Ultraviolet reflectance of great spotted cuckoo eggs and egg discrimination by magpies

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Hosts of obligate avian brood parasites use visual cues to distinguish between their own eggs and those of the parasite. Despite major differences between human and bird vision, most previous studies on cuckoo egg mimicry estimated color matching based on human color vision. Undetected by humans, ultraviolet reflectance (UVR) may play a previously ignored role for rejection behavior in avian brood parasite systems. We explored this possibility by manipulating UVR of great spotted cuckoo *Clamator glandarius* eggs and assessing the response of magpie *Pica pica* hosts. We coated cuckoo eggs with an ultraviolet (UV) light blocker that reduced UVR but left the human visible reflectance (400–700 nm) unaltered. The first control treatment also coated the eggs but did not alter their reflectance. A second control group of cuckoo eggs was maintained uncoated to control for handling effects on magpie discrimination. We artificially parasitized a third of a breeding magpie population with each type of experimental egg and studied the rejection of cuckoo eggs. We failed to find significant differences between rejection rate of cuckoo eggs with and without reduced reflectance in the UV region. Our results indicate that artificial reduction of UVR of cuckoo eggs does not affect the probability of ejection by magpie hosts. *Key words:* avian brood parasitism, egg discrimination, egg mimicry, great spotted cuckoo, magpie, ultraviolet vision. *[Behav Ecol 17:310–314 (2006)]*

Avian brood parasitism constitutes a well-studied example of the coevolutionary process (Rothstein, 1990). Brood parasite offspring receive parental care from unrelated hosts, and parasitized hosts usually experience a reduced reproductive output (Payne, 1997; Røkaft et al., 1990). Therefore, brood parasitism selection pressure favors the evolution of host defenses, which, in turn, select for cuckoo counterdefenses (Davies and Brooke, 1988; Soler and Møller, 1990; Soler JJ and Soler M, 2000). This coevolutionary "arms race" leads to intricate adaptations and counteradaptations by both sides, where each party responds to the selective forces imposed by the other (Dawkins and Krebs, 1979). For instance, selection for cuckoo eggs mimicking those of their host results from increasing host ability to discriminate between parasitic and their own eggs (Davies and Brooke, 1988).

A long-standing paradox within this coevolutionary scenario is why many host species accept nonmimetic eggs (Davies, 2000; Rothstein and Robinson, 1998). Two major evolutionary hypotheses have been proposed to explain why rejection is not universal among host species. Firstly, the absence of rejection behavior may be due to an evolutionary lag in the development of defensive mechanisms by the host (e.g., Davies and Brooke, 1988; Dawkins and Krebs, 1979; Hoover, 2003; Moksnes et al., 1990; Rothstein, 1975a). Lag may be due to an absence of genetic variants (Rothstein, 1975b) or the time it takes it to spread in a host population (Kelly, 1987). Secondly, rejection of parasitic eggs may be costly for hosts (Davies and Brooke, 1988; Marchetti, 1992), and such costs may sometimes exceed the benefits of rejection. Acceptance of the cuckoo egg might be adaptive according to this scenario (Brooker M and Brooker L, 1996; Lotem and Nakamura, 1998; Lotem et al., 1992; Rohwer and Spaw, 1988; Zahavi, 1979).

More recently, Cherry and Bennett (2001) have proposed a third possibility, which is related to methodological problems when estimating similarities between parasitic and host eggs. This is because using ultraviolet (UV)-visible reflectance spectrophotometry, they found that the eggs of the red-chested cuckoo *Cuculus solitarius* matched those of its African hosts for chromatic aspects of eggs invisible to humans. They argued that, due to different color sensitivity by birds and humans, egg discrimination by hosts and cuckoo-host egg mimicry might partially or totally occur for wavelengths not detectable to humans. Most diurnal birds have at least four kinds of photopigments in the cones of their eyes (Bowmaker et al., 1997), including a spectrally sensitive peak near the UV, which is absent in the human eye (Chen et al., 1984). Therefore, although birds can detect wavelengths in the same range as humans (400–700 nm), most species can also detect UV wavelengths in the range 360–400 nm (Cuthill et al., 2000). In this way, cuckoo eggs that appear mimetic to the human eye may be perceived as nonmimetic by the host (Cherry and Bennett, 2001).

Although different color sensitivity by humans and birds may be a plausible explanation for acceptance of nonmimetic cuckoo eggs, to our knowledge, this possibility has not been experimentally tested. Cherry and Bennett (2001) based their argument on differences in degree of mimicry between cuckoo and host eggs as estimated from human and bird vision, but they did not report whether hosts faced with a parasite egg in their nests behaved differently when the color of the parasite eggs was manipulated in a way imperceptible to the human eye. Here, we address this issue by assessing the role of ultraviolet reflectance (UVR) of great spotted cuckoo *Clamator glandarius* eggs in determining discrimination and ejection of parasitic eggs by the corvid magpie *Pica pica* hosts in a field experiment. The magpie is the main host of the great spotted cuckoo in Europe (Soler, 1990). In this parasite-host system, the magpie is able to discriminate and eject cuckoo eggs from its nest (Alvarez et al., 1976; Soler, 1990). Magpies use visual cues to distinguish between their own eggs

and those of great spotted cuckoos, with cuckoo eggs that are poorly mimetic to the human eye being more frequently rejected than good mimetic eggs (e.g., Soler and Møller, 1990; Soler JJ and Soler M, 2000). Here, we coated experimental cuckoo eggs with a smear that absorbed UV (360–400 nm), but left the human visible reflectance (400–700 nm) unaltered, and with horse fat as a control treatment that caused cuckoo eggs to retain their color but with a similar level of handling as the former. Finally, a second control group of non-manipulated cuckoo eggs was established to control for the effect of handling on magpie egg discrimination. We artificially parasitized a third of a breeding magpie population with each of these three kinds of experimental eggs. Because experimental eggs with reduced reflectance in the UV region show poorer mimicry with host eggs in the avian spectrum of vision than control eggs, a high rejection rate of these eggs is predicted if magpies used UV vision to assess the degree of dissimilarity between their own eggs and parasitic eggs. Nonetheless, information on whether magpies can actually see in the UV range is absent. The analysis of spectral sensitivities of retinas through microspectrophotometry provides widespread evidence of UV vision among passerines, although it has not been made for the corvid magpie (reviewed in Cuthill et al., 2000). Recent genetic analyses have revealed that the short-wavelength cone pigment of the corvid jackdaw *Corvus monedula* and the hooded crow *Corvus corone* has a wavelength of maximum absorbance (λ-max hereafter) at 408 and 406 nm, respectively (Ödeen and Håstad, 2003). However, pigments are also functional at wavelengths others than λ-max (Cuthill et al., 2000). Bird sensitivity beyond the λ-max is due to the ocular media (cornea, aqueous humor, lens, and vitreous humor) of all the birds (except the mallard) studied to our days having a relatively high transmission of short wavelengths. This anatomical organization permits to violet sensitive/ultra violet sensitive pigment of birds to confer considerable UV sensitivity (Cuthill et al., 2000). Consequently, the fact that the λ-max for the two corvid species for which information is currently available is at 406 and 408 nm does not mean that these two corvid species are not able to perceive UV information. This value means that, because sensitivity to a target wavelength decreases as differences with the λ-max increase (see Figure 1 in Cuthill et al., 2000), sensitivity at the UV (<400 nm) would be weaker, but detected, than that at 407 nm. Therefore, it is more than possible that magpies detected UVR above 360 nm, which was the minimum reflectance we measured in our experiment.

METHODS

The study was carried out in Hoya de Guadix, southern Spain (37° 18′ N, 3° 11′ W) during April and May 2004 (see Soler et al., 1998, for a detailed description). The magpie is a monogamous passerine typically used as a host by the brood parasitic great spotted cuckoo in this area (mean parasitism rate = 54.83%, *n* = 766 nests [Soler et al., 1998]). Magpies in our study area usually lay six or seven eggs (range 2–10 eggs [Soler et al., 2001]). The female incubates the eggs some days after the first egg is laid. Magpies in the study area reject model cuckoo eggs with a frequency around 40–50% (Soler JJ and Soler M, 2000).

The study of host response against experimentally inserted parasitic eggs is the most frequently used methodology in avian brood parasitism studies to distinguish between individuals with respect to their discrimination abilities within a host population (e.g., Davies, 2000; Davies and Brooke, 1988; Lotem et al., 1992; Rothstein, 1975a; Soler and Møller, 1990; Soler et al., 1998). According to this methodology, hosts are classified as rejecters (nest deserters or egg ejectors) or accep-
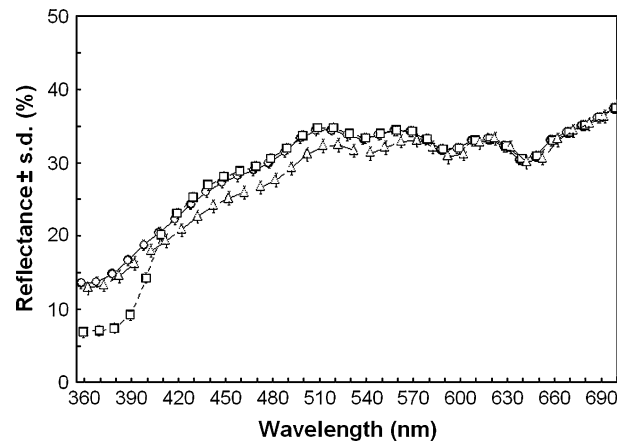
Figure 1
Reflectance spectra (mean ± SD) of great spotted cuckoo eggs before (*n* = 86 eggs, open circles) and after (*n* = 43 eggs, open squares) treatment with an UV light blocker and a control treatment (i.e., coated with horse fat) (*n* = 43 eggs, open triangles). Reflectance spectra of noncoated control eggs are included in that of cuckoo eggs before treatment.

tors of parasitic eggs. We searched for magpie nests at the beginning of the 2004 breeding season. We found 132 nests at different stages of building, which were randomly assigned to one of the following three groups: experimentally reduced UVR (UVR, *n* = 43), control coated with horse fat (*n* = 43), and uncoated control (*n* = 46). We introduced in each group of nests one unhatched great spotted cuckoo egg collected in the study area. Cuckoo eggs were taken from magpie nests before magpies started to lay in the same season in which this experiment was performed. Cuckoo eggs were used fresh after collection and saved to experiment time in closed boxes to diminish possible fading. We got a number of cuckoo eggs lower than the number of experimental nests, then experimental cuckoo eggs were reutilized twice with the same experimental treatment.

The time interval between laying of the first magpie egg and the beginning of the experiment varied. However, magpie response to artificial parasitism does not vary between laying and incubation (Alvarez et al., 1976; Soler and Møller, 1990). As in previous studies with this system, host response to the foreign eggs was assessed 72 h after manipulation (see Avilés et al., 2004; Soler and Møller, 1990; Soler et al., 1998, 1999).

The UVR treatment consisted of coating real cuckoo eggs with an UV light blocker (50/50 w/w blend of Parsol 1789 and MCX, Roche, Basel, Switzerland), while cuckoo eggs in the first control group were coated with horse fat. Changes in the spectral shape of cuckoo eggs for the two treatments are shown in Figure 1. Reflectance spectra in the range 360–700 nm were obtained from all experimental cuckoo eggs using a spectroradiometer (Konica Minolta Sensing [Seoul, South Korea], CM-2600d) measuring at 10-nm intervals. Eggs with reduced UVR and those in the first control group were measured before and after coating with their respective treatments. As in Cherry and Bennett (2001), 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. Color was measured once in each of these five bands. The egg was illuminated at 90° to the measuring surface by a xenon light source and the reflected light captured at the same angle. The measurements were relative and referred to a standard white reference (CM-A145, Konica Minolta Sensing) and to a standard dark (CM-A32, Konica Minolta Sensing). A reference

and dark calibration was made before the beginning of measurement of each egg. The mean reflectance spectrum for each egg was calculated from the five spectra taken from each cuckoo egg. The UVR and control treatments minimally affected the reflectance of magpie eggs in the human visible spectrum, while the UVR treatment markedly reduced magpie egg reflectance in the range below 400 nm (Figure 1). We detected a limited time for persistence of the UV-block effect on cuckoo eggs (i.e., UVR increased to an intermediate value at 48 h after coating). Therefore, we revisited experimental magpie nests every day for coating cuckoo eggs with UV blocker and also with horse fat in control nests. In addition, cuckoo egg treatments did not alter the spectral shape of magpie eggs at the experimental nests after 48 h, suggesting that the treatment was not transferred to adjacent eggs of the host.

Statistical methods

We used generalized linear models (GENMOD procedure; SAS Institute, 1996) to test for an association between the occurrence of rejection and experimental treatment. Probability of rejection of each experimental egg was modeled as a binomial response variable (1 = rejection, 0 = acceptance) using a logistic link function. Because proneness to rejection of cuckoo eggs could vary during the season (e.g., Lotem et al., 1992), we also entered magpie laying date as an independent variable in the model. The starting model contained the main effects plus the only possible interaction. Model selection was carried out by removing, one by one, the effects that were the furthest from statistical significance, starting with the highest order interaction down to the main effects.

Ethical note

The hypothesis under investigation required the insertion of great spotted cuckoo eggs into experimental magpie nests. Cuckoo eggs were taken from magpie nests before magpies started to lay in the same season in which this experiment was performed. Every year, many great spotted cuckoo eggs mislaid before magpies even start laying and depredated, ejected, or buried by magpies when they start to lay are (Avilés JM, personal observation). Therefore, these cuckoo eggs would have been doomed to failure even if they had not been used in the experiment. As far as we were able to determine, no magpie eggs were crushed because of our manipulations, and no effect on magpie egg-hatching success relative to nonexperimentally treated nests was detected. Our experiment was carried out under special licences from the Spanish government and Junta de Andalucía for animal experiments.

RESULTS

Magpie responses to experimental eggs are summarized in Figure 2. Eighteen out of 132 (13.6%) magpie pairs rejected the experimental eggs (Figure 2).

Blocking of UVR of cuckoo eggs had no perceptible effect on the probability of rejection of real cuckoo eggs by magpies (Figure 2, logistic regression model, treatment effect: $\chi^2 = 1.10$, df = 2, $p = .58$). It is important to note that magpies rejected coated and uncoated experimental eggs at similar rates irrespective of UVR, suggesting no bias by handling in our experiment (Figure 2). No effect of laying date was detected on magpie response to experimental treatments (logistic regression model, treatment × laying date effect: $\chi^2 = 0.18$, df = 2, $p = .91$).
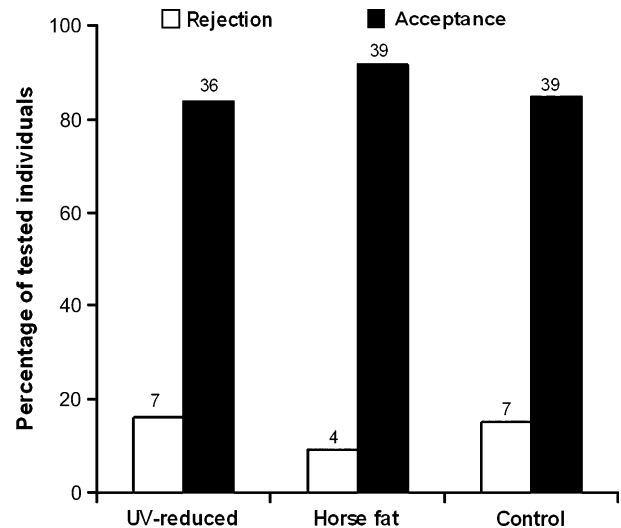
Figure 2
Magpie responses to great spotted cuckoo eggs with reduced UVR and controls (coated with horse fat or uncoated).

DISCUSSION

Cherry and Bennett (2001) have recently provided evidence for a new hypothesis that may potentially explain why some host species accept parasitic eggs. They found that eggs of the red-chested cuckoo and its African hosts were highly matched in chromatic aspects invisible to humans. From this result, they hypothesized that cuckoo eggs that appear nonmimetic to the human eye could be perceived as mimetic by the host. This hypothesis would clearly predict changes in host rejection behavior as color of cuckoo eggs is manipulated in a way imperceptible to the human eye but perceptible to birds.

This study constitutes, to our knowledge, the first experimental test of the role of differences between human and bird vision on egg rejection behavior of a host of an obligate avian brood parasite. The results of this study do not provide evidence for magpie rejection behavior being determined by variation in UVR, which is imperceptible to humans but detectable by birds (Bowmaker et al., 1997; Chen et al., 1984). We manipulated cuckoo eggs by reducing reflectance at wavelengths below 400 nm and with a control treatment that minimally affected original reflectance. Differences in reflectance between experimental eggs with reduced reflectance in the UV region and control eggs did not explain magpie rejection behavior. Therefore, our result suggests that the UV reduction treatment did not affect egg discrimination behavior by magpies and thus that UV cues alone did not effectively signal parasitism to the host in this system.

Differences between human and avian color vision are typically assumed because the existence of UV-sensitive cones has been confirmed in eight Passeriformes species in which avian pigments were assessed by microspectrophotometry (see review in Cuthill et al., 2000). Moreover, electrophysiological and behavioral experiments provide widespread evidence of UV vision in birds (Cuthill et al., 2000). Recent genetic findings would suggest that corvids may be special among the passerines with respect to their vision because they had the wavelength of maximum absorbance for the short-wavelength cone pigment slightly more than 400 nm (Ödeen and Håstad, 2003). That is, although they perceive UV light (see Introduction), they would show a wavelength sensitivity bias toward violet with a peak of maximal sensitivity between 406 and 408 nm. Therefore, a plausible explanation for the fact that magpies, which are corvids, do not appear to use UV signals in this context would be that

they have low sensitivity to UVR. Another possible explanation for our results is that UV cues were too weak to be considered by hosts. Great spotted cuckoo, but also magpie, eggs reach their minimum spectral value at the UV wavelength (Avilés et al., 2004, Figure 1). Following this train of thought, magpies should be unable to distinguish between parasitic eggs modified in the UV part of the spectrum just because differences between UV reduction and control manipulation of reflectance were unimportant considered over the complete visual range of this bird. This is a plausible explanation because it is known that, for magpies, probability of rejection of nonmimetic eggs is much larger than that of mimetic model eggs as judged by humans (Alvarez et al., 1976; Soler and Møller, 1990; Soler JJ and Soler M, 2000; Soler et al., 1999).

We performed 132 cuckoo egg–recognition experiments, and only 18 magpie pairs rejected the experimental eggs. Moreover, percentage of rejecter magpies did not differ between experimental groups and, thus, the null hypothesis (i.e., UV does not affect egg recognition and rejection decision by magpies) was not rejected. However, taking into account the small effect size induced by our manipulation (UV versus horse fat treatment, effect size = 0.1; UV versus control treatment, effect size = 0.015; and horse fat versus control treatment, effect size = 0.09), our sample size was quite small for accepting the null hypothesis. With that sample size, our experiment yielded a maximum detectable effect size of 0.27 for a power of 0.8 (see Cohen, 1988). Soler JJ and Soler M (2000) have reported rejection rates of mimetic and nonmimetic model eggs at Guadix of, respectively, 44% and 97%, which results in an effect size of 0.67, far above our detectable effect size. Thus, our sample size would be large enough to detect significant statistical differences even with less than half of the effect size detected when comparing rejection rate of mimetic and nonmimetic model eggs.

Alternatively, it could be argued that magpies could mainly use certain wavelengths within the human vision range (400–700 nm) for parasitic egg recognition, disregarding UV information. This result would be compatible with Cherry and Bennett (2001) hypothesis stating that hosts may use certain combinations of wavelengths in cuckoo egg discrimination. In a previous study, we have shown that the largest differences between the spectral shapes of magpie and great spotted cuckoo eggs were in the visible part of the spectrum, while cuckoo eggs showed a relative better matching with magpie eggs in the UV region (Avilés et al., 2004). If great spotted cuckoos have a lower potential for mimicking magpie eggs in the visible part of the spectrum, selection may have favored magpies using information from that color rather than from the complete spectrum.

In conclusion, our study reveals that UVR had a negligible effect on host perception of mimicry in at least one avian host-brood parasite system. Therefore, our results do not support the hypothesis that differences between human and avian vision may explain acceptance of nonmimetic cuckoo eggs to the human eye for a corvid. Because most hosts of avian brood parasites are, of course, passerines and not corvids, which may vary in their UV signal perception, future studies should test the generality of this finding by using a similar experimental approach in other noncorvid passerines.

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