



## Red-crested cardinal defences against shiny cowbird parasitism

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### Abstract

Avian brood parasites reduce the reproductive success of the host, which favours the evolution of antiparasitic defences, such as aggression towards parasites or rejection of their eggs. The red-crested cardinal, *Paroaria coronata*, is a potential good-quality host of the shiny cowbird, *Molothrus bonariensis*. However, the frequency of cowbird parasitism in cardinal nests is very low and there are no records of this host raising parasite's chicks, which suggest that it may have evolved effective antiparasitic defences. We studied cardinal antiparasitic defences by: (1) presenting dummy models of a female cowbird and non-predator and predator control species to nests during laying and incubation, and (2) conducting experiments of artificial parasitism with natural cowbird eggs of different morphs and conspecific eggs during laying and early and late incubation. We found that: (1) the frequency of cowbird parasitism in cardinal nests was 7%, (2) cardinals did not exhibit aggressive behaviours towards cowbird or nonpredator models but responded aggressively towards a predator model, (3) they rejected parasite eggs in 98.5% of the cases (mostly through egg ejection), but conspecific eggs in only 6% of the cases, (4) there were no costs (breakage or ejection of their own eggs) associated with ejection of the parasite's eggs, and (5) a relatively low frequency of parasitism is enough selection pressure to maintain egg rejection at a high level. The antagonistic expression of antiparasitic defences in red-crested cardinals suggests that they may have lost the behaviour of aggression towards the parasite as a result of associated costs.

### Keywords

antiparasitic defences, brood parasitism, egg rejection, *Molothrus bonariensis*, *Paroaria coronata*.

## 1. Introduction

Avian brood parasites, such as cuckoos and cowbirds, lay eggs in nests of other species (hosts) that incubate eggs and feed parasite chicks (Ortega, 1998; Davies, 2000). Detrimental effects of brood parasitism on host fitness result in selection pressures that favour the evolution of antiparasitic defences (Rothstein, 1990). Most common antiparasitic defences are aggression towards parasites to deter them from accessing the nest (Mosknes et al., 1990; Briskie et al., 1992; Røskaft et al., 2002; Gill et al., 2008) and rejection of parasite eggs, by deserting the nest (Goguen & Mathews, 1996; Hosoi & Rothstein, 2000), or by burying (Sealy, 1995; Moskát & Honza, 2002) or ejecting (Sealy & Neudorf, 1995; Moskát et al., 2002) the parasite egg. The cue more frequently used by hosts to recognize and reject parasite eggs is the disparity in colour and pattern between the parasite's eggs and the host's own eggs (Rothstein, 1974; Davies & Brooke, 1988; Spottiswoode & Stevens, 2010), although differences in size may also improve discrimination (Rothstein, 1982; Marchetti, 2000). Other antiparasitic defences, such as rejecting parasite chicks (Langmore et al., 2003; Sato et al., 2010), or not feeding cowbird chicks or fledglings (De Mársico et al., 2010; Delhey et al., 2011) are less common.

Egg ejection has been described as the most frequent and least costly antiparasitic defence (Davies & Brooke, 1989; Rothstein, 1990; Krüger, 2007). However, some authors have argued that recognition and ejection of parasitic eggs may be costly for hosts because they can occasionally break or mistakenly eject their own eggs when trying to eject the parasite egg (Rothstein, 1990; Davies et al., 1996). Aggression directed at the parasite is the first line of defence and may reduce parasitism when hosts are considerably larger in body mass than the parasite and may prevent them from accessing the nest (Mermoz & Fernández, 1999; Fiorini et al., 2009), but not when they are smaller or similar in body mass than the parasite (Tewksbury et al., 2002; Ellison & Sealy, 2007; Friesen et al., 2007). Besides, aggression may provide parasites with a cue to locate host nests (Robertson & Norman, 1977; Smith et al., 1984; Uyehara & Narins, 1995; but see Gill et al., 1997; Clotfelter, 1998), which may select against this trait.

Soler et al. (1999) hypothesized that when aggression is not effective to deter the parasite from accessing the nest, hosts that recognize and reject parasite eggs should defend their nests at a lower level than non-recognizers. These authors proposed that hosts specialize in one antiparasitic defence and

that different kinds of defence (aggression towards the parasite and egg rejection) are antagonistically expressed. Evidence in support of this hypothesis was found in magpies, *Pica pica*, parasitized by great spotted cuckoos, *Clamator glandarius* (Soler et al., 1999) and in four hosts of the brown-headed cowbirds, *Molothrus ater* (Neudorf & Sealy, 1992). On the contrary, Røskoft et al. (2002) found a strong correlation between the aggression level of potential host species towards dummy models of the common cuckoo, *Cuculus canorus*, and the rate at which they reject non-mimetic cuckoo eggs, supporting previous results of Moksnes et al. (1990), who suggested that the cuckoo–host arms race would lead to the evolution of both traits: aggression towards the parasite and rejection of non-mimetic parasite eggs.

The shiny cowbird, *M. bonariensis*, is an extreme generalist brood parasite whose eggs have been found in nests of more than 250 species (Ortega, 1998; Lowther, 2011) including the red-crested cardinal, *Paroaria coronata* (Friedmann, 1929; Friedmann & Kiff, 1985; De la Peña, 2005). Several features make the red-crested cardinal a potential good quality host for shiny cowbirds. Cardinals are sympatric and breed at the same time as shiny cowbirds, have accessible nests and similar body mass, and feed their young with a diet appropriate for shiny cowbirds (i.e., animal proteins). However, the frequency of shiny cowbird parasitism on cardinal nests is very low (De la Peña, 2005; Segura, 2011) and there are no records of this host successfully raising parasite chicks (Lowther, 2011; Segura, 2011). One explanation for the absence or low frequency of parasitism in potential good quality hosts is that they have well-developed antiparasitic defences (Scott, 1977; Sealy & Neudorf, 1995; Peer & Bollinger, 1997; Davis et al., 2002; but see Peer et al., 2000). Therefore, we expect that red-crested cardinals have evolved effective antiparasitic defences against shiny cowbirds. Because red-crested cardinals are similar in body mass to shiny cowbird females, it is unlikely that they can effectively deter them from gaining access to their nests. Thus, according with Soler et al. (1999) hypothesis, we expect that they have evolved egg rejection as the main antiparasitic defence. In addition, we also expect stronger antiparasitic defences during laying than during incubation (Hobson & Sealy, 1989; Gill & Sealy, 1996; Moskát, 2005; Campobello & Sealy, 2010). We experimentally tested these predictions by: (1) presenting dummy models of a female shiny cowbird and nonpredator and predator control species to red-crested cardinal nests during laying and incubation, and (2) parasitizing red-crested cardinal nests with natural shiny cowbird eggs of different

morphs (see below) and with conspecific eggs during laying and early and late incubation.

## 2. Methods

### 2.1. Study area

The study was conducted at 'Estancia La Matilde' (35°20'S, 57°11'W) near the town of Punta Indio, province of Buenos Aires, Argentina. The study site is a flat area of approximately 400 ha within the Biosphere Reserve 'Parque Costero del Sur' (MAB-UNESCO). It is semi-open grassland with patches of woodlands mainly dominated by native tree species such as *Celtis tala* (tala), *Scutia buxifolia* (coronillo) and *Schinus longifolius* (molle).

### 2.2. Study species

The red-crested cardinal (Thraupidae; Burns et al., 2002) is a sexually monomorphic species that inhabits semi-open areas with scattered trees and shrubs from east central Argentina to southern Brazil, Paraguay, eastern Bolivia and Uruguay (Ridgely & Tudor, 1994). At our study site, they breed from early October to late February nesting primarily in talas and secondarily in coronillos and molles at a height of 2–6 m (Segura & Arturi, 2009). They build open-cup nests with external and internal diameters of 13 and 6.5 cm, respectively, and a depth of 4.5 cm (Segura, 2011). Modal clutch size is three eggs; incubation starts with the laying of the penultimate egg and nestlings hatch after 12 days of incubation and fledge 14 days after hatching (Segura, 2011). Adult body mass is 45–48 g (Segura, 2011).

Shiny cowbirds are sexually dimorphic and dichromatic. Males are glossy black and weigh 50–55 g, whereas females are brownish-grey and weigh 40–45 g (Mason, 1987). At our study site, they lay eggs from late September to mid January. In eastern Argentina and neighbouring parts of Uruguay and Brazil, shiny cowbird eggs are white immaculate or spotted, and highly variable in the colour and size of the spots (Mahler et al., 2008). Some shiny cowbird hosts accept immaculate and spotted eggs (Mason, 1986; Massoni & Reboreda, 1998), others accept spotted eggs only (Mason, 1986; Mermoz & Reboreda, 1994; Sackmann & Reboreda, 2003; Astié & Reboreda, 2005), and there is one host (the rufous hornero, *Furnarius rufus*) that ejects eggs of both morphs if they are smaller than their own eggs (Mason & Rothstein, 1986).

### 2.3. Data collection

We found nests by searching systematically in potential nest sites and by observing nesting behaviour of territorial pairs (Martin & Geupel, 1993). We monitored 108, 120, 139, 49 and 41 nests during the 2005–2006, 2006–2007, 2007–2008, 2008–2009 and 2009–2010 breeding seasons, respectively. Experiments of artificial parasitism were conducted during the 2008–2009 and 2009–2010 breeding seasons, while experiments of nest defence were conducted during the 2009–2010 breeding season.

#### 2.3.1. Nest defence experiments

We tested species-specific agonistic behaviours towards shiny cowbirds by presenting taxidermic models mounted in a life like position (i.e., perched on a branch) to pairs of nesting cardinals. We conducted our experiments during egg laying ( $N = 11$  nests) and incubation (4–9 days after clutch completion,  $N = 11$  nests). Each nest was tested with models of: (1) female shiny cowbird, (2) baywing (*Agelaioides badius*) and (3) guira cuckoo (*Guira guira*). Baywings are sympatric with cardinals and similar in body mass to female shiny cowbirds (40 versus 40–45 g), but pose no threat to cardinals. Guira cuckoos are considerably larger than cardinals (body mass 150 g) and are nest predators during the egg and chick stages. We presented the models sequentially with an interval between presentations of 20 min to avoid habituation or positive reinforcement (Sealy et al., 1998). To minimize the possible effect of the order of presentation of the stimuli, we used three different sequences: shiny cowbird–baywing–guira cuckoo, baywing–guira cuckoo–shiny cowbird, and guira cuckoo–shiny–baywing (approximately one-third of the nests for each sequence). We conducted the experiments during early morning (07:00–10:00), time at which shiny cowbirds visit frequently host nests (Fiorini et al., 2009). We attached the models to a branch 0.8 m from the nest at the same height and pointing to it. Each trial began when one of the nest owners returned to the nest and lasted for 5 min or until one of the members of the pair struck down the model. We video recorded host behaviours with a video camera placed 10–15 m from the nest. Because, the difficulty in obtaining taxidermic models (they were prepared from birds found dead in the wild) we could use one model per species only. Therefore, we cannot discriminate whether the differential responses towards models were because they were different species or different models.

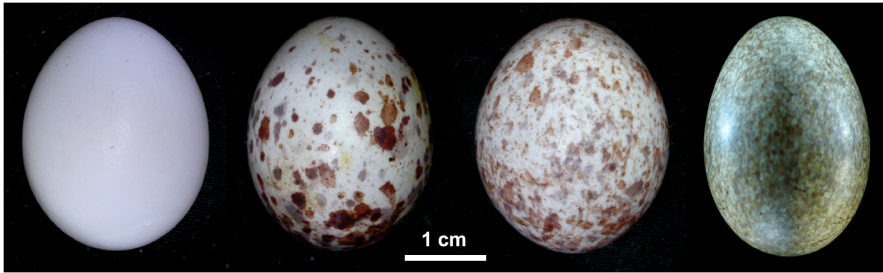
We analyzed the videotapes in the laboratory using EthoLog (Ottoni, 2000) and quantified the following behaviours for both members of the pair

combined: (1) frequency of attacks and close passes directed to the model (direct aggression), (2) proportion of time at least one member of the pair was perched less than 2 m from the model (approach), (3) frequency of distress calls (distress calls) and (4) proportion of time cardinals spent sitting in the nest (time in the nest). We chose these variables to characterize host responses according to the level of aggressiveness or degree of risk taken (aggressive attacks > approach > distress calls > time in the nest) (Sealy et al., 1998). We excluded from the analyses one nest in incubation where the members of the pair did not respond to any of the models.

### *2.3.2. Artificial parasitism experiments*

We experimentally parasitized 68 nests with natural shiny cowbird eggs (interspecific parasitism) and 16 nests with natural cardinal eggs (intraspecific parasitism). We collected 68 fresh shiny cowbird eggs (i.e., without incubation) from active chalk-browed mockingbirds nests and 9 fresh red-crested cardinal eggs from nests that had been deserted during laying. We conducted interspecific parasitism experiments during egg laying ( $N = 35$  nests), early incubation (1–4 days after clutch completion,  $N = 21$  nests) and mid-late incubation (6–9 days after clutch completion,  $N = 12$  nests) and intraspecific parasitism experiments during egg laying ( $N = 9$  nests) and early incubation ( $N = 7$  nests). For the experiments on interspecific parasitism, we used shiny cowbird eggs of three different morphs (Figure 1): (1) white immaculate ( $N = 18$  nests), (2) lightly spotted ( $N = 30$  nests) and (3) highly spotted ( $N = 20$  nests). Shiny cowbird eggs vary markedly in spotting pattern and in ground colour (Mahler et al., 2008). To control for this variation, in our experiments we only used spotted eggs with white ground colour. As regard the spotting pattern, we selected eggs of two categories based on visual observation: lightly spotted (eggs with spots covering only part of the egg's surface) and heavily spotted (eggs with spots covering most of the egg surface, Figure 1).

Shiny cowbird eggs used in interspecific parasitism experiments were  $24.6 \pm 0.1$  mm in length and  $17.2 \pm 0.1$  mm in width and there were no significant differences in length and width among egg morphs (Kruskal–Wallis tests; length:  $H_2 = 2.5$ ,  $p = 0.31$ ; width:  $H_2 = 3.7$ ,  $p = 0.15$ ,  $N = 68$  eggs). Preliminary observations indicated that cardinals are puncture-ejecters. To evaluate if they abandon the nest when they cannot puncture the parasite egg we also experimentally parasitized 15 nests with model eggs made of plaster of Paris and painted with acrylic paints to simulate the appearance



**Figure 1.** Photographs of parasite and conspecific eggs used in the artificial parasitism experiments. From left to right: white immaculate, lightly spotted and highly spotted shiny cowbird eggs and red-crested cardinal egg. This figure is published in colour in the online edition of this journal, which can be accessed via <http://www.brill.nl/beh>

of shiny cowbird spotted eggs. We conducted these experiments during egg laying ( $N = 9$  nests) and early incubation ( $N = 6$  nests). Plaster eggs used in these experiments were  $24.4 \pm 0.1$  mm in length and  $17.4 \pm 0.1$  mm in width ( $N = 10$  eggs). No host eggs were removed in conjunction with experimental parasitism. We did not remove a host egg at the time of experimental parasitism because shiny cowbirds do not remove host eggs. Instead, they peck (and sometimes puncture) one or more host eggs, which are afterwards removed by the host (Astié & Reboreda, 2006). For the experiments of interspecific parasitism with natural eggs we used a different egg for each nest, while for the experiments of interspecific parasitism with plaster eggs we used 10 different eggs for the 15 nests, and for the experiments of intraspecific parasitism we used 9 different eggs for the 16 nests.

We checked nests for egg ejection daily and at each visit we carefully examined host and parasite eggs for cracks or punctures and determined whether the nest was active or if it had been abandoned. We considered a parasitic egg ejected if it disappeared from the nest, and accepted if it remained in the nest for at least five days after the experimental introduction (Rothstein, 1975; Sealy, 1996).

#### 2.4. Statistical analysis

We used non-parametric tests to analyze the responses of hosts to the stimuli, as the assumptions of normality and equality of variances were not met in original or transformed data sets. We analyzed differences in aggressive responses towards dummy models with Friedman's nonparametric ANOVAs and post-hoc contrasts and compared differences in frequency of rejection

among treatments using Chi-square tests. Statistical tests were performed using Statistica 7.0 with an alpha level = 0.05 (two-tailed). Data are presented as means  $\pm$  SE.

### **3. Results**

#### *3.1. Frequency of natural parasitism*

The frequency of shiny cowbird parasitism in red-crested cardinal nests was 7% (9 of 130 nests found during nest building or egg laying) and in all cases nests were parasitized with a single egg. The first parasitic event occurred on October 15 and the last one on December 15. One parasite egg was white immaculate, 5 were lightly spotted and 3 were heavily spotted. Seven nests were parasitized during laying and two in early incubation. In six cases, the parasite egg was ejected and in the other three the nest was abandoned. In three nests, one host egg was punctured and in two nests one host egg disappeared in association with parasitism.

#### *3.2. Aggression towards parasites*

We did not detect significant differences between models in the frequency of attacks and close passes, proportion of time close to the model, and proportion of time birds sat in the nest when models were presented during host laying, but the guira cuckoo model elicited a higher frequency of distress calls than female shiny cowbird or baywing models (Table 1). During incubation, the guira cuckoo model elicited higher frequencies of attacks and close passes, higher proportion of time close to the model, higher frequency of distress calls, and lower proportion of time sitting in the nest than either the shiny cowbird or baywing models (Table 1).

#### *3.3. Rejection of parasite eggs*

Cardinal eggs in experimental nests were  $25.5 \pm 0.1$  mm in length and  $17.2 \pm 0.1$  mm in width (average of the mean values of 68 clutches) and were significantly larger and narrower than the shiny cowbird eggs used in the experiments (Wilcoxon signed-rank tests; length:  $Z = 6.3$ ,  $p < 0.001$ ; width:  $Z = 7.1$ ,  $p < 0.001$ ;  $N = 68$ ). Rejection rate of natural shiny cowbird eggs was 98.5% (67 of 68 nests) and occurred at similar frequencies regardless of the stage at which experimental parasitism occurred (i.e., laying, early incubation or late incubation;  $\chi^2_2 = 2.2$ ,  $p = 0.32$ ) or the egg morph used (i.e.,



**Table 1.**  
Responses of red-crested cardinal (11 nests in laying and 10 in incubation) to the presence of dummy models of female shiny cowbird, baywing (non-predator control species) and guira cuckoo (predator control species) close to their nests.

		Shiny cowbird	Baywing	Guira cuckoo	Friedman test	Comparisons
Egg laying	Time in the nest	0.28 ± 0.09	0.28 ± 0.08	0.09 ± 0.06	$\chi^2 = 4.7, p = 0.09$	SC = Bw = GC
	Approach to model	0.50 ± 0.10	0.48 ± 0.07	0.65 ± 0.09	$\chi^2 = 1.4, p = 0.48$	SC = Bw = GC
	Distress calls	0.90 ± 0.39	1.10 ± 0.49	5.91 ± 0.61	$\chi^2 = 17.7, p < 0.001$	SC & Bw < GC
Incubation	Direct aggression	0.64 ± 0.36	0.18 ± 0.12	0.64 ± 0.31	$\chi^2 = 2.6, p = 0.26$	SC & Bw = GC
	Time in the nest	0.39 ± 0.10	0.23 ± 0.06	0.02 ± 0.02	$\chi^2 = 10.1, p = 0.006$	SC & Bw > GC
	Approach to model	0.48 ± 0.08	0.61 ± 0.07	0.84 ± 0.05	$\chi^2 = 7.8, p = 0.02$	SC & Bw < GC
	Distress calls	2.00 ± 0.80	4.00 ± 1.80	11.60 ± 3.10	$\chi^2 = 16.7, p < 0.001$	SC & Bw < GC
	Direct aggression	0.50 ± 0.50	1.60 ± 0.90	4.50 ± 1.90	$\chi^2 = 10.9, p = 0.004$	SC & Bw < GC

The variables analyzed were: proportion of time spent in the nest (time in the nest), proportion of time at least one member of the pair was perched to less than 2 m from the model (approach to model), frequency of distress calls (distress calls) and frequency of attacks and close passes directed to the model (direct aggression). Values indicate mean ± SE and results of Friedman's ANOVAs.

**Table 2.**  
Results of experiments of artificial parasitism.

		Eject and continue	Eject and abandon	Accept and continue	Accept and abandon	Total
Egg laying	White immaculate eggs	12 (100)	0 (0)	0 (0)	0 (0)	12
	Lightly spotted eggs	11 (85)	2 (15)	0 (0)	0 (0)	13
	Highly spotted eggs	9 (90)	0 (0)	0 (0)	1 (10)	10
	Conspecific eggs	0 (0)	0 (0)	8 (89)	1 (11)	9
Early incubation	White immaculate eggs	5 (100)	0 (0)	0 (0)	0 (0)	5
	Lightly spotted eggs	8 (89)	1 (11)	0 (0)	0 (0)	9
	Highly spotted eggs	6 (86)	0 (0)	1 (14)	0 (0)	7
	Conspecific eggs	0 (0)	0 (0)	7 (100)	0 (0)	7
Late incubation	White immaculate eggs	1 (100)	0 (0)	0 (0)	0 (0)	1
	Lightly spotted eggs	8 (100)	0 (0)	0 (0)	0 (0)	8
	Highly spotted eggs	3 (100)	0 (0)	0 (0)	0 (0)	3

Red-crested cardinal nests were parasitized with natural shiny cowbird eggs (white immaculate, lightly spotted or highly spotty) and natural conspecific eggs during laying, early incubation (1–4 days after clutch completion) and late incubation (6–9 days after clutch completion). Values indicate number of nests (percentages).

white immaculate, lightly spotted or highly spotted;  $\chi^2_2 = 2.4$ ,  $p = 0.29$ , Table 2). All but one case of rejection was by egg ejection (66 of 67 nests). In 63 cases, cardinals ejected the parasite egg and continued incubation, in three cases they ejected the parasite egg and abandoned the nest and in one case they abandoned the nest without previous egg ejection. In 64 of 66 nests, ejection occurred within 24 h following experimental parasitism and in 2 of 66 nests between 24 and 48 h following experimental parasitism. Video records of three experiments of artificial parasitism with natural cowbird eggs showed that cardinals first pecked and punctured the parasite egg and then carry it with the bill away from the nest by grasping the eggshell. We did not observe any case of damage of host eggs in association with the ejection of the parasite egg. Similarly to the experiments with shiny cowbird natural eggs, cardinals rejected parasite plaster eggs in 100% of the cases (in 13 of 15 nests by ejection and in the other two by desertion). All ejections occurred within 24 h after experimental parasitism. In two nests we observed the damage of one host egg in association with the ejection of the parasite plaster egg. In five nests, we found the plaster egg on the grass within a radius of 3 m around the nest and in all cases the egg showed signs of have been intensely pecked (they presented cracks and small holes that could have been

used to grasp and eject the egg). Contrary to the high frequencies of rejection of natural and plaster parasite eggs, the frequency of rejection of conspecific eggs was only 6% (1 of 16 nests, Table 2). This frequency was significantly lower than that of parasite eggs (all stages combined;  $\chi^2_1 = 71.2$ ,  $p < 0.001$ ).

#### 4. Discussion

We show that: (1) red-crested cardinals do not display agonistic behaviours towards shiny cowbird or non-predator control models, but they respond to the presence of a predator control model, (2) they eject shiny cowbird eggs of all morphs, but accept conspecific eggs, (3) the ejection of shiny cowbird eggs has no cost in terms of destroying or ejecting their own eggs, and (4) ejection behaviour is as strong during incubation as during laying.

Responses of red-crested cardinal towards a female shiny cowbird model placed close to the nest did not differ from those directed towards a non-predator control species. In contrast, cardinals responded differentially to the presence of a predator control model during laying by increasing the frequency of distress calls, and during incubation by spending more time close to the model and by increasing the frequencies of direct attacks and distress calls. These positive results towards a predator control model indicate that our experimental condition was appropriate to study aggressive responses in cardinals, but the presence of a female cowbird close to the nest did not elicit aggression as an antiparasitic defence.

On the contrary, experiments of artificial parasitism with natural and plaster shiny cowbird and conspecific eggs indicate that egg rejection in cardinals would be an antiparasitic defence, as they rejected parasite eggs in 98.5% of the cases (in all but one case through egg ejection), but conspecific eggs in only 6% of the cases. An alternative explanation for egg rejection is that cardinals express this behaviour because it has been selected in the context of conspecific instead of interspecific brood parasitism. However, we did not observe any indirect evidence of conspecific parasitism (i.e., appearance of two eggs the same day, or abnormal clutch sizes) in more than 180 nests followed daily during laying. Another possibility is that red-crested cardinal behaviour of rejecting cowbird eggs is not an antiparasitic defence, but it has evolved from benefits obtained by cleaning the nest (it would be an exaptation instead of an adaptation). This explanation (nest sanitation hypothesis, Guigueno & Sealy, 2009, 2012) has been suggested by some authors to

account for some rejection behaviours (mostly egg burial) of hosts of the common cuckoo, *Cuculus canorus* (Bartol et al., 2003; Moskát et al., 2003) and the brown-headed cowbird (Guigueno & Sealy, 2009), and less likely, as an explanation for the maintenance of egg rejection in host populations that are not sympatric with parasite ones (Peer et al., 2007). Some of our results (i.e., lack of aggression towards the parasite and similar frequency of egg ejection during laying, early and late incubation, see below) are consistent with predictions of this hypothesis. However, although our experiments were not designed to test this hypothesis (i.e., we did not parasitize nests with objects that resemble debris), we consider that this explanation is unlikely in our case as almost all cases of rejection were through ejecting the parasite egg (likely after puncturing the parasite egg) and there were no cases of egg burial, which is the more frequent example of rejection associated to nest sanitation behaviour.

Nest-defence mechanisms are usually considered ancestral, whereas egg recognition and rejection would be a subsequent stage in the co-evolutionary process between hosts and parasites (Rothstein, 1990; Soler et al., 1999). In this context, after cardinals evolved egg rejection, they may have lost aggressive behaviours towards cowbirds because the costs (i.e., increase in the probability of nest detection by parasites or predators) exceeded the benefits (prevent parasites from gaining access to the nest). Thus, our results are consistent with Soler et al.'s (1999) hypothesis, which proposes that when aggression is not effective to deter the parasite from accessing the nest; hosts that recognize and reject parasite eggs should defend their nests at a lower level than non-recognizers (but see Røskaft et al., 2002). Further experiments in rejecter and non-rejecter hosts, larger and smaller in body mass than shiny cowbirds, may help to disentangle whether aggression towards the parasite and rejection of parasite eggs are antagonistically expressed or highly correlated.

Contrary to our prediction, egg ejection was as frequent during laying than during early and late incubation. Although parasite eggs laid during incubation are unlikely to hatch, they could reduce the hatchability of cardinal eggs, which may have selected for the maintenance of this behaviour. Alternatively, because egg ejection is cost free (see below) there might be no selection pressure on hosts to reduce the intensity of this behaviour during incubation.

This is the first well-documented study reporting a shiny cowbird host that recognizes and ejects parasite eggs of both immaculate and spotted morphs. Previous studies showed that some hosts eject eggs of the white immaculate morph but accept spotted ones (Mason, 1986; Mermoz & Reboreda, 1994; Sackmann & Reboreda, 2003; Astié & Reboreda, 2005). One exception is the rufous hornero, which ejects white immaculate and spotted eggs using the discrepancy in size between parasite and their own eggs as a cue (Mason & Rothstein, 1986). Another species that potentially could be an ejector of spotted shiny cowbird eggs is the fork-tailed flycatcher (*Tyrannus savana*), but the experimental evidence is weak (2 of 2 spotted eggs ejected, Mason, 1986). Our results suggest that the cue used by red-crested cardinals to recognize shiny cowbird eggs is discrepancy in colour between their own and parasite eggs, but we cannot rule out the possibility that they use other cues, such as size or shape of the egg, to recognize parasite eggs. Also, the red-crested cardinal is the first species reported as puncture ejector of shiny cowbird eggs. All previously described ejector species were grasp ejectors: rufous horners (Mason & Rothstein, 1986), brown and yellow marshbird, *Pseudoleistes virescens* (Mermoz & Reboreda, 1994), chalk-browed mockingbird, *Mimus saturninus*, and rufous-bellied thrush, *Turdus rufiventris* (Sackmann & Reboreda, 2003), and creamy-bellied thrush, *Turdus amaurochalinus* (Astié & Reboreda, 2005). All these hosts are considerably larger in body size than shiny cowbirds and therefore they have a bill large enough to grasp the parasite egg. In contrast, most hosts smaller than shiny cowbirds do not eject parasite eggs despite being considerably different in size and colour compared to host eggs (Mason, 1986). Most of these hosts have a similar history of sympatry with shiny cowbirds as do ejector species and, therefore, they could have evolved egg ejection behaviour. One possible explanation for the difference in ejection behaviour between ‘small’ and ‘large’ hosts of shiny cowbirds is that the former cannot grasp the cowbird egg because of a bill size constrain and they cannot puncture the cowbird egg because of its thicker eggshell (Spaw & Rohwer, 1987; Mermoz & Ornelas, 2004; Peer & Sealy, 2004). Red-crested cardinals are similar in size to shiny cowbirds and although they appear to have a beak not large enough to grasp and remove the cowbird egg, they can puncture cowbird eggs.

Our results also show that cardinals did not have any cost associated with the ejection of parasite eggs. Lorenzana & Sealy (2001) found that in the gray catbird (*Dumetella carolinensis*) the cost of puncture ejection, in terms

of hosts breaking their own eggs, is four times greater than that of grasp ejection. In our experiments, however, cardinals removed parasitic eggs without damaging their own eggs. Even in the experiments with artificial plaster eggs, where cardinals pecked the model egg more intensively, the damage to their own eggs was relatively infrequent (two host egg damaged in 15 nests). In addition, we did not detect any case of disappearance of host eggs in association with the ejection of the parasite egg (either in experiments with natural or in those with plaster eggs), which indicate that there were no cases where the host mistakenly ejected its own eggs. Thus, our results indicate that in this host, the cost of egg ejection is very low.

Finally, our study indicates that a relatively low frequency of parasitism is enough selection pressure to maintain an antiparasitic defence at a high level. The frequency of parasitism we reported (7%) is probably an underestimate of the real one, because in some cases cardinals could have ejected the parasite egg before we visited their nest. However, we visited host nests during the morning (and parasitism occurs early in the morning) and we did not find indirect evidence of shiny cowbird parasitism in unparasitized nests (i.e., host eggs with punctures or disappearance of host eggs between visits; Massoni & Reboreda, 2002; Astié & Reboreda, 2006). Therefore, we are confident that the actual frequency of parasitism was close to the one we measured. Other studies have proposed that hosts can retain ejection behaviour for long periods because in the absence of parasitism its adaptive value is close to neutral (Rothstein, 2001). Consistently some studies have shown that populations currently unparasitized but that are descended from lineages likely to have been parasitized in the past, have maintained egg ejection behaviour for periods from at least 10 000 years (Peer et al., 2007) to 2.8–3.0 million years (Peer et al., 2011). To summarize, our study shows that in the red-crested cardinal aggression towards shiny cowbirds and rejection of shiny cowbird eggs are antagonistically expressed, ejection of parasite eggs is cost-free and a relatively low frequency of parasitism appears to be enough selection pressure to maintain high levels of egg rejection.

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