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Rejection of brood-parasitic shiny cowbird *Molothrus bonariensis* nestlings by the firewood-gatherer *Anumbius annumbi*?

Kaspar Delhey, Martín Carrizo, Lucas Verniere, Bettina Mahler and Anne Peters

K. Delhey (kaspar.delhey@monash.edu) and A. Peters, Max Planck Inst for Ornithology, Schlossallee 2, DE - 78315, Radolfzell, Germany. Present address of KD and AP: School of Biological Sciences, Monash Univ., Melbourne, VIC 3168, Australia. – M. Carrizo, Alvarado 845, 5°A, 8000, Bahía Blanca, Argentina. – L. Verniere, Félix San Martín 576, Dto. 10, 8371, Junín de los Andes, Argentina. – B. Mahler, Depto de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Univ. de Buenos Aires, Pabellón II Ciudad Universitaria, C1428EHA Buenos Aires, Argentina.

Costs imposed by brood parasitic birds exert strong selection on their hosts to avoid parasitism. While egg rejection is a common defence, nestling rejection is rarer and less well understood. Theoretical models suggest that among non-evicting parasites such as cowbirds nestling rejection can only evolve when levels of parasitism are high. Here we describe a possible case of early rejection of cowbird nestlings, by an infrequently parasitised host, the firewood-gatherer *Anumbius annumbi*. Firewood-gatherers accepted most shiny cowbird *Molothrus bonariensis* eggs despite clear differences in coloration. Cowbird eggs usually hatched 4–5 d before host eggs. All parasitic nestlings died within 48 h, and hosts continued their breeding attempts. Nestling death was most likely due to neglect since little food was found in the stomach of dead nestlings. Feeding neglect could be due to differences in visual or acoustic appearance between host and parasite hatchlings. Alternatively, hosts may refrain from feeding nestlings that hatch too early compared to their normal incubation time. At the moment our data do not allow distinction between active nestling recognition or cowbird nestling failure due to the unsuitability of the firewood-gatherer as a host (i.e. too long incubation). Experiments are needed to tease these alternatives apart.

Brood parasitic birds impose severe reproductive costs on hosts and these are thus strongly selected to reject parasitism attempts (Davies 2000). While many hosts show finely tuned egg rejection mechanisms, the lack of nestling rejection, even if the parasitic chick is an obvious aberration, is an evolutionary puzzle (Grim 2006). One possibility is that the evolution of nestling rejection is constrained by efficient egg rejection, making parasitic nestlings too rare to elicit strong selection pressure (Grim 2006). Even if parasitic nestlings do occur sufficiently often, the evolution of (learned) nestling rejection may be constrained by the risk of mis-imprinting on a parasitic chick in the first breeding attempt, and rejecting future own offspring (Lotem 1993). Therefore, nestling rejection should be most common in host species that are egg acceptors, with high incidence of parasitism, and low cost of recognition errors through mis-imprinting (Langmore et al. 2003, Lawes and Marthews 2003, Grim 2006).

If recognition of parasite nestlings happens through imprinting, nestling rejection is more likely to evolve in non-evicting brood parasites (such as cowbirds *Molothrus* spp., Fam. Icteridae), where host and parasite nestlings share the nest, than in evicting parasites (such as many cuckoos, Fam. Cuculidae) where the likelihood of mis-imprinting on a parasitic chick in the first breeding attempt is too costly (Lotem 1993, Lawes and Marthews 2003). However, so far, the most convincing observational and experimental evidence of parasite chick rejection is by hosts of evicting cuckoos (Langmore et al. 2003, Grim 2007, Sato et al. 2009, Langmore and Kilner 2010, Tokue and Ueda 2010) and possibly, both, an innate template and learning are part of the recognition process (Langmore et al. 2009). There are only two examples of cowbird chick discrimination by hosts, both involving shiny cowbirds *M. bonariensis*. Parasitic chicks are fed less compared to host chicks in nests of rufous-bellied thrushes *Turdus rufiventris* and often starve (Lichtenstein 2001), and the same happens to cowbird fledglings after leaving the nests of bay-winged cowbirds *Agelaioides badius* (Fraga 1998). The surprising absence of records of cowbird nestling rejection may be due to low selection pressures for the evolution of chick rejection due to low frequencies of cowbird parasitism, high frequency of egg rejection, insufficient reduction in host reproductive success by these non-evicting chicks (Lawes and Marthews 2003), or high chances of mis-imprinting. Here we describe a possible case of shiny cowbird nestling rejection shortly after hatching and discuss alternative explanations for this observation.

Material and methods

We studied shiny cowbird parasitism in a colour-banded population of 10–25 breeding pairs of firewood-gatherers *Anumbius annumbi* in a low scrubland plot (~170 ha) near the town of Bahía Blanca (38°35′S, 62°04′W), Argentina, during four breeding seasons between August 2006 and March 2010. Firewood-gatherers (family Furnariidae) are medium sized insectivorous birds (32.5–47.5 g, unpubl.) that breed in large (45 by 40 cm, unpubl.) roughly ovoid enclosed nests placed on shrubs and trees. Nests are made of sticks and consist of a spiralling entrance tunnel that leads to the brood chamber which is lined throughout with a dense layer of soft material (de la Peña 2005). Thus, in most cases the brood chamber is dark, although light levels were not measured. At our study site this species breeds between August and March making up to 6 breeding attempts per season (median = 3) and laying clutches of 5 eggs on average (Delhey et al. 2010). Incubation takes 15.5 d on average (SE = 0.32, range = 12–19 d, Delhey et al. 2010). Firewood-gatherers are not often parasitized by cowbirds, and we have observed no overt aggression during the study (unpubl.). No cases of parasitism have been recorded in three populations studied in Argentina (Salvador 1983, Mason 1986, de la Peña 2005) although shiny cowbird eggs (Friedmann et al. 1977) and presumably also nestlings (Castellanos 1934) have occasionally been found in firewood-gatherer nests. Shiny-cowbirds are medium-sized (average body mass from the subspecies present at our study site *M. b. bonariensis*, males = 55.5 g, females = 44.9 g, Lowther 2011) obligate brood-parasites that have been shown to lay eggs in nests of around 250 species (Lowther 2011). Shiny-cowbird eggs require 11–12 d of incubation on average (Lowther 2011), which usually gives them a developmental advantage in nests of hosts with longer incubation times (Briskie and Sealy 1990). In our study site the breeding season of cowbirds is shorter than that of firewood-gatherers starting about a month later (second half of September) and finishing two months earlier (February, unpubl.). This matches the breeding season of the most important shiny cowbird hosts at the study site: mockingbirds (*Mimus saturninus* and *M. triurus*, incubation 14–15 d; de la Peña 2005) and the diuca finch (*Diuca diuca*, incubation 10–13 d, unpubl.).

To determine the initiation and outcome of brood parasitism, nest contents were monitored regularly (on average every 3–4 d); 4 parasitized nests were not found until incubation, and thus the exact timing of laying of the parasitic egg could not be determined. We collected three recently dead cowbird nestlings and preserved them in 70% ethanol until dissection. For a more detailed description of general field methods and study site see Delhey et al. (2010).

Results

Shiny cowbird brood parasitism varied between 13 and 19% of nests over the years (all years pooled, 27/164 nests). Most (23/27) parasitized nests had only one cowbird egg, the rest had two. All cowbird eggs belonged to the spotted morph (Fig. 1). White cowbird eggs are rare in the study area (2 out of 55 eggs in nests of other hosts, unpubl.) and thus unlikely to have biased our estimate of parasitism. Cowbird eggs of both morphs are very similar to firewood-gatherer eggs in size (averages (SE, range): cowbird: 24.4 (0.18, 23.5–26.5) × 18.8 (0.15, 17.8–19.9) mm, n = 15, firewood-gatherer: 23.6 (0.05, 19.9–26.1) × 17.4 (0.03, 15.3–19.3) mm, n = 407) and white-morph cowbird eggs also resemble firewood-gatherer eggs in colour (de la Peña 2005, Delhey et al. 2010).

Table 1 summarizes the information on the 27 parasitised nests found. Cowbird eggs were laid during the egg laying period of the firewood-gatherer (21 eggs, 19 nests), or during incubation (5 eggs, 5 nests; 3 during the first half of the incubation period, 2 during the second half). We followed the fate of 31 eggs in 27 nests (Table 1). Cowbird eggs were generally accepted: only 3 eggs (3 nests) were presumed to have been rejected before hatching, since the time interval between the start of incubation and disappearance date was shorter than the average incubation period of cowbirds (11–12 d, Lowther 2011). For 5 eggs (4 nests) that disappeared after or around 11–12 d of incubation we cannot distinguish whether these represented egg rejection events or cases of undetected early nestling rejection (see below). Eleven parasitized nests were depredated before hatching (12 eggs) while 4 cowbird eggs remained un-hatched (4 nests). As a result, 7 cowbird eggs hatched in 6 nests.

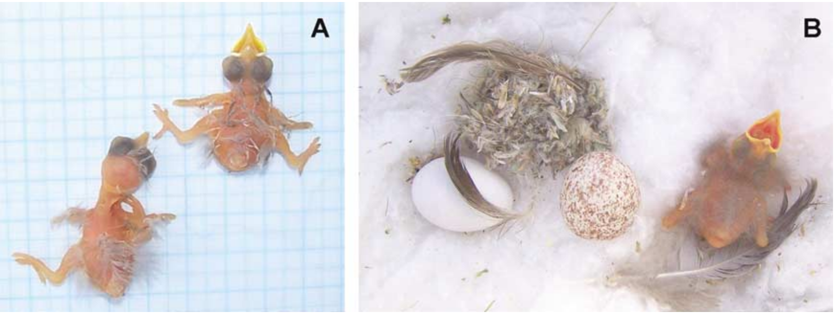

Figure 1. (A) Newly hatched firewood-gatherer nestlings and (B) shiny cowbird nestling with the rest of its spotted egg shell (centre) and a white unhatched firewood-gatherer egg (left). Fotos by M. Carrizo.

Table 1. Summary of the twenty-seven firewood-gatherer nests that were parasitized by shiny-cowbirds between 2006 and 2010.

Nest	Season	No. cowbird eggs	No. host eggs	Cowbird egg laid during host	Fate cowbird egg	Fate cowbird chick	Fate host eggs	Fate host chicks
N115A	2006-2007	1	6	laying	dissapeared (hatching not possible)[1]	–	hatched	fledged
N253a	2006-2007	1	5	laying	hatched	found dead	hatched	fledged
N250	2006-2007	1	6	laying	hatched	found alive, dead 1 d later	depredated	–
N256	2006-2007	1	6	laying	hatched	found dead	hatched	depredated
N288	2006-2007	1	4	laying	depredated	–	depredated	–
N35	2006-2007	1	6	laying	unhatched	–	hatched	fledged
N289a	2006-2007	2	6	both laying	both depredated	–	depredated	–
N289b	2006-2007	1	6	?	depredated	–	depredated	–
N303	2006-2007	1	4	?	dissapeared (hatching possible)[2]	?	hatched	depredated
N288	2007-2008	1	5	?	unhatched	–	hatched	depredated
N533	2007-2008	1	7	incubation	depredated	–	depredated	–
N535	2007-2008	2	5	?	both dissapeared (hatching possible)[2]	?	?	?
N509A	2007-2008	1	3	laying	depredated	–	depredated	–
N530	2007-2008	1	3	laying	depredated	–	depredated	–
N534	2007-2008	1	5	laying	dissapeared (hatching not possible)[1]	–	depredated	–
N540	2007-2008	1	6	incubation	depredated	–	depredated	–
N657	2008-2009	1	6	incubation	dissapeared (hatching possible)[2]	?	?	?
N293Ba	2008-2009	1	6	laying	depredated	–	hatched	fledged
N494A	2008-2009	1	4	laying	depredated	–	depredated	–
N528B	2008-2009	1	4	laying	hatched	found dead[3]	hatched	fledged
N634b	2008-2009	1	6	laying	dissapeared (hatching not possible)[1]	–	hatched	fledged
N641	2008-2009	1	?	laying	depredated	–	depredated	–
N728	2009-2010	1	6	laying	unhatched	–	hatched	depredated
N715b	2009-2010	1	?	laying	depredated	–	depredated	–
N718a	2009-2010	1	6	incubation	unhatched	–	hatched	fledged
N718b	2009-2010	2	5	laying; incubation	dissapeared (hatching possible)[2]; hatched (10 d after host)	?; found alive, gone 3 d later	hatched	fledged
N543Ab	2009-2010	2	6	both laying	both hatched	both found alive, dead 1-2 d later[3]	depredated	–

[1] dissapeared before 11–12 d of incubation; [2] dissapeared around 11–12 d of incubation; [3] collected for dissection.

No cowbird nestlings survived. Cowbird eggs hatched 4–5 d before the host eggs and they were the only chicks in the nest at this time (except in one case, see below). Three newly hatched cowbird nestlings were found dead inside the nest. Three were found alive just after hatching and were dead inside the nest on our next visit (1–3 d later). Lastly, one cowbird chick that hatched ten days later than the host chicks (and thus shared the nest with host nestlings) had disappeared from the nest on our next visit three days later. Of the dead nestlings we found, one seemed slightly crushed while the others did not show external damage. Dissection of three dead cowbird nestlings revealed that their gizzards contained only hair and fibrous nest lining. In one gizzard we found a small chitinous particle that could have been a fragment of an arthropod. The mortality levels (7 out of 7 nests) of cowbird nestlings in firewood-gatherer nests contrasts with the low levels of host nestling mortality due starvation or parasitism (excluding predation). This type of nestling mortality was only recorded in 7 out of 72 unparasitised host nests (two-sided Fisher's exact test, $p < 0.001$ following the method in Bailey (1995)).

Cowbird nestling failure was not due to host parents being unable to raise chicks: in 4 of 6 nests in which the cowbird nestling died, host eggs hatched and nestlings were raised until fledging or predation (two were depredated before hatching). Based on our limited sample, parasite failure did not appear to be dependent on previous breeding experience: in one nest cowbird parasitism took place during the first breeding attempt of the first breeding season of both pair members. The other 5 cases of early cowbird failure were by pairs that had previous experience with firewood-gatherer hatchlings.

Discussion

While most cowbird eggs laid in firewood-gatherer nests were accepted and incubated, all nestlings died within 48 h of hatching. Our data show that death of the parasitic chick can take place very early in the nestling phase and thus could easily go undetected. If this is also the case in other hosts it could help to explain the apparent overall scarcity of nestling rejection (Grim 2006), especially in cowbirds.

Most (>70%) of cowbird eggs were incubated. Whether cowbird eggs disappeared because they were recognized as alien is unclear. Many host eggs also disappear from the nest during laying and incubation (Delhey et al. 2010) and this is related to egg puncture by cowbirds prompting the host to remove the damaged eggs (Reboreda et al. 2003). In our population, egg-puncturing causes the loss of 9.5% of host eggs and affects 23% of nests (Delhey et al. 2010). Egg-puncturing could also account for the disappearance of cowbird eggs which are also occasionally found punctured (unpubl.). Experimental evidence from other populations (not parasitized by cowbirds) shows that the firewood-gatherer does not reject spotted cowbird eggs (Mason 1986, S. Salvador pers. comm.). Rejecting foreign eggs may be difficult due to nest type constraints such as dim light which hinders recognition (Langmore et al. 2003) or the long spiralling entrance tunnel which may prevent rolling out intact eggs if eggs are too large relative to gape size (Mason and Rothstein 1986). Thus, perhaps only punctured eggs can be held in the bill and carried out of the nest.

All cowbird nestlings died shortly after hatching. Nestling death seemed to happen through neglect rather than active ejection (Sato et al. 2009), since in most cases we found nestlings dead in the nest. Nestling death is unlikely to be a consequence of inadequate diet. While cowbird nestlings die of starvation in nests of species that feed their nestlings with seeds such as finches and siskins (Lowther 2011), firewood-gatherers feed their nestlings with arthropods and other invertebrates (unpubl.) which constitute a suitable diet for cowbirds (Mason 1986). Moreover, examination of stomach contents of dead nestlings showed that nestlings were hardly fed, if at all, and mainly had remains of nest lining in their stomachs, which may have been ingested accidentally. The scant food remains found belonged to arthropods indicating that at least in one case suitable food was provided.

Insufficient or no parental feeding rather than unsuitable diet could instead be the cause of swift hatchling death. Such neglect could result from hosts recognizing cowbirds nestlings as foreign, perhaps based on visual and acoustic differences between hosts and parasite nestlings. Newly hatched chicks of cowbirds and firewood-gatherers are similarly sized, shiny cowbirds: 2.5–3.5 g (Lowther 2011), firewood-gatherers: 2.75–4 g (unpubl.), but cowbirds have a reddish mouth instead of the yellow of the firewood-gatherer (Fig. 1). Begging calls also differ, those of the host being higher pitched than the parasite (Fraga 1985, unpubl.). Alternatively, cowbird nestlings may fail to elicit feeding behaviour from their hosts simply due to communicational constraints, for example if the reddish mouth lining is not sufficiently visible in the dark brood chamber (Heeb et al. 2003). This limitation would be worsened by the fact that cowbird nestlings cannot rely on host nestlings to elicit feeding behaviour (Kilner et al. 2004), since they hatch much earlier and are thus alone in the nest.

Large differences in hatching time may also allow firewood-gatherers to discriminate against nestlings that hatch too early. This could be analogous to the 'discrimination without recognition' mechanism observed in reed warblers *Acrocephalus scirpaceus* where some pairs desert cuckoo chicks if they do not fledge before the reed warbler-specific fledging date (Grim 2007). This notion is supported by an experiment (Mason 1986) which placed one cowbird chick (age unknown) in a brood of similar-sized firewood-gatherer chicks. The parasitic chick survived for 3–4 d before dying together with its nest mates from botfly (*Philornis* sp.) parasitism and was considered to have been accepted by the hosts (Mason 1986). In our population, however, the one case where the cowbird nestling hatched later than its nest mates and died could instead suggest that active recognition takes place. But, as hatching happened around 10 d later than the host chicks, such a large developmental disadvantage may be difficult to overcome, even for a brood parasite.

At this stage it is unclear whether the swift death of the cowbird nestlings represents a case of active nestling rejection or the unsuitability of the firewood-gatherer as a host due to different incubation period or other life history traits. These alternatives could thus either represent cases of failed 'trickery' or 'tuning' (Davies 2011). Failed 'trickery' would imply that at the moment co-evolution between host and parasite has led to an effective defense against cowbird-parasitism. Failed 'tuning' on the other hand would not require a co-evolutionary response of the firewood-gatherer, but may simply reflect the lack of cowbird adaptations to parasitise this particular host. Experiments are needed to tease the alternatives apart.

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