

# Eavesdropping cuckoos: further insights on great spotted cuckoo preference by magpie nests and egg colour

Juan J. Soler · Jesús M. Avilés · David Martín-Gálvez ·  
Liesbeth de Neve · Manuel Soler

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**Abstract** Reproductive success of brood parasites largely depends on appropriate host selection and, although the use of inadvertent social information emitted by hosts may be of selective advantage for cuckoos, this possibility has rarely been experimentally tested. Here, we manipulated nest size and clutch colouration of magpies (*Pica pica*), the main host of great spotted cuckoos (*Clamator glandarius*). These phenotypic traits may potentially reveal information about magpie territory and/or parental quality and could hence influence the cuckoo's choice of host nests. Experimentally reduced magpie nests suffered higher predation rate, and prevalence of cuckoo parasitism was higher in magpie nests with the densest roofs, which suggests a

direct advantage for great spotted cuckoos choosing this type of magpie nest. Colouration of magpie clutches was manipulated by adding one artificial egg (blue or cream colouration) at the beginning of the egg-laying period. We found that host nests holding an experimental cream egg experienced a higher prevalence of cuckoo parasitism than those holding a blue-coloured egg. Results from these two experiments suggest that great spotted cuckoos cue on magpie nest characteristics and the appearance of eggs to decide parasitism, and confirm, for the first time, the ability of cuckoos to distinguish between eggs of different colours within the nest of their hosts. Several hypothetical scenarios explaining these results are discussed.

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J. J. Soler (✉) · J. M. Avilés · D. Martín-Gálvez  
Departamento de Ecología Funcional y Evolutiva,  
Estación Experimental de Zonas Áridas (CSIC),  
4120 Almería, Spain  
e-mail: jsoler@eeza.csic.es

J. M. Avilés  
e-mail: javiles@eeza.csic.es

J. J. Soler · J. M. Avilés · M. Soler  
Grupo Coevolución, Unidad Asociada al CSIC,  
Universidad de Granada, Granada, Spain  
e-mail: msoler@ugr.es

**Present Address:**  
D. Martín-Gálvez  
European Molecular Biology Laboratory, European  
Bioinformatics Institute, Wellcome Trust Genome Campus,  
Hinxton, Cambridge CB10 1SD, UK  
e-mail: damargal@ebi.ac.uk

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L. de Neve  
Terrestrial Ecology Unit, Department of Biology,  
Ghent University, 9000 Ghent, Belgium  
e-mail: liesbeth.deneve@UGent.be

M. Soler  
Departamento de Zoología, Facultad de Ciencias,  
Universidad de Granada, 18071 Granada, Spain

## Introduction

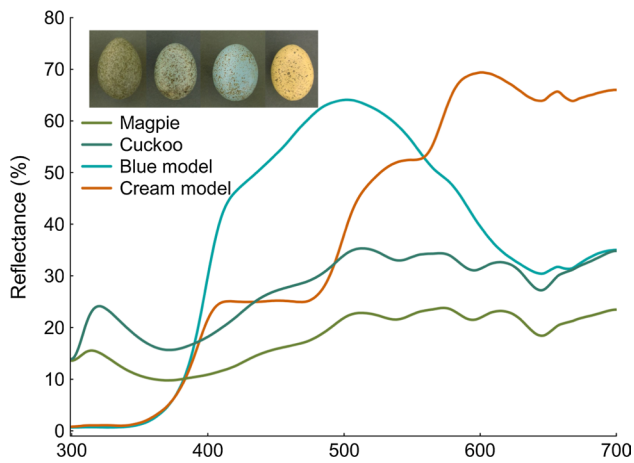
Avian brood parasites lay their eggs in the nests of their hosts, which incubate and rear parasitic offspring. Reproductive success of obligate brood parasites therefore depends on finding an appropriate host and brood parasite cognitive abilities allowing the detection of a suitable host nest where parasite offspring will experience high probability of survival will thus rapidly spread. Appropriate hosts may be those breeding in suitable habitats (Røskaft et al. 2002; Álvarez 1993; Martín-Vivaldi et al. 2012), with nests and diets that respectively allow brood parasitism and a proper development of parasite nestlings (Moksnes and Røskaft 1995; Grim et al. 2011), with relatively lower probability of nest predation (Avilés et al. 2006b) and/or lower ability to detect and avoid parasitism in their nests, either by mobbing parasites (Welbergen and Davies 2009) or rejecting eggs (Procházka and Honza 2003; Honza et al. 2004; Martín-Vivaldi et al. 2012) or nestlings (Langmore et al. 2003; Sato et al. 2010).

Within a given population of a host species, brood parasites may lay their eggs randomly in available host nests or, alternatively, may base their nest choice on inadvertent social information (ISI; sensu Danchin et al. 2004) provided by individual host phenotypes and/or activities that are related to territory quality, willingness to invest in reproduction (i.e. parental ability) (Soler et al. 1995a) or even probability of nest predation (Avilés et al. 2006b). Brood parasites may thus evaluate host phenotypes and select those host nests that offer the best chance of survival for their offspring (review in Parejo and Avilés 2007). Empirical work has shown that some nest attributes such as nest size or the density of the nest roof are likely related to nest accessibility or visual location by predators in magpies (*Pica pica*) and other birds (Hansell 2000; Quesada 2007), thereby suggesting an adaptive advantage in terms of predation avoidance for brood parasites that eavesdropped on those attributes. Nest characteristics can also reveal information about host willingness to invest in reproduction (Soler et al. 1998b) as nest size may function as a sexual signal of parental quality (Moreno et al. 1994; Soler et al. 1995a, 1996, 1998a, 2001; Palomino et al. 1998; De Neve and Soler 2002; De Neve et al. 2004; Szentirmai et al. 2005; Sanz and García-Navas 2011). Nowadays, evidence supporting the hypothesis that brood parasites may evaluate host's nest characteristics (e.g. size) for host selection is only correlative and not consistent between different brood parasite–host systems. For instance, the probability of brood parasitism was related to nest size in magpies (Soler et al. 1995a; Molina-Morales et al. 2012), the main host species of the great spotted cuckoo (*Clamator glandarius*) in Europe. The selection of host nest by the brood parasitic Asian koel (*Eudynamis scolopacea*) is also related to nest size (Begum et al. 2011a). However, the association between probability of parasitism and nest size was

not detected for some host species of the European cuckoo *Cuculus canorus* (Antonov et al. 2007; Avilés et al. 2009). Large host nests may, however, be more easily detected by brood parasites which may also explain the detected relationship between risk of parasitism and nest size. Thus, for testing the possibility that brood parasites eavesdrop on characteristics of host nests revealing parental quality for host selection, evidence supporting the association between nest size and host traits that enhance reproductive success of parasites is necessary.

Brood parasites may also benefit from eavesdropping on characteristics of eggs found in host nests because of several reasons. Gathering information on colour characteristics of eggs in host nests would allow female parasites to detect other parasitic eggs in host nests, and, thus, either copy previous parasitism and assure the use of hosts that accept parasitic eggs (White et al. 2007), or avoid nests that already included parasitic eggs in order to reduce competition with co-specific offspring (Ortega et al. 1994). Brood parasites may even recognize and destroy previously laid parasitic eggs, which would select for mimetic eggs (Brooker et al. 1990; Spottiswoode 2013). Moreover, particular colour patterns of host eggs may increase the likelihood of predation or brood parasitism and act as blackmail for males to increase their nest attendance during the incubation phase to keep eggs covered (Hanley et al. 2013). In fact, blue-green colouration of eggshells was related to the probability of parasitism (Hanley et al. 2013) and rejection of cuckoo eggs by hosts (e.g. Avilés et al. 2004; Soler et al. 2012), which may potentially add benefits for cuckoos which were able to eavesdrop on egg characteristics (e.g. colouration) aiming to reduce the chance of egg-rejecter hosts. Eavesdropping cuckoos may also gain information about the level of parental investment that their offspring will receive by cueing on host egg colouration. This possibility is based on the hypothesis that blue-green colour intensity of eggs might function as a sexually selected signal of females that would be positively related to the level of parental investment received by the offspring (Moreno and Osorno 2003; Soler et al. 2005; Moreno et al. 2008; Soler et al. 2008; Hanley and Doucet 2009; Sanz and García-Navas 2009; English and Montgomerie 2011; but see, Krist and Grim 2007; Reynolds et al. 2009; Honza et al. 2011; Hanley et al. 2013).

As far as we know, the association between the appearance of eggs in host nests and the probability of parasitism has only been tested experimentally in a captive population of the brown-headed cowbird (*Molothrus ater*). Juvenile females, but not adults, preferred to parasitize nests that contained cowbird model eggs with particular size and colour patterns (White et al. 2007). In the European cuckoo, a number of studies have reported non-random laying in relation to host egg colour attributes (Avilés et al. 2006a; Cherry et al. 2007; Polacikova et al. 2009; Begum et al.



**Fig. 1** Reflectance spectra of magpie (*Pica pica*) (green line), great spotted cuckoo (*Clamator glandarius*) (green–blue line) and experimental blue (blue line) and cream (orange line) model eggs. Pictures of each egg type and in the same legend order are also shown (color figure online)

2011b), which may suggest a possible preference of female cuckoos for particular host egg colour shades. However, egg colouration may be related to other host characteristics and thus experimental manipulations of colouration are necessary to test whether cuckoo parasites are using the appearance of eggs of their hosts as ISI for host selection. Here, we provide the first experimental test of whether cuckoos use inadvertent social information of their hosts (i.e. the eavesdropping hypothesis) by experimentally manipulating attributes of nests and clutch colouration, and explore its effects on cuckoo parasitism.

The great spotted cuckoo–magpie system is ideal for testing this idea experimentally. First, the size of the roof in magpie nests is a sexually selected trait (Soler et al. 2001), and it is positively related to the probability of great spotted cuckoo parasitism (see above) and negatively to the probability of predation (Baeyens 1981; Quesada 2007). Since offspring of great spotted cuckoos parasitizing larger and denser magpie nests could benefit from a lower probability of predation and/or a better nutritional environment during development, we predict that magpie nests with enlarged domes will experience the highest probability of parasitism. Concerning the egg colour experiment, we used model eggs of two different colourations: blue and cream, with reflectance curves that on average differed equally from that of magpie eggs, but the blue model was more similar to the average reflectance curves of great spotted cuckoo eggs (see Fig. 1). Thus, cream eggs can be considered the control treatment of the hypothesis of attractive blue eggs, and we can predict that adding a blue-coloured egg to magpie nests should increase the likelihood of parasitism because bluer eggs could be positively related to lower egg rejection

rates and/or higher levels of parental investment in magpies (see above). In any case, if cuckoos showed a non-random egg-laying pattern in relation to model egg colouration, this would confirm that, independent of the evolutionary mechanism behind preference bias, cuckoos are able to discriminate egg colour traits in magpie nests.

## Materials and methods

### Study area, fieldwork and experimental design

The magpie population of Guadix (37°18'N, 3°11'W, southern Spain) is situated in a high-altitude plateau (approx. 1,000 m a.s.l.). The vegetation is sparse, including cultivated cereals (especially barley) and many groves of almond trees (*Prunus dulcis*), pines (*Pinus halepensis* and *P. pinaster*), and oaks (*Quercus rotundifolia*) in which magpies build their nests (Soler 1990). Magpies build domed, almost spherical, large nests with a stick framework. After the framework is finished, a bowl of mud is built inside and lined with fibrous roots, hair, and grass (Birkhead 1991). Both members of the pair participate in nest building, but the male makes significantly more trips to collect mud and large twigs than the female (Birkhead 1991). Fieldwork was conducted during the breeding season of 2009 at three magpie subpopulations present in the study area (Martín-Gálvez et al. 2007; Soler et al. 2013). The probability of parasitism differed among these subpopulations [Carretera (38.9 %,  $n = 18$ ), Huéneja (77.8 %,  $n = 18$ ) and Charches (63.16 %,  $n = 76$ ); maximum likelihood  $\chi^2 = 5.99$ ,  $df = 2$ ,  $P = 0.0499$ ]. The probability of predation did not differ among study plots [Carretera (5.3 %,  $n = 19$ ), Huéneja (20.0 %,  $n = 20$ ) and Charches (18.3 %,  $n = 82$ ); maximum likelihood  $\chi^2 = 2.61$ ,  $df = 2$ ,  $P = 0.27$ ]. Thus, the identity of study plots was included in the statistical models that explored the effects of nest and clutch characteristics on brood parasitism but not on the probability of predation (see below).

### Nest size manipulation: field procedures

At the beginning of spring (mid March–early April), we intensely searched for new magpie nests during the nest-building stage. We performed the nest size manipulation in the final phase of construction, namely when magpies start to cover the cup with soft material (Birkhead 1991). This manipulation consisted in removing a bag (80 × 80 × 50 cm) of sticks from 51 nests (hereafter reduced nests) and adding these previously collected sticks to the nest roof of another 53 nests (hereafter enlarged nests). Another group of nests ( $n = 55$ ) were left as manipulation control (i.e. some of the nest roof sticks were removed and included again in the nest structure without affecting nest size) and were visited at the

same rate as the enlarged and reduced ones. Before and after manipulation, we measured the size of nests using a flexible steel tape (precision  $\pm 1$  cm) and calculated nest volume (in litres), and estimated density of branches in the nest roof using semi-quantitative scales. Visibility of the nests was also estimated in a semi quantitative scale during the egg laying stage (see electronic supplementary material, ESM) and, thus, was not estimated for all nests (Table 1 in ESM). On average, volume, stick density, and visibility of reduced and enlarged nests decreased and increased, respectively as consequence of our manipulation (see Figure in the ESM).

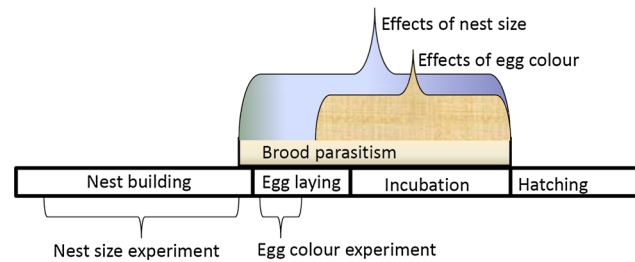
Magpies laid eggs in 76.1 % of the manipulated nests and the frequency of nests that were abandoned before egg laying was not biased in relation to the experimental treatment (enlarged: 20.75 %, control: 23.64 %, and reduced: 27.45 %,  $\chi^2 = 0.64$ ,  $df = 2$ ,  $P = 0.72$ ). On average, magpies started reproduction (i.e., laying date) 16.5 (SE = 0.76) days after the nest size manipulation.

During the egg-stage period, nests were visited regularly to assess great spotted cuckoo parasitism and to perform the egg colour experiment (see below). We recorded parasitism until the time of hatching because great spotted cuckoos may parasitise magpie nests through the whole incubation period. We used the nest parasitism status (i.e. parasitised or non-parasitised) and the number of parasite eggs detected in parasitised magpie nests (i.e. intensity of parasitism hereafter) as an estimation of great spotted cuckoo preference when exploring the effect of nest size manipulation.

#### Egg colour experiment: field procedures

Before the start of egg incubation (i.e. usually when the fourth–fifth egg was laid) we added an artificial egg to each magpie nest (number of magpie eggs present in the nest at the time of the experiment: mode = 1, median = 3).

Artificial eggs were made of plaster of Paris resembling the shape of great spotted cuckoo eggs but either intensely blue or cream coloured (Fig. 1). While blue model eggs reflected strongly at the blue–green wavelength, cream eggs mostly reflected at the yellow–red wavelength interval (Fig. 1). A blue or cream model egg was randomly introduced in each of the nests used for the nest size manipulation experiment. At the time of artificial egg introduction some magpie nests were already parasitized (30.6 %,  $N = 108$ ). However, since egg colour treatment (i.e. the addition of one artificial blue or cream coloured egg) was randomly assigned (see above), it was not associated to the probability of parasitism ( $\chi^2 = 0.14$ ,  $df = 1$ ,  $P = 0.71$ ) or the number of parasite eggs found in a nest before the egg manipulation [nests with a cream model, mean (SE) = 0.54 (0.15), nests with blue model, mean (SE) = 0.57 (0.13),  $F = 0.03$ ,  $df = 1, 106$ ,  $P = 0.87$ ]. Estimates of great spotted cuckoo preference (nest parasitism status and intensity) in



**Fig. 2** Timeline of the performed experiments at different stages of magpie reproduction. The figure shows that the response to each experiment does not coincide temporally. While the effect of nest size manipulation on brood parasitism can be estimated during the complete egg period, the effect of the egg colour experiment (and the interaction with the nest size experiment) can only be explored on parasitism that occurred after the egg colour experiment was performed

relation to the egg colour experiment were therefore calculated for the period after the experiment was performed and extended to the entire incubation period (Fig. 2).

#### Statistical analyses

Laying date and laying date squared were included in all statistical models as covariates to account for the possible linear and nonlinear influence of laying date on the probability and intensity of parasitism and nest characteristics. The identity of study plots was also included in all models as an additional factor to control for variation in the probability of parasitism (see above).

Experimental effects of nest size and egg colour manipulations were explored in separate models because the estimates of the probability and intensity of parasitism (i.e. response variables) were not the same for the two experiments (Fig. 2). Parasitism estimates for the nest size experiment refer to the entire egg-laying plus incubation period of magpies, while those for the egg colour experiment were restricted to the period after the artificial egg was introduced (i.e. whether or not a magpie nest was parasitized after the egg colour experiment and how many cuckoo eggs it received after the experiment) (Fig. 2). Because the two different time intervals used for parasitism estimates included periods for which the magpie nests were influenced by the two experiments, when exploring the effect of nests size, the statistical models were ran with and without information of the egg colour experiment. Similarly, when analysing the effect of egg colour experiments the statistical models were ran with and without information of the nest size experiment. Consequently, the effect of the interaction between the two experimental treatments can only be estimated in models explaining the probability and intensity of parasitism estimated for the time period after the egg colour experiment (i.e. when effects of both manipulations occurred simultaneously during the same time period) (Fig. 2; see below).

**Table 1** Generalized linear models testing for the effect of the experimental manipulation of nest size on the probability of parasitism by great spotted cuckoo (*Clamator glandarius*) on magpie (*Pica pica*)(binomial distribution and logit link function,  $n = 109$ ) and on the intensity of parasitism (Poisson distribution and log link function, only parasitised nests were considered,  $n = 65$ )

Factors	Probability of parasitism				Intensity of parasitism			
	Estimate (SE)	Wald statistic	df	P	Estimate (SE)	Wald statistic	df	P
Including nest experimental treatment								
Laying date	0.276 (0.199)	1.93	1	0.15	<b>-0.163 (0.055)</b>	<b>11.61</b>	1	<b>0.0007</b>
Laying date <sup>2</sup>	-0.005 (0.005)	2.01	1	0.17	<b>0.004 (0.001)</b>	<b>7.70</b>	1	<b>0.006</b>
Nest experiment		1.14	2	0.57		3.26	2	0.88
Study plot		<b>5.55</b>	<b>2</b>	<b>0.062</b>		3.03	2	0.22
Including nest measurement after the experiment								
Laying date	0.225 (0.221)	1.03	1	0.31	<b>-0.163 (0.055)</b>	<b>10.43</b>	1	<b>0.001</b>
Laying date <sup>2</sup>	-0.005 (0.005)	1.17	1	0.28	<b>0.004 (0.001)</b>	<b>8.21</b>	1	<b>0.004</b>
Nest volume	1.06 (1.227)	0.75	1	0.39	0.519 (0.522)	1.06	1	0.30
Roof density	<b>-0.503 (0.259)</b>	<b>3.77</b>	<b>1</b>	<b>0.052</b>	-0.013 (0.143)	0.01	1	0.96
Nest visibility	0.064 (0.294)	0.05	1	0.83	-0.194 (0.133)	2.19	1	0.14
Study plot		<b>5.16</b>	<b>2</b>	<b>0.076</b>		<b>95.34</b>	1	<b>0.069</b>

Results of different full models are presented, either including the experimental treatment or the nests characteristics (nest volume, roof density and nest visibility) after the experiment as independent factors (sample size for models analysing probability and intensity of parasitism, respectively,  $n = 89$ ,  $n = 52$ ). Laying date and laying date squared were included to statistically control for their potential effects on parasitism. The identity of study plots was also included as a fixed factor because the probability of parasitism differed among study plots (see “Materials and methods”)

For facilitating visualisation of the detected tendencies, variables and statistics with associated  $P$  values lower than 0.1 are shown in bold

Because we did not find a significant effect of the nest size experimental manipulation on the probability of parasitism (see “Results”), we further explored the association between nest characteristics (i.e. log-10-transformed nest volume, roof density and nest visibility) after manipulation on the probability and intensity of parasitism. Variables describing magpie nests were not strongly related to each other, the shared variance was always lower than 50 % ( $n = 89$ , including magpie nests with estimations for all three variables); nest volume–roof density:  $R^2 = 0.41$ ,  $t = 7.77$ ,  $P < 0.0001$ ; nest visibility–nest volume:  $R^2 = 0.01$ ,  $t = 1.09$ ,  $P = 0.28$ ; nest visibility and roof density:  $R^2 = 0.11$ ,  $t = 3.31$ ,  $P = 0.001$ . Thus, including all these variables in the same model does not imply problems of collinearity (Quinn and Keough 2002). In any case, we ran the models including the variables describing magpie nests one by one and, except for the probability of predation, results did not differ qualitatively.

Magpies are able to eject experimental model eggs from their nests (Soler and Soler 2000), which may occur before great spotted cuckoos visited magpie nests, which could affect our results. To statistically account for this possibility, the response of magpies to experimental model eggs (eject or accept, estimated 7 days after the introduction of the model egg) was included in the model exploring the effect of egg colour manipulation on the probability and intensity of parasitism.

The effects of the two experimental manipulations (nest size and egg colouration) and nest size characteristics on the

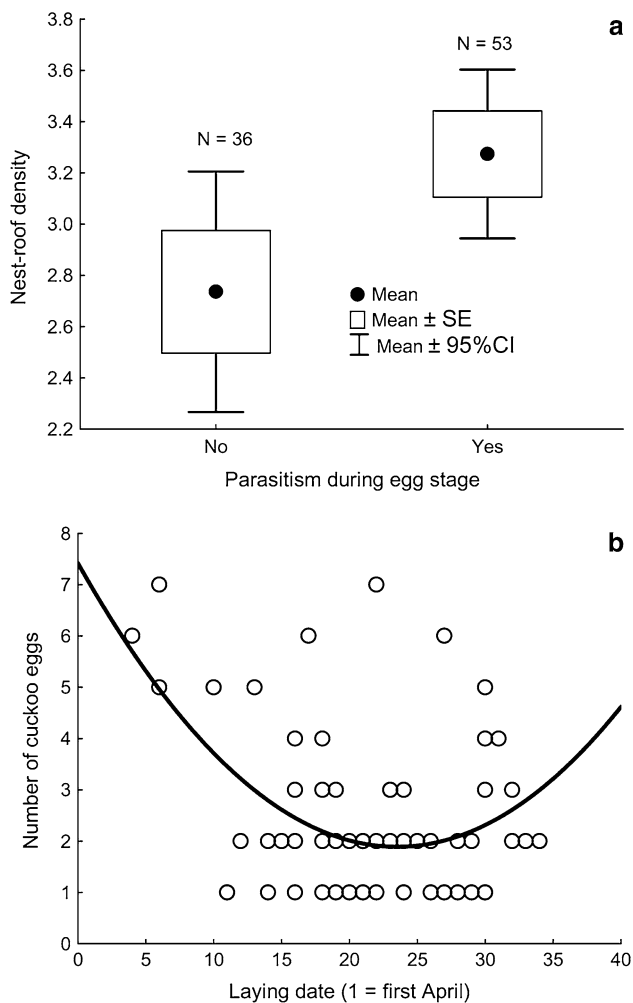
probability of parasitism or predation were separately explored by means of generalised linear models (GLM) with binomial error and logit link function, while the effects on intensity of parasitism were explored only considering parasitised magpie nests by means of GLM with Poisson distribution and log-link function. The effect of the interaction between the two performed experiments on the probability and intensity of parasitism was explored for the period after the egg-colour experiment (see above, Fig. 2). All statistical tests were two-tailed and performed in Statistica 10.1 (Statsoft 2011). Reported values are mean  $\pm$  SE in the text and mean  $\pm$  95 % confidence intervals in the figures. The dataset used for these analyses is available as ESM.

## Results

### Magpie nest characteristics and the probability and intensity of parasitism

We did not find any evidence that the nest size manipulation affected the probability of great spotted cuckoo parasitism (reduced: 56.25 %,  $n = 32$ ; enlarged: 64.10 %,  $n = 39$ , control: 63.41 %,  $n = 41$ ) or its intensity (i.e. number of cuckoo eggs laid per parasitised nest) (reduced:  $2.28 \pm 0.24$ ,  $n = 18$ ; enlarged:  $2.48 \pm 0.34$ ,  $n = 23$ , control:  $2.46 \pm 0.39$ ,  $n = 26$ ) (Table 1). Roof density estimated after the manipulation was the only factor tending to explain the probability of parasitism in a separate model (Table 1), and this tendency did not change when removing





**Fig. 3** **a** Mean  $\pm$  SE and 95 % CI of residual nests roof density of parasitised and non-parasitised magpie nests. **b** The curvilinear relationship between laying date and the intensity of parasitism

all other terms with associated  $P$  values larger than 0.1 from the model (study plot: Wald's stat = 6.29,  $P = 0.043$ , roof density: Wald's stat = 3.81,  $P = 0.051$ ). In short, magpie nests with a denser roof tended to be more likely parasitised (Fig. 3a). Including the egg colour experiment did not affect results qualitatively (results not shown).

The intensity of parasitism decreased as the season progressed although a small peak appeared at the end of the breeding season (Table 1; Fig. 3b). None of the nest characteristic variables did associate with the intensity of parasitism (Table 1). Again, including the egg colour experiment did not affect the results qualitatively (results not shown).

#### Magpie nest characteristics and the probability of nest predation

Experimental manipulation of nest size, nest volume and roof density explained the probability of predation of

magpie nests before hatching (Fig. 4). These effects, however, disappeared when including all variables in the same model, suggesting that all these factors share explained variance of the probability of predation (GLZ, with binomial function and logit link function, experimental treatment: Wald statistic 1.68,  $P = 0.19$ ; nest size: Wald statistic 1.69,  $P = 0.19$ , roof density: Wald statistic 1.14,  $P = 0.28$ , nest visibility: Wald statistic 0.97,  $P = 0.33$ ). Thus, experimentally reduced nests, nests with a small volume or nests with lower roof density suffered the highest probability of predation (Fig. 4). The effect of nest visibility did not reach statistical significance (Fig. 4).

#### Artificial egg colour in magpie nests and the probability of parasitism

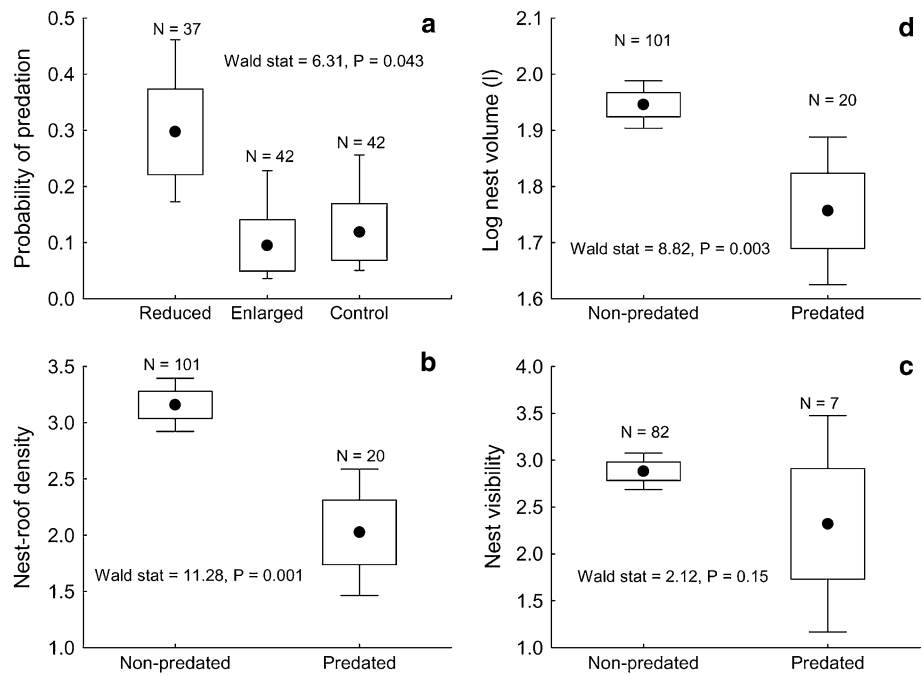
The probability of parasitism after the introduction of the coloured artificial egg was higher for nests with experimental cream eggs than for those with blue eggs (Table 2; Fig. 5a). No other variable in the model explained an additional significant proportion of the variance in the probability of parasitism (Table 2). The addition of the nest size manipulation experiment and its interaction with the egg colour experiment did not affect this conclusion (egg colour experiment: Wald statistic 4.09,  $P = 0.043$ ; nest experiment: Wald statistic 1.31,  $P = 0.52$ ; interaction between experimental treatments: Wald statistic 3.15,  $P = 0.21$ ; main effects estimated without the non-significant interaction).

None of the variables included in the model explained variation in the intensity of parasitism of parasitised magpie nests (Table 2). When the nest-size manipulation experiment and its interaction with the egg colour experiment were included in the model, neither, the effect of egg colour experiment (Wald statistic 0.72,  $P = 0.40$ ), that of nest experiment (Wald statistic 2.05,  $P = 0.36$ ), or their interaction (Wald statistic 4.29,  $P = 0.12$ ) (all main effects estimated without including the non-significant interaction) reached statistical significance. However, enlarged nests that received blue coloured eggs were more intensely parasitised than enlarged nests with experimental cream eggs (Wald statistic 3.89,  $P = 0.049$ ). In reduced and control nests, the intensity of parasitism did not differ between nests with blue or colour cream eggs (both Wald statistic  $\geq 2.62$ ,  $P \geq 0.11$ ; Fig. 5b).

#### Discussion

The experimental modification of nest size strongly affected size, visibility and roof density of magpie nests. We did not find an experimental effect of nest size manipulation on brood parasitism, but great spotted cuckoos

**Fig. 4** Means  $\pm$  SE (rectangles) and 95 % CI (whiskers) of the probability of predation experienced by reduced, enlarged and control nests (a). Means and dispersion values of nest volume (b), nest-roof density (c) and nest visibility (d) of predated and non-predated nests. Wald’s statistics and associated *P* values from the GLZ models exploring separately the influence of the experimental treatment and nest characteristics on the probability of predation (binomial distribution and logit-link function), after excluding non-significant terms (i.e. study plot, laying date and laying date squared; *P* > 0.1 in all cases) are shown



**Table 2** Generalized linear models testing for the effect of the experimental addition of a model egg of blue or cream colouration on the probability (binomial distribution and logit link function, *n* = 109)

Factors	Probability of parasitism				Intensity of parasitism			
	Estimate (SE)	Wald statistic	df	<i>P</i>	Estimate (SE)	Wald statistic	df	<i>P</i>
Laying date	0.294 (0.182)	2.59	1	0.11	−0.078 (0.059)	1.76	1	0.18
Laying date <sup>2</sup>	−0.001 (0.004)	2.70	1	0.10	0.002 (0.002)	1.21	1	0.27
<b>Egg experiment</b>	<b>−0.472 (0.225)</b>	<b>4.38</b>	<b>1</b>	<b>0.036</b>	−0.112 (0.116)	0.93	1	0.33
Host response	−0.368 (0.308)	2.66	1	0.10	0.125 (0.118)	1.12	1	0.29
Study plot		1.63	2	0.44		0.95	2	0.62

Laying date, laying date squared and whether or not experimental model eggs disappeared from magpie nests within the following 7 days of the experiment were included in order to statistically control for their potential effects on brood parasitism. The identity of study plots was included as a fixed factor because the probability of parasitism differed among study plots

For facilitating visualisation of the detected tendencies, variables and statistics with associated *P* values lower than 0.1 are shown in bold

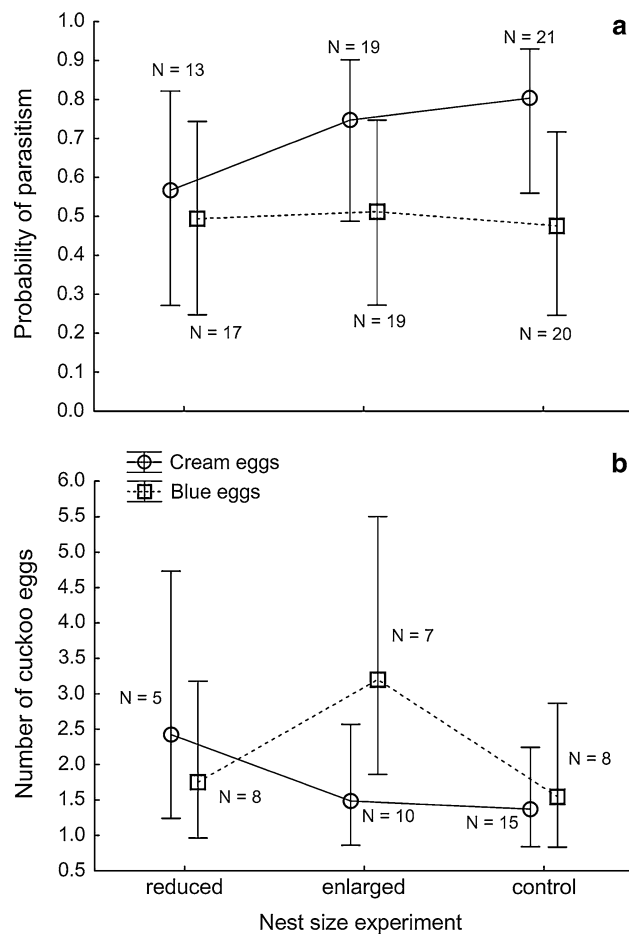
tended to preferentially parasitise magpie nests with a dense roof. Because denser nests were less frequently predated, selecting nests with a dense roof to parasitise might be of selective advantage for cuckoos in terms of predation avoidance. On the other hand, the addition of an artificial blue or cream coloured egg to magpie nests did affect the probability of parasitism. Specifically, nests with experimental cream eggs were preferentially parasitised by great spotted cuckoos. These results provide some support for our main expectation, namely, that great spotted cuckoos might use inadvertent social information of magpies (i.e. nest characteristics and egg coloration) for host selection. Below, we discuss the strength of these experimental and correlative evidences under several non-exclusive scenarios.

and the intensity of parasitism (Poisson distribution and log-link function; only parasitised nests were considered, *n* = 53)

Nest size manipulation, brood parasitism and nest predation

Nest size was manipulated on average 2 weeks before the first host eggs were laid, thus allowing enough time for the effects to be detected by great spotted cuckoos. However, we failed to detect a direct effect of the experiment on the probability and intensity of parasitism. Rather, nest characteristics after manipulation, which were closely related to the experimental treatment (ESM, Fig. 1), were associated with the probability and/or intensity of parasitism.

Choosing to parasitise large magpie nests may be important, mainly for those cuckoos that parasitise nests after the incubation started, due to the positive association between magpie nest size and parental quality (see “Introduction”).



**Fig. 5** Predicted means  $\pm$  confidence intervals (95 %) of probability (a) and intensity (b) of parasitism of magpie nests with shortened, enlarged or not manipulated roofs and that received either cream or blue experimental model eggs. Sample sizes of each experimental treatment are also shown

Cuckoo eggs laid before the start of incubation hatch at least 4 days earlier than those of the host, normally resulting in the starvation of all magpie hatchlings (Soler and Soler 2000). However, cuckoos hatching from eggs laid during magpie incubation often have to compete with similar aged or even older host nestlings (Soler et al. 1995b), sometimes provoking starvation of cuckoo nestlings (Soler et al. 1998c; Soler and De Neve 2013). Thus, it is possible that using ISI of host traits that signal parental quality can especially be beneficial when parasitism occurs during magpie incubation. Good magpie foster parents could raise a larger number of fledglings, and hence increase survival probabilities of cuckoo nestlings. In accordance with the above suggestion, Molina-Morales et al. (2012) found that the predicted positive association between nest size and parasitism was only detected at the end of the breeding season (2012), when food availability for nestling cuckoos is likely less abundant and multi-parasitism is more frequent

as the breeding season progresses (Fig. 3b). Intriguingly, when restricting the estimates of parasitism to those after incubation had started (i.e. after the egg colour experiment), enlarged nests that received blue eggs were more intensely parasitised than those with cream eggs, while in control and reduced nests, this pattern was not detected. Blue eggs in host nests may also signal parental quality (see “Introduction”) and, thus, this result might also be in accordance with the hypothesis that great spotted cuckoos eavesdrop on host traits related to parental quality. However, the interaction effect between the nest size and the egg colour experimental treatments on the intensity of parasitism did not reach statistical significance and, thus, these results should be considered with caution. We also found that nests with denser roofs experienced the highest probability of parasitism (Fig. 3a). Roof density is closely related to the experimental nest size manipulation (ESM, Fig. 1) and, thus, this result might be in accordance with the hypothesis that great spotted cuckoos eavesdrop on magpie nest characteristics that signal parental quality (De Neve et al. 2004).

Reduced probability of predation may also be an important advantage for cuckoos selecting large and dense magpie nests (Quesada 2007). In fact, we have detected that experimentally reduced nests or those with lower density or volume suffered a higher predation risk compared to enlarged or control nests or those with a larger volume and denser roof (Fig. 4). Therefore, the results presented here suggest that cuckoos selecting nests of larger size have the advantage of reducing the probability of predation of their offspring.

The high risk of parasitism experienced by nests with dense roofs might simply be the consequence of being more easily detected by cuckoos and not due to the effect of eavesdropping cuckoos. However, even if that were the case, the negative association between nest size and the risk of predation (Fig. 4), and the previously shown positive relationship between nests size and parental quality of magpies (see “Introduction”) would rapidly select for cuckoos choosing dense and more visible nests for parasitism. Thus, eavesdropping cuckoos should at least partially explain our results.

#### Egg colour manipulation and brood parasitism

One of the hypothetical scenarios predicting a biased selection of magpie nests for parasitism was related to the possibility that cuckoo females identified the experimental blue egg as one of the host eggs and that the blue–green colour intensity of magpie eggs would reflect parental quality of the magpie pair. In this scenario, cuckoos would benefit by considering host egg colouration when selecting which nest to parasitise (Soler et al. 2012). Although we do not know if egg



coloration of magpie eggs is related with parental quality, we found that cuckoos preferred nests with cream coloured eggs for parasitism which is not in accordance with the hypothesis.

Another hypothetical scenario predicting a biased selection of magpie nest for parasitism (see “[Introduction](#)”) was related to the possibility that cuckoos, after checking available nests, decide to parasitise those nests with egg coloration matching that of the cuckoo female to make egg recognition by magpies more difficult (Avilés et al. 2006a; Cherry et al. 2007). Great spotted cuckoos, however, preferred magpie nests with experimental eggs of cream colour which is not in accordance with that possibility.

Multiparasitism of magpie nests by the same or different great spotted cuckoo females is common in the area (see “[Introduction](#)”). Thus, another possibility explaining the detected association is that cuckoos identified the cream model egg as a parasitic egg and copied nest selection by previous parasitic females (White et al. 2007), which may increase the probability of selecting magpies unable to detect and remove parasitic eggs. An alternative scenario of interpreting the results is that female cuckoos identified experimental blue eggs as brood parasitic eggs and avoided nests with eggs with such colouration, since it could reduce the likelihood of successful hatching and fledging of great spotted cuckoo eggs (Soler et al. 1998c). Our experiment, however, does not allow us to distinguish between these two possibilities.

Although further investigations are clearly necessary to establish the mechanisms behind the detected biased parasitism towards magpie nests with experimental cream eggs, the egg colouration experiment did confirm that a brood parasite, the great spotted cuckoo, is able to discriminate and evaluate variation in colouration of eggs within the nests of its host and that it uses this information to decide parasitism. The possibility that brood parasites are able to evaluate the colouration of their own eggs and those of their hosts has been suggested previously (Avilés et al. 2006a; Cherry et al. 2007; Soler et al. 2012), and results from some previous experiments with great spotted cuckoos and brown-headed cowbirds (*Molothrus ater*) have already pointed in this direction. In both species, nests from which parasitic eggs were removed experimentally suffered a higher risk of predation by brood parasites (Soler et al. 1995c; Hoover and Robinson 2007) than control nests in which parasitic eggs were not removed, invoking a mechanism for egg recognition at the parasite side. Moreover, host nests with experimental parasitic cowbird eggs were preferentially selected by first-time breeding female cowbirds, but avoided by adult females (White et al. 2007). Our experiment goes a step further and uses two different coloured experimental model eggs and results support the hypothesis that great spotted cuckoos are able to detect variation in egg coloration.

Detecting variation in egg coloration in host nests by brood parasites may have important consequences for parasite–host coevolutionary interactions if, as suggested above, it allows cuckoos to evaluate egg colour matching, previous parasitism, and/or parental quality of potential hosts. We hope that these results encourage further research in other brood parasite–host systems to confirm that egg recognition has been selected not only in hosts, but also in their brood parasites.

In summary, our study for the first time provides evidence suggesting that a brood parasite, the great spotted cuckoo, might use inadvertent social information of host nest characteristics that are related with the probability of predation. Furthermore, our results suggest that great spotted cuckoos can distinguish between eggs of different colours within the nests of their hosts, which opens the possibility for the use of colour egg information by cuckoos when deciding where to parasitize. Further work is needed on this and other systems to confirm and understand the role of ISI of hosts for brood parasites.

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