

Identification of the Sex Responsible for Recognition and the Method of Ejection of Parasitic Eggs in Some Potential Common Cuckoo Hosts

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Abstract

Egg rejection is the most common defence used by hosts against avian brood parasites and experimental studies have provided some of the best documented demonstrations of the coevolutionary process. However, the sex responsible for egg ejection and whether eggs are grasped or punctured are two essential questions that remain unanswered for most host species. In this paper, by filming the behaviour of individuals of three different species confronted with a foreign egg experimentally introduced into their nests, we first determine the relationship between recognition (when the birds aggressively pecked the experimental egg) and ejection. Secondly, we demonstrate that in the species where only the female incubates, only the female recognizes and ejects the model egg, whereas in the two species where both sexes incubate, both sexes eject the foreign egg. Finally, the large host species ejected the model egg by grasping it with the bill, whereas the two small species ejected it by puncturing it first. Furthermore, our data suggest that puncture ejection is more costly than grasping ejection considered both in terms of energetic and ejection costs.

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Introduction

The most common defence directly used by hosts against an avian brood parasite is rejection of the parasitic egg (Rothstein 1990; Winfree 1999). Egg-rejection behaviour is the most efficient method to eliminate parasitism and the most extensively studied of host defences (Rothstein 1990; Winfree 1999). Many experiments performed by introducing model eggs into host nests have provided

some of the best-documented examples of the coevolutionary process (Brooke and Davies 1988; Davies and Brooke 1988; 1989; Soler and Møller 1990; Briskie et al. 1992; Sealy 1996; Soler et al. 1999).

However, two essential questions concerning the evolution of egg rejection as a defence used by hosts against brood parasites remain unanswered for most host species: (1) which sex ejects parasitic eggs and (2) how are the eggs ejected, by 'grasping' or 'puncturing'?

The question of which sex ejects parasitic eggs has important implications because if both sexes are responsible, the rejecter trait will spread faster in the population than if only one sex is responsible (Rothstein 1975b; Sealy and Neudorf 1995). Usually, it is assumed that only females eject parasitic eggs (e.g. Rothstein 1975b; Davies and Brooke 1988; Lotem et al. 1992), an assumption that has been supported by observations (Rothstein 1977; Rohwer et al. 1989; Moksnes et al. 1994) and experiments (Palomino et al. 1998). However, there are observations of males ejecting experimentally introduced parasitic eggs in a host species of the brown-headed cowbird *Molothrus ater* (Sealy and Neudorf 1995) and in a species with conspecific brood parasitism (Pinxten et al. 1991). As females lay and usually incubate the clutch, it is not surprising that they eject foreign eggs, given that they spend more time than males in the nest caring for the eggs. However, in species where both sexes incubate, males also should be expected to recognize and eject the foreign egg (Hypothesis 1).

The second question of how individuals eject the parasitic egg is relevant because the method of ejection is directly related to rejection costs (Rohwer and Spaw 1988; Moksnes et al. 1991, 1994). Thicker shells and round eggs of many brood parasites may have evolved to resist puncture ejection by small host species (Spaw and Rohwer 1987; Rohwer and Spaw 1988; Moksnes et al. 1991).

Rejection costs involve the accidental destruction of the host's own eggs when trying to eject the foreign egg (Davies and Brooke 1988, 1989; Rohwer et al. 1989). 'Grasp' ejection allows to remove the parasite's egg with little chance of damaging any host eggs, whereas 'puncture' ejection (when the host has to puncture the foreign egg before removing it from the nest) frequently causes accidental damage to some of the host's own eggs (Rohwer and Spaw 1988; Moksnes et al. 1991, 1994). However, this is a circular argument, because grasp ejection is inferred when model eggs are removed and all host eggs remain intact after ejection, and puncture ejection is assumed when host eggs are damaged in the process (Rothstein 1975a; Rohwer and Spaw 1988; Moksnes et al. 1991). Actually, puncture ejection of brood parasitic eggs by hosts has been documented only sporadically (Rothstein 1977; Moksnes and Røskaft 1988; Lotem et al. 1992; Moksnes et al. 1994; Sealy 1996).

The most important factor in ejection behaviour is the relationship between host bill size and parasitic egg size. Hosts with large bills are grasp ejectors, whereas hosts with bills too small to grasp parasitic eggs must puncture-eject or reject by desertion (Rohwer and Spaw 1988; Davies and Brooke 1989; Moksnes et al. 1991). Hence, it is expected that large species should be grasp ejectors, whereas small ones should be puncture ejectors (Hypothesis 2).

To test the two hypotheses described above, we performed egg-recognition experiments filming the behaviour of individuals in three sexually dimorphic species: one large species where only the female incubates, the blackbird *Turdus merula* (80–110 g), and two small species where both sexes incubate: blackcap *Sylvia atricapilla* (14–20 g) and sub-Alpine warbler *S. cantillans* (9–13 g). These species are not (or are rarely) used by cuckoos as hosts nowadays in Europe (Glue and Murray 1984; Moksnes and Røskaft 1995), but they have probably been used previously because they have evolved highly effective recognition and rejection of parasitic eggs (Davies and Brooke 1989; Moksnes et al. 1990; Moksnes and Røskaft 1992; this paper). This is considered as an indication of an old history of interactions between hosts and parasites (Davies and Brooke 1989; Soler and Møller 1990).

The aim of the present paper is to test the two above-mentioned hypotheses by quantifying recognition and ejection activities in order to determine their inter-relationship.

Methods

The study was performed in the springs of 1998 and 1999 in Sierra Nevada mountains (S Spain) in oak and holm oak woods, on the banks of the Genil River, and in high-mountain scrubland (mainly *Genista versicolor* and *Astragalus granatensis*) at 800–2000 m a.s.l. in two adjacent valleys (Monachil and Genil Rivers). Common cuckoos were present in all the study plots throughout the breeding season, although apparently only the robin (*Erithacus rubecula*) is parasitized in these areas (M. Martín-Vivaldi, M. Soler & T. Pérez-Contreras, unpubl. data). Between Mar. and Jul. we looked for nests of the potential host species by observing nest-building behaviour, or carefully checking shrubs and trees in the study area. All nests were numbered and visited as needed to establish laying date, clutch size and fledging success. On some visits, when the full clutch was found, we introduced a model egg into the nest. At the same time a Sony CCD-TRO5E PAL video camera was placed 1.5–5 m in a way that the sex and behaviour of incubating birds could be recorded. We recorded the time needed for the birds to eject the model egg (see below). Sometimes, the birds did not eject the model during recording. In such cases we revisited the nests the following days to determine whether the model was rejected or accepted. All model eggs were ejected before 7 d, the time a priori decided for a model egg to be considered as accepted.

We used two kinds of models. In all experiments performed in 1998, we used artificial eggs made of plaster-of-Paris using latex moulds made from real cuckoo eggs. Later they were painted with acrylic paints to produce non-mimetic or mimetic eggs. Blackbirds easily grasp-ejected the model egg in all cases. However, in the two experiments conducted in nests of each of blackcaps and sub-Alpine warblers, birds failed to eject the model egg and, after destroying most of their own eggs, deserted the nest. For this reason, in subsequent tests with these two species, we used real eggs taken from a captive population of house sparrows, *Passer domesticus*, breeding in aviaries at the Animal Biology and Ecology

Department at the University of Granada. We used only fresh sparrow eggs that were painted with acrylic paints in the same way as artificial eggs. The real eggs were refrigerated at 4–5°C until used, which was invariably shortly after being laid. House sparrow eggs (mean \pm SE = 20.7 \pm 0.9 \times 15.0 \pm 0.7 mm, n = 31) were of a similar size to those of the common cuckoo (mean \pm SE = 21.0 \pm 0.7 \times 15.5 \pm 0.3 mm, n = 3), and shell thickness measured using an IDC Digimatic Indicator was also very similar (house sparrow: mean \pm SE = 0.109 \pm 0.08 mm, n = 12; common cuckoo: mean \pm SE = 0.112, n = 1). The value of shell thickness of the cuckoo egg is similar to that reported by Moksnes et al. (1991) (mean = 0.108 mm).

The behaviour of birds confronted with an experimental egg was filmed in 25 nests (eight blackcap, eight sub-Alpine warbler and nine blackbird nests). We did not use a control because we showed previously for another small passerine that before introducing the model egg in the nest (control), the number of pecks and time spent pecking were zero (M. Soler, J. Fernández-Morante, M. Martín-Vivaldi, pers. comm.). Thus, it is unlikely that video recording affects host behaviour. The length of time of filming depended on when the model egg was ejected (from 12 min to 8.5 h; mean \pm SE = 12 373 \pm 2426 s, n = 25). We scored a bird as having recognized the experimental egg if we observed it aggressively pecking the experimental egg or if it ejected the egg. Pecking an egg implies recognition, because a study on another small passerine showed that no host egg was pecked and, after ejection of the experimental egg, no other egg was pecked (for details see M. Soler, J. Fernández-Morante, M. Martín-Vivaldi, pers. comm.). Recognition was evident because birds aggressively pecked the experimental egg, which implies recognition because no host egg was pecked and, after ejection of the experimental egg, no other egg was pecked. A more detailed discussion of this issue can be found in M. Soler, J. Fernández-Morante, M. Martín-Vivaldi (pers. comm.).

The information recorded in each experiment from the video records included, for each sex: (1) time to arrival at nest; (2) time from arrival to first peck; (3) time from recognition to ejection; (4) number of pecks at the model egg; (5) time spent pecking the model egg; (6) time incubating and (7) time spent moving the eggs with the bill. Here we considered the entire period of filming if the experimental egg was not ejected, or, if it was, only the period until ejection.

We used non-parametric statistics following Siegel and Castellan (1988). All statistical tests are two-tailed and values reported are mean (\pm SE).

Results

Recognition and Ejection

Blackcaps pecked or ejected the experimental egg in six of eight nests (75%), sub-Alpine warblers in eight of eight nests (100%) and blackbirds in eight of nine nests (88.9%) (Table 1). Incubation is important in egg recognition because parameters such as time of incubation and time spent moving the eggs are usually

Table 1: Observations of recognition and ejection by males and females of three potential host species. 'Recognition' shows the number of individuals that were observed pecking the egg (see Methods)

Species	n	Recognition by			Ejection during filming by		Ejected final
		Male	Female	Pair ^a	Male	Female	
Blackcap	8	4 (6)	4 (4)	6	2	3	7 ^b
sub-Alpine warbler	8	4 (6)	8 (8)	8	0	3	7 ^b
Blackbird	9	0 (0)	8 (9)	8	0	5	9

^aAt least one pair member.

^bOne nest was depredated.

The number of individuals that were observed incubating is shown between brackets. 'n' indicates the total number of nests.

higher for recognizer than for non-recognizer individuals (Table 2). The difference was significant in the case of sub-Alpine warbler males but was not significant for blackcap males (Table 2).

All model eggs were eventually ejected (Table 1) and in two cases nests were deserted after the model egg was pecked (see above). However, during filming (maximum 3.5 h), blackcaps ejected five of eight (62.5%) model eggs, sub-Alpine warblers three of eight eggs (37.5%) and blackbirds five of nine eggs (55.6%).

Table 2: Comparison (Mann–Whitney U-test) of duration of incubation and time spent moving the eggs between recognizer and non-recognizer individuals in blackcaps and sub-Alpine warbler males and females

	Duration of incubation (s)			Time spent moving the eggs (s)			
	Mean ± SE	n	U p	Mean ± SE	n	U p	
Blackcap males							
Recognizers	3809 ± 1456	4		136 ± 45	4		
Non-recognizers	3803 ± 1466	3	6 1	84 ± 34	3	4	0.84
Blackcap females ^a							
Recognizers	6136 ± 2042	4		399 ± 168	4		
sub-Alpine warbler males							
Recognizers	5385 ± 801	4		375 ± 163	4		
Non-recognizers	1670 ± 869	3	0 0.03	10 ± 4	3	0	0.03
sub-Alpine warbler females ^b							
Recognizers	6000 ± 1456	8		168 ± 141	8		

^aAll blackcap females that were observed incubating were recognizers, the other four females were not seen on the nest.

^bAll sub-Alpine warbler females were recognizers.

Sex Responsible for Recognition and Ejection

In the blackbird, the species where only the female incubates, and in the sub-Alpine warbler, females were responsible for egg ejection in all events recorded (Table 1). In the blackcap, in two cases the ejector was the male and in three the female (Table 1). Considering recognition ability, in blackcaps, both sexes show a similar recognition rate (50%), but if we consider only individuals that were able to see the eggs (those that incubated during filming time), recognition rate was 66.7% for males and 100% for females (Table 1). In sub-Alpine warblers all females were able to recognize but only 50% of the males (66.7% of males that incubated) did so (Table 1). In the blackbird, eight of nine females recognized the foreign egg (Table 1), but no males approached the nests during filming.

In blackcaps, in the three cases where females ejected, females incubated for a longer time (7092 ± 4421 s) and spent more time moving the eggs (496 ± 337 s) than did males (2195 ± 2303 s and 69.7 ± 60.8 s, respectively for duration of incubation and time spent moving the eggs). However, only in one of the two cases when the male ejected the model egg, did the male incubate for a longer time than did the female.

These results support Hypothesis 1, which stated that in species where both sexes incubate, both sexes should be able to recognize the foreign egg. Furthermore, individuals that ejected usually had incubated more.

Method of Ejection

Blackbird females invariably ejected the model egg by grasping it in their bill. Nevertheless, they frequently pecked the model egg before ejecting it: in five of eight cases of egg recognition the egg was pecked, but in the other three the model egg was ejected without previously pecking it. Blackcaps and sub-Alpine warblers, the smaller species, always ejected the model egg by puncturing the egg before ejection. Therefore, the large species is a grasp ejector, whereas the two small ones are puncture ejectors, as stated in Hypothesis 2 (see above).

Puncture ejectors pecked the foreign egg more than did grasp ejectors (number of pecks during ejection process: blackcaps plus sub-Alpine warblers 514 ± 256 , $n = 16$, blackbirds 18 ± 14 , $n = 9$; Mann–Whitney U-test, $z = -2.75$, $p = 0.006$) and also spent more time pecking (blackcaps plus sub-Alpine warblers 456 ± 151 s, $n = 16$, blackbirds 34 ± 28 s, $n = 9$; Mann–Whitney U-test, $z = -2.66$, $p = 0.008$). Blackbirds pecked the egg softly, but pecking seemed energetically more costly for blackcaps and sub-Alpine warblers because they pecked the egg vigorously, sometimes causing it to jump above the rim of the nest. Egg jumping followed by falling on the host eggs broke four eggs in two sub-Alpine warbler nests. As the cuckoo egg is stronger than house sparrow eggs used in our experiments (see Methods), we assume that rejection costs in naturally parasitized nests of small species are higher than in experimental tests.

Discussion

Ejection and Recognition

Pecking eggs prior to nest desertion or ejection by hosts of brood parasites has been reported as a constant behaviour (100% of the observations) in three cases, in two puncture-ejector species (Brooke and Davies 1988; Sealy 1996) and in one grasp-ejector species (M. Soler, J. Fernández-Morante, M. Martín-Vivaldi, pers. comm.). We found that the two puncture-ejector species pecked the experimental egg repeatedly (house sparrow egg pecked 4004 times by a male sub-Alpine warbler), and the blackbird, a large grasp-ejector, pecked the egg in five of eight cases. This means that pecking behaviour is a frequent response against a foreign egg.

The three species ejected all the model eggs, even mimetic ones (see Methods). These results parallel previous studies on ejection behaviour which reported high ejection rates by blackcaps (Moksnes et al. 1990; Moksnes and Røskaft 1992) and blackbirds (Davies and Brooke 1989; Moksnes et al. 1990). Sub-Alpine warblers, as far as we know, have never been tested before.

Sex Responsible for Recognition and Ejection

Our results support the idea that in species where only the female incubates, the male does not recognize or eject foreign eggs. In the blackbird, as previously recorded in other passerine species where only the female incubates [i.e. the rufous-tailed scrub robin *Cercotrichas galactotes* (M. Soler, J. Fernández-Morante, M. Martín-Vivaldi, pers. comm.)], males did not see the eggs during the filming time. Our results also support the hypothesis that in species where both sexes incubate, both males and females are able to recognize and eject the foreign egg (Hypothesis 1). Although ejection was performed only by blackcap males, it was clear that sub-Alpine warbler males were able to recognize the foreign eggs. Egg pecking (recognition) by males was also observed by Davies and Brooke (1988) in the reed warbler *Acrocephalus scirpaceus*, a species where both sexes incubate. In 10 of 17 nests where pecking was observed, both males and females were seen doing so (Davies and Brooke 1988).

However, our data suggest that females might be more effective at recognizing and ejecting foreign eggs even in species where both sexes incubate (Table 1). This fact is clear from our results (Table 1) and Sealy (1996), studying warbling vireos *Vireo gilvus*, found that all four ejections observed were by the female, whereas the male was responsible for two of three unsuccessful ejection attempts.

Frequently, it is assumed that only host females eject parasitic eggs (e.g. Rothstein 1975b; Davies and Brooke 1988; Lotem et al. 1992). Our results are important because we have found that in the two species where both sexes incubate males are able to eject the foreign eggs, and if both sexes are responsible for egg recognition and rejection the rejecter trait will spread faster among the population than if only one sex is responsible.

Method of Ejection

In accordance with Hypothesis 2, the large species (the blackbird) should be a grasp-ejector, whereas small-sized ones (the blackcap and the sub-Alpine warbler) are puncture-ejectors.

Our results support the idea that 'puncture' ejection is more costly than grasp ejection because of frequent accidental damage to some of the host's own eggs (Rohwer and Spaw 1988; Moksnes et al. 1991, 1994). Our observations also suggest that puncture ejection can involve a higher energetic cost because hosts repeatedly and vigorously pecked the parasitic egg. Furthermore, our preliminary tests made during 1998 demonstrated that experiments using artificial model eggs (more difficult or impossible to puncture) provoked nest desertion instead of ejection and higher rejection costs, as previously suggested by Moksnes et al. (1994).

In conclusion, our data, although based on small sample sizes, clearly suggest that in species that share incubation, males and females have evolved the ability to recognize and eject odd eggs. This should be considered as a rule when modelling the evolution of rejection behaviour in potential host species.

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