

Rapid increase in cuckoo egg matching in a recently parasitized reed warbler population

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Keywords:

avian brood parasitism;
coevolution;
cuckoo preference hypothesis;
egg appearance;
egg mimicry change;
host selection.

Abstract

Parasitic cuckoos lay eggs that mimic those of their hosts, and such close phenotypic matching may arise from coevolutionary interactions between parasite and host. However, cuckoos may also explicitly choose hosts in a way that increases degree of matching between eggs of cuckoos and parasites, with female preference for specific host phenotypes increasing the degree of matching. We tested for temporal change in degree of matching between eggs of the parasitic European cuckoo (*Cuculus canorus*) and its reed warbler (*Acrocephalus scirpaceus*) host during 24 consecutive years in a recently parasitized reed warbler population. Cuckoo–host egg matching in an ultraviolet-brownness component yielding most of the chromatic variance of eggs improved during the study period. Improved matching was not due to changes in cuckoo egg phenotype. Cuckoo eggs matched host eggs for ultraviolet-brownness within nests irrespective of duration of sympatry. Ultraviolet-brownness of cuckoo eggs was similar to that of reed warbler eggs at parasitized nests, but differed from that of reed warbler eggs at unparasitized nests. These findings provide tentative support for the cuckoo preference hypothesis suggesting that cuckoo–host egg matching could partially be due to cuckoo females selecting host nests based on the appearance of their eggs.

Introduction

The eggs of the common cuckoo (*Cuculus canorus*) are surprisingly variable. This variation and the close resemblance between cuckoo eggs and those of some of its favourite hosts have attracted the interest of scientists for a long time (review in Davies, 2000). Matching between cuckoo and host eggs has largely been explained as the result of a long coevolutionary relationship between the cuckoo and different host species (Brooke & Davies, 1988). Cuckoo parasitism greatly reduces reproductive success of hosts, and thus is a powerful agent selecting for anti-parasite defence mechanisms by hosts, which at the same time selects for more sophisticated trickeries by the cuckoo to overcome host defences (Brooke & Davies, 1988; Davies & Brooke, 1988; Rothstein, 1990). In this coevolutionary arms race scenario recognition and rejection

of parasite eggs from host nests is a generalized host defence against cuckoo parasitism that selects for more acute host egg matching by the cuckoo.

Although host rejection of odd cuckoo eggs is a main factor favouring egg matching there must also be some kind of cuckoo host preference to allow mimicry to evolve and be maintained. Indeed, pioneer observational studies reporting cuckoo female laying histories showed remarkable host specificity (e.g. Chance, 1940; Baker, 1942). In addition, genetic analyses have revealed that cuckoo genets are restricted to female lineages that specialize on specific host species (Marchetti *et al.*, 1998), with cross-mating by males maintaining cuckoos as a single species (Gibbs *et al.*, 2000). Moreover, Avilés & Møller (2004) have reported a high degree of resemblance between the appearance of cuckoo eggs and those of different hosts within the same habitat, suggesting a high level of host specificity (see also Moksnes & Røskaft, 1995; Honza *et al.*, 2001). More convincing is the evidence from radio-telemetry studies showing that radio-tagged cuckoo females show strict host preferences when different host species coexist in the same area

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(Wyllie, 1981; Dröscher, 1988; Nakamura & Miyazawa, 1997; Nakamura *et al.*, 1998; Honza *et al.*, 2002).

Beyond species-specific preferences cuckoos may also prefer certain host individuals of a single species. Indeed, great spotted cuckoos (*Clamator glandarius*) in Spain do not lay their eggs at random among available host magpie (*Pica pica*) nests, but select larger nests, which increases their fitness because nest size correlates with parental abilities of magpie hosts (Soler *et al.*, 1995). In addition, the level of parasitism positively correlates with host song activity at nests in four North American passerines parasitized by the brown-headed cowbird (*Molothrus ater*) (Banks & Martin, 2001; see also Garamszegi & Avilés, 2005). Álvarez (2000) found that common cuckoo females parasitized rufous bush chat (*Cercotrichas galactotes*) nests with larger eggs. Therefore, current evidence suggests that obligate avian brood parasites in general and the common cuckoo in particular may have evolved fine cognitive capacities improving host selection. Cuckoo host preference is something likely to have evolved early in the evolutionary history of cuckoos. If so, this implies that when cuckoos start to parasitize a new host, they have already developed a general preference. Therefore, at the beginning of an 'arms race' all host eggs are more or less unlike those of the cuckoo. However, cuckoo selection of host nests based on host phenotype (e.g. nest size, egg size, egg colour) may act together with host discrimination and speed up the evolutionary process of developing a good match between eggs of the parasite and its host.

The aim of this study was to test whether host egg matching by cuckoos could partially be the consequence of cuckoos actively selecting host nests with eggs that are closer in appearance to their own eggs than expected by chance (cuckoo preference hypothesis). It is obvious that cuckoos benefit by selecting host nests with eggs closer in appearance to their own eggs. Indeed a large number of experimental studies have documented that cuckoo chick fitness is positively related to degree of matching between cuckoo and host eggs (review in Davies, 2000). That is, cuckoo eggs laid in nests with host eggs closer in appearance to those of the parasite are likely to be less frequently rejected by hosts than those laid in host nests with dissimilar eggs. Here we analyse changes in the degree of matching between cuckoo and host eggs across 24 years (1918–1941) in a reed warbler (*Acrocephalus scirpaceus*) population on the island of Zealand (Denmark) recently parasitized by the common cuckoo. This population has apparently been parasitized by the common cuckoo for less than a century. The first breeding records for reed warblers on Zealand are from the mid-19th century (Løppenthin, 1967), being an abundant breeder in the island after this time. No report exists of cuckoo parasitism on reed warblers before 1902 and uninterrupted records of cuckoo parasitism on reed warblers exist after 1916 (J.M. Avilés, A. Moksnes, E. Røskaft, B.G. Stokke & A.P. Møller, unpublished data). Cuckoos;

however, arrived at Zealand before 1900 because records exist of cuckoo parasitism of whitethroat *Sylvia communis*, garden warbler *Sylvia borin* and yellow wagtail *Motacilla flava* before that time (J.M. Avilés, A. Moksnes, E. Røskaft, B.G. Stokke & A.P. Møller, unpublished data). Hence the reed warbler can reasonably be considered as a recent cuckoo host on Zealand in 1918.

Recent sympatry between cuckoos and reed warblers makes the system particularly suitable for testing predictions from the cuckoo preference hypothesis regarding egg mimicry. The additive effects of host selection for particular cuckoo egg phenotypes and of cuckoo selection for a particular host egg phenotype would predict (i) a rapid improvement of matching between cuckoo and reed warbler eggs in the population. Noteworthy is that this prediction is not ascribable to the cuckoo preference hypothesis itself because cuckoo preference just may reinforce the evolution of increasingly mimetic cuckoo eggs through host rejection of nonmatching cuckoo eggs. However, the cuckoo preference hypothesis (ii) does not predict a change in cuckoo egg phenotype over time as would be expected by host removal of odd cuckoo eggs. Rather it predicts (iii) a correspondence between cuckoo and host egg traits within nests due to nonrandom laying by cuckoos. Finally, we studied cuckoo and host egg phenotypes at parasitized and unparasitized reed warbler nests in the population to determine whether cuckoos actively selected host nests on the basis of their egg appearance. According to the cuckoo preference hypothesis, we predicted (iv) that parasitized reed warbler nests should have eggs that are closer in appearance to that of cuckoo eggs.

Materials and methods

Museum data

Cuckoo–host egg matching was assessed for parasitized reed warbler clutches stored 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 the testing of evolutionary and ecological hypotheses related to egg coloration of birds (e.g. Brooke & Davies, 1988; Davies & Brooke, 1989; Moksnes & Røskaft, 1995; Øien *et al.*, 1995; Cherry & Bennett, 2001; Stokke *et al.*, 2002; Avilés & Møller, 2003, 2004; Soler *et al.*, 2003, 2005). Matching was assessed for a sample of 483 reed warbler clutches parasitized by the cuckoo assembled 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 (total number of clutches is about 800). However, we randomly selected a representative sample of clutches for every breeding season which ensured that our sample was a representative subset of the collection. All sampled clutches were

collected by three persons within an area of more than 1200 km² with a radius of 40 km. The mean (\pm SD) number of clutches measured per year was 20.12 (\pm 9.86) (range = 7–42). All clutches were collected in May or June [mean (\pm SD) collection date was 27 May (\pm 12 days)] and the mean collection date was delayed by 0.23 days every year across the study ($F_{5,18} = 4.25$, $P = 0.05$, $R^2 = 0.16$). The number of clutches collected from each 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 the pattern of clutch collection across years. The mean (\pm SD) number of host eggs per parasitized clutch was 2.34 (\pm 0.91), whereas the mean number of cuckoo eggs per clutch was 1.03 (\pm 0.19).

Field data

Cuckoo and reed warbler egg appearance in parasitized and unparasitized nests was estimated in the field at lake Arresø (Northern Zealand, 55°58'N, 12°04'E) during June 2004. The littoral zone surrounding this natural lake consists of dense but narrow belts of reed (*Phragmites australis*), and holds a high density of reed warblers (about 25 pairs ha⁻¹) (B. G. Stokke, unpublished data).

We systematically searched for reed warbler nests and in total 62 nests were found before egg laying or with eggs. All nests were numbered and mapped. The nests were visited daily and each cuckoo and host egg was numbered with waterproof ink in consecutive order as laid. Seven of 62 nests (11.29%) were parasitized by the cuckoo. Spectrometric measurements of cuckoo and host eggs were taken the day after clutch completion or immediately after the nest was found if the clutch was completed and being incubated. Three of the seven parasitized nests were deserted and contained no reed warbler eggs. Therefore, we finally estimated reed warbler egg coloration at four parasitized and 55 unparasitized reed warbler nests respectively. However, cuckoo egg coloration was estimated for all the seven parasitized nests, but the results remained unchanged compared to when analyses were based on cuckoo eggs found together with reed warbler eggs (results not shown). The parasite egg turned out to be accepted in all four reed warbler clutches containing both parasite and host eggs.

Egg colour and cuckoo egg matching measurements

Egg matching was estimated using spectrophotometry (field and museum) and photography (only in museum). The former technique allows objective assessment of egg coloration and accounts for ultraviolet information to which humans are blind (Cherry & Bennett, 2001; Avilés & Møller, 2003, 2004; Langmore *et al.*, 2003; Avilés *et al.*, 2004, 2006). However, spectrophotometric techniques

may have flaws when quantifying overall egg appearance of a spotted egg as it may be difficult to distinguish between different patterns of spottiness, which obviously could be important for birds in distinguishing between own and foreign eggs. Therefore, we also quantified differences in the pattern of spottiness between cuckoo and host eggs based on photos.

Reflectance spectra in the range 300–700 nm were obtained from all eggs of the analysed clutches using a spectro-radiometer (Ocean Optics Europe, Duiven, the Netherlands) measured 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 & Bennett, 2001; Langmore *et al.*, 2003; Avilés *et al.*, 2006). Colour was measured once in 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 fibre-optic probe that provides illumination at a 45° angle from the light source and transfers reflected light to the spectrometer. Data from the spectrometer were passed into a computer, where a software package (OOIBase 32; Ocean Optics Europe) 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 the mean value of five adjacent measurements. The mean reflectance spectrum for each egg in a clutch was calculated from the five spectra taken for each egg. This was justified because coloration was repeatable within eggs [repeatability of colour estimates (see below for further details on colour information provided by PCs) was calculated on 500 randomly selected eggs: PC1: $r = 0.20$, $F_{499,1999} = 2.22$, $P < 0.00001$; PC2: $r = 0.66$, $F_{499,1999} = 10.84$, $P < 0.00001$; PC3: $r = 0.56$, $F_{499,1999} = 7.45$, $P < 0.00001$]. In addition, egg coloration was also repeatable among the different eggs of a clutch (PC1: $r = 0.52$, $F_{482,643} = 3.55$, $P < 0.00001$; PC2: $r = 0.25$, $F_{482,643} = 1.78$, $P < 0.00001$; PC3: $r = 0.51$, $F_{482,643} = 3.37$, $P < 0.00001$), and a mean host spectrum for each clutch was calculated because there was a variable number of host eggs per clutch (see Cherry & Bennett, 2001). The following matching analyses were therefore based on 483 pairs of spectra (one mean host and one cuckoo spectrum for each clutch in the museum). Analyses on reed warbler and cuckoo egg colour in the field were based on 66 spectra (one mean reed warbler spectrum for each clutch and one cuckoo spectrum for each of the seven clutches with a cuckoo egg).

Clutches in the museum were also photographed in their boxes together with a colour reference of the type 'Polaroid High Definition DFF1/HDF1 Reference Print', using a Canon Eos 50 (Canon Norge SA, Oslo, Norway) with film type Fujicolor 100 ISO (Fujicolor, Skärholmen, Sweden). Matching in the pattern of spottiness between cuckoo and host eggs was later assessed based on these photos on a 1–5 scale (1 = perfect

matching, 5 = no matching) by three experienced persons unaware of the hypothesis being tested, using a protocol defined for common cuckoo eggs (Moksnes & Røskaft, 1995). When more than a single cuckoo egg was found in a clutch ($n = 14$ clutches), one of the parasitic eggs was randomly chosen for the analysis. The mean value of the scores attained by the test persons were used in the subsequent analyses that is justified by a high consistency among the various assessments ($r = 0.59$, $F_{17,18} = 3.85$, $P = 0.003$).

Confounding factors

A number of factors may potentially influence matching between host and cuckoo eggs over time irrespective of the duration of sympatry. Although current evidence suggests that variation in egg coloration is highly heritable in birds (Collias, 1993), we have noted that variation in egg colour in our reed warbler population was associated with climatic conditions during the laying period of the host (J.M. Avilés, A. Moksnes, E. Røskaft, B.G. Stokke & A.P. Møller, unpublished data). Therefore, we controlled for the effects of climatic conditions when studying the association between the degree of matching and duration of sympatry. Local weather variables used were mean daily temperature and the accumulated precipitation from 1 to 31 May collected by the Meteorological Institute of Copenhagen within the study area at Stege permanent meteorological station (55°12'N, 12°18'E). We selected May because this was the period when most of the sampled clutches were laid (see above). From these data two indices of spring weather were calculated and their possible effects on cuckoo–host egg matching estimated: (a) the mean spring temperature calculated on mean daily temperatures from 1 to 31 May and (b) the accumulated rainfall in the same period.

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 colour 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. This assumption is justified by the holes in eggs through which the egg contents were removed all being small, suggesting that all embryos must have been very young.

Cuckoo–host egg matching could be affected by degree of variation in the appearance of host eggs. Because a low intra-clutch variation in egg appearance results in high levels of egg variation among host females, cuckoos may have more difficulty laying highly mimetic eggs with low intra-clutch variation at the

population level. To control for this potential bias we estimated intra-clutch variation in egg colour traits for all clutches in the museum having more than one host egg and considered it in the full models describing cuckoo–host egg matching.

Statistical analyses

Principal component analysis (PCA) was performed on reflectance data to reduce the number of correlated variables (reflectance at 1.86 nm) into a few orthogonal variables summarizing colour variation (Cuthill *et al.*, 1999). The average reflectance spectra of cuckoo and reed warbler eggs corresponded to the generally brown pale appearance perceived by humans (Fig. 1). PCA

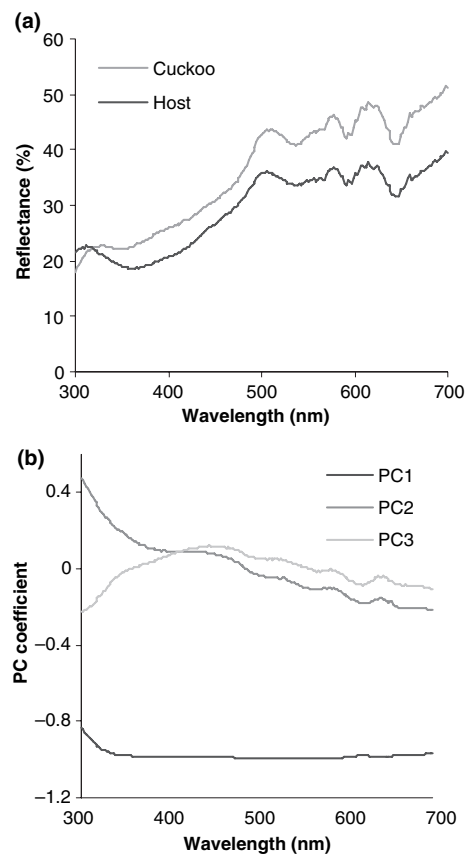


Fig. 1 Reflectance of cuckoo and reed warbler eggs ($n = 483$ clutches). (a) Average reflectance spectra of cuckoo and reed warbler eggs. Values are means of the egg means of each clutch shown separately for cuckoos and hosts. (b) Principal components in relation to wavelength, derived from reflectance 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. PC2 (ultraviolet-brownness) and PC3 (greenness), respectively, explain 66.7% and 17.4% of the chromatic variance.

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). Together these three first components explained 99.4% of the total variance in spectra (Fig. 1). PC1 was flat and described achromatic variation explaining 96.1% of the overall variation. PC2 and PC3 were not spectrally flat and together they accounted for 84.1% of the chromatic variance (Fig. 1). PC2 had high and positive loadings at short wavelengths and high negative ones at long wavelengths and could therefore classify the sampled eggs along a gradient of ultraviolet-brownness. PC3, however, had high positive loadings approximately at the green (475–550 nm) wavelength and thus could be described as a greenness gradient. Differences in PC1, PC2 and PC3 score between the cuckoo eggs and the mean value of host eggs within a clutch, as well as mean human estimates of matching in spottiness, were used as measures of matching between eggs of cuckoos and those of their hosts. The standard deviation of PC1, PC2 and PC3 scores attained for all host eggs in a clutch was used as an index of intra-clutch variation in egg coloration.

Variation in degree of matching between cuckoo and host eggs at the clutch level and in cuckoo egg phenotype 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), environmental conditions (amount of precipitation in mm and mean monthly temperature for May; Rainfall and temperature terms in the model, respectively) and host intra-clutch variation (Intra-clutch variation term in the model) 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

Prediction 1: Cuckoo–host egg matching improves with duration of sympatry

Cuckoo–host egg matching improved over the 24-year study period for the main chromatic component of egg coloration (Table 1). In particular, general linear mixed models revealed that differences in ultraviolet-brownness (defined by PC2 scores) between cuckoo and host eggs decreased with duration of sympatry after accounting for the environmental conditions prevailing during laying, laying date, host intra-clutch variation in egg appearance

and the significant random effect of study year (Table 1, Fig. 2a). Cuckoo–host egg differences in achromatic brightness (defined by PC1 scores), greenness (defined by PC3 scores) and pattern of spottiness did not relate to duration of sympatry between cuckoos and reed warblers in Zealand (Table 1).

Some of the additional independent variables originally included in the full model were significantly related to matching between cuckoo and host eggs after accounting for the significant random effect of study year (Table 1). For example, laying date was positively related to differences in achromatic brightness and negatively related to differences in greenness between cuckoo and host eggs (Table 1). In addition, reed warbler clutches with larger intra-clutch variation in ultraviolet-brownness of their eggs were parasitized by relatively less mimetic cuckoo eggs for that chromatic component (Table 1).

Prediction 2: Cuckoo egg phenotype does not change with duration of sympatry

The analyses of temporal changes in ultraviolet-brownness of cuckoo eggs allowed us to determine whether improved mimicry was due to changes in cuckoo egg phenotype (Fig. 2b). General linear mixed models showed that variation in ultraviolet-brownness (i.e. the main chromatic component of egg coloration) of cuckoo eggs was unrelated to duration of sympatry between cuckoos and hosts in Zealand (Table 2) once we took the environmental conditions prevailing at laying, laying date, host intra-clutch variation in egg appearance and the significant random effect of study year into account (Table 2, Fig. 2b).

Prediction 3: Cuckoo egg phenotype matches host egg phenotype within nests

A similar analysis including ultraviolet-brownness of host eggs as independent fixed factor together with the above determinants of ultraviolet-brownness coloration of cuckoo eggs revealed a significant positive relationship between PC2 (i.e. ultraviolet-brownness) cuckoo scores and PC2 host scores after accounting for the significant random effect of year (Table 2). Therefore, among clutch variation in the main chromatic colour feature of host eggs is matched by variation in the same feature of cuckoo eggs (Fig. 3).

Prediction 4: Parasitized nests have reed warbler eggs more similar in appearance to that of cuckoo eggs

Field data supported the prediction of a cuckoo preference because reed warbler eggs at parasitized nests looked similar in terms of ultraviolet-brownness to cuckoo eggs, whereas reed warbler eggs at unparasitized

Table 1 Determinants of egg matching (i.e. host–cuckoo difference in PC1, PC2 and PC3 scores from the PCA on spectrometric measures and human scores for similarity) as revealed by general linear mixed models with stepwise backward model selection involving duration of sympatry, laying date (collection date), environmental conditions (amount of precipitation in mm and mean monthly temperature for May), and host intra-clutch variation as independent fixed factors and study year as a random factor.

Dependent variable	Independent variables	β (SE)	Statistic
Host–cuckoo differences in PC1 Overall final model: $F_{24,458} = 4.58$, $P < 0.00001$, $R^2 = 0.19$	Intercept		$F_{1,20.97} = 203.64$, $P < 0.0001$
	Laying date (fixed)	0.15 (0.04)	$F_{1,186.97} = 6.57$, $P = 0.011$
	Duration of sympatry (fixed)		Not in the model*
	Rainfall (fixed)		Not in the model*
	Temperature (fixed)		Not in the model*
	Intra-clutch variation in PC1 (fixed)		Not in the model*
	Study year (random)		$F_{23,458} = 4.40$, $P < 0.0001$
Host–cuckoo differences in PC2 Overall final model: $F_{24,458} = 2.70$, $P < 0.00003$, $R^2 = 0.15$	Intercept		$F_{1,16.57} = 806.81$, $P < 0.0001$
	Laying date (fixed)		Not in the model*
	Duration of sympatry (fixed)	-1.10 (1.94)	$F_{1,17.47} = 6.73$, $P = 0.018$
	Rainfall (fixed)		Not in the model*
	Temperature (fixed)		Not in the model*
	Intra-clutch variation in PC2 (fixed)	0.16 (0.05)	$F_{1,300.62} = 14.00$, $P = 0.0002$
	Study year (random)		$F_{22,361.00} = 1.69$, $P = 0.025$
Host–cuckoo differences in PC3 Overall final model: $F_{24,458} = 2.66$, $P < 0.00004$, $R^2 = 0.12$	Intercept		$F_{1,19.40} = 575.60$, $P < 0.0001$
	Laying date (fixed)	-0.11 (0.04)	$F_{1,314.38} = 6.85$, $P = 0.009$
	Duration of sympatry (fixed)		Not in the model*
	Rainfall (fixed)		Not in the model*
	Temperature (fixed)		Not in the model*
	Intra-clutch variation in PC3 (fixed)		Not in the model*
	Study year (random)		$F_{23,458} = 2.43$, $P = 0.0002$
Host–cuckoo differences in spottiness Overall final model: $F_{23,459} = 3.30$, $P < 0.00001$, $R^2 = 0.14$	Intercept		$F_{1,20.36} = 2221.95$, $P < 0.00001$
	Laying date (fixed)		Not in the model*
	Duration of sympatry (fixed)		Not in the model*
	Rainfall (fixed)		Not in the model*
	Temperature (fixed)		Not in the model*
	Intra-clutch variation (fixed)		Not in the model*
	Study year (random)		$F_{23,459} = 3.30$, $P = 0.0001$

*Not selected by the stepwise procedure

nests differed significantly from cuckoo eggs for that chromatic colour component (Fig. 4).

Discussion

Evolutionary changes in parasite–host interactions may be sufficiently rapid to be demonstrated by long-term studies. However, there are relatively few well-documented examples from natural populations of brood parasitic cuckoos and their hosts, probably due to few studies being conducted over more than a few years (e.g. Soler *et al.*, 1994; Nakamura *et al.*, 1998; Lahti, 2005). To our knowledge, this is the first study in which perceptible temporal changes in matching between the eggs of an obligate avian brood parasite and those of its host have been documented. The degree of egg matching between cuckoo and host in terms of ultraviolet-brownness (which explained 66.7% of the chromatic variance of eggs) improved from 1918 to 1941 in a reed warbler population recently parasitized by the common cuckoo on the island of Zealand (Prediction 1, Fig. 2). We

consider two nonexclusive mechanisms for the temporal improvement of egg matching.

Improved matching could be a rapid micro-evolutionary response to host removal of odd cuckoo eggs. Cuckoo egg appearance coevolves with host rejection behaviour given examples showing that dissimilar cuckoo eggs are more likely to be discarded by hosts (review in Davies, 2000). Reed warblers should have the potential to discriminate and reject cuckoo eggs differing in ultraviolet-brownness from their eggs. Ultraviolet vision is widely demonstrated among passerines (review in Cuthill *et al.*, 2000), and, more supportive, Davies & Brooke (1988) have reported differences in rejection of cuckoo model eggs that tenuously differed in brown coloration in a British reed warbler population. However, we may ask the question whether 23 years suffice for a micro-evolutionary response by cuckoos to host selection of cuckoo egg phenotypes. We lack information about reed warbler rejection abilities when the museum clutches were collected. However, in a nearby reed warbler population in southern Norway in which cuckoo

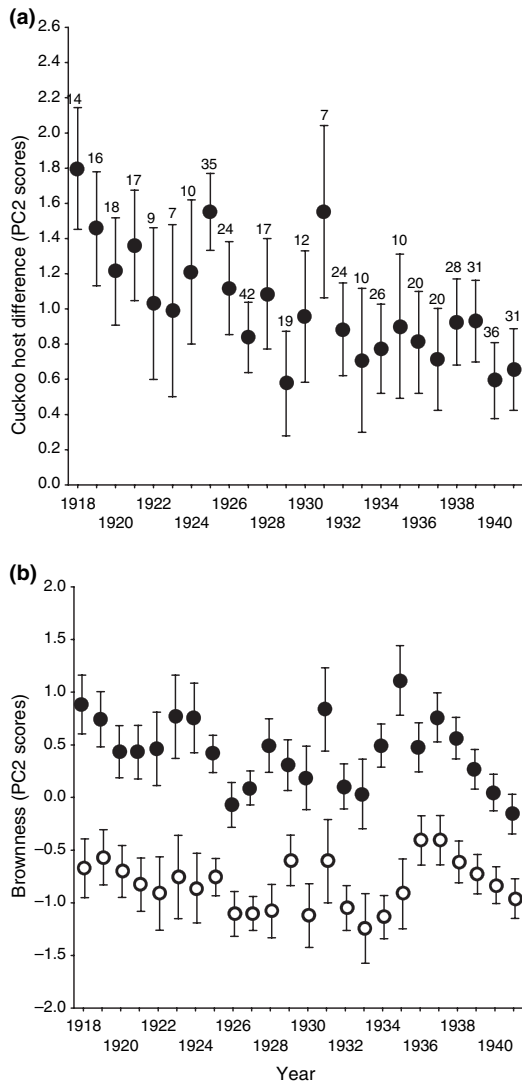


Fig. 2 Temporal changes in mean egg matching between eggs of cuckoos and hosts and cuckoo and host egg phenotype during 1918–1941. (a) Differences in ultraviolet-brownness (PC2 scores) between cuckoo and reed warbler eggs and (b) variation in ultraviolet-brownness in cuckoo (open circles) and reed warbler (closed circles) eggs. Values are means with SE (error bars). Sample sizes above each error bar indicate the measured number of clutches each year.

parasitism has not yet been reported, three (11.5%) of 26 artificially parasitized nests rejected nonmimetic cuckoo model eggs ($n = 26$ experimental nests in 2002, I. Hafstad, A. Moksnes, E. Røskaft & B.G. Stokke, unpublished data). Thus, it is likely that reed warblers on Zealand may have come from a gene pool of reed warblers that was exposed to cuckoo parasitism, and that hosts rejected cuckoo eggs already in 1918. Persistence of egg recognition in the absence of cuckoo brood parasitism has been widely reported among hosts of other obligate avian brood parasites (e.g. Rothstein, 2001;

Avilés, 2004; Lahti, 2006), including the closely related Australian reed warbler *Acrocephalus australis* (Welbergen *et al.*, 2001). The absence of a temporal trend in cuckoo egg phenotype for ‘ultraviolet-brownness’ during the study period (Prediction 2) would suggest, however, that it is unlikely that improved discrimination by reed warblers was the sole mechanism involved in the change in egg matching that we have observed during the course of the study.

The second mechanism likely to favour a rapid improvement of matching is based on cuckoo abilities to select closely-matching clutches of reed warbler eggs (cuckoo preference hypothesis). This mechanism predicts a close match between cuckoo and hosts eggs within the same nest for the egg traits in which matching improved. We found that cuckoo and host eggs were matched for the ultraviolet-brownness component within nests irrespective of duration of sympatry between cuckoos and hosts (Prediction 3, Fig. 3). The correlation between cuckoo and host egg features within nests is consistent with expectations from the cuckoo preference hypothesis. Nonetheless, it seems unlikely that cuckoo ability to select well matched clutches of host eggs alone could explain the improvement of matching that we have reported in this study. If a cuckoo female learns her own egg type during her first clutch, and thereafter seeks out matching host clutches, we should see evidence of the cuckoo preference hypothesis immediately in newly parasitized populations, and there should be no improvement in matching with time. Hence, although cuckoo preference may favour improved matching over time, reed warbler rejection of nonmatching eggs should always be the ultimate selection factor leading to the evolution of increasingly mimetic cuckoo eggs.

Some alternative explanations are possible for the recorded within-nest correlation between cuckoo and reed warbler egg phenotype. It could be argued that museum collections may be a biased source of data for the study of mimicry because they refer to cuckoo eggs collected and not to cuckoo eggs laid. Thus, if hosts have ejected a proportion of cuckoo eggs before collection, and ejection of poorly mimetic eggs is more frequent than that of mimetic ones, bias may create a correlation between egg appearance of cuckoo and host eggs within nests. We cannot discard this possibility, although several indirect sources of evidence would suggest that bias caused by rapid ejection by the reed warbler of the poorest matched cuckoo eggs should be weak. Experiments performed in the study area about 100 years after cuckoos started to use reed warblers as host resulted in only two (5.9%) of 34 artificially parasitized nests ejecting nonmimetic cuckoo model eggs (B.G. Stokke, A. Moksnes & E. Røskaft, unpublished data). Furthermore, the three Norwegian reed warbler pairs in sympatry with the cuckoo that rejected model cuckoo eggs did so by desertion of their nests, and cuckoo eggs were found in the nests ($n = 26$ experimental nests in

Table 2 Determinants of cuckoo egg coloration (ultraviolet-brownness, PC2 scores from the PCA on spectrometric measures) as revealed by general linear mixed models with stepwise backward model selection involving duration of sympatry, laying date (collection date), environmental conditions (amount of precipitation in mm and mean monthly temperature for May), and host intra-clutch variation as independent fixed factors and study year as a random factor.

Dependent variable	Independent variables	β (SE)	Statistic
UV-brownness of cuckoo eggs without including UV-brownness of host eggs Overall final model: $F_{23,459} = 3.37$, $P < 0.00001$, $R^2 = 0.14$	Intercept		$F_{1,20.40} = 262.63$, $P < 0.0001$
	Laying date (fixed)		Not in the model*
	Duration of sympatry (fixed)		Not in the model*
	Rainfall (fixed)		Not in the model*
	Temperature (fixed)		Not in the model*
	Intra-clutch variation in PC2 (fixed)		Not in the model*
	Study year (random)		$F_{23,459} = 3.37$, $P < 0.0001$
UV-brownness of cuckoo eggs including UV-brownness of host eggs Overall final model: $F_{24,458} = 4.46$, $P < 0.00001$, $R^2 = 0.19$	Intercept		$F_{1,19.49} = 361.53$, $P < 0.0001$
	Laying date (fixed)		Not in the model*
	Duration of sympatry (fixed)		Not in the model*
	Rainfall (fixed)		Not in the model*
	Temperature (fixed)		Not in the model*
	Intra-clutch variation in PC2 (fixed)		Not in the model*
	UV-brownness of host eggs (fixed)	0.25 (0.04)	$F_{1,74.43} = 31.33$, $P < 0.0001$
	Study year (random)		$F_{23,458} = 2.60$, $P < 0.0001$

*Not selected by the stepwise procedure.

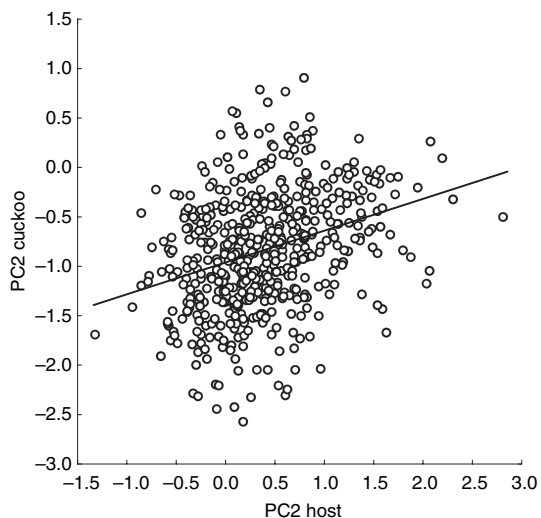


Fig. 3 Similarities between host and cuckoo egg ultraviolet-brownness (i.e. PC2 scores) within a clutch ($n = 483$ clutches). The regression line is derived from the univariate regressions of the respective color features. Results of full analysis are shown in Table 2.

2002, I. Hafstad, A. Moksnes, E. Røskaft & B.G. Stokke, unpublished data). Davies & Brooke (1988) found that only eight (19%) of 42 naturally parasitized reed warbler nests in a long-term parasitized population in Britain rejected the cuckoo egg, four by desertion and four by ejection (i.e. only 9.5% of all cuckoo eggs may have been missed because of rapid ejection). Similarly, Øien *et al.* (1998) reported 8% ejection of cuckoo eggs in a naturally parasitized reed warbler population in the Czech Repub-

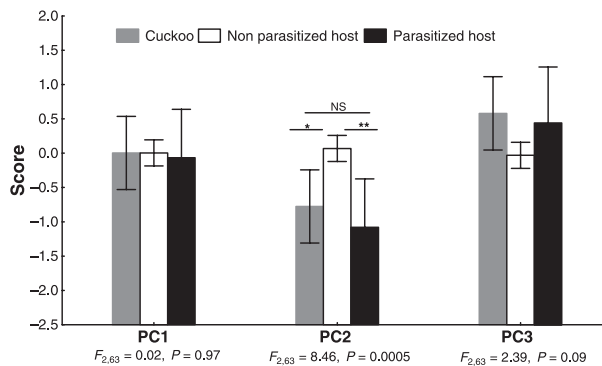


Fig. 4 Similarities between cuckoo and reed warbler egg color features at parasitized and unparasitized nests. Analyses are general linear models with egg colour feature (i.e. PC1, PC2 and PC3 scores) as dependent variables and egg type (i.e. cuckoo ($n = 7$), reed warbler at parasitized nests ($n = 4$) and reed warbler at unparasitized nests ($n = 55$), as independent fixed effects. Asterisks show significant differences between egg groups (Scheffe test: * $P < 0.05$, ** $P < 0.001$).

lic. Finally, evidence from our field data showed that reed warbler eggs in parasitized nests did not differ in ultraviolet-brownness from cuckoo eggs, but that cuckoo eggs differed greatly for the chromatic component of egg coloration from reed warbler eggs in unparasitized nests (Prediction 4, Fig. 4). In other words, in a field study in which rapid ejection was controlled by daily monitoring of nests we found that cuckoos seemed to actively select host nests containing eggs with close similarity in appearance to their own eggs. We have analysed three different colour variables (i.e. PC1, PC2 and PC3), and,

therefore, the probability of finding the same result in museum collections and in the field assuming that at least one would be significant is low ($P = 0.11$). Hence, the importance of UV-brownness in explaining mimicry and the fact that cuckoos preferred the same feature of colour of their hosts is difficult to explain by chance. However, although our field data supported the cuckoo preference hypothesis only four nests contained both cuckoo and host eggs, which is probably an insufficient sample for making firm conclusions.

Alternatively, the correlation between cuckoo and host egg features within the same nests could be the consequence of spatial autocorrelation in the diet of cuckoos and hosts (Cherry & Bennett, 2001). According to this possibility, egg matching between cuckoos and hosts would be due to cuckoos and hosts sharing similar local or ecological resources influencing egg coloration. This possibility may perhaps lead to regional differences in matching when habitats are heterogeneous. Reed warblers breed in reed beds that are patchily distributed. However, it seems unlikely that spatial autocorrelation may induce the pattern that we found at the population level because a single cuckoo female may parasitize many different available nests of the same host, which are likely to use similar resources (Wyllie, 1981). In addition, cuckoos and reed warblers rely on different food collected in different sites because cuckoos mostly feed on butterfly caterpillars that are not found in reed beds, whereas reed warblers feed on insects, spiders and small snails captured mostly at middle height in reeds (Wyllie, 1981). Another explanation for the correlation between cuckoo and host scores could be that there are storage or handling effects on individual clutches, leading to similar impacts on cuckoo and host eggs within the same clutch and differences between clutches. However, no changes in cuckoo egg coloration were detected in our museum data (Table 2), and this possibility would not explain why female cuckoos in our field study selected the best matching host clutches.

How may individual female cuckoos acquire a recognition template for the appearance of their own eggs that they presumably have limited opportunity to view? Female cuckoos may learn what their own eggs look like during their first breeding season, as suggested for rejecter hosts of avian brood parasites (e.g. Victoria, 1972; Rothstein, 1975; Lotem *et al.*, 1992). Learning would be promoted by the high consistency of egg coloration reported for females of this and other species throughout their life (e.g. Punnett & Bailey, 1920; Punnett, 1933; Collias, 1993). In addition, visits to host nests without laying by female cuckoos are frequent (Honza *et al.*, 2002). Indeed, recent radio-telemetry studies and video recordings have shown that cuckoo females lay eggs during only 70% of their visits to reed warbler nests during the host laying period (Moksnes *et al.*, 2000; Honza *et al.*, 2002). Therefore, a cuckoo female may decide where to lay based on differences in coloration between host eggs and the

learned recognition templates of their eggs that she detected during her visits to host nests.

In conclusion, we have found that the level of matching between cuckoo and reed warbler eggs increased very rapidly in a reed warbler population recently parasitized by the cuckoo. We have also provided tentative support to the hypothesis that cuckoos select nests in which to lay, rather than laying at random in the population. Cuckoo selection of host nests on the basis of the appearance of their eggs may be an important mechanism influencing matching at the earliest stages of an arms race (i.e. when selection on cuckoo egg phenotype by hosts is weak). These findings would be in agreement with previous studies suggesting that avian brood parasites have evolved extremely acute auditory and visual mechanisms leading to successful exploitation of hosts.

Acknowledgments

We thank J. Fjelds  and J. Bolding of the ornithological section of the Zoological Museum of the University of Copenhagen for help and facilities during data collection. J.J. Soler and M. Sorenson made very useful suggestions on a previous draft. This research was funded by a European Community Postdoctoral Grant (MCFI-2000-00023) to J. M. A. and by the Research Council of Norway (grant no. 151641/432) to B. G. S. The Danish Meteorological Institute kindly provided access to weather data.

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Received 16 March 2006; accepted 3 May 2006