Egg rejection by Iberian azure-winged magpies *Cyanopica cyanus* in the absence of brood parasitism

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Avilés, J. M. 2004. Egg rejection by Iberian azure-winged magpies *Cyanopica cyanus* in the absence of brood parasitism. – J. Avian Biol. 35: 295–299.

The Iberian azure-winged magpie *Cyanopica cyanus* shows a remarkable ability to discriminate against great spotted cuckoo *Clamator glandarius* eggs. Here, I studied whether egg recognition in this species could be a derived feature resulting from intraspecific brood parasitism. Azure-winged magpies showed a very high level of discrimination and rejection of great spotted cuckoo models (73.7%), and of conspecific eggs (42.8%), even when no evidence of great spotted cuckoo or conspecific brood parasitism has been found in the population. Azure-winged magpie discriminated more readily than magpies, the current favourite host of the great spotted cuckoo. The high rejection rate of conspecific eggs by the azure-winged magpie suggests that it is quite possible that egg discrimination in this species evolved in response to conspecific brood parasitism rather than to cuckoo parasitism.

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The azure-winged magpie *Cyanopica cyanus* is a social small corvid with a remarkable disjunct distribution. It occurs widely in the eastern Palearctic, and it is also found in a smaller geographical area in Spain and Portugal (Cramp, S. 1988). Fok et al. (2002), have recently demonstrated that the azure-winged magpie is principally divided in two genetically distinct clades, an Asiatic and an Iberian that diverged approximately 1.2 million years ago. Parasitism of the azure-winged magpie by the common cuckoo *Cuculus canorus* has become very frequent in some parts of the eastern Palearctic. In central Japan cuckoo parasitism started 25 years ago and spread rapidly with some local magpie populations suffering rates of parasitism exceeding 50% (Yamagishi and Fujioka 1986, Nakamura et al. 1998). Interestingly, an increase in a relatively short time of 10–20 years in the frequency of egg rejection of cuckoo eggs by the azure-winged magpie has occurred since the cuckoo started to exploit this new host in Japan (Nakamura et al. 1998).

On the Iberian peninsula one obligate brood parasite species, namely the great spotted cuckoo *Clamator glandarius*, may potentially exploit the azure-winged magpie as host since they are largely sympatric in many areas (Cramp, S. 1988). Nowadays, evidence of great spotted cuckoo parasitism on the Iberian azure-winged magpie is lacking from studies in which the nests were daily monitored and the eggs numbered. During a 12 year study, inter-specific brood parasitism was never reported in an azure-winged magpie population in Central Spain (Valencia et al. 2004, n = 690 nests). Moreover, Arias de Reyna and co-workers failed to find clear evidence of great spotted cuckoo parasitism in three azure-winged magpie populations near Sierra Morena (n = 54 nests for Cordoba population; Arias de Reyna and Hidalgo 1982) and sample sizes was not provided for Andújar and Montoro populations (Arias de Reyna 1998). However, different sources of evidence suggests that the azure-winged magpie may have been used as host by the great spotted cuckoo in the recent

past. Friedman (1964) reported 11 cases of parasitized clutches by the great spotted cuckoo in a sample of 172 parasitized clutches of different hosts parasitized by the great spotted cuckoo. Moreover, 6 clutches of azure-winged magpies parasitized by the great spotted cuckoo are currently kept in the British Museum of Natural History (n = 4), and the Walter Koenig Museum at Bonn (n = 2; Soler et al. 2003). These clutches were collected from distant areas of Spain and Portugal at the end of the 19th century, suggesting that great spotted cuckoo parasitism of azure-winged magpies was widespread a century ago.

Redondo and Arias de Reyna (1989) concluded that the current absence of great spotted cuckoo parasitism was not due to the host being unsuitable, since experimentally introduced great spotted cuckoo eggs and chicks had similar success in azure-winged magpie and in magpie nests (the current favourite great spotted cuckoo host in Iberia (Soler 1990)). Also, the breeding success of the azure-winged magpie in experimentally parasitized nests was not significantly lowered compared to unparasitized control nests (Redondo and Arias de Reyna 1989). Although their results were based on a very sparse data set (6 magpie vs 4 azure-winged nests; Redondo and Arias de Reyna (1989), altogether their results would suggest that azure-winged magpies have the potential to raise great spotted cuckoo chicks.

Previous studies have found that the Iberian azure-winged magpie show a remarkable ability to discriminate against great spotted cuckoo models (Arias de Reyna and Hidalgo 1982, Arias de Reyna 1998). The aim of this study was to investigate how the discrimination and rejection of parasitic eggs evolved in the azure-winged magpie. In assessing the adaptive basis of egg recognition in the azure-winged magpie previous studies focused only on interspecific brood parasitism (Arias de Reyna and Hidalgo 1982, Arias de Reyna 1998). Here I experimentally parasitized azure-winged magpie nests with conspecific eggs for testing whether egg recognition in this species could be a derived feature resulting from intra-specific brood parasitism. I also compare rejection abilities and discrimination costs of the azure-winged magpie to those of the magpie, current favourite great spotted cuckoo host in the region.

Methods

Study species

The azure-winged magpie is a flexible cooperative breeder including helping at different stages of the breeding cycle (Valencia et al. 2003). Azure-winged magpies usually lay five to six eggs (range 2–8), and most first clutches at the study area were found in April and during the first week of May (J. M. Avilés unpubl. data). No signs of parasitism by the great spotted cuckoo

were reported in the 58 breeding attempts studied. In contrast, the magpie is a monogamous passerine typically used as a favourite host by the brood parasitic great spotted cuckoo in southern Spain (Soler 1990). Magpies in southern Spain usually lay six or seven eggs (range 2–10; Soler et al. 1996), and laying in the study area occurred in the two last weeks of March and April (J. M. Avilés unpubl. data). The prevalence of great spotted cuckoo parasitism in the study population of magpies was 14.8% (n = 54 nests, J. M. Avilés unpubl. data).

On the Iberian Peninsula, the eggs of the great spotted cuckoo found in the nests of the azure-winged magpie one century ago looked different than those of the host (Soler et al. 2003). Similarly, spectrophotometric analyses have revealed that great spotted cuckoo egg coloration and patterns are not the result of coevolution with their hosts since changes in the appearance of great spotted cuckoo eggs do not parallel those of their hosts (Soler et al. 2003). Thus the capacity of the great spotted cuckoo to evolve highly mimetic eggs seems to be very low which renders a reduction of intra-clutch variation in egg appearance non-adaptive for this host-parasite system (Avilés et al. 2004).

Study area

The field study was carried out in the surroundings of Guadiloba reservoir near Cáceres (37°18′N, 3°11′W), southern Spain during March–June 2003. The study area approximately comprised 400 ha in which azure-winged magpies and magpies breed sympatrically with the great spotted cuckoo in a wooded plot of holm oak trees *Quercus ilex*. Both the azure-winged magpie and the magpie nests were mostly found in holm oak trees.

Host discrimination against avian brood parasitism

I searched for nests at the beginning of the 2003 breeding season. Thirty-eight azure-winged magpie nests and 37 magpie nests, respectively, were used for this experiment. Nests were found at different stages of building, and were numbered and mapped. The nests were visited daily and each egg was numbered with waterproof ink in consecutive laying order. No evidence of intraspecific parasitism was found since not more than one egg appeared on a single day.

A total of five azure-winged magpie nests, and three magpies nests were depredated before responses to artificial parasitism could be assessed. The remaining azure-winged magpie nests were randomly assigned to one of the following groups: experimentally parasitized with great spotted cuckoo models (n = 19), and experimentally parasitized with conespecifc eggs (n = 14). All magpie nests were artificially parasitized with great spotted cuckoo models (n = 34).

I experimentally examined host responses to great spotted cuckoo models by introducing quail eggs painted with acrylic paint to mimic the colour and spotted pattern of real cuckoo eggs in the area (see Soler and Møller 1990). Mean dimensions of quail eggs were 32.5 mm × 25.1 mm and 8.5 g, thus being within the natural range of variation of those of the great spotted cuckoo (Cramp and Perrins 1994). Azure-winged magpies response to conspecific brood parasitism was tested by using eggs collected from abandoned nests in the study area. All parasitic eggs were in good condition, and no host eggs were removed during experimental parasitism to simulate great spotted cuckoo laying tactics (Soler 1990). Similarly, since conspecific parasitism attempts have not be reported for the azure-winged magpie, and, hence, information on costs of conspecific brood parasitism is currently unavailable for this species, no host eggs were removed when testing the azure-winged magpie response to conspecific eggs.

The time interval between the laying of the first host egg and the beginning of the experiment varied. While 67.6% of the azure-winged magpie nests were artificially parasitised during host egg laying, the remaining 32.4% were parasitised after completion of the clutch. A total of 82.4% of the magpie nests were parasitized during host egg laying. Responses to artificial parastism in azure-winged magpies did not vary between laying and incubation (Fisher exact test: $P = 0.32$), and magpie responses to artificial parasitism have been widely shown to be unaffected by the breeding phase (Soler and Møller 1990). No artificially parasitized nests were naturally parasitized by great spotted cuckoos, and no naturally parasitized nests were used in the experiments. In no case did artificial parasitism cause host eggs to be removed during the subsequent days. All nests were checked every second day, and responses to parasitism were finally assessed six days from artificial parasitism as rejection (eggs ejected or nest deserted), or acceptance of parasitic eggs (all others).

Results

Rejection behaviour of great spotted cuckoo eggs

Azure-winged magpie showed a significantly higher level or rejection against great spotted cuckoo models than magpies (Table 1, chi-square test: $\chi_1^2 = 5.0$, $P = 0.03$).

The method of rejection of great spotted cuckoo eggs did not differ between magpies and azure-winged magpies since all rejections were by ejection (Table 1). Moreover, the proportion of rejected pairs suffering some costs by rejecting a great spotted cuckoo model was similar since two (28.6%) of the seven rejecter magpies, and four (28.6%) of the 10 rejecter azure-winged magpies suffered the loss of at least one of their own eggs while rejecting the parasitic one.

Results were unaffected by host laying date since the probability of rejecting parasitic eggs was not significantly associated with laying date for the azure-winged magpie (mean = 120.00, s.d. = 6.84, n = 8 in acceptors vs mean = 115.26, s.d. = 6.25, n = 15 in rejecters, logistic regression: $\chi^2 = 2.76$, d.f. = 1, $P = 0.10$), nor for the magpie (mean = 16.89, s.d. = 4.06, n = 19 in acceptors vs mean = 15.84, s.d. = 5.09, n = 4 in rejecters, logistic regression: $\chi^2 = 0.42$, d.f. = 1, $P = 0.51$).

Azure-winged magpie rejection behaviour of conspecific eggs

42.8% azure-winged magpie pairs rejected conspecific eggs (Table 1). This percentage did not vary significantly from that when azure-winged magpies were parasitized with great spotted cuckoo models (chi-square test: $\chi_1^2 = 2.05$, $P = 0.15$), but the statistical power to detect this difference was low (b = 0.4). The method of rejection used by azure-winged magpies did not differ between the kinds of parasitic egg. A total of 2 of 6 (33.3%) rejecters of conspecific eggs deserted their nests, while all rejections were by ejection when azure-winged magpies were parasitized with great spotted cuckoo models (Fisher's exact test: $P = 0.08$).

Azure-winged magpie did not lose any eggs in the 4 ejections of conspecific eggs. Instead, 2 of 14 (14.3%) azure-winged magpies parasitized with great spotted cuckoo models broke one of their own eggs, and 2 pairs (14.3%) broke three of their own eggs.

Discussion

Egg rejection in the absence of parasitism

Interestingly, azure-winged magpies rejected great spotted cuckoo models at a very high rate even when

Table 1. Reactions of azure-winged magpies and magpies to artificial parasitism with great spotted cuckoo eggs and conspecific eggs, respectively.

Host species	Parasitic egg	Accepted	Ejected	Deserted	% rejection
Azure winged magpie	Great spotted cuckoo	5	14	0	73.7
	Conspecific	8	4	2	42.8
Magpie	Great spotted cuckoo	27	7	0	20.6

no evidence of great spotted cuckoo parasitism was found in the population. Similar results were reported by Arias de Reyna (1998) in three azure-winged magpie populations in southern Spain in which no evidence of great spotted cuckoo parasitism were found. The retention of host defenses in the absence of parasitism implies that the maintenance of this behavioral trait should be cost-free, or nearly so, otherwise rejection behaviour would be lost. Recognition errors when azure-winged magpies were parasitised with the highly non-mimetic great spotted cuckoo models eggs seemed to be negligible in my study, since in 14 ejections no single case of azure-winged magpies ejecting their own eggs instead of the artificial ones was recorded. Moreover, the loss of their own eggs by azure-winged magpies was also negligible in the 4 cases of ejection of conspecifc eggs. Although my results would support non-costly retention by the azure-winged magpie, the fact that: a) this species was parasitized 100 years ago, b) that it is a long lived bird, and c) that the lack of discrimination costs is based on a reduced sample, undermines any firm conclusion on the retention linked to discrimination costs.

An alternative explanation for retention of anti-parasite defences in the azure-winged magpie could be related to egg recognition currently having an adaptive value in some other scenario than interspecific brood parasitism. Intraspecific brood parasitism is a widely used breeding strategy among colonially breeding birds (Brown and Brown 1988, Yom-Tov 2001) and is known to be a factor in the maintenance of egg rejection in weavers (*Ploceus* spp.; Freeman 1988, Jackson 1998). The azure-winged magpie is a flexible cooperative breeder, which may suggest that it is suitable for conspecific brood parasitism. However, in long-term studies of the azure-winged magpie in Spain (J. Valencia and C. de la Cruz pers. comm., n = 690 nests) in which eggs were numbered, no evidence of intraspecific brood parasitism was found. Furthermore, I failed to find evidence of intraspecific brood parasitism in any of the 58 nests I studied.

Gene flow from azure-winged magpie populations exposed to great spotted cuckoo parasitism is another possible explanation for the rejection behaviour shown by azure-winged magpies. However I find this possibility unlikely for several reasons: First, no signs of great spotted cuckoo parasitism have been found in all the studies dealing with the breeding biology of the azure-winged magpie in the Iberian Peninsula during the last 30 years (Álvarez and Arias de Reyna 1974, Pacheco et al. 1975, Araujo 1975, Arias de Reyna and Hidalgo 1982, Muñoz-Pulido et al. 1990, Arias de Reyna 1998, Valencia 2003, this study). Second, azure-winged magpies are highly sedentary with dispersal among colonies being negligible (Nakamura et al. 1998), thus reducing the probability of gene flow.

In conclusion, I failed to find support for retention of the rejection behaviour currently being adaptive in a different scenario than interspecific brood parasitism. However, more exhaustive studies dealing with discrimination costs are needed to establish firm conclusions on the adaptive value of egg discrimination in the absence of brood parasitism in the azure-winged magpie.

How did rejection evolve in azure-winged magpies?

Great spotted cuckoo parasitism seems to be an unlikely source of selection for the evolution of recognition in the azure-winged magpie. Azure-winged magpie acutely discriminated the highly mimetic conspecific eggs. It is difficult to imagine that such a high level of discrimination evolved from rejection of the highly non-mimetic great spotted cuckoo eggs (see Soler et al. 2003). Moreover, breeding success of the azure-winged magpie, experimentally parasitized with great spotted cuckoo chicks, remained unchanged compared to unparasitized nests (Redondo and Arias de Reyna 1989), which would make the evolution of this behavioural trait non-adaptive.

The azure-winged magpie might show egg recognition because it is adaptive in terms of conspecific brood parasitism. Accordingly, the azure-winged magpie efficiently rejected conspecific eggs when these were artificially introduced into their nests. Although no evidence of conspecific brood parasitism has been reported for the azure-winged magpie (see above), it may still be prevalent in other populations. Thus genetic analyses would be necessary to prove the absence of egg dumping in a number of populations before one could exclude its existence.

Alternatively, the original source of selection for egg recognition in the azure-winged magpie might be the common cuckoo. Thus rejection by Iberian azure-winged magpies would be a relic behaviour evolved long time ago and retained because of its low cost. There are fossil records from the Pleistocene of azure-winged magpies (Cooper 2000) and common cuckoo from the Iberian Peninsula (Tyrberg 1998), and the opportunity for co-evolution between cuckoos and azure-winged magpies is at least possible. Also, the azure-winged magpie is a common host of the cuckoo in Japan (Yamagishi and Fujioka 1986, Nakamura et al. 1998), which suggests that it could be a suitable cuckoo host also In Iberia. Finally, the adaptive value of cuckoo egg recognition by azure-winged magpies is clear because acceptor azure-winged magpies are unlikely to fledge their own offspring (Nakamura et al. 1998).

In conclusion, I found weak support for the hypothesis that recognition abilities in the azure-winged magpie evolved as a response to great spotted cuckoo parasitism. However, exhaustive analyses of parentage are clearly

needed to discriminate between conspecific brood parasitism and cuckoo parasitism as selective agents of rejection in the azure-winged magpie.

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