Prolactin mediates behavioural rejection responses to avian brood parasitism

Francisco Ruiz-Raya^{*1,2}, Juan Diego Ibáñez-Álamo¹, Charline Parenteau³, Olivier Chastel³
and Manuel Soler¹

¹*Departamento de Zoología, Universidad de Granada, E-18071 Granada, Spain*

²*Present address: Departamento de Ecología y Biología Animal, Universidad de Vigo, 36310 Vigo, Spain*

³*Centre d'Études Biologiques de Chizé (CEBC), UMR7372 CNRS - Université de La Rochelle, France.*

*Corresponding author: francisco.ruiz.raya@uvigo.es

25 **Abstract**

26 Even though adaptations resulting from co-evolutionary interactions between avian
 27 brood parasites and their hosts have been well studied, the hormonal mechanisms
 28 underlying behavioural host defences remain largely unexplored. Prolactin, the main
 29 hormone mediating avian parental behaviour, has been hypothesized to play a key role
 30 in the orchestration of host responses to brood parasitic eggs. Based on the positive
 31 association between plasma prolactin and parental attachment to eggs, decreasing levels
 32 of this hormone are expected to facilitate egg-rejection decisions in parasitized clutches.
 33 We tested this prediction by implanting Eurasian blackbirds (*Turdus merula*) females
 34 with an inhibitor of prolactin secretion, bromocriptine mesylate, to experimentally low
 35 their prolactin levels. We found that bromocriptine-treated females rejected mimetic
 36 model eggs at higher rates than placebo-treated individuals. To our knowledge, this is
 37 the first experimental evidence that host responses to brood parasitism are mediated by
 38 the primary endocrine pathway that orchestrates the expression of avian parental care.

39

40 **Keywords:** Acceptance threshold, egg rejection, hormones, parental behaviour

41

42

43

44

45

46

47 **Introduction**

48 Obligate avian brood parasites lay their eggs in the nest of host species, thus exploiting
 49 the parental care that other birds provide to their offspring (Roldán and Soler, 2011).
 50 This reproductive strategy imposes severe fitness costs on hosts since the newly-hatched
 51 parasitic nestling often kills or outcompetes all host's offspring (Feeney et al., 2014;
 52 Soler, 2014). In turn, hosts have evolved a wide range of behavioural, morphological
 53 and life-story adaptations to counteract brood parasitism at all stages of the breeding
 54 cycle (Feeney et al., 2014; Soler, 2014; Soler, 2017). Surprisingly, while behavioural,
 55 cognitive and conditional aspects of host resistance are well known (Soler, 2017), we
 56 still have a very limited understanding of the physiological mechanisms underlying anti-
 57 parasitic host responses to avian brood parasitism.

58 Among anti-parasitic behaviours, the recognition and subsequent rejection of parasitic
 59 eggs is the most widespread defence used by hosts to counteract brood parasitism
 60 (Davies and Brooke, 1989; Feeney et al., 2014; Soler, 2014). At a mechanistic level,
 61 egg rejection involves the selective disruption of typical parental behaviours to actively
 62 respond against a specific stimulus from the own clutch (i.e. parasitic eggs), so it has
 63 been hypothesized that host responses to parasitic eggs might be mediated by hormonal
 64 pathways involved in the regulation of parental decisions (Abolins-Abols and Hauber,
 65 2018; Ruiz-Raya and Soler, 2020). Recent evidence suggests that physiological and
 66 behavioural host responses to foreign eggs may be mediated by the neuroendocrine
 67 pathways underlying stress physiology (i.e. glucocorticoids (Abolins-Abols and Hauber,
 68 2020; Ruiz-Raya et al., 2018)); however, whether rejection decisions are regulated by
 69 the endocrine pathways mediating the expression of avian parental behaviours remains
 70 unexplored.

71 Prolactin, classically known as the “parental hormone” (Buntin, 1996; Sockman et al.,
 72 2006), is the primary candidate for orchestrating anti-parasitic defences associated with
 73 parental decisions (Abolins-Abols and Hauber, 2018; Ruiz-Raya et al., 2018). This
 74 peptide hormone mediates the transition from sexual to parental activity in birds
 75 (Buntin, 1996; Smiley, 2019; Sockman et al., 2006), playing a key role in the regulation
 76 of major aspects of avian parental care: prolactin levels are positively related with the
 77 initiation and maintenance of associative parental behaviours such as incubation or post-
 78 hatching parental care (Angelier and Chastel, 2009; Angelier et al., 2016; Smiley,
 79 2019). Importantly, the presence of foreign eggs has been found to lead to changes in
 80 the prolactin response to stressors (Ruiz-Raya et al., 2018), which suggests that this
 81 hormone might play a crucial role in mediating host responses to parasitic eggs
 82 (Abolins-Abols and Hauber, 2018; Ruiz-Raya and Soler, 2020). Based on the positive
 83 association between circulating prolactin and the expression of avian parental
 84 behaviours (Angelier et al., 2016; Smiley, 2019), it may be hypothesized that a
 85 reduction in prolactin levels would affect rejection decisions. Specifically, decreasing
 86 prolactin levels would be expected to result in more restrictive egg acceptance
 87 thresholds and facilitate rejection decisions (Abolins-Abols and Hauber, 2018). Here,
 88 we tested this hypothesis for the first time by implanting host females with
 89 bromocriptine-mesylate pellets, a dopamine receptor agonist, to experimentally decrease
 90 their prolactin levels. Afterwards, we assessed their response to experimental parasitism
 91 compared to placebo-implanted individuals in order to determine whether changes in
 92 prolactin profiles affect hosts’ rejection decisions.

93 **Material and methods**

94 *Study site and species*

95 This study was conducted in a Eurasian blackbird (*Turdus merula*) population located in
 96 the Valley of Lecrín, Southern Spain, from late March to June 2018. The Eurasian
 97 blackbird (hereafter blackbird) is a potential common cuckoo (*Cuculus canorus*) host
 98 frequently used in egg-rejection studies (e.g. Grim et al., 2011; Roncalli et al., 2019;
 99 Ruiz-Raya et al., 2015; Samas et al., 2011; Soler et al., 2015; Soler et al., 2017).
 100 Blackbirds typically remove foreign eggs by grasping them with their bill (Soler et al.,
 101 2015), showing high egg-ejection abilities (see references above).

102 *Hormone manipulation*

103 Females (N = 36) were captured just after clutch completion (mean $\pm$ se = 2.3 ± 0.19
 104 days) by using a mist net placed near the nest (6:00 - 8:00 am). Blood samples were
 105 collected from the brachial vein with a 25-gauge needle and 80- μ l heparinized
 106 microhematocrit tubes within 3 minutes after capture. Through the breeding season, we
 107 sequentially assigned females to one of two experimental groups. Half of the females (n
 108 = 18) were implanted with bromocriptine mesylate (BRC) time-release pellets
 109 (according to the characteristics of our study species: C-231, 0.5 mg, 10-days release,
 110 3.1 mm diameter; Innovative Research of America), a dopamine receptor agonist which
 111 has been widely used to lower circulating prolactin in avian species (e.g. Angelier et al.,
 112 2006; Cottin et al., 2014; Smiley and Adkins-Regan, 2018; Thierry et al., 2013).
 113 Another half of the individuals (n = 18) were assigned to the control group and therefore
 114 implanted with placebo pellets (C-111, 0.5 mg, 10-day release, 3.1 mm diameter; same
 115 company). Time-release dosage pellets have been frequently used to modify hormone
 116 levels in ecophysiological studies, showing no negative long-term effect for birds'
 117 welfare (e.g. Cottin et al., 2014; Müller et al., 2009; Thierry et al., 2013). Even so, in
 118 order to discard the existence of unintended side effects of our BRC treatment on avian
 119 feeding behaviour (Buntin, 1989), females were weighted to the nearest 1 g before and

120 after the hormonal treatment to assess potential changes in their body mass (Smiley and
121 Adkins-Regan, 2018). Additionally, although nest desertion is not an egg-rejection
122 mechanism in blackbirds (Soler et al., 2015), we evaluated whether our BRC
123 manipulation affected the probability of nest abandonment.

124 BRC and placebo-implanted individuals underwent identical procedures. Pellets were
125 implanted subcutaneously in the female's back through a small incision (5 mm) that was
126 subsequently sutured with surgical tissue adhesive (Vetbond, 3M). In our study
127 population, there was no seasonal variation in baseline prolactin in both experimental
128 groups (both cases $p > 0.57$; Fig. S1; see statistical methods). Individuals were marked
129 with a combination of coloured rings to verify their identity. All birds were released 10
130 min after implantation. All females returned to their nest to resume incubation within
131 the next two hours. To test the effectiveness of the 10-day release BRC pellets, females
132 ($N = 9$) were recaptured six days after implantation (i.e. at the end of the experimental
133 parasitism trials; see below) and a second blood sample was collected from the opposite
134 wing. Similar sample sizes have been proved valid to verify hormone implants/injection
135 effectiveness in previous studies (Abolins-Abols and Hauber, 2020; Goutte et al., 2010;
136 Ouyang et al., 2013), so we minimized the number of re-sampled females to reduce the
137 stress linked to recapture and handling for breeding pairs. The visual examination of
138 recaptured individuals revealed that incisions were successfully sutured in all cases.
139 Blood samples were kept cold for up to 6 hours until centrifugation. Afterwards, plasma
140 was extracted and stored at -20°C until the hormonal assay. Prolactin plasma
141 concentration was determined by a heterologous radioimmunoassay at the Centre
142 d'Etudes Biologiques de Chizé. The assay was conducted using chicken prolactin and
143 antibody against chicken prolactin (supplied by Dr. A. F. Parlow, Harbor-UCLA
144 Medical Center, Torrance, CA, USA). All samples were run in one assay, in duplicate,

145 with 25 μ l of plasma for each duplicate and a limit of detection of 0.43 ng/ml. The intra-
146 assay coefficient of variation was 10.3%.

147 *Brood parasitism experiments*

148 Nests were parasitized with mimetic eggs 24 hours after pellet implantation. As model
149 eggs, we used natural blackbird eggs collected from deserted clutches, which were
150 painted mimetic to simulate interspecific parasitism (Fig. 2b; see (Soler et al., 2015) for
151 additional details). This type of mimetic model eggs elicits intermediate ejection rates in
152 blackbirds (26.8 - 50% (Roncalli et al., 2019; Soler et al., 2015)), thus allowing to detect
153 bi-directional changes in egg-rejection rates in response to the experimental
154 manipulation. All nests were checked every 24 h to determine egg ejection. Recent
155 research shows that nest desertion is not an egg-rejection mechanism in medium-sized
156 hosts such as blackbirds (Soler et al. 2015), so nest abandonment was not considered a
157 genuine response to experimental brood parasitism. Experimental eggs were consider as
158 accepted if they remained in active nests for 5 days (Roncalli et al., 2019; Ruiz-Raya et
159 al., 2015; Ruiz-Raya et al., 2016; Soler et al., 2017).

160 *Statistical analysis*

161 Between-groups differences in prolactin levels prior to our hormonal manipulation were
162 assessed through a linear model including treatment, clutch size, and their interaction as
163 predictors. We used linear regression to explore seasonal variations in baseline prolactin
164 levels before implantation. Linear mixed-effect models were fitted by using *nlme* (R
165 package v.3.1-117 (Pinheiro et al., 2014)) to assess the effect of our manipulation on
166 circulating prolactin and body mass. As fixed factors, our mixed models included:
167 implant treatment (BRC or placebo), sample day, and their interaction, while female ID
168 was included as random term. A generalized linear model with binomial error was

performed to assess the effect of our experimental treatment on egg ejection. As predictors, our binomial regression model included: implant treatment, clutch size (3-4 eggs), the interaction between these two terms, baseline prolactin levels before implantation and laying date. We assessed whether our BRC treatment affected nest abandonment through a binomial regression model including implant treatment and clutch size as predictors. Stepwise procedures were avoided to minimize the chances for type I errors, and full models were used for inference (Whittingham et al., 2006). Assumptions for normality of residuals and homogeneity of variances were verified by the visual inspection of the residual graphs. All analysis and graphs were performed using R version 3.6.1.

Results and Discussion

Pre-treatment prolactin levels did not differ between BRC and placebo-implanted females (estimate $\pm$ se = 3.459 ± 6.493 ; $t = 0.533$; $p = 0.60$; $N = 36$; Fig. 1a); however, both groups differentially varied their prolactin levels in response to our BRC treatment (implant treatment $\times$ sample day: $F_{1,7} = 52.17$; $p < 0.001$; Fig. 1b). Specifically, while BRC-treated individuals showed a significant reduction in their prolactin levels after implantation (Tukey's test: estimate $\pm$ se = 35.39 ± 4.02 ; $df = 7$; $t = 8.81$; $p < 0.001$), circulating prolactin did not vary in placebo-implanted females (Tukey's test: estimate $\pm$ se = -8.12 ± 4.49 ; $df = 7$; $t = -1.81$; $p = 0.34$). These results confirm the effectiveness of our hormonal manipulation, and supports previous studies using BCR to experimentally decrease plasma prolactin levels in birds (e.g. Angelier et al., 2006; Cottin et al., 2014; Smiley and Adkins-Regan, 2018; Thierry et al., 2013).

Prolactin is the main hormone orchestrating the expression and maintenance of avian parental behaviours during incubation (Angelier and Chastel, 2009; Smiley, 2019), so egg-rejection decisions might be affected by circulating levels of this hormone

(Abolins-Abols and Hauber, 2018; Ruiz-Raya and Soler, 2020). As expected, the experimental decrease in circulating prolactin carried out in our study significantly affected the host response to experimental brood parasitism: BRC-implanted females ejected mimetic model eggs at significantly higher rates than placebo-implanted individuals (implant treatment: $\chi^2 = 5.59$; $df = 1$; $p = 0.018$; Fig. 2a), independently of clutch size (implant treatment x clutch size: $\chi^2 = 0.24$; $df = 1$; $p = 0.62$). Laying date and baseline prolactin levels did not affect the egg-ejection behaviour of females (both cases $p > 0.76$). These results show that the experimental reduction of plasma prolactin increases the probabilities of egg rejection in blackbirds, while placebo-treated individuals ejected mimetic model eggs at similar rates to those found in previous studies in this species where no hormonal manipulation was performed (Soler et al., 2015). To the extent of our knowledge, this is the first experimental demonstration that host responses to brood parasitism may be mediated by the main endocrine pathway involved in the expression of avian parental care.

A better knowledge of the endocrine bases of host responses to brood parasitism will be crucial to further understand individual variation in the expression and evolution of rejection decisions (Abolins-Abols and Hauber, 2018). Since hosts might mistakenly eject an own egg (recognition costs), or break their clutch during the process (ejection costs) (Marchetti, 1992; Stokke et al., 2002), parasitized individuals are expected to optimize their individual performance by adjusting their acceptance thresholds according to host-parasite egg phenotype dissimilarity, previous experience or parasitism risk (Hauber et al., 2006; Ruiz-Raya and Soler, 2017; Ruiz-Raya and Soler, 2020). While the endocrine mechanisms underlying conditional responses to avian brood parasitism have been little studied, our results indicate that flexible shifts in hosts' acceptance thresholds may be linked to variations in circulating prolactin.

219 Interestingly, Abolins-Abols and Hauber (2020) have recently shown that an
 220 experimental reduction in plasma corticosterone increases the acceptance of parasitic
 221 model eggs in American robins (*Turdus migratorius*), a brown-headed cowbird
 222 (*Molothrus ater*) host closely related to Eurasian blackbirds. Taken together, these
 223 findings suggest that egg-rejection decisions are under the control of multiple endocrine
 224 mechanisms, and draw attention to the need for integrative studies combining prolactin
 225 and corticosterone, two hormones that may interact under certain circumstances to
 226 maintain parent homeostasis, providing complementary information on parental
 227 decisions (Angelier et al., 2016).

228 Beyond individual differences in baseline prolactin levels, interindividual variation in
 229 egg-rejection decisions might be determined by differences in hormonal sensitivity to
 230 environmental stimuli. Plasma prolactin has been found to decrease in response to
 231 stressors in many bird species (Angelier and Chastel, 2009; Angelier et al., 2016), so the
 232 host response to brood parasitism might be to some extent modulated by the strength of
 233 the prolactin response to environmental stressors. For example, an increase in the
 234 perceived risk of brood parasitism (e.g. witnessing a parasite near the nest) might lead
 235 to a drop in host's prolactin levels, which would explain why the sight of a parasite at
 236 the nest facilitates egg rejection by some hosts (Bartol et al., 2002; Moksnes et al.,
 237 1993; Moksnes et al., 2000). Under certain circumstances, hosts might be expected to
 238 modulate their prolactin response to stressors according to current environmental
 239 conditions, which could result in an adaptive prolactin stress response (Angelier and
 240 Chastel, 2009). Some hosts rely on the combined use of personal and social information
 241 about local parasitism risk to plastically adjust their acceptance thresholds to the current
 242 environmental context (Thorogood and Davies, 2016), or they require from two stimuli
 243 of brood parasitism (a parasitic egg in the nest and female parasite presence) to reach

the threshold for egg rejection (Moksnes et al., 1993). Interestingly, the reduction in plasma prolactin in response to stressors is more pronounced in individuals experimentally parasitized with non-mimetic eggs (Ruiz-Raya et al., 2018), which could suggest that prolactin responsiveness to parasite presence might be higher after perceiving a first cue of brood parasitism (e.g. an odd egg in the nest). While brood parasitism has been shown to impact the endocrine profiles of adult hosts during incubation (Ruiz-Raya et al., 2018), nestling (Antonson et al., 2020) and fledgling stages (Mark and Rubenstein, 2013), the extent to which adult parasite presence affects the hormonal levels of hosts still needs further study. Furthermore, since circulating prolactin often vary according to different life-history contexts (e.g. in response to predation risk or unpredictable environmental conditions (Angelier and Chastel, 2009; Angelier et al., 2016)), our understanding of the physiological mechanisms underlying anti-parasitic defences would benefit from the study of the endocrine response of hosts to their entire environmental context.

In our study, five out of 36 females (13.8%) deserted their nest but, most importantly, this abandonment probability was independent of the hormonal manipulation ($\chi^2 = 0.23$; $df = 1$; $p = 0.63$) and clutch size ($\chi^2 = 0.07$; $df = 1$; $p = 0.80$). Decreasing prolactin levels is associated with nest abandonment and lower breeding success in some bird species (Angelier et al., 2016), although this link is not clear in other avian species (Angelier et al., 2016; Kosztolányi et al., 2012; Wojczulanis-Jakubas et al., 2013). Our results confirm previous studies showing that nest desertion is not predicted by changes in circulating prolactin in Eurasian blackbirds (Ruiz-Raya et al., 2018), a potential host that does not use nest desertion as an anti-parasitic defence (Soler et al., 2015). These results suggest that nest abandonment is likely under the control of different neuroendocrine pathways, as well as other important aspects of avian breeding biology

269 such as energy storage (Angelier et al., 2016; Kosztolanyi et al., 2012). Future studies
 270 should explore whether prolactin changes are linked to nest desertion decisions in
 271 small-sized hosts that use nest abandonment as anti-parasitic defence. Our experimental
 272 manipulation had no impact on females' body mass (sample day: $F_{1,7} = 1.98$; $p = 0.20$)
 273 independently on the hormonal treatment (implant treatment x sample day: $F_{1,7} = 1.02$; p
 274 $= 0.35$). These results indicate that our BRC treatment did not effected females' feeding
 275 behaviour (Buntin, 1989), as well as supports previous results showing absence of
 276 unintended side effects on feeding behaviour in BRC-treated zebra finches (*Taeniopygia*
 277 *guttata*) (Smiley and Adkins-Regan, 2018).

278 In conclusion, our findings show that prolactin may play an important role as a mediator
 279 of host resistance to avian brood parasitism. While decreasing prolactin levels likely
 280 lead to more restrictive acceptance thresholds by facilitating host rejection decisions, the
 281 mechanism through which hormonal variations mediate egg-rejection decisions need
 282 further exploration. The fact that BRC-treated individuals ejected model eggs at higher
 283 rates, as well as the absence of ejection costs, suggests that a reduction in circulating
 284 prolactin does not impact the cognitive abilities of hosts. Indeed, changes in the
 285 individual cognitive performance are likely mediated by alternative endocrine pathways
 286 such as glucocorticoids (Maille and Schradin, 2016). Future studies addressing
 287 individual variations in baseline prolactin levels, the strength of the prolactin response
 288 to stressors, or differences in target tissue sensitivity to hormones (e.g. prolactin
 289 receptor density) would be particularly helpful to further explore the hormonal
 290 mechanisms mediating differences in egg-rejection behaviour within and among host
 291 populations. Finally, prolactin might also play a decisive role in the regulation of other
 292 anti-parasitic host defences linked to parental decisions, such as the rejection of
 293 parasitic nestlings (Grim, 2017), so future research on the role of prolactin as mediator

294 of host defences to brood parasitism should be extended to other stages of the breeding
295 cycle.

296 **Ethics.** This study follows all relevant Spanish national (Decreto 105/2011, 19 de
297 Abril) and regional guidelines. No individual exhibited long-term negative effects as a
298 consequence of our treatment.

299 **Competing interests.** We declare we have no conflict of interest.

300 **Authors' contribution.** FRR, JDIA and MS designed the study. FRR conducted the
301 fieldwork. CP and OC performed the hormonal assays. FRR analysed the data and
302 wrote the first draft. All authors critically contributed to drafts and gave final approval
303 for publication.

304 **Funding.** Financial support was provided by Consejería de Economía, Innovación,
305 Ciencia y Empleo; Junta de Andalucía (research project CVI-6653 to MS).

306 **References**

307 **Abolins-Abols, M. and Hauber, M. E.** (2018). Host defences against avian brood
308 parasitism: an endocrine perspective. *Proc. R. Soc. B Biol. Sci.* **285**, 20180980.

309 **Abolins-Abols, M. and Hauber, M. E.** (2020). Endocrine regulation of egg rejection in
310 an avian brood parasite host. *Biol. Lett.* **16**, 20200225.

311 **Angelier, F. and Chastel, O.** (2009). Stress, prolactin and parental investment in birds:
312 A review. *Gen. Comp. Endocrinol.* **163**, 142–148.

313 **Angelier, F., Barbraud, C., Lormée, H., Prud'homme, F. and Chastel, O.** (2006).
314 Kidnapping of chicks in emperor penguins: A hormonal by-product? *J. Exp. Biol.*
315 **209**, 1413–1420.

- 316 **Angelier, F., Wingfield, J. C., Tartu, S. and Chastel, O.** (2016). Does prolactin
317 mediate parental and life-history decisions in response to environmental conditions
318 in birds? A review. *Horm. Behav.* **77**, 18–29.
- 319 **Antonson, N. D., Hauber, M. E., Mommer, B. C., Hoover, J. P. and Schelsky, W.**
320 **M.** (2020). Physiological responses of host parents to rearing an avian brood
321 parasite: An experimental study. *Horm. Behav.* **125**, 104812.
- 322 **Bartol, I., Karcza, Z., Moskát, C., Røskft, E. and Kisbenedek, T.** (2002).
323 Responses of great reed warblers *Acrocephalus arundinaceus* to experimental
324 brood parasitism: the effects of a cuckoo *Cuculus canorus* dummy and egg
325 mimicry. *J. Avian Biol.* **33**, 420–425.
- 326 **Buntin, J. D.** (1989). Time course and response specificity of prolactin-induced
327 hyperphagia in ring doves. *Physiol. Behav.* **45**, 903–909.
- 328 **Buntin, J. D.** (1996). Neural and hormonal control of parental behavior in birds. *Adv.*
329 *Study Behav.* **25**, 161–213.
- 330 **Cottin, M., Chastel, O., Kato, A., Debin, M., Takahashi, A., Ropert-Coudert, Y.**
331 **and Raclot, T.** (2014). Decreasing prolactin levels leads to a lower diving effort
332 but does not affect breeding success in Adélie penguins. *Horm. Behav.* **65**, 134–
333 141.
- 334 **Davies, N. B. and Brooke, M. D. L.** (1989). An experimental study of co-evolution
335 between the cuckoo, *Cuculus canorus*, and its hosts. I. Host egg discrimination. *J.*
336 *Anim. Ecol.* **58**, 207–224.
- 337 **Feeney, W. E., Welbergen, J. A. and Langmore, N. E.** (2014). Advances in the study
338 of coevolution between avian brood parasites and their hosts. *Annu. Rev. Ecol.*

- 339 *Evol. Syst.* **45**, 227–246.
- 340 **Goutte, A., Angelier, F., Welcker, J., Moe, B., Clément-Chastel, C., Gabrielsen, G.**
 341 **W., Bech, C. and Chastel, O.** (2010). Long-term survival effect of corticosterone
 342 manipulation in Black-legged kittiwakes. *Gen. Comp. Endocrinol.* **167**, 246–251.
- 343 **Grim, T.** (2017). Host defences against brood parasite nestlings: theoretical
 344 expectations and empirical evidence. In *Avian Brood Parasitism Behaviour,*
 345 *Ecology, Evolution and Coevolution* (ed. Soler, M.), pp. 539–556. Springer.
- 346 **Grim, T., Samaš, P., Moskát, C., Kleven, O., Honza, M., Moksnes, A., Røskft, E.**
 347 **and Stokke, B. G.** (2011). Constraints on host choice: why do parasitic birds
 348 rarely exploit some common potential hosts? *J. Anim. Ecol.* **80**, 508–518.
- 349 **Hauber, M. E., Moskát, C. and Bán, M.** (2006). Experimental shift in hosts’
 350 acceptance threshold of inaccurate-mimic brood parasite eggs. *Biol. Lett.* **2**, 177–
 351 180.
- 352 **Kosztolanyi, A., Küpper, C., Chastel, O., Parenteau, C., Yilmaz, K. T., Miklósi, A.,**
 353 **Székely, T. and Lendvai, A. Z.** (2012). Prolactin stress response does not predict
 354 brood desertion in a polyandrous shorebird. *Horm. Behav.* **61**, 734–740.
- 355 **Kosztolányi, A., Küpper, C., Chastel, O., Parenteau, C., Yilmaz, K. T., Miklósi, Á.,**
 356 **Székely, T. and Lendvai, Á. Z.** (2012). Prolactin stress response does not predict
 357 brood desertion in a polyandrous shorebird. *Horm. Behav.* **61**, 734–740.
- 358 **Maille, A. and Schradin, C.** (2016). Ecophysiology of cognition: How do
 359 environmentally induced changes in physiology affect cognitive performance?
 360 *Biol. Rev.* **1102**, 1101–1112.
- 361 **Marchetti, K.** (1992). Costs to host defence and the persistence of parasitic cuckoos.

- 362 *Proc. R. Soc. - Biol. Sci.* **248**, 41–45.
- 363 **Mark, M. M. and Rubenstein, D. R.** (2013). Physiological costs and carry-over effects
364 of avian interspecific brood parasitism influence reproductive tradeoffs. *Horm.*
365 *Behav.* **63**, 717–722.
- 366 **Moksnes, A., Røskoft, E. and Korsnes, L.** (1993). Rejection of cuckoo (*Cuculus*
367 *canorus*) eggs by meadow pipits (*Anthus pratensis*). *Behav. Ecol.* **4**, 120–127.
- 368 **Moksnes, A., Røskoft, E., Hagen, L. G., Honza, M., Mork, C. and Olsen, P. H.**
369 (2000). Common cuckoo *Cuculus canorus* and host behaviour at reed warbler
370 *Acrocephalus scirpaceus* nests. *Ibis (Lond. 1859)*. **142**, 247–258.
- 371 **Müller, C., Almasi, B., Roulin, A., Breuner, C. W., Jenni-Eiermann, S. and Jenni,**
372 **L.** (2009). Effects of corticosterone pellets on baseline and stress-induced
373 corticosterone and corticosteroid-binding-globulin. *Gen. Comp. Endocrinol.* **160**,
374 59–66.
- 375 **Ouyang, J. Q., Muturi, M., Quetting, M. and Hau, M.** (2013). Small increases in
376 corticosterone before the breeding season increase parental investment but not
377 fitness in a wild passerine bird. *Horm. Behav.* **63**, 776–781.
- 378 **Pinheiro, J., Bates, D., Debroy, S. and Sarkar, D.** (2014). nlme: Linear and nonlinear
379 mixed effects models. R package version 3.1-117. Available: [http://CRAN.R](http://CRAN.R-project.org/package=nlme)
380 [project.org/package=nlme](http://CRAN.R-project.org/package=nlme)>.
- 381 **Roldán, M. and Soler, M.** (2011). Parental-care parasitism: how do unrelated offspring
382 attain acceptance by foster parents? *Behav. Ecol.* **22**, 679–691.
- 383 **Roncalli, G., Soler, M., Ruiz-Raya, F., Serrano-Martín, A. J. and Ibáñez-Álamo, J.**
384 **D.** (2019). Predation risk affects egg-ejection but not recognition in blackbirds.

- 385 *Behav. Ecol. Sociobiol.* **73**,.
- 386 **Ruiz-Raya, F. and Soler, M.** (2017). Phenotypic plasticity in egg rejection: evidence
387 and evolutionary consequences. In *Avian Brood Parasitism - Behaviour, Ecology,*
388 *Evolution and Coevolution* (ed. Soler, M.), pp. 449–471. Springer.
- 389 **Ruiz-Raya, F. and Soler, M.** (2020). Signal detection and optimal acceptance
390 thresholds in avian brood parasite-host systems: implications for egg rejection.
391 *Philos. Trans. R. Soc. B* **375**, 1–9.
- 392 **Ruiz-Raya, F., Soler, M., Sánchez-Pérez, L. L. and Ibáñez-Álamo, J. D.** (2015).
393 Could a factor that does not affect egg recognition influence the decision of
394 rejection? *PLoS One* **10**, 1–10.
- 395 **Ruiz-Raya, F., Soler, M., Roncalli, G., Abaurrea, T. and Ibáñez-Álamo, J. D.**
396 (2016). Egg rejection in blackbirds *Turdus merula*: a by-product of conspecific
397 parasitism or successful resistance against interspecific brood parasites? *Front.*
398 *Zool.* **13**, 16.
- 399 **Ruiz-Raya, F., Soler, M., Abaurrea, T., Chastel, O., Roncalli, G. and Ibáñez-**
400 **Álamo, J. D.** (2018). Hormonal responses to non-mimetic eggs: is brood
401 parasitism a physiological stressor during incubation? *Behav. Ecol. Sociobiol.* **72**,.
- 402 **Samas, P., Hauber, M. E., Cassey, P. and Grim, T.** (2011). Repeatability of foreign
403 egg rejection: Testing the assumptions of co-evolutionary theory. *Ethology* **117**,
404 606–619.
- 405 **Smiley, K. O.** (2019). Prolactin and avian parental care: New insights and unanswered
406 questions. *Horm. Behav.* **111**, 114–130.
- 407 **Smiley, K. O. and Adkins-Regan, E.** (2018). Lowering prolactin reduces post-hatch

- 408 parental care in male and female zebra finches (*Taeniopygia guttata*). *Horm.*
409 *Behav.* **98**, 103–114.
- 410 **Sockman, K. W., Sharp, P. J. and Schwabl, H.** (2006). Orchestration of avian
411 reproductive effort: an integration of the ultimate and proximate bases for
412 flexibility in clutch size, incubation behaviour, and yolk androgen deposition. *Biol.*
413 *Rev. Camb. Philos. Soc.* **81**, 629–666.
- 414 **Soler, M.** (2014). Long-term coevolution between avian brood parasites and their hosts.
415 *Biol. Rev.* **89**, 688–704.
- 416 **Soler, M.** (2017). *Avian Brood Parasitism - Behaviour, Ecology, Evolution and*
417 *Coevolution*. (ed. Soler, M.) Springer.
- 418 **Soler, M., Ruiz-Raya, F., Roncalli, G. and Ibáñez-Álamo, J. D.** (2015). Nest
419 desertion cannot be considered an egg-rejection mechanism in a medium-sized
420 host: an experimental study with the common blackbird *Turdus merula*. *J. Avian*
421 *Biol.* **46**, 369–377.
- 422 **Soler, M., Ruiz-Raya, F., Roncalli, G. and Ibáñez-Álamo, J. D.** (2017). Relationships
423 between egg-recognition and egg-ejection in a grasp-ejector species. *PLoS One* **12**,
424 e0166283.
- 425 **Stokke, B. G., Honza, M., Moksnes, A., Røskoft, E. and Rudolfson, G.** (2002). Costs
426 associated with recognition and rejection of parasitic eggs in two European
427 passerines. *Behaviour* **139**, 629–644.
- 428 **Thierry, A. M., Brajon, S., Massemin, S., Handrich, Y., Chastel, O. and Raclot, T.**
429 (2013). Decreased prolactin levels reduce parental commitment, egg temperatures,
430 and breeding success of incubating male Adélie penguins. *Horm. Behav.* **64**, 737–

431 747.

432 **Thorogood, R. and Davies, N. B.** (2016). Combining personal with social information
433 facilitates host defences and explains why cuckoos should be secretive. *Sci. Rep.* **6**,
434 10–15.

435 **Whittingham, M. J., Stephens, P. A., Bradbury, R. B. and Freckleton, R. P.** (2006).
436 Why do we still use stepwise modelling in ecology and behaviour? *J. Anim. Ecol.*
437 **75**, 1182–1189.

438 **Wojczulanis-Jakubas, K., Jakubas, D. and Chastel, O.** (2013). Behavioural and
439 hormonal stress responses during chick rearing do not predict brood desertion by
440 female in a small Arctic seabird. *Horm. Behav.* **64**, 448–453.

441

442

443

444

445

446

447

448

449

450

451

Figure Legends

Figure 1: (a) Pre-treatment prolactin levels (i.e. before implantation) showed by placebo (n = 18) and bromocriptine-implanted (BRC; n = 18) females. (b) Effect of the bromocriptine (BRC) 10-days-release pellets on females' prolactin levels 6-days after implantation: BRC-implanted females showed a significant reduction in circulating prolactin post-treatment compared to placebo-implanted individuals.

468

469 **Figure 2:** (a) Probability of egg ejection in both bromocriptine (BRC; yellow; N = 16)
 470 and placebo-implanted females (Placebo; blue; N = 15). (b) Blackbird clutch
 471 experimentally parasitized with a mimetic model egg (white arrow).