Intraspecific avian brood parasites avoid host nests infested by ectoparasites

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Abstract

Intraspecific brood parasitism is widespread among birds and provides clues for elucidating evolutionary origin of interspecific brood parasitism. Studies suggest that brood parasitism does not occur at random, but parasitic females select nests with advantages such as higher physical stability, reduced predation risk, or lower ectoparasite infestations. However, this evidence is sparse and mainly correlative. By experimentally increasing abundance of *Carnus hemapterus* (a common, generalist and widespread ectoparasitic nest-fly of a multitude of bird species) in half of the nests, we show that parasitic Spotless Starlings (*Sturnus unicolor*) avoid conspecific nests infested by ectoparasites. Since *Carnus* ectoparasites impinge costs on their avian nestling hosts, this avoidance response would be adaptive for parasitic starlings. Further, we suggest a mechanism by which parasite females may assess the level of ectoparasite infestation to select host nests accordingly: by using parasite cues such as faeces and blood remains. Additionally, these cues may be used by parasite females for synchronization with the reproductive cycle of host females because ectoparasite cues also indicate that incubation has already commenced. Whatever the functionality, the mechanism suggested here may be employed by intra- and interspecific brood parasites, so it might represent a widespread strategy in nature.

Keywords: Conspecific brood parasitism, host selection mechanisms, risk of ectoparasitism, host-parasite synchronization, eggshell spottiness, intraspecific nest parasitism

Introduction

Intraspecific brood parasitism is widespread among birds (Yom-Tov 2001). It has received much less attention than interspecific parasitism (Lyon and Eadie 2008), although it may provide clues for elucidating evolutionary origin of the later (Payne 1977; Davies 2000). Identifying possible host cues used by brood parasites for host selection that maximize reproductive success is central for understanding the evolution of intra- (Lyon and Eadie 2008) and inter-specific brood parasitism (Parejo and Avilés 2007; Soler et al. 2014).

In the case of intraspecific brood parasitism, some stimulating evidence points out that birds do not parasitize nests randomly but use cues associated with breeding success (Pöysä et al. 2014). At least six of such cues have been suggested, though some may just be breeding outcomes of potential host nest sites and the putative cues have yet to be identified. First, parasitic females select host nests at the appropriate timing of host reproductive cycle (e.g., during egg laying; Brown and Brown 1989; Schielzeth and Bolund 2010). Second, Cliff Swallows (*Petrochelidon pyrrhonota*) prefer to parasitize old rather than new nests, which may be a guarantee of its structural stability (Brown and Brown 1991). Third, probability of parasitism in nests increases with the number of previous nesting attempts and occupation rate in Common Goldeneyes (*Bucephala clangula*) (Paasivaara et al. 2010). Fourth, Common Goldeneyes select host nests with reduced predation risk (Pöysä 2006; Pöysä et al. 2010). Fifth, Wood Ducks *Aix sponsa* preferentially parasitize nests with smaller clutch sizes, which may increase hatching success (Odell and Eadie 2010). Finally, intraspecific brood parasitism in Cliff Swallows was mainly detected in nests that are most successful at producing fledglings (Brown and Brown 1991). Interestingly, this nest-selection bias of parasitic Cliff Swallows was associated with a low probability of infestation by blood-sucking ectoparasites (fleas and bugs), a major source of nestling mortality in this species (Brown and Brown 1991). Nevertheless, mechanisms for assessment of ectoparasite loads of conspecific host nests were unknown (Brown and Brown 1991; Pöysä et al. 2014). In addition, most of this evidence is correlational and experimental

manipulation of host nest features is necessary to understand the detected non-random selection of conspecific host nests (Pöysä et al. 2014).

We took advantage of a study closely monitoring egg laying and incubation in Spotless Starlings (*Sturnus unicolor*) to detect events of intraspecific parasitism once incubation has commenced. We aimed to test whether ectoparasite infestation of a nest predicts its probability of being parasitized by conspecific females, for which purpose we experimentally increased abundance of the ectoparasitic nest-fly *Carnus hemapterus* in half of the nests. The abundance of this ectoparasite is revealed by conspicuous spottiness on eggs, which are the product of parasite faeces and blood remains (López-Rull et al. 2007; Avilés et al. 2009; Tomás et al. submitted). We expected that parasite females would avoid experimental nests with an increased load of *Carnus hemapterus* flies. Additionally, ectoparasite cues may be used also by parasite females for a better synchronization with the reproductive cycle of host females. Parasite females would avoid nests with ectoparasite cues because it indicates that incubation has already commenced.

Methods

The Spotless Starling is a hole-nesting passerine that mostly breeds in colonies, where intraspecific parasitism is a common phenomenon occurring in around 25% of nests (Calvo et al. 2000). Overall, 17% of nests are parasitized during host's egg laying stage and 9% during incubation, with the later representing 32 - 53% of parasitism depending on year (Calvo et al. 2000). Our study was carried out in 2011 in a starling colony breeding in nest-boxes in Guadix, Spain (37°18' N, 3°11' W). Typical clutch sizes are four to five eggs in the population, laid one per day. The incubation lasts 12-13 days and usually starts before clutch completion, resulting in asynchronous hatching (Soler et al. 2008). Soon after the onset of incubation, immaculate blue eggs sometimes become brownish-spotted as a result of the viscous faeces (and blood remains) that adults of the ectoparasite *Carnus hemapterus* (hereafter *Carnus*) deposit

attached to substrates, including birds' skin, feathers, and eggs (López-Rull et al. 2007; Avilés et al. 2009; Tomás et al. submitted; Fig. 1). *Carnus* is a 2 mm blood-sucking fly found in nests of an extremely wide diversity of birds. It has been found parasitizing 64 host species from 24 avian families, from raptors to passerines (Grimaldi 1997; Brake 2011; Calero-Torralbo 2011). It has been recorded throughout most of North America and Europe, with more scarce records in Asia and North Africa (Grimaldi 1997; Brake 2011). After emergence from overwintering pupae inside nests, winged adults may disperse, and lose their wings once a suitable nest is found (Mercier 1928; Grimaldi 1997; Calero-Torralbo 2011). *Carnus* feeds exclusively on birds while at the nests, mainly on nestlings, but also on incubating birds (López-Rull et al. 2007; Avilés et al. 2009; Tomás et al. submitted). *Carnus* flies are hardly visible in nests as they dwell within the nest matrix. Infestation at the incubation stage can therefore be cued almost exclusively from egg spottiness and is positively correlated with infestation at the nestling stage (López-Rull et al. 2007).

Nest-boxes were inspected every 4 days to detect initiation of egg laying, and eggs were individually marked. Five days after laying of the first egg, we measured length and breadth of all eggs with a digital calliper (± 0.01 mm) and estimated eggshell spottiness (see below). Eggshell surface area was estimated according to Narushin (2005). Then, nests were alternately assigned to experimental or control treatments (33 nests each). In experimental nests, 10 unwinged *Carnus* flies collected from nearby nests were added (which is within the natural infestation level at the beginning of incubation; López-Rull et al. 2007; Avilés et al. 2009), while no flies were added in control nests. Ectoparasite cues were already visible on eggs within 24h after experimental infestation (authors pers. obs.).

Nests were visited 4 and 7 days after manipulation to estimate eggshell spottiness and to ascertain whether parasitic eggs were present. Estimations of eggshell spottiness were performed three times during the incubation period: (1) when incubation had already commenced (day 5 after laying of the first egg, immediately before treatment assignment), (2)

at middle (day 9), and (3) at late (day 12) incubation period. Egg spots, as indication of ectoparasite abundance, were counted in every egg of each nest at the three visits. In some nests where egg spots were so abundant that counting them became unreliable, we counted spots within a 1cm² on a random position along the shortest axis of the egg (López-Rull et al. 2007), which was extrapolated to eggshell surface. Both estimates were correlated on a subsample of eggs ($r^2 = 0.25$, $P < 0.0001$, $n = 53$). On each visit, a randomly selected egg was cleaned of spots with a sterile swab to sample bacterial loads for other purposes (Tomás et al. submitted). These eggs cleaned in previous visits were not computed when calculating within-nest mean eggshell spottiness per egg for analyses.

Although intraspecific parasitism may occur earlier, we specifically focused on events occurring after the experimental treatment. Since eggs were individually marked, parasitic eggs were recognized when new eggs appeared in nests after clutch completion (no eggs laid in two days and incubation started; Yom-Tov 1980; McRae 1997). Events of intraspecific parasitism were always visually confirmed by differential colour and morphology between parasitic and host eggs (Yom-Tov 1980; Evans 1988; McRae 1997). Laying dates of the parasitic eggs used in this study were spread over a period of 17 days and distance between host nests ranged from 50 to 200m. Parasitism in colonial birds usually occurs among close neighbours (Møller 1987; Brown and Brown 1989), so we are confident that parasitic eggs were laid by different females.

To explore the effectiveness of the experiment in increasing ectoparasite abundance (and ectoparasite cues), a repeated-measures ANOVA (rmANOVA) was carried out with values of eggshell spottiness (log10-transformed) at early, middle and late incubation as dependent repeated-measures variable, with treatment as factor. The probability of nests being parasitized was compared between treatments with a G-test with Williams' correction, indicated for contingency tables with empty cells (Sokal and Rohlf 1995). A value of 0.1 was

added to all cells to eliminate zeros (e.g., Fallon and Ricklefs 2008). Analyses were performed with Statistica 8.0 (StatSoft 2007).

Results

Nests under different treatments did not differ in laying date ($F_{1,64} = 0.40$, $P = 0.53$), clutch size ($F_{1,64} = 0.10$, $P = 0.76$), or eggshell spottiness before the experiment ($F_{1,64} = 0.10$, $P = 0.76$). Later on, as expected, eggshell spottiness was higher in *Carnus*-infested than in control nests (rmANOVA: $F_{1,63} = 279.32$, $P < 0.0001$). Eggshell spottiness increased during incubation ($F_{2,126} = 268.03$, $P < 0.0001$), with this increase being pronounced in *Carnus*-infested (post-hoc LSD tests: all $P < 0.0001$) but not in control nests (all $P > 0.05$) (Time*Treatment interaction: $F_{2,126} = 196.77$, $P < 0.0001$) (Fig. 2). Ectoparasitism, and therefore egg spottiness, greatly varied among study areas and years (unpublished data), and the non-significant detected increase in eggshell spottiness of control nests indicates relatively low incidence of ectoparasitism during the study year.

During incubation, none of the 33 experimental *Carnus*-infested nests were parasitized by starlings, while 4 out of 33 control nests (12.1%) received a parasite egg. Thus, probability that nests were parasitized was lower in experimental *Carnus*-infested nests (G adjusted = 4.59, $df = 1$, $P = 0.0322$).

Discussion

A quarter of a century ago, Brown and Brown (1991) first suggested that conspecific parasite birds may actively select host nests with lower ectoparasite loads and higher overall success. Their study was however correlative and also the mechanism(s) employed by birds to assess parasitic loads, if any, remained unknown. We present one of the first experimental evidence for non-random selection of conspecific host nests by brood parasitic females and the first one regarding risk of ectoparasitism. By manipulating abundance of a common, generalist and

widespread ectoparasite of a multitude of bird species, we have shown that parasitic birds avoid conspecific nests experimentally infested. Because *Carnus* ectoparasites impinge costs on their avian hosts (Avilés et al. 2009), this avoidance response would be adaptive for parasitic starlings. Furthermore, we suggest one mechanism by which parasite females may assess the level of ectoparasite infestation to perform an active selection of host nests: by using parasite cues such as faeces and blood remains. Ectoparasite cues may also be used by parasite females for a better synchronization with the reproductive cycle of host females. The most evident cue of *Carnus* parasitism available to an external observer is egg spottiness (see Fig. 1 and Online Resource 1), as *Carnus* parasites remain hidden within the nest materials. In fact, researchers have long been intrigued by egg spottiness in many bird species, ignoring which parasite, if any, may be the causative agent (Jackson 1970, Feare and Constantine 1980; Hornsby et al. 2013). Utilization of these cues may be widespread in brood parasitism systems during the incubation stage, as other nest-dwelling ectoparasites also develop within the nest matrix in close contact with eggs, thereby producing similar cues (e.g., fleas: McNeil and Clark 1987; bugs: Krinsky 2002; or mites: G. Tomás, pers. obs.). An experimental manipulation of egg spottiness in the absence of parasites would be necessary for demonstration that brood parasites cue mostly, if not solely, on egg spottiness rather than something else by the ectoparasite.

We consider three scenarios in which this non-random choice of host nests would be adaptive for conspecific and interspecific brood parasites. First, by selecting nests with lower ectoparasite infestation, parasitic birds would increase the survival prospects of their offspring (Brown and Brown 1991), as nest ectoparasites are deleterious for nestling birds (Møller et al. 2009). Furthermore, nests with high level of ectoparasitism may indicate females and/or nest sites of overall inferior quality, which influences quality of parental care received by developing offspring (Avilés et al. 2009).

Second, by selecting nests with no or few spots in eggs, females may better synchronize parasitism with host egg-laying or with beginning of incubation, because egg spottiness is low at the beginning of incubation and increases as incubation progresses (this study; López-Rull et al. 2007). Success of parasitic eggs is higher when laid during the egg laying period of the host or at early incubation, but it declines gradually with advancing incubation because of increased mismatch with host hatching time (Calvo et al. 2000). It is likely that parasitic females do make use of egg spottiness not only for avoiding nests infested by ectoparasites, but also for avoiding nests where incubation is much advanced. It should be noted that dependence on egg spottiness to infer reproductive timing of hosts would not be effective in years or populations with low level of ectoparasite infestation.

Third, parasitic females may preferentially lay eggs in nests with no or fewer egg spottiness to difficult foreign egg recognition by host birds, because newly laid parasitic eggs would appear conspicuous in nests with more egg spottiness. However, several studies with related starling species have shown that starlings do not eject parasitic eggs once they have begun egg laying (Stouffer et al. 1987; Pinxten et al. 1991; Yamaguchi 1997). In accordance, we found that starlings never evicted eggs cleaned of spots (see Materials and methods), despite having an appearance of newly laid eggs. In addition, we know from another experiment that Spotless Starlings do not eject red-painted model eggs when placed in nests during incubation (Soler et al. unpublished data). Therefore, this third possibility is not likely to account for our results.

We can consider several shortcomings in the interpretation of our results. It could be argued that we only detect parasitic eggs in control nests because any parasite egg laid in an experimental nest is quickly recognized and evicted by host females. However, as stated above, starlings do not evict parasitic eggs during egg laying and incubation. On the other hand, it could be questioned whether the mechanism employed by parasitic females to assess ectoparasite load is egg spottiness or any other cue, such as direct observation of

ectoparasites or differential behaviour of females from parasitized nests. We are confident that egg spottiness is the most patent and plausible cue because, as stated above, *Carnus* flies are hardly observed in nests and the only apparent cue is egg spottiness (see Fig. 1 and Online Resource). In addition, because *Carnus* flies do not crawl onto birds when out of the nest (Grimaldi 1997), it is unlikely that female birds from parasitized nests behave differently. Additionally, it could be argued that manipulation of ectoparasites may have affected overall health state of incubating females and consequently its nest defence capabilities against conspecific parasites. Nevertheless, if females from experimental nests were less efficient defending their nests against brood parasites, we should have found that *Carnus*-infested nests were more parasitized than control nests, just the opposite outcome of what we found.

It should be noted that rate of intraspecific parasitism in our starling population is likely larger than reported here, as we did not record parasitic eggs laid before or during the laying period of the host because it was out of scope of the present study. Our specific purpose was to record intraspecific parasitism in relation to experimental ectoparasite infestation and, in order to resemble natural ectoparasitism timing, this manipulation was carried out at the beginning of incubation and not before. Therefore, we exclusively focused on intraspecific parasitism occurring during incubation period. Parasitism during incubation stage of the host is a common phenomenon for this (see Materials and methods) and many other species (Pöysä et al. 2014). It should be noted that the mechanism for host selection that we suggest here will work for parasite females parasitizing nests during host incubation stage, but there might be other cues that parasitic birds use for nest selection during host egg laying stage, when ectoparasite cues are not present. Nevertheless, egg laying and incubation stages overlap for many avian species, which usually start incubation before clutch completion. This provides a time-window when the suggested mechanism for host selection may be effective. Although incurring costs, parasitism during incubation may be advantageous in giving the parasite more time for assessment and appropriate choice of better hosts (Brown and Brown 1991).

Appropriate timing of parasitism to match host reproductive cycle may therefore be to some extent traded-off against waiting to gather more cues to select better host nests.

In our study, none of the parasitic eggs produced fledglings. However, it is likely that success of parasitic eggs laid during incubation shows strong geographical and seasonal variability. In another Spotless Starling population, 9% of the eggs laid during incubation produced fledglings (Calvo et al. 2000). Similarly, in the closely related European Starling, 22% of parasitic eggs laid after host's laying stage succeed to hatch and, of those, 40% produced fledglings (Evans 1988).

To conclude, we have experimentally shown that conspecific brood parasites avoid host nests infested by ectoparasites. We further suggest that parasite females may use ectoparasite cues such as faeces and blood remains to assess level of ectoparasitism and/or to time egg laying with the laying period of the host. Both instances would be adaptive for parasitic females by increasing survival prospects for their offspring. This suggested mechanism may be commonly employed not only by conspecific, but also by interspecific brood parasites, so it might represent a widespread strategy in nature and merits further research.

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Figure captions

Fig. 1: Representative starling clutches uninfested (left) and infested (right) by *Carnus*

hemapterus flies. Note that none *Carnus* fly is seen on the pictures, giving support to our suggested mechanism that spottiness is a likely cue used by parasitic females to infer ectoparasite infestation level.

Fig. 2: Effect of experimental addition of *Carnus hemapterus* flies on spottiness (mean number of spots per egg) of starling eggshells along the incubation period. Mean $\pm$ SE values at early (before treatment), middle, and late incubation, for *Carnus*-infested ($n = 33$; full dots) and control nests ($n = 33$; open dots) are shown.

Fig. 1

Fig. 2