

Environmental conditions influence egg color of reed warblers *Acrocephalus scirpaceus* and their parasite, the common cuckoo *Cuculus canorus*

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Abstract The outer layer of the eggshell in birds is in many cases covered by pigments that are assumed to be genetically determined traits with a negligible environmental component. To test the hypothesis that spring environmental conditions (i.e., temperature and rainfall) may affect bird egg pigmentation, we measured by spectrophotometry and photography egg coloration and spottiness on reed warbler (*Acrocephalus scirpaceus* L.) clutches parasitized by the common cuckoo (*Cuculus canorus* L.) collected over a period of 24 years and preserved in the Zoological Museum, Copenhagen, Denmark. In addition, we investigated whether spring environmental conditions may influence the coevolutionary relationship between the cuckoo and its host via changes in cuckoo–host egg matching. Generalized mixed models revealed that reed warbler eggs were more brilliant in those springs with a higher rainfall and tended to be bluer and greener in springs with a lower relative temperature. On the other hand, cuckoo eggs were bluer and greener in springs with a higher rainfall. Cuckoo–

host egg matching in blue-greenness and spottiness was better in springs with a higher rainfall. These results provide support for the existence of an environmental component on bird egg coloration and suggest that environmental factors may potentially affect the outcome of important features of the arms race between cuckoos and reed warblers.

Keywords Avian egg coloration · Cuckoo–host interaction · Environmental effect

Introduction

The outer layer of the eggshell in birds is in many cases covered by pigments. Shell pigmentation may have an adaptive value in terms of predation since it may increase crypsis when eggs are laid directly on the ground (e.g., Tinbergen et al. 1962; Solis and de Lope 1995). Yet the role of nest predation on egg coloration remains controversial for nesting cup passerines (e.g., Weidinger 2001; Underwood and Sealy 2002; Soler et al. 2005; Avilés et al. 2006a). Another functional explanation for variation in eggshell coloration is provided by brood parasitism studies. Cuckoo eggs showing a poor match with those of the host have a higher probability of being rejected, thus leading to evolution of host egg mimicry by cuckoos (e.g., Davies and Brooke 1989; Soler and Møller 1990; Davies 2000; Avilés et al. 2006b). Also, a low variation in egg color within a host clutch may increase the probability of detecting an odd parasite egg (e.g., Øien et al. 1995; Soler and Møller 1996; Stokke et al. 1999, 2002), although recent studies have revealed that it is not necessarily so that rejecter individuals have a lower intraclutch variation than acceptors (e.g., Karcza et al. 2003; Avilés et al. 2004; Honza et al. 2004; Stokke et al. 2004;

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Antonov et al. 2006). Alternatively, the inclusion of particular pigment patterns in the shell may help in identification of own eggs by parents at colonies with high density (Birkhead 1978). More recently, the proposed signaling hypothesis states that blue and green egg colors may function as a postmating sexually selected signal of female phenotypic quality to their mates in order to induce a higher allocation of parental care (Moreno and Osorno 2003; Soler et al. 2005). 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 proposed functions of eggshell pigmentation are outcomes of evolutionary processes and thus implicitly assume a genetic basis for bird egg coloration. Although evidence supports that eggshell pigmentation has a major genetic basis in birds (Punnett and Bailey 1920; Punnett 1933; Collias 1984; Joseph et al. 1999; Gosler et al. 2000), very few phenotypic traits are wholly dependent on genetic control, and environmental factors are likely to mediate their expression. Environmental factors may influence bird egg coloration by their direct effects on the physiological status of laying females, but also indirectly via their effects on food availability. Direct environmental effects on egg coloration have never been shown in natural populations, but rather in poultry studies in which hens were exposed to unnatural environmental conditions (e.g., Hughes et al. 1986; Joseph et al. 1999).

The first aim of this study was to investigate the influence of two environmental factors (temperature and rainfall) on coloration of reed warbler *Acrocephalus scirpaceus* and common cuckoo *Cuculus canorus* eggs. Both temperature and rainfall are likely to influence on food availability for insectivorous birds directly via their effects on development of insects and indirectly via their effects on vegetation (Jones et al. 2003). Hence, the link between food abundance and egg coloration may ultimately be driven by local temperature and rainfall patterns. The present study was carried out in a reed warbler population parasitized by the common cuckoo across 24 consecutive years. If eggshell coloration is related to female condition at laying and/or indirectly dependent on food availability, we predicted associations between environmental conditions and egg coloration across years for reed warblers and cuckoos. However, we have not a priori clear species specific predictions concerning environmental effects on egg coloration. Indeed, reed warblers and cuckoos greatly differ in their diets. Cuckoos mostly feed on butterfly caterpillars that are avoided by reed warblers (Wyllie 1981), while adult reed warblers feed on insects, spiders, and small snails captured mostly in reeds (Cramp 1998). Abundance of these prey types for cuckoos and reed warblers can be affected by climatological conditions (e.g., Polis et al. 1997; Sillett et al. 2000). Differences in diet between

cuckoos and reed warblers would predict species-specific susceptibility to temperature and rainfall if these environmental factors affect food availability. However, direct effects of environmental factors may also induce species-specific environmental effects on egg color if cuckoo and reed warbler females had different oxidative costs associated with the inclusion of blue–green related pigments in their eggs. This is likely because pigments for egg coloration cannot be used to prevent cellular oxidative stress processes (see Moreno and Osorno 2003) and there exists evidence suggesting a limitation of blue–green related pigment to color the eggs (Moreno et al. 2005). In addition, cuckoos lay many more eggs than nonparasitic birds (e.g., Payne 1973, 1974), and thus environmental factors could be particularly relevant for cuckoo females.

Regardless of the actual mechanism behind, any evidence of an association between environmental factors and reed warbler and/or cuckoo egg coloration would suggest that environmental factors may potentially influence the coevolutionary relationship between the cuckoo and its host via changes in cuckoo–host egg matching. The reed warbler is among the favorite hosts of the cuckoo in Europe (Moksnes and Røskaft 1995). Parasitism by the cuckoo is costly because the newly hatched cuckoo chick ejects all host eggs from the nest. To overcome this cost, reed warblers have evolved a main line of defense, namely egg rejection that favors the evolution of cuckoo eggs mimicking reed warbler eggs (Davies and Brooke 1988). Gosler et al. (2000) hypothesized that partial environmental control of eggshell pigmentation might be particularly beneficial for reed warblers since it may hamper the ability of the cuckoo to mimic their eggs. On the other hand, a major environmental influence on cuckoo egg coloration may lead to imperfect levels of cuckoo–host egg matching. The second aim of this study was to explore these possibilities and to determine whether environmental conditions may induce perceptible changes in the level of mimicry between cuckoo and host eggs.

Materials and methods

Data collection

Egg coloration was assessed on reed warbler clutches parasitized by the cuckoo preserved at the egg collection in the Zoological Museum in Copenhagen (Denmark). Museum egg collections provide researchers with a unique and extensive set of data allowing tests of evolutionary and ecological hypotheses related to egg coloration of birds (e.g., Brooke and Davies 1988; Davies and Brooke 1989; Moksnes and Røskaft 1995; Soler and Møller 1996; Cherry and Bennett 2001; Stokke et al. 2002; Avilés and Møller 2003, 2004; Soler

et al. 2005). Host and cuckoo egg coloration was assessed for a sample of 483 reed warbler clutches parasitized by the cuckoo collected over 24 consecutive breeding seasons between 1918 and 1941 on the island of Zealand, Denmark. There were a number of parasitized clutches from the study population that were not measured due to time limitation (whole number of clutches is about 800 clutches). However, we randomly selected a representative sample of clutches for every breeding season which assured that our sample was a representative subset of the collection. All clutches were collected mostly by three persons within an area with a radius of 40 km, in an area of more than 1,200 km². All clutches were collected in May or June [mean (\pm SD) collection date was May 27 (\pm 12 days) and the mean \pm SD number of clutches measured per year was 20.12 \pm 9.86] (range 7–42). The number of clutches collected per year was unrelated to spring environmental conditions in the study area (see below for sources, Pearson correlations: mean spring temperature $r=-0.06$, $N=24$, $P=0.77$; accumulated precipitation: $r=0.11$, $N=24$, $P=0.59$), suggesting that weather did not influence pattern of clutch collection across years. The mean (\pm SD) number of host eggs per parasitized clutch was 2.34 \pm 0.91, while the mean number of cuckoo eggs per clutch was 1.03 \pm 0.19.

Egg color and cuckoo egg matching measurements

Egg matching between host and cuckoo was estimated using spectrophotometry and human-based assessment of dissimilarity. The former technique allows objective assessment of egg coloration and accounts for ultraviolet information to which humans are blind (Cherry and Bennett 2001; Avilés and Møller 2003, 2004; Langmore et al. 2003; Avilés et al. 2004, 2006b,c). However, spectrometric techniques may have flaws when quantifying overall egg appearance of a spotted egg since it may be difficult to distinguish between different patterns of spottiness, which obviously could be important for birds distinguishing between own and foreign eggs. Therefore, we also quantified differences in pattern of spottiness between cuckoo and host eggs based on photos (Avilés et al. 2006b).

Reflectance spectra in the range 300–700 nm were obtained from all eggs of the 483 analyzed clutches using a spectroradiometer (Ocean Optics Europe) measuring at 0.37 nm intervals. A stratified random sample of spectra from all regions of the eggs was obtained by dividing each egg in five bands around the long axis of the egg (Cherry and Bennett 2001). Color was measured once within each of these five bands. The illuminant was a deuterium and a halogen light source (DH 2000). The light was transferred to the eggs with a fiber-optic probe that provides illumination at 45° angle from the light source and transfers reflected light to the spectrometer. Data from the spectrom-

eter were passed into a computer, where a software package (OOIBase 32) calculated reflectance spectra relative to a standard white reference (WS-2). Reflectance at 0.37 nm was transformed to 1.86 nm intervals by calculating mean value for the five adjacent measurements. The mean reflectance spectra for each egg in a clutch were calculated from the five spectra taken for each egg. This was justified because coloration of reed warblers and cuckoos was repeatable within eggs and among the different eggs of a clutch (see Avilés et al. 2006b), and mean host spectra for each clutch were calculated as there were a variable number of host eggs per clutch (see Cherry and Bennett 2001). Therefore, the following analyses were based on 483 pairs of spectra (one mean host and one mean cuckoo series of spectra per clutch).

To account for matching in spottiness between cuckoo and host eggs in a clutch, clutches were also photographed in their boxes together with a color reference of the type “Polaroid High Definition DFF1/HDF1 Reference Print”. A Canon Eos 50 with film type Fujicolor 100 ISO was used. Matching in the pattern of spottiness was later assessed on a 1–5 scale (1=perfect matching, 5=no matching) by three experienced persons unaware of the hypothesis being checked, and using a protocol defined for common cuckoo eggs (Moksnes and Røskaft 1995). When more than a single cuckoo egg was found in a clutch, which occurred in 15 out of the 483 clutches, one of the parasitic eggs was randomly chosen for the analysis. The mean value of the scores attained by these persons was used in the subsequent analyses, which is justified by a high consistency among the various assessments (Kendall Tau correlation: $\tau=0.50$, $Z=5.41$, $P<0.0001$, $N=56$).

Weather variables

Local weather variables used to identify links between environmental conditions and egg coloration in birds were mean monthly temperature and the accumulated precipitation from 1 May to 30 June. We arbitrarily selected this period because it was the period when all sampled clutches were laid. Temperature and rainfall data were collected by the Meteorological Institute of Copenhagen within the study area at Stege permanent meteorological station (55°12' N, 12°18' E). From these data, two indices of spring weather were calculated (see Dunn and Winkler 1999; Torti and Dunn 2005) and their relationship with egg coloration investigated: the mean spring temperature calculated on mean monthly temperatures from May and June, and the accumulated rainfall in the same period. We have chosen this period for calculating the climatic indexes because we did not know whether egg color was related to female condition at laying and/or indirectly dependent on food availability, which can be affected by longer time

periods. We assume that our measures of spring climatic conditions truly reflect the strength at which climate affects egg coloration in this system. Indeed, “spring rainfall” was significantly and positively correlated with accumulated rainfall in May (Pearson correlation, $r=0.6$, $P=0.003$, $N=24$ years) and June (Pearson correlation, $r=0.45$, $P=0.03$, $N=24$ years). Also “spring temperature” was significantly and positively correlated with mean temperature in May (Pearson correlation, $r=0.75$, $P=0.001$, $n=24$ years) and June (Pearson correlation, $r=0.78$, $P=0.00001$, $N=24$ years). Therefore, any further noise in the data should be random for the questions at hand, and should not confound the pattern we have reported in this study.

Confounding factors

A number of factors may potentially mediate the focused association between egg coloration and weather variables. For instance, the duration of sympatry between reed warblers and cuckoos is likely to determine the degree of matching between parasite and host eggs (e.g., Davies 2000; Avilés et al. 2006b). In addition, it could be argued that 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. Therefore, the coevolutionary process between cuckoos and reed warblers and egg fading may potentially affect the relationship between egg colors with weather variables if duration of sympatry and/or time since clutches were collected was related to weather

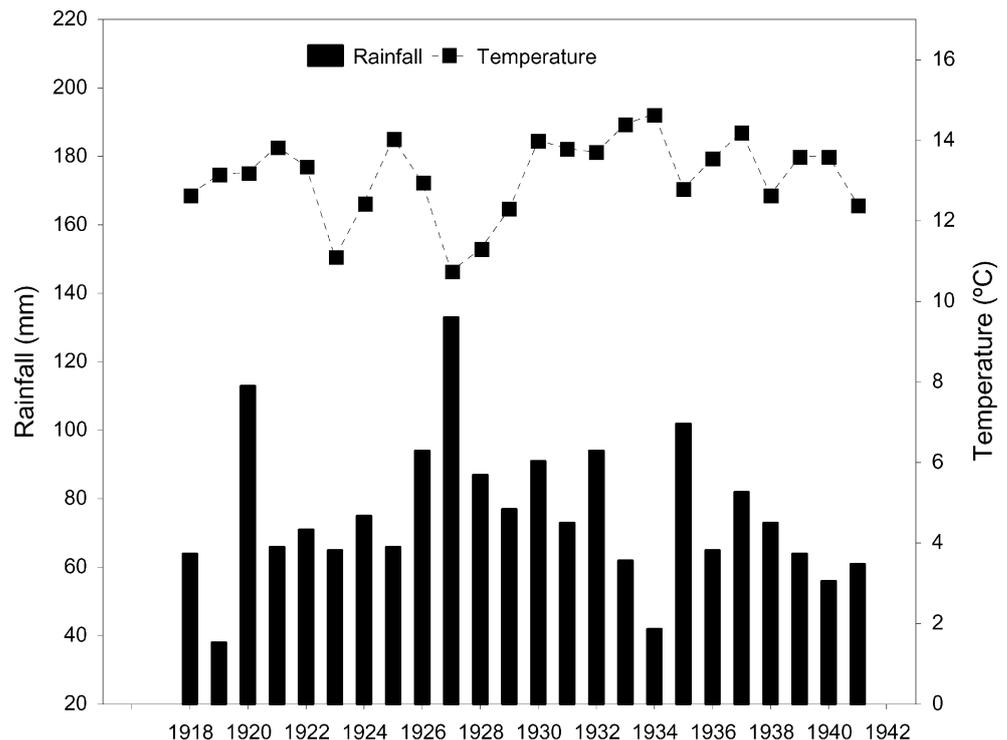
conditions. To control these potential sources of biases, information on year of collection as an estimator of duration of sympatry and/or time since clutch collection was simultaneously considered with the traits of interest in our analyses. Year of collection is a reliable predictor of the duration of sympatry between cuckoos and reed warblers at the studied population because the reed warbler is just a recent host of the cuckoo in southern Scandinavia, since it expanded during the 20th century. In addition, year of collection can be considered as a surrogate of probability of egg fading since older eggs will more likely become discolored.

The sampled clutches were collected on increasingly later dates during the course of the study (see above), which may influence the degree of matching because of bias towards later breeders among hosts. This could be a problem because age-related laying and egg color features have been described for the closely related great reed warbler *Acrocephalus arundinaceus* (Lotem et al. 1992). Therefore, to account for differences in laying date, we entered this variable in our analyses. Laying date was assumed to correspond to collection dates for each clutch that were retrieved from labels in the museum collection.

Statistical analyses

Principal components analysis (PCA) was performed on reflectance data (i.e., reflectance at the 215 possible 1.86 nm intervals between 300 and 700 nm) to reduce the number of correlated variables into a few orthogonal

Fig. 1 Long-term variation in average climatic conditions during 1918–1941 on the island of Zealand, Denmark. Data are amount of precipitation in millimeter and mean monthly temperature calculated from daily precipitation and temperatures in the period May 1 to June 30 from the Stege meteorological station



variables summarizing color variation (Cuthill et al. 1999; Cherry and Bennett 2001). Invariably, the first principal component (PC1) obtained from reflectance spectra on natural objects describes achromatic variation, essentially brightness, and this often explains more than 90% of the spectral variation (Endler and Thery 1996). Principal components 2 and 3 (PC2 and PC3, respectively) represent variation in hue and saturation (i.e., chromatic variation). Differences in PC1, PC2, and PC3 scores between the cuckoo and the mean value of host eggs within a clutch, as well as mean human estimates of matching, were used as measures of phenotypic egg matching between cuckoo and host.

Variation in degree of matching between cuckoo and host eggs at the clutch level and in cuckoo and host egg phenotype in relation to weather conditions was studied with general linear mixed models with stepwise backward model selection involving duration of sympatry (i.e., from 1 for 1918 to 24 for 1941; duration of sympatry term in the model), laying date (i.e., collection date; laying date term in the model), and environmental conditions (amount of precipitation in millimeter and mean monthly temperature; rainfall and temperature terms in the model, respectively) as independent fixed factors. Study year (study year term in the model) was also entered in the models as a random factor to control for the fact that clutches collected in a single year were not independent. Nonsignificant effects were excluded from the model following a stepwise deletion procedure in which the threshold P value was set at 0.05. We removed nonsignificant effects, starting with the least significant term.

Results

Weather

Across the study period the weather was highly variable during the breeding season of the reed warbler. The mean temperature varied between 10.7 and 14.6°C, while accumulated precipitation ranged between 38 and 133 mm, respectively (Fig. 1). Neither accumulated precipitation nor the mean temperature exhibited an overall increase or decrease over the course of the study (precipitation: $r=0.10$, $F=0.22$, $df=22$, $P=0.64$; temperature: $r=0.24$, $F=1.31$, $df=22$, $P=0.26$). Thus, possible associations between egg coloration and weather variables in this study cannot be attributed to nonrandom climatic effects on egg color (i.e., microevolutionary change in host or cuckoo egg phenotype and/or egg fading) across the study period.

Cuckoo and host egg spectral properties

The average reflectance spectra of cuckoo and reed warbler eggs (Fig. 2) corresponded with the generally brown pale

appearance perceived by humans. Principal components analyses allowed us to distinguish between achromatic “brightness” variation represented by the first principal component (PC1) and chromatic variation represented by PC2 and PC3. Together these first three components explained 99.4% of the total variance in spectra (Fig. 2).

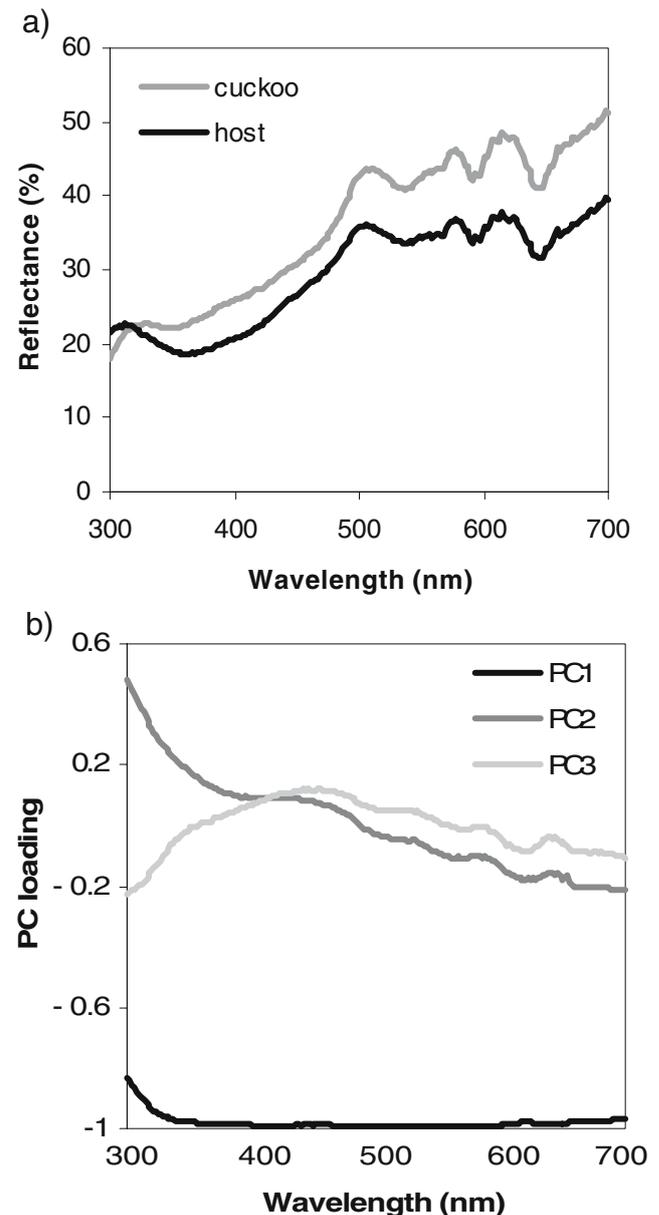


Fig. 2 Reflectance of cuckoo and reed warbler eggs ($N=483$ clutches). **a** Average reflectance spectra of cuckoo and reed warbler eggs. Values are means of the egg means of each clutch shown separately for cuckoos and hosts. **b** Factor loadings in relation to wavelength, derived from reflectance spectra from cuckoo and host eggs in each clutch. *PC1* indicates principal component 1, *PC2* principal component 2, and *PC3* principal component 3. *PC1* describes achromatic variation and explains 96.1% of overall variation (eigenvalue 220.03). *PC2* and *PC3* explain 66.7 and 17.4% of the chromatic variance, which represent 2.61% (eigenvalue 6.00) and 0.68% (eigenvalue 1.57) of overall variation, respectively

PC1 was flat, and it described achromatic variation, explaining 96.1% of overall variation. PC2 and PC3 were not spectrally flat (Fig. 2) and together accounted for 84.1% of chromatic variance, which is the spectral variance remaining after discounting achromatic variance explained by PC1. PC2 had and positive loadings at short wavelengths and negative ones at long wavelengths and therefore could be described as an ultraviolet-brownness gradient. PC3, however, had positive loadings approximately at the blue–green (400–550 nm) wavelength, and it could be described as a blue–greenness gradient.

Paired *t* tests revealed significant achromatic and chromatic differences between cuckoo and host eggs (Fig. 3). Cuckoo eggs were significantly brighter (lower PC1; $t=12.39$, $df=964$, $P<0.00001$) than reed warbler eggs. In addition, cuckoo eggs were browner and less ultraviolet (lower PC2; $t=31.52$, $df=964$, $P<0.00001$) and showed a generally less blue–green appearance (lower PC3; $t=4.87$, $df=964$, $P<0.00001$) than reed warbler eggs (Fig. 3).

Effects of environmental conditions on reed warbler and cuckoo egg coloration

Generalized mixed models revealed that brightness (defined by PC1 scores) was associated with environmental conditions in reed warblers but not in cuckoos (Table 1). In particular, once we controlled for duration of sympatry and laying time, brightness of reed warbler eggs was positively and significantly associated with spring rainfall (Fig. 4a). In addition, variation in blue–green coloration (defined by PC3 scores) was associated with environmental conditions for the two species (Table 1): there was a negative, though statistically not significant,

relationship between intensity of the blue–green coloration of reed warbler eggs and spring temperature (Table 1, Fig. 4b). On the other hand, intensity of the blue–green coloration of cuckoo eggs was positively and significantly associated with spring rainfall (Fig. 4c). UV-brownness (defined by PC2 scores) of reed warbler and cuckoo eggs were unrelated to the prevailing environmental conditions in the study area (Table 1).

Effects of environmental conditions on cuckoo–host egg matching

Spring environmental conditions explained a significant amount of variance in matching between cuckoos and hosts eggs (Table 2). In particular, generalized mixed models revealed that a higher spring rainfall was associated with a better matching in blue–greenness (Table 2, Fig. 5a). In addition, a higher spring rainfall was significantly associated with a better general matching in spottiness between cuckoo and host eggs (Table 2, Fig. 5b).

Discussion

Our results show that spring climatic conditions affected egg coloration of reed warblers and cuckoos. More specifically, reed warbler eggs were more brilliant those springs with a higher rainfall and were bluer and greener in years with a lower spring temperature. On the other hand, cuckoo eggs were bluer and greener in years with a high relative spring rainfall once we controlled for duration of sympatry (Fig. 4). Therefore, these results offer support for an environmental effect on bird egg coloration.

Fig. 3 Achromatic and chromatic differences between cuckoo and reed warbler eggs as expressed by their scores on PC1, PC2, and PC3 ($N=483$ clutches). Values are means \pm SE

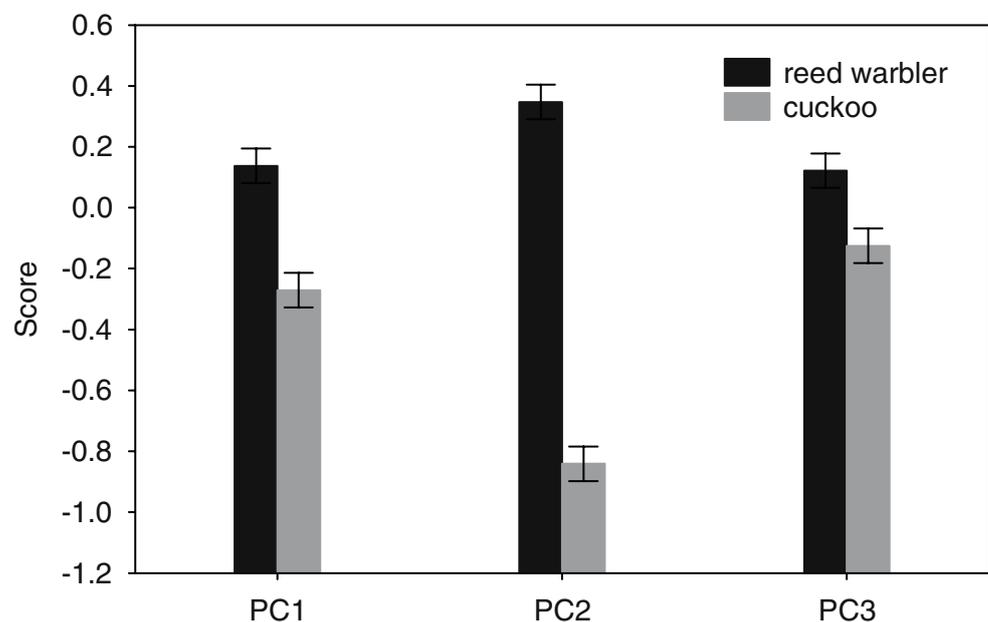


Table 1 Relationships between reed warbler and cuckoo egg coloration (i.e., PC1, PC2, and PC3 scores on spectrometric measures) and environmental conditions during laying

Dependent variable	Retained independent variables	Statistic
Reed warbler		
PC1 (brightness)	Laying date (fixed)	Not in the model ^a
	Duration of sympatry (fixed)	$F_{1,458}=5.84$, $P=0.016$
	Rainfall (fixed)	$F_{1,458}=6.23$, $P=0.012$
	Temperature (fixed)	Not in the model ^a
	Study year (random)	$Z=0.26$, $P=0.39$
PC2 (UV-brownness)	Laying date (fixed)	Not in the model ^a
	Duration of sympatry (fixed)	Not in the model ^a
	Rainfall (fixed)	Not in the model ^a
	Temperature (fixed)	Not in the model ^a
PC3 (blue–green)	Study year (random)	$Z=0.26$, $P=0.39$
	Laying date (fixed)	Not in the model ^a
	Duration of sympatry (fixed)	$F_{1,458}=7.34$, $P=0.007$
	Rainfall (fixed)	Not in the model ^a
Cuckoo	Temperature (fixed)	Not in the model ^a
	Study year (random)	$Z=2.62$, $P=0.004$
	Temperature (fixed)	$F_{1,458}=3.26$, $P=0.07$
PC1 (brightness)	Laying date (fixed)	Not in the model ^a
	Duration of sympatry (fixed)	$F_{1,458}=8.95$, $P=0.002$
	Rainfall (fixed)	Not in the model ^a
	Temperature (fixed)	Not in the model ^a
	Study year (random)	$Z=2.26$, $P=0.01$
PC2 (UV-brownness)	Laying date (fixed)	Not in the model ^a
	Duration of sympatry (fixed)	Not in the model ^a
	Rainfall (fixed)	Not in the model ^a
	Temperature (fixed)	Not in the model ^a
PC3 (blue–green)	Study year (random)	$Z=2.24$, $P=0.01$
	Laying date (fixed)	$F_{1,457}=14.29$, $P=0.0002$
	Duration of sympatry (fixed)	$F_{1,457}=9.39$, $P=0.002$
	Rainfall (fixed)	$F_{1,457}=5.21$, $P=0.02$
	Temperature (fixed)	Not in the model ^a
	Study year (random)	$Z=2.57$, $P=0.005$

Analyses are generalized mixed models with stepwise backward model selection involving duration of sympatry, laying date (collection date), and environmental conditions (amount of precipitation in mm and mean monthly temperature for May and June) as independent fixed factors and study year as a random factor.

^a Not selected by the stepwise procedure.

It is interesting to note that blue–green pigmentation of cuckoo and reed warbler eggs was affected by the spring environmental conditions. A recent hypothesis has proposed that the blue and green coloration of bird eggs of

many species with biparental care may function as sexual signals indicating female phenotypic quality to their mates (Moreno and Osorno 2003). The hypothesis is based on the strong antioxidant capacities of the blue–green egg pigment biliverdin (Neuzil and Stocker 1993; McDonagh 2001; Kaur et al. 2003). Deposition of blue–green pigments in the eggshell may signal female capacity to control free radicals despite allocating antioxidants to eggshells (Moreno and Osorno 2003). Therefore, the documented relationship between the intensity of blue and green egg coloration and climatic conditions may reflect the condition-dependence of blue–green egg coloration (see also Moreno et al. 2005). Accordingly, if female condition is somehow related to antioxidant capacity, they should show a better condition at laying and may allocate more blue–green pigments in the eggshell in years with better environmental conditions. Alternatively, selection for signaling condition in reed warbler females may be stronger in poor years, when male help is more important.

Female condition at laying could be directly or indirectly affected by climatic effects. Climatic conditions may influence bird egg coloration by their direct effects on the physiological status of laying females (e.g., Hughes et al. 1986; Joseph et al. 1999). Stress experienced by poultry result in the release of the hormone epinephrine, which is responsible for causing a delay in oviposition and the cessation of shell gland cuticle formation (Solomon et al. 1994). In addition, laying is a particularly stressful period for females due to high levels of oxidative stress (von Schantz et al. 1999). Hence, stress induced by extreme climatic conditions at laying may interfere with the appropriate inclusion of pigments by epithelial cells in the shell gland and thus contribute to the observed relationship between environmental conditions and egg coloration. Alternatively, climatic conditions may affect bird egg coloration, primarily through its effects on food availability. Reed warblers and cuckoos mainly prey on insects (Cramp 1998). Temperature and rainfall fluctuations may potentially influence insect availabilities at a local scale (e.g., Sillett et al. 2000), thereby affecting the condition of the laying females and/or the capacity of females to include in the eggshell pigments ingested via insect consumption.

An alternative possibility is that the found patterns were not due to direct or indirect effects of climatic factors on female condition but rather to dispersal behavior of reed warblers and/or cuckoos being affected by environmental conditions. In such a scenario, individuals from other subpopulations varying in egg color could settle on Zealand causing changes in egg coloration at the population level. We have no direct information on dispersal linked to weather conditions in reed warblers and/or cuckoos. However, the Zealand subpopulation of the reed warbler is at the edge of the northern distribution of the reed

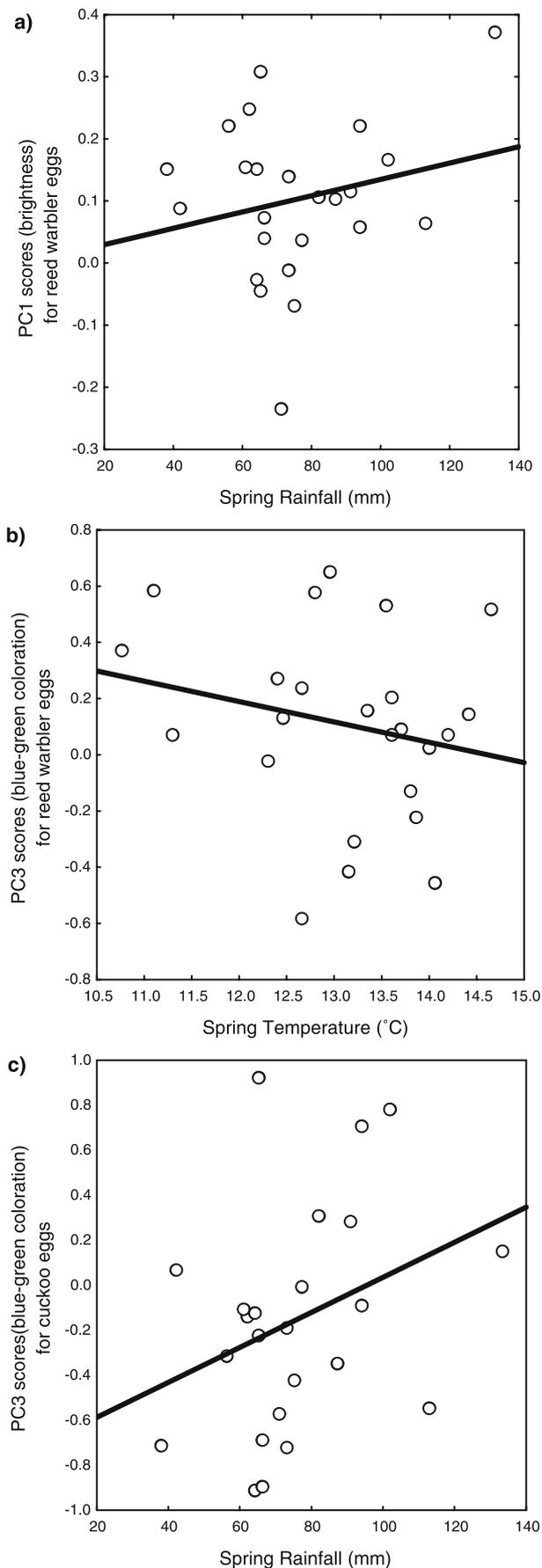


Fig. 4 Relationship between egg coloration and environmental conditions. **a** Host egg phenotype (PC1 scores) vs spring rainfall. **b** Host egg phenotype (PC3 scores) vs mean spring temperature. **c** Cuckoo egg phenotype (PC3 scores) vs spring rainfall. Regression lines are derived from the univariate regression of the respective features. Figures were based on mean values per year ($N=24$ years) although analyses were based on clutches (see “Materials and methods” and Table 1)

warbler metapopulation (Cramp 1998), which would reduce the probability of settlement from distant subpopulations. Furthermore, cuckoo arrival dates in different European breeding areas seemed unaffected by changes in climatic conditions (Lehikoinen et al. 2004).

The intensity of blue–green coloration of cuckoo and reed warbler eggs was differently affected by temperature and rainfall. Species-specific environmental susceptibility of egg color could be the consequence of cuckoo and reed warbler females having different oxidative costs associated with the inclusion of blue–green pigments in the eggshell (see Moreno and Osorno 2003). Although the signaling hypothesis would not affect female cuckoos directly (because either, it is highly unlikely that a cuckoo male may evaluate the signal, or because no evidence of parental care by cuckoo males has been reported so far), they should include blue–green pigments in their eggs to mimic reed warbler eggs and to avoid rejection (Davies and Brooke 1988). Cuckoo females lay more eggs than their reed warbler hosts (e.g., Payne 1973, 1974). Therefore, they should be more constrained by oxidative costs associated with the inclusion of blue–green related pigments in their eggshells. This possibility would lead to the prediction that cuckoo egg coloration is more environment-dependent than reed warbler egg coloration. Although we found that both cuckoo and reed warbler blue–green egg colors were related to climatic factors, perceptible changes in matching for that color trait were due to a rainfall effect on cuckoo blue–green egg color. Alternatively, species-specific differences in the environmental effects on egg coloration would be expected because cuckoos and reed warblers greatly differ in their diets (Cramp 1998). Then, different environmental susceptibility of butterfly caterpillars consumed by cuckoos and of insects, spiders, and small snails captured by reed warblers may ultimately induce species-specific susceptibility of egg coloration to climatic factors.

Our study showed a significant association between spring environmental conditions during the egg-laying period and the degree of mimicry between cuckoo and reed warbler eggs (Fig. 5). This constitutes the first support for the hypothesis that environmental factors may affect important features of the arms race between cuckoos and their hosts. We found that differences in chromatic aspects of color (but not in achromatic brightness) between cuckoo and host eggs measured by UV–visible spectrophotometry

Table 2 Relationships between matching of cuckoo and reed warbler eggs (i.e., difference in PC1, PC2, and PC3 scores from the PCA on spectrometric measures and human scores for spottiness) and environmental conditions during laying

Dependent variable	Retained independent variables	Statistic
Host-cuckoo differences in PC1 (brightness)	Laying date (fixed)	Not in the model ^a
	Duration of sympatry (fixed)	$F_{1,458}=9.08$, $P=0.003$
	Rainfall (fixed)	Not in the model ^a
	Temperature (fixed)	Not in the model ^a
	Study year (random)	$Z=1.13$, $P=0.12$
Host-cuckoo differences in PC2 (UV-brownness)	Laying date (fixed)	Not in the model ^a
	Duration of sympatry (fixed)	Not in the model ^a
	Rainfall (fixed)	Not in the model ^a
	Temperature (fixed)	Not in the model ^a
Host-cuckoo differences in PC3 (blue–green)	Study year (random)	$Z=2.15$, $P=0.01$
	Laying date (fixed)	Not in the model ^a
	Duration of sympatry (fixed)	Not in the model ^a
	Rainfall (fixed)	$F_{1,459}=4.81$, $P=0.03$
Host-cuckoo differences in spottiness	Temperature (fixed)	Not in the model ^a
	Study year (random)	$Z=2.53$, $P=0.005$
	Laying date (fixed)	Not in the model ^a
	Duration of sympatry (fixed)	$F_{1,458}=3.61$, $P=0.06$
	Rainfall (fixed)	$F_{1,458}=4.87$, $P=0.03$
	Temperature (fixed)	Not in the model ^a
	Study year (random)	$Z=2.04$, $P=0.02$

Analyses are generalized mixed models with stepwise backward model selection involving duration of sympatry, laying date (collection date), and environmental conditions (amount of precipitation in millimeter and mean monthly temperature for May and June) as independent fixed factors and study year as a random factor.

^a Not selected by the stepwise procedure.

and spottiness were associated with climatic conditions prevailing at the time of laying after controlling for time since collection (Table 2). Specifically, cuckoo–host egg mimicry in blue–green color and spottiness improved in those years with a higher spring rainfall (Fig. 5). A higher spring rainfall also associates with bluer and greener cuckoo eggs (Fig. 4). Therefore, the rainfall effect on cuckoo–host egg matching in blue–green coloration was due to increased intensity of blue and green coloration of cuckoo eggs in rainy seasons. It has been previously suggested that a large environmental component to phenotypic variation in host eggshell pigmentation may reduce the ability of the cuckoo to produce mimetic eggs (Gosler et al. 2000). Alternatively, it could be hypothesized that a higher environmental component in cuckoo egg coloration may prevent cuckoos from reaching a perfect match of the

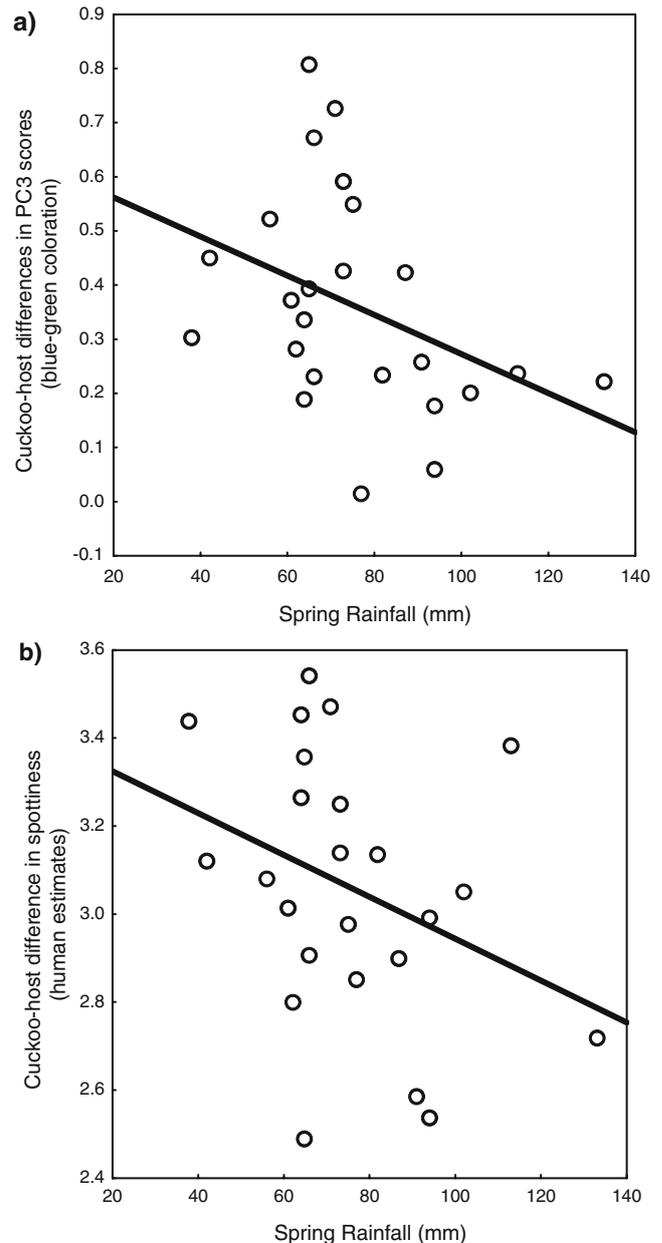


Fig. 5 Relationships between spring rainfall (amount of precipitation in mm accumulated in May and June) and cuckoo host egg matching. **a** Cuckoo–host differences in PC3 scores vs spring rainfall. **b** Cuckoo–host differences in spottiness scores vs spring rainfall. Regression lines are derived from the univariate regression of the respective features. Figures were based on mean values per year ($N=24$ years) although analyses were based on clutches (see “Materials and methods” and Table 2)

host eggs. In this study, we have found that both cuckoo and reed warbler variation in egg phenotype were affected by different environmental components. However, changes in matching for blue–green coloration were due to a higher environmental effect on cuckoo egg coloration for that color component. Therefore, cuckoos could be constrained by oxidative costs associated with the inclusion of blue–green related pigments in their eggs.

In summary, we found that spring climatic conditions affected chromatic aspects of both reed warbler and cuckoo egg coloration. In addition, our study suggests complexities in the cuckoo–host arms race hitherto not considered because we have found consistent support for the hypothesis that environmental conditions may affect phenotypic egg matching between cuckoo and host egg. Therefore, environmental conditions may play a previously ignored role in the dynamic of the coevolutionary arms race between cuckoos and their hosts.

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