.212	1.972	2.754	11.187
<i>Chloris chloris</i> (1)	F	U	0.05	24.14	Open	Mono-polyg	14	15	-0.716	-0.521	0.233	-0.904	-0.517	-0.012	0.188	0.872
<i>Emberiza citrinella</i> (5)	F	S	0.01	100	Open	Monogamous	13	13	0.314	-0.580	1.589	0.588	-0.426	0.268	2.480	0.773
<i>Emberiza schoeniclus</i> (2)	FM	S	0.15	95	Open	Mono-polyg	14	13	1.301	-0.166	2.365	1.270	-0.076	2.024	0.943	0.222
<i>Erethacus rubecula</i> (7)	F	S	0.29	20	Semi-	Mono-polyg	14	14	-0.643	-1.548	-1.170	0.407	0.566	0.440	5.090	6.168
<i>Ficedula hypoleuca</i> (9)	F	U	0	0	Hole	Mono-polyg	13	15	-1.036	0.965	-0.216	0.368	1.908	-0.034	3.445	6.868
<i>Fringilla coelebs</i> (6)	F	S	0.01	61.29	Open	Monogamous	13	14	0.039	-0.251	1.177	0.683	0.143	0.468	1.610	3.533
<i>Lanius collurio</i> (6)	F	S	0.01	71.2	Open	Monogamous	15	14	-1.222	-0.987	-1.144	-0.323	-0.595	-0.191	1.043	4.362
<i>Motacilla alba</i> (27)	F	S	0.42	73.17	Semi-	Monogamous	14	15	-0.586	-0.222	1.236	0.763	-0.152	0.194	3.467	6.868
<i>Motacilla flava</i> (23)	F	S	0.13	80	Open	Monogamous	13	13	0.612	-1.342	1.246	0.936	-0.685	0.418	1.887	1.731
<i>Muscicapa striata</i> (12)	FM	S	0.12	72.22	Semi-	Monogamous	13	14	0.145	-1.746	-0.538	0.486	0.479	0.364	6.688	2.428
<i>Oenanthe oenanthe</i> (3)	F	U	0	5.9	Hole	Mono-polyg	14	15	-1.884	0.827	0.893	0.142	1.664	-0.683	4.140	8.209
<i>Phoenicurus phoenicurus</i> (43)	F	S	0.03	31.5	Hole	Mono-polyg	14	15	-0.462	1.683	0.803	0.176	2.162	0.259	1.891	2.892
<i>Phylloscopus collybita</i> (3)	F	S	0	100	Semi-	Mono-polyg	13	14	-2.463	-0.211	2.324	0.431	0.244	0.110	4.871	11.749
<i>Phylloscopus trochilus</i> (6)	F	S	0.06	90	Semi-	Mono-polyg	13	14	-2.542	-0.895	0.739	-0.088	0.774	0.164	3.560	9.760
<i>Prunella modularis</i> (8)	F	S	1.93	3.13	Open	Polynandrous	12	12	0.219	2.204	2.094	0.483	-0.513	0.542	10.761	0.178
<i>Sylvia atricapilla</i> (10)	FM	S	0.17	76.92	Open	Monogamous	11	12	-0.841	-1.691	0.917	-0.206	-0.817	0.338	1.029	2.942
<i>Sylvia borin</i> (7)	FM	S	0.32	66.7	Open	Mono-polyg	12	10	0.042	-1.362	1.150	0.093	0.944	0.170	7.082	0.583
<i>Sylvia communis</i> (13)	FM	S	0.07	100	Open	Mono-polyg	12	12	0.081	-1.172	0.387	0.316	-0.572	-0.932	1.042	1.097
<i>Troglodytes troglodytes</i> (1)	F	S	0.04	0	Semi-	Mono-polyg	15	17	-3.332	0.198	2.438	-0.133	-0.666	0.587	5.765	11.019
<i>Turdus philomelos</i> (1)	F	U	0.01	62.74	Open	Monogamous	13	14	0.623	1.854	0.131	0.690	-0.680	0.169	9.970	0.296

APPENDIX 2

Phylogenetic relationships amongst passerine cuckoo-host species included in the analyses.

