Intraspecific avian brood parasites avoid host nests infested by ectoparasites

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1 **Abstract**

2 Intraspecific brood parasitism is widespread among birds and provides clues for elucidating

3 evolutionary origin of interspecific brood parasitism. Studies suggest that brood parasitism

4 does not occur at random, but parasitic females select nests with advantages such as higher

5 physical stability, reduced predation risk, or lower ectoparasite infestations. However, this

6 evidence is sparse and mainly correlative. By experimentally increasing abundance of *Carnus*

7 *hemapterus* (a common, generalist and widespread ectoparasitic nest-fly of a multitude of bird

8 species) in half of the nests, we show that parasitic Spotless Starlings (*Sturnus unicolor*) avoid

9 conspecific nests infested by ectoparasites. Since *Carnus* ectoparasites impinge costs on their

10 avian nestling hosts, this avoidance response would be adaptive for parasitic starlings. Further,

11 we suggest a mechanism by which parasite females may assess the level of ectoparasite

12 infestation to select host nests accordingly: by using parasite cues such as faeces and blood

13 remains. Additionally, these cues may be used by parasite females for synchronization with the

14 reproductive cycle of host females because ectoparasite cues also indicate that incubation has

15 already commenced. Whatever the functionality, the mechanism suggested here may be

16 employed by intra- and interspecific brood parasites, so it might represent a widespread

17 strategy in nature.

18

19 **Keywords:** Conspecific brood parasitism, host selection mechanisms, risk of ectoparasitism,

20 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

47   manipulation of host nest features is necessary to understand the detected non-random

48   selection of conspecific host nests (Pöysä et al. 2014).

49   We took advantage of a study closely monitoring egg laying and incubation in Spotless

50   Starlings (*Sturnus unicolor)* to detect events of intraspecific parasitism once incubation has

51   commenced. We aimed to test whether ectoparasite infestation of a nest predicts its

52   probability of being parasitized by conspecific females, for which purpose we experimentally

53   increased abundance of the ectoparasitic nest-fly *Carnus hemapterus* in half of the nests. The

54   abundance of this ectoparasite is revealed by conspicuous spottiness on eggs, which are the

55   product of parasite faeces and blood remains (López-Rull et al. 2007; Avilés et al. 2009; Tomás

56   et al. submitted). We expected that parasite females would avoid experimental nests with an

57   increased load of *Carnus hemapterus* flies. Additionally, ectoparasite cues may be used also by

58   parasite females for a better synchronization with the reproductive cycle of host females.

59   Parasite females would avoid nests with ectoparasite cues because it indicates that incubation

60   has already commenced.

61

62   **Methods**

63   The Spotless Starling is a hole-nesting passerine that mostly breeds in colonies, where

64   intraspecific parasitism is a common phenomenon occurring in around 25% of nests (Calvo et

65   al. 2000). Overall, 17% of nests are parasitized during host´s egg laying stage and 9% during

66   incubation, with the later representing 32 - 53% of parasitism depending on year (Calvo et al.

67   2000). Our study was carried out in 2011 in a starling colony breeding in nest-boxes in Guadix,

68   Spain (37º18' N, 3º11' W). Typical clutch sizes are four to five eggs in the population, laid one

69   per day. The incubation lasts 12-13 days and usually starts before clutch completion, resulting

70   in asynchronous hatching (Soler et al. 2008). Soon after the onset of incubation, immaculate

71   blue eggs sometimes become brownish-spotted as a result of the viscous faeces (and blood

72   remains) that adults of the ectoparasite *Carnus hemapterus* (hereafter *Carnus*) deposit

73 attached to substrates, including birds' skin, feathers, and eggs (López-Rull et al. 2007; Avilés

74 et al. 2009; Tomás et al. submitted; Fig. 1). *Carnus* is a 2 mm blood-sucking fly found in nests of

75 an extremely wide diversity of birds. It has been found parasitizing 64 host species from 24

76 avian families, from raptors to passerines (Grimaldi 1997; Brake 2011; Calero-Torralbo 2011). It

77 has been recorded throughout most of North America and Europe, with more scarce records in

78 Asia and North Africa (Grimaldi 1997; Brake 2011). After emergence from overwintering pupae

79 inside nests, winged adults may disperse, and lose their wings once a suitable nest is found

80 (Mercier 1928; Grimaldi 1997; Calero-Torralbo 2011). *Carnus* feeds exclusively on birds while

81 at the nests, mainly on nestlings, but also on incubating birds (López-Rull et al. 2007; Avilés et

82 al. 2009; Tomás et al. submitted). *Carnus* flies are hardly visible in nests as they dwell within

83 the nest matrix. Infestation at the incubation stage can therefore be cued almost exclusively

84 from egg spottiness and is positively correlated with infestation at the nestling stage (López-

85 Rull et al. 2007).

86   Nest-boxes were inspected every 4 days to detect initiation of egg laying, and eggs were

87 individually marked. Five days after laying of the first egg, we measured length and breadth of

88 all eggs with a digital calliper (± 0.01 mm) and estimated eggshell spottiness (see below).

89 Eggshell surface area was estimated according to Narushin (2005). Then, nests were

90 alternately assigned to experimental or control treatments (33 nests each). In experimental

91 nests, 10 unwinged *Carnus* flies collected from nearby nests were added (which is within the

92 natural infestation level at the beginning of incubation; López-Rull et al. 2007; Avilés et al.

93 2009), while no flies were added in control nests. Ectoparasite cues were already visible on

94 eggs within 24h after experimental infestation (authors pers. obs.).

95   Nests were visited 4 and 7 days after manipulation to estimate eggshell spottiness and

96 to ascertain whether parasitic eggs were present. Estimations of eggshell spottiness were

97 performed three times during the incubation period: (1) when incubation had already

98 commenced (day 5 after laying of the first egg, immediately before treatment assignment), (2)

99   at middle (day 9), and (3) at late (day 12) incubation period. Egg spots, as indication of

100   ectoparasite abundance, were counted in every egg of each nest at the three visits. In some

101   nests where egg spots were so abundant that counting them became unreliable, we counted

102   spots within a $1cm^2$ on a random position along the shortest axis of the egg (López-Rull et al.

103   2007), which was extrapolated to eggshell surface. Both estimates were correlated on a

104   subsample of eggs ($r^2 = 0.25$, $P < 0.0001$, $n = 53$). On each visit, a randomly selected egg was

105   cleaned of spots with a sterile swab to sample bacterial loads for other purposes (Tomás et al.

106   submitted). These eggs cleaned in previous visits were not computed when calculating within-

107   nest mean eggshell spottiness per egg for analyses.

108   Although intraspecific parasitism may occur earlier, we specifically focused on events

109   occurring after the experimental treatment. Since eggs were individually marked, parasitic

110   eggs were recognized when new eggs appeared in nests after clutch completion (no eggs laid

111   in two days and incubation started; Yom-Tov 1980; McRae 1997). Events of intraspecific

112   parasitism were always visually confirmed by differential colour and morphology between

113   parasitic and host eggs (Yom-Tov 1980; Evans 1988; McRae 1997). Laying dates of the parasitic

114   eggs used in this study were spread over a period of 17 days and distance between host nests

115   ranged from 50 to 200m. Parasitism in colonial birds usually occurs among close neighbours

116   (Møller 1987; Brown and Brown 1989), so we are confident that parasitic eggs were laid by

117   different females.

118   To explore the effectiveness of the experiment in increasing ectoparasite abundance

119   (and ectoparasite cues), a repeated-measures ANOVA (rmANOVA) was carried out with values

120   of eggshell spottiness (log10-transformed) at early, middle and late incubation as dependent

121   repeated-measures variable, with treatment as factor. The probability of nests being

122   parasitized was compared between treatments with a *G*-test with Williams' correction,

123   indicated for contingency tables with empty cells (Sokal and Rohlf 1995). A value of 0.1 was

124   added to all cells to eliminate zeros (e.g., Fallon and Ricklefs 2008). Analyses were performed

125   with Statistica 8.0 (StatSoft 2007).

126

127   **Results**

128   Nests under different treatments did not differ in laying date ($F_{1,64}$ = 0.40, P = 0.53), clutch size

129   ($F_{1,64}$ = 0.10, P = 0.76), or eggshell spottiness before the experiment ($F_{1,64}$ = 0.10, P = 0.76). Later

130   on, as expected, eggshell spottiness was higher in *Carnus*-infested than in control nests

131   (rmANOVA: $F_{1,63}$ = 279.32, P < 0.0001). Eggshell spottiness increased during incubation ($F_{2,126}$ =

132   268.03, P < 0.0001), with this increase being pronounced in *Carnus*-infested (post-hoc LSD

133   tests: all P < 0.0001) but not in control nests (all P > 0.05) (Time*Treatment interaction: $F_{2,126}$ =

134   196.77, P < 0.0001) (Fig. 2). Ectoparasitism, and therefore egg spottiness, greatly varied among

135   study areas and years (unpublished data), and the non-significant detected increase in eggshell

136   spottiness of control nests indicates relatively low incidence of ectoparasitism during the study

137   year.

138   During incubation, none of the 33 experimental *Carnus*-infested nests were parasitized

139   by starlings, while 4 out of 33 control nests (12.1%) received a parasite egg. Thus, probability

140   that nests were parasitized was lower in experimental *Carnus*-infested nests (*G* adjusted =

141   4.59, *df* = 1, P = 0.0322).

142

143   **Discussion**

144   A quarter of a century ago, Brown and Brown (1991) first suggested that conspecific parasite

145   birds may actively select host nests with lower ectoparasite loads and higher overall success.

146   Their study was however correlative and also the mechanism(s) employed by birds to assess

147   parasitic loads, if any, remained unknown. We present one of the first experimental evidence

148   for non-random selection of conspecific host nests by brood parasitic females and the first one

149   regarding risk of ectoparasitism. By manipulating abundance of a common, generalist and

150 widespread ectoparasite of a multitude of bird species, we have shown that parasitic birds

151 avoid conspecific nests experimentally infested. Because *Carnus* ectoparasites impinge costs

152 on their avian hosts (Avilés et al. 2009), this avoidance response would be adaptive for

153 parasitic starlings. Furthermore, we suggest one mechanism by which parasite females may

154 assess the level of ectoparasite infestation to perform an active selection of host nests: by

155 using parasite cues such as faeces and blood remains. Ectoparasite cues may also be used by

156 parasite females for a better synchronization with the reproductive cycle of host females. The

157 most evident cue of *Carnus* parasitism available to an external observer is egg spottiness (see

158 Fig. 1 and Online Resource 1), as *Carnus* parasites remain hidden within the nest materials. In

159 fact, researchers have long been intrigued by egg spottiness in many bird species, ignoring

160 which parasite, if any, may be the causative agent (Jackson 1970, Feare and Constantine 1980;

161 Hornsby et al. 2013). Utilization of these cues may be widespread in brood parasitism systems

162 during the incubation stage, as other nest-dwelling ectoparasites also develop within the nest

163 matrix in close contact with eggs, thereby producing similar cues (e.g., fleas: McNeil and Clark

164 1987; bugs: Krinsky 2002; or mites: G. Tomás, pers. obs.). An experimental manipulation of egg

165 spottiness in the absence of parasites would be necessary for demonstration that brood

166 parasites cue mostly, if not solely, on egg spottiness rather than something else by the

167 ectoparasite.

168 We consider three scenarios in which this non-random choice of host nests would be

169 adaptive for conspecific and interspecific brood parasites. First, by selecting nests with lower

170 ectoparasite infestation, parasitic birds would increase the survival prospects of their offspring

171 (Brown and Brown 1991), as nest ectoparasites are deleterious for nestling birds (Møller et al.

172 2009). Furthermore, nests with high level of ectoparasitism may indicate females and/or nest

173 sites of overall inferior quality, which influences quality of parental care received by

174 developing offspring (Avilés et al. 2009).

175   Second, by selecting nests with no or few spots in eggs, females may better synchronize

176   parasitism with host egg-laying or with beginning of incubation, because egg spottiness is low

177   at the beginning of incubation and increases as incubation progresses (this study; López-Rull et

178   al. 2007). Success of parasitic eggs is higher when laid during the egg laying period of the host

179   or at early incubation, but it declines gradually with advancing incubation because of increased

180   mismatch with host hatching time (Calvo et al. 2000). It is likely that parasitic females do make

181   use of egg spottiness not only for avoiding nests infested by ectoparasites, but also for

182   avoiding nests where incubation is much advanced. It should be noted that dependence on

183   egg spottiness to infer reproductive timing of hosts would not be effective in years or

184   populations with low level of ectoparasite infestation.

185   Third, parasitic females may preferentially lay eggs in nests with no or fewer egg

186   spottiness to difficult foreign egg recognition by host birds, because newly laid parasitic eggs

187   would appear conspicuous in nests with more egg spottiness. However, several studies with

188   related starling species have shown that starlings do not eject parasitic eggs once they have

189   begun egg laying (Stouffer et al. 1987; Pinxten et al. 1991; Yamaguchi 1997). In accordance, we

190   found that starlings never evicted eggs cleaned of spots (see Materials and methods), despite

191   having an appearance of newly laid eggs. In addition, we know from another experiment that

192   Spotless Starlings do not eject red-painted model eggs when placed in nests during incubation

193   (Soler et al. unpublished data). Therefore, this third possibility is not likely to account for our

194   results.

195   We can consider several shortcomings in the interpretation of our results. It could be

196   argued that we only detect parasitic eggs in control nests because any parasite egg laid in an

197   experimental nest is quickly recognized and evicted by host females. However, as stated

198   above, starlings do not evict parasitic eggs during egg laying and incubation. On the other

199   hand, it could be questioned whether the mechanism employed by parasitic females to assess

200   ectoparasite load is egg spottiness or any other cue, such as direct observation of

201   ectoparasites or differential behaviour of females from parasitized nests. We are confident

202   that egg spottiness is the most patent and plausible cue because, as stated above, *Carnus* flies

203   are hardly observed in nests and the only apparent cue is egg spottiness (see Fig. 1 and Online

204   Resource). In addition, because *Carnus* flies do not crawl onto birds when out of the nest

205   (Grimaldi 1997), it is unlikely that female birds from parasitized nests behave differently.

206   Additionally, it could be argued that manipulation of ectoparasites may have affected overall

207   health state of incubating females and consequently its nest defence capabilities against

208   conspecific parasites. Nevertheless, if females from experimental nests were less efficient

209   defending their nests against brood parasites, we should have found that *Carnus*-infested

210   nests were more parasitized than control nests, just the opposite outcome of what we found.

211   It should be noted that rate of intraspecific parasitism in our starling population is likely

212   larger than reported here, as we did not record parasitic eggs laid before or during the laying

213   period of the host because it was out of scope of the present study. Our specific purpose was

214   to record intraspecific parasitism in relation to experimental ectoparasite infestation and, in

215   order to resemble natural ectoparasitism timing, this manipulation was carried out at the

216   beginning of incubation and not before. Therefore, we exclusively focused on intraspecific

217   parasitism occurring during incubation period. Parasitism during incubation stage of the host is

218   a common phenomenon for this (see Materials and methods) and many other species (Pöysä

219   et al. 2014). It should be noted that the mechanism for host selection that we suggest here will

220   work for parasite females parasitizing nests during host incubation stage, but there might be

221   other cues that parasitic birds use for nest selection during host egg laying stage, when

222   ectoparasite cues are not present. Nevertheless, egg laying and incubation stages overlap for

223   many avian species, which usually start incubation before clutch completion. This provides a

224   time-window when the suggested mechanism for host selection may be effective. Although

225   incurring costs, parasitism during incubation may be advantageous in giving the parasite more

226   time for assessment and appropriate choice of better hosts (Brown and Brown 1991).

227   Appropriate timing of parasitism to match host reproductive cycle may therefore be to some

228   extent traded-off against waiting to gather more cues to select better host nests.

229   In our study, none of the parasitic eggs produced fledglings. However, it is likely that

230   success of parasitic eggs laid during incubation shows strong geographical and seasonal

231   variability. In another Spotless Starling population, 9% of the eggs laid during incubation

232   produced fledglings (Calvo et al. 2000). Similarly, in the closely related European Starling, 22%

233   of parasitic eggs laid after host´s laying stage succeed to hatch and, of those, 40% produced

234   fledglings (Evans 1988).

235   To conclude, we have experimentally shown that conspecific brood parasites avoid host

236   nests infested by ectoparasites. We further suggest that parasite females may use ectoparasite

237   cues such as faeces and blood remains to assess level of ectoparasitism and/or to time egg

238   laying with the laying period of the host. Both instances would be adaptive for parasitic

239   females by increasing survival prospects for their offspring. This suggested mechanism may be

240   commonly employed not only by conspecific, but also by interspecific brood parasites, so it

241   might represent a widespread strategy in nature and merits further research.

242

253 **Ethics statement:** All procedures were conducted under licence from the Environmental

254 Department of the Regional Government of Andalucía, Spain (ref. SGYB/FOA/AFR). All

255 applicable international, national, and/or institutional guidelines for the care and use of

256 animals were followed.

257

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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 ± 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