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7 Proximate Predictors of Variation in Egg Rejection Behavior by Hosts of Avian Brood Parasites

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10 Mikus Abolins-Abols and Mark E. Hauber

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12 Author Note

13 Mikus Abolins-Abols, Department of Evolution, Ecology, and Behavior, School of Integrative Biology,

14 University of Illinois, Urbana-Champaign, IL 61801, USA

15 Mark Hauber, Department of Evolution, Ecology, and Behavior, School of Integrative Biology, University

16 of Illinois, Urbana-Champaign, IL 61801, USA

17

18 Correspondence should be addressed to Mikus Abolins-Abols, m.abolins.abols@gmail.com

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Abstract

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29 The rejection of parasitic eggs by hosts of avian brood parasites is one of the most common and

30 effective defenses against parasitism. Despite its adaptive significance, egg rejection often

31 shows substantial intraspecific variation: some individuals are more likely to remove or abandon

32 parasitic eggs than others. Understanding variation in egg rejection requires that we study

33 factors linked to both the ability to perceive parasitic eggs, as well as factors that may influence

34 the rejection of a foreign egg once it has been recognized. Here we asked what cognitive,

35 physiological, and life-history factors explained variation in the rejection of model eggs by

36 American Robin *Turdus migratorius* females. We found that the probability of egg rejection was

37 related to the clutch size at the time of parasitism: in support of Weber's law, females with fewer

38 eggs were more likely to reject the model eggs. In turn, females with greater mass and higher

39 corticosterone levels were less likely to reject eggs, and egg rejection probability was negatively

40 related to incubation progress. Our data thus suggest that proximate predictors of an individual's

41 egg rejection behavior include components of the nest's perceptual environment, life-history

42 factors, as well as the physiological state of the animal. However, much of the variation in the

43 responses of robins to the model eggs remained unexplained. Future experiments should aim to

44 understand the causal roles of these and other factors in generating within- and among-

45 individual variation in the rejection of parasitic eggs.

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47 **Keywords:** brood parasitism; Brown-headed Cowbird, corticosterone; egg rejection; host

48 defenses, American Robin

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Introduction

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51   In obligate avian brood parasitic systems, parasites lay their eggs in the nests of host species,

52   often causing a substantial reproductive loss to the affected hosts (Davies, 2000; Feeney,

53   Welbergen, & Langmore, 2014; Soler, 2017). Many hosts have evolved a variety of adaptations

54   that allow them to mitigate or overcome the negative fitness effects of brood parasitism. One of

55   the most effective defenses against parasitism is the rejection of foreign eggs from the nest

56   (Davies & Brooke, 1988; Moksnes & Røskaft, 1992; Rothstein, 1982). Decades of research

57   have demonstrated that some hosts can recognize parasitic eggs using a variety of cognitive

58   rules, which include memory-based templates as well as direct comparisons of egg colors

59   (reviewed by Manna, Moskát, & Hauber, 2017). Brood parasites, on the other hand, have

60   evolved to overcome these defenses by laying mimetic (Davies & Brooke, 1988) or cryptic

61   (Langmore, Stevens, Maurer, & Kilner, 2009) eggs that limit the ability of hosts to recognize and

62   reject the parasitic eggs (Davies, Brooke, & Kacelnik, 1996; Feeney, Welbergen, & Langmore,

63   2012; Moskát & Hauber, 2007).

64   Variation in the host visual systems, morphology, and coevolutionary history with brood

65   parasites has led to divergent abilities of host species to recognize and reject parasitic eggs

66   (Avilés, 2008; Peer & Sealy, 2004; Rothstein, 1975; Stoddard & Stevens, 2011). Importantly,

67   however, differences in the egg rejection behavior exist not only among, but also within species

68   (Croston & Hauber, 2014a; Grim, Samaš, & Hauber, 2014; Molina-Morales et al., 2014; Samaš,

69   Hauber, Cassey, & Grim, 2011). While some of the intraspecific variation in egg rejection is

70   linked to the variation in the degree of mimicry of the parasitic egg in a particular clutch (Abolins-

71   Abols, Hanley, Moskát, Grim, & Hauber, 2019; Moskát et al., 2014), host individuals

72   nevertheless often show variation in the response to the *same* foreign egg stimuli (Grim et al.,

73   2014; Luro & Hauber, 2017). From a mechanistic standpoint, differences in responses to the

74   same parasitic stimuli could be caused by differences in the ability of hosts to perceive the

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75   parasitic egg stimulus, or differences in the probability of rejection once the parasitic egg has

76   been recognized.

77   Differences in the ability to perceive and identify a parasitic egg as non-self may be

78   driven either by individual variation in sensory systems (Stoddard & Stevens, 2011) or variation

79   of the perceptual environment of the parasitized nest (Honza, Procházka, Morongová, Čapek, &

80   Jelínek, 2011). For example, some hosts use the range and distribution of egg colors and

81   patterns in the nest to make rejection decisions (Bán, Moskát, Barta, & Hauber, 2013; Moskát,

82   Avilés, Bán, Hargitai, & Zölei, 2008). The variation of egg color in a clutch may be driven by the

83   extent of the host's intraclutch variability (Moskát et al., 2008) or differences in the parasite-to-

84   host egg ratio, due to single vs. multiple parasitism and/or variation in host clutch size (Manna et

85   al., 2019; Moskát et al., 2009; Stevens, Troscianko, & Spottiswoode, 2013, but see Lang,

86   Bollinger, & Peer, 2014). Hosts may respond differently to nests with varying parasite-to-host

87   egg ratio not only because of the variation in color, but also because of the Weber's law, which

88   posits that the ability of animals to discriminate between stimuli depends on the stimulus

89   proportionality (Stevens, 1975). Weber's law states that, when the proportional change in a

90   stimulus is low (e.g. a single parasitic egg added to a large clutch), the ability of individuals to

91   detect that change is lower relative to situations when the proportional change is larger (e.g. a

92   single parasitic egg in a small clutch). The ability of hosts to recognize brood parasitism may

93   thus depend on the size of the host clutch (Akre & Johnsen, 2014).

94   The probability of parasitic egg rejection may depend not only on the variation in the

95   host's ability to perceive the parasitic egg, but also on factors that affect the likelihood of egg

96   rejection *after* sensory and cognitive processes have processed the parasitic egg stimulus. For

97   example, hosts are more likely to reject foreign eggs of the same phenotype following a prior

98   experience with brood parasitism (Hauber, Moskát, & Bán, 2006) or earlier in the incubation

99   stage (Moskát & Hauber, 2007). Such experience- and life history-dependent plasticity in

100   parasitic egg rejection reflects the complexity of the changing costs associated with brood

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101   parasitic egg rejection in relation to host fitness (Avilés, 2018; Hauber et al., 2014; Molina-

102   Morales et al., 2014). For example, a brood parasitic chick hatched from an egg laid in the host

103   nest prior to the onset of incubation is more likely to negatively affect host fitness than a

104   parasitic chick hatched from an egg laid late in incubation (Moskát, 2005). Variation in the

105   likelihood of egg rejection across different life history stages may in turn be linked to changes in

106   the physiological state of the host (Abolins-Abols & Hauber, 2018): for instance, hormones that

107   affect maternal motivation and attachment in females (Bridges, 2015; Richard-Yris, Leboucher,

108   Chadwick, & Garnier, 1987) may cause changes in the readiness of hosts to accept or reject

109   any eggs in the nest (Abolins-Abols & Hauber, 2018). Specifically, corticosterone, a

110   glucocorticoid hormone that is involved in the regulation of the stress-response and metabolism

111   (MacDougall-Shackleton, Bonier, Romero, & Moore, 2019; Romero & Butler, 2007) can

112   suppress maternal behavior (Horton & Holberton, 2009). Importantly, corticosterone has been

113   shown to increase in hosts when they are exposed to brood parasitism (Mark & Rubenstein,

114   2013; Ruiz-Raya et al., 2018), suggesting that it may affect the likelihood of parasitic egg

115   rejection by hosts (Abolins-Abols & Hauber, 2018). In addition to context-specific, plastic

116   differences in egg rejection, hormones may also explain stable and repeatable individual

117   variation (i.e., personalities) in egg rejection irrespective of the context (Avilés & Parejo, 2011).

118   Indeed, in many cases hosts show repeatable individual variation in parasitic egg rejection

119   (Croston & Hauber, 2014a; Samaš et al., 2011) often across different contexts (Grim et al.,

120   2014). Importantly, hormonal levels also often show consistent differences between individuals

121   (Baugh et al., 2017; Rensel & Schoech, 2011; Romero & Reed, 2008), suggesting that

122   individual differences in egg rejection may be hormonally mediated (Abolins-Abols & Hauber,

123   2018).

124   In this study, we took a holistic approach and asked what cognitive, physiological, and

125   life-history factors explained variation in the egg rejection behavior of American Robins

126   (hereafter: robin), hosts to the obligate brood-parasitic Brown-headed Cowbird *Molothrus ater*

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127   (hereafter: cowbird) in North America. Robins have served as a productive model system in

128   which to investigate the perceptual mechanisms underlying parasitic egg rejection (e.g. Hanley

129   et al., 2017). While only a minority of robin individuals accept naturally laid parasitic cowbird

130   eggs (Rothstein, 1975, 1982), they show repeatable individual variation in the acceptance or

131   rejection of model eggs (Croston & Hauber, 2014a; Luro & Hauber, 2017).

132   Psychophysical theory (Weber's law; Akre & Johnsen, 2014) and empirical data (Moskát

133   et al., 2008) suggest that hosts should reject parasitic eggs more frequently when they have

134   smaller clutches. We therefore asked whether the probability of rejection of model parasitic eggs

135   by robins was explained by clutch size at the time of experimentation. We also tested if egg

136   rejection was related to the life history context (Hauber et al., 2014) by assessing whether

137   model egg rejection by robin hosts depended on female age, the progression of the breeding

138   season, and incubation stage. We predicted that older, more experienced breeders may be

139   more likely to reject model eggs (Moskát, Bán, & Hauber, 2014), that females would be more

140   likely to accept model eggs added later in incubation (Alvarez, 1996; Moskát, 2005) and later in

141   the breeding season (Thorogood & Davies, 2013). Finally, we asked if the probability of egg

142   rejection depended on host internal state as assessed by baseline corticosterone levels and

143   body mass, predicting that females with higher corticosterone levels (Abolins-Abols & Hauber,

144   2018) and higher body mass (Peer & Sealy, 2004) would be more likely to reject model eggs.

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146   **Method**

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148   **Study Site and Species**

149   All procedures were approved by the University of Illinois IACUC protocol #17259. We

150   studied American Robin females at Wandell's Nursery, a deciduous tree farm near Urbana, IL,

151   USA (lat: 40.128184; long: -88.105349), between April 26 and July 8, 2018. We surveyed the

152   study area every 3 days to search for nests and monitor their status. We assumed a deposition

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153   of one new egg per day during the laying period, and clutches with 2 or more consecutive days

154   of constant egg numbers were deemed completed (Vanderhoff, Pyle, Patten, Sallabanks, &

155   James, 2016). The median clutch size is 3 eggs in this population (mean: 3.38, range: 2-5).

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157   **Capture and Sample Collection**

158   We captured female robins (n=52) using mist nets as close to the predicted clutch

159   completion date as possible (range: 2 days before, 4 days after; median 1.5 days after

160   completion). In bird species with female-only incubation, including the American Robin

161   (Vanderhoff et al., 2016), the female is known to be the egg rejecter sex (Honza, Požgayová,

162   Procházka, & Tkadlec, 2007; Palomino, Martín-vivaldi, Soler, & Soler, 1998). Subjects were

163   captured between 6:00 am and 11:30 am using a 12 m long mist net (eye size 38 mm) that was

164   bent around a middle pole, resulting in a V-shaped net around the focal female's nest. Robin

165   females were either caught in the net while attempting to land in their nests, or, if the females

166   avoided the net and started incubating, by flushing the incubating females into the net.

167   Upon capture, we collected a ~75 µl blood sample from the brachial vein using a 26 G

168   needle and heparinized capillary tubes. The mean start time of blood collection was 131 s after

169   capture (range 70-220 s, standard deviation (SD) = 37.7). We typically ended blood collection at

170   180 s (3 min) after capture if a sufficient blood volume was collected (i.e., the "3 minute rule" for

171   baseline corticosterone analysis: Romero & Reed, 2005). If not, we continued to collect blood

172   after the 3 min cutoff (mean: 200 s, range for ending blood collection 143-245 s, SD = 18.9; see

173   below for the effect of the timing of blood collection on corticosterone levels). Blood was

174   transferred to a centrifuge tube and stored on ice until centrifugation (3-9 hrs after collection).

175   We centrifuged samples at 13,000 rpm for 10 min at 4 ºC to separate plasma from blood cells,

176   and stored the plasma samples at -80 ºC until analysis.

177   After blood collection, we banded each bird with a USGS aluminum band as well as 3

178   unique plastic color bands for individual identification in the field. We measured the mass of

179   birds to the nearest 0.5 g. We estimated the age of the females (second year or after second

180   year) by comparing the coloration of the wing feathers according to published guidelines (Pyle,

181   1997). Birds were then released at the site of capture (~10 min after capture), and the net was

182   removed.

183   One day after the capture, we returned to the focal nest to confirm female identity and

184   nest attendance by verifying the band colors. We found that 6 out of 52 (12 %) of females

185   abandoned their nests overnight and 5 out of 52 (10%) nests were depredated overnight

186   following the capture.

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188   **Experimental Parasitism**

189   If the nest was still active on the day after capture, we placed a dark blue 3D-printed

190   model cowbird egg in the nest (Figure 1). Our 3D-printed smooth nylon eggs (sourced

191   commercially from Shapeways.com) resembled natural Brown-headed Cowbird eggs in size

192   and weight (details given in Igic et al., 2015). We painted the eggs with acrylic dark blue paint

193   (Winsor & Newton Galeria Acrylic Ultramarine) applying three paint coats to each egg. The

194   resulting reflectance spectra of the model eggs and those of natural robin eggs have been

195   reported previously (Croston & Hauber, 2014b). We did not remove a robin egg from the nest as

196   cowbirds do when they parasitize nests (Davies, 2000), because prior studies had suggested

197   that *Turdus* thrushes respond to model parasitic eggs similarly, irrespectively of whether clutch

198   size is maintained or increased as part of the experiment (Grim et al., 2011). We chose this

199   artificial egg color type because cowbirds are natural brood parasites of robins (Rothstein, 1982)

200   and because eggs of this color have been shown to be rejected at intermediate rates by robin

201   females (Croston & Hauber, 2014b). Furthermore, differences in the propensity of rejection or

202   acceptance of these eggs are significantly repeatable among robin individuals across multiple

203   exposures within a breeding attempt (Luro & Hauber, 2017).

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204   Following the addition of the model egg, we counted the number of natural robin eggs in

205   the nest. We then returned and recorded the presence/absence of the blue egg on the next day,

206   visiting the nest at the same time of day compared to when the model egg was added. Robins

207   that reject dark blue model eggs nearly always do so on the first day after experimental

208   parasitism (Croston & Hauber, 2014a), therefore we used the rejection at day 1 as our response

209   variable. We found that a further 3 nests were abandoned and 4 depredated overnight following

210   the experimental parasitism. Due to the abandonment and predation of nests, and because 3

211   birds escaped during the initial processing, we had complete data on the rejection behavior of

212   31 birds (60% of the initial captures).

213   Because our aim was to begin to explore correlates of individual variation in rejection

214   behavior in response to a model egg of known intermediate rejection rate, we did not

215   manipulate any of the factors that were predicted to explain variation in egg rejection. We also

216   did not investigate the difference in the rejection rates of the dark blue model eggs in

217   comparison with control model egg treatments, such as robin-mimetic or cowbird-mimetic

218   colors: previous work had already shown these other egg colors to be consistently rejected or

219   accepted, respectively, independent of the individual identity in robins (Croston & Hauber,

220   2014b; Hanley et al., 2017; Igic et al., 2015). However, as an internal control, we checked on

221   each of the robin's own eggs in the nest (i.e. their number and whether intact) throughout our

222   monitoring period, and found none of these eggs to be missing or broken, indicating that robins

223   responded specifically to the dark blue model eggs.

224

Hormone Assays

226   We used an enzyme immune assay to measure the circulating baseline corticosterone

227   levels in blood samples from females caught before the experimental parasitism (Cayman

228   Chemical, catalogue number 501320). Hormones were extracted from plasma using diethyl

229   ether: 10 µl plasma was suspended in 200 µl double-distilled water and mixed with 1 ml diethyl

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230   ether. The aqueous phase was flash-frozen, and the ether phase decanted. The extraction

231   procedure was repeated 3 times, following which the ether was evaporated under a gentle

232   stream of nitrogen using an in-house constructed evaporation manifold (Nevins et al., 2005).

233   This extraction technique has been shown by MA-A to have high extraction efficiency for

234   songbird plasma (96%, Abolins-Abols, Hope, & Ketterson, 2016). Therefore, to limit the

235   logistical challenges associated radioactivity-based efficiency measurements, we did not

236   measure extraction efficiency in this study (Virgin & Rosvall, 2018).

237   The plasma extract was suspended in 600 µl assay buffer provided with the kit by

238   vortexing for 1 min before storing it at 4 ºC overnight. The corticosterone concentration of the

239   extract was measured following the instructions from the kit manufacturer. We first validated the

240   Cayman corticosterone assay to determine that robin plasma extracts reacted with the assay as

241   expected. The assay showed good parallelism (slope=1.00; r^2=0.99), sensitivity (10.7 pg/mL),

242   and recovery (88.9%). We then ran the extracted samples in triplicate, using a pooled robin

243   plasma extract as a within- and across-plate control. We used a Biotek 800TS plate reader to

244   record assay absorbance at 405 nm. Data were analyzed using a 4-point logistic curve using

245   the analysis spreadsheet provided by Cayman Chemical. All samples fell within 20-80% B/Bo

246   range. The among-plate coefficient of variation (CV) was 16.4%, whereas the within-plate CV

247   was 5.17%. We averaged the data from the 3 replicates, and standardized sample

248   concentrations across plates using the mean concentration of the control samples.

249

250   **Data Analysis**

251   We first investigated if corticosterone levels in robin females were related to the timing of

252   capture and blood sampling. While in most animals corticosterone levels start rising after 3 min,

253   and therefore a blood sample taken under 3 min post capture can be considered a "baseline"

254   sample (Romero & Reed, 2005), in some cases corticosterone levels start rising earlier (Small

255   et al., 2017). In our data, corticosterone levels were positively correlated with the blood

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256   collection start time - circulating corticosterone in robins started to increase soon after capture

257   (estimate=0.02 ng/ml increase per s; r^2=0.16; p=0.02, Figure S1). We therefore corrected for the

258   variation in corticosterone as a function of the blood collection start time by running a linear

259   regression between these variables, and used the residuals as a bleed time-independent

260   measure of baseline corticosterone levels. We chose to use the blood collection start time as

261   opposed to the end time, because in the majority of cases we ended blood collection at exactly

262   3 min. No other covariates (time of day, time since nest approach, time taken to set up the mist

263   net) were related to corticosterone levels (all p > 0.05, data not shown).

264   We used generalized linear models (GLMs) with binomial error structure in R (R Core

265   Team, 2017) to ask if rejection of the model eggs was related to the following ecological and

266   proximate factors, all of which were known to be linked to egg rejection in brood parasite hosts,

267   including robins: the date of model egg addition (Dainson, Hauber, López, Grim, & Hanley,

268   2017), age (Moskát et al., 2014), mass (Peer & Sealy, 2004), incubation stage relative to clutch

269   completion (further as "days since clutch completion"; Marchetti, 2000), the number of eggs in

270   the nest at the time of experimentation (Moskát & Hauber, 2007), and baseline corticosterone

271   (Ruiz-Raya et al., 2018). We did not have any *a priori* expectations for interactions between

272   these variables. All predictors were standardized to mean 0 and standard deviation of 1.

273   To investigative which of our observed predictor variables best explained egg rejection

274   we used information-theoretic (IT) approach, combined with model averaging (Burnham,

275   Anderson, & Huyvaert, 2011; Symonds & Moussalli, 2011). The emerging consensus for animal

276   behavior-relevant studies is that IT-based model averaging approaches are better suited for

277   hypothesis testing with correlative datasets from natural study systems compared to the more

278   traditional p-value based approaches (Aho, Derryberry, & Peterson, 2014). In short, an IT-based

279   approach allows one to compare the strength of evidence for various competing alternative

280   models that may explain the variation in a variable (here: egg rejection), while a frequentist p-

281   value-based approach is a better fit for controlled experiments with a defined null hypothesis

282   (Murtaugh, 2014; Valpine, 2014). An IT approach is especially useful when, as is the case in our

283   data, there are a number of independent candidate predictors that may explain variation the

284   response variable, but where a model with all of the covariates may not necessarily generate

285   the best model. Model averaging of competing models, in turn, allows one to evaluate the

286   strength of evidence supporting a relationship between a particular independent variable and

287   the dependent variable (here, the probability of egg rejection). Nevertheless, we have also

288   provided p-values for the variables included in the best models to aid in data interpretation.

289   We used the package *MuMIn* (Bartoń, 2018) to calculate the corrected Akaike

290   information criterion (AICc) scores for the models that contained all combinations of predictors

291   (without interaction terms) to determine the model that was most likely to be the best model.

292   Ten models had AICc scores higher than the null model. We calculated the goodness of fit of

293   each of these top models in relation to the null model using the package *lmtest* (Zeileis &

294   Hothorn, 2002). To estimate how much variance the top models explained, we used the

295   package *pscl* (Jackman, 2017) to calculate McFadden's pseudo-R^2. Seven models had AICc

296   scores that were within 2.0 units of the top model, indicating a level of uncertainty of which

297   variables contributed to the best model. We, therefore, also followed a model averaging

298   approach (Burnham et al., 2011; Symonds & Moussalli, 2011): we calculated the relative

299   importance of each independent variable within the models with cumulative AICc weights of

300   0.95, i.e. a set that had a 95% probability of including the best model.

301

302   **Results**

303

304   Among the 31 female American Robins that were included in the analyses, 18 (58.1%)

305   ejected the model egg within 24 hrs.

306   Out of candidate 64 statistical models testing the predictors of the probability of egg

307   rejection, 10 had AICc-s higher than the null model, which included no predictor variables (Table

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308   1). However, these models were highly competitive, with seven top models being within 2.0

309   AICc-s of each other. The seven top models included egg number, days since clutch

310   completion, corticosterone levels, and mass as the predictors of egg rejection. The model with

311   the lowest AICc (41.80) suggested that females with more eggs in the nest at the time of

312   experimentation were significantly less likely to reject the model egg (Figure 2). However, based

313   on the corrected Akaike weights (w_i=0.11), this model only had 11% overall probability of being

314   the best model for explaining egg rejection behavior, and it explained only 11% of overall

315   variation in egg rejection (r^2=0.11). An equally competitive model (AICc=42.75, ΔAICc=0.95,

316   w_i=0.07, r^2=0.28), included all of the covariates present in the 7 top models, and suggested that

317   females with smaller clutch sizes, lower mass, lower corticosterone levels, and those whose

318   nests were parasitized earlier with respect to their clutch completion were more likely to reject

319   the model eggs (Table 2).

320   Despite the uncertainty of the composition of the best model, likelihood ratio tests

321   showed that all except two of the top 7 models with ΔAICc < 2.0 were significantly better

322   predictors of egg rejection behavior compared to the null model (Table 1). The number of eggs

323   term was present in 6 out of 7 top models, with the only exception being a model that only

324   contained corticosterone levels. Other predictors among the 7 models with ΔAICc < 2.0 -

325   corticosterone, mass, and days since clutch completion - were present in 3 out of the 7 top

326   models (Figure 2). The full model which included all of the covariates had a much lower AICc

327   than the null model (Table 1).

328   Model averaging of all models with cumulative Akaike weight of 0.95 echoed the

329   patterns of individual top models and suggested that the number of eggs at the time of the

330   experimental parasitism was consistently the strongest predictor of egg rejection – birds with

331   less eggs in the nest had a higher probability of egg rejection. This metric was present in 26 out

332   of 45 models with a relative importance of 0.72 (Table 3). The other 3 predictors occurring in

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333   some of the top 7 models, had a weaker relationship to egg rejection: birds with lower mass

334   (importance: 0.46, present in 24 out of 45 models), lower corticosterone levels (importance:

335   0.44, present in 21 out of 45 models), and birds experimentally parasitized earlier with respect

336   to the clutch completion date (importance: 0.37, present in 18 of 45 models) were more likely to

337   reject the model eggs.

338

339   **Discussion**

340   Rejection of parasitic eggs by hosts is one of the most widespread and effective

341   defenses against avian brood parasites (Davies, 2000; Soler, 2017). However, different host

342   species and, at an intraspecific level, different individuals show substantial variation in the

343   propensity to reject foreign eggs from their nests (Alvarez, 1996; Brooke, Davies, & Noble,

344   1998; Bård G. Stokke, Moksnes, & Røskaft, 2005). Here we asked if cognitive, physiological,

345   and life-history factors predict variation in egg rejection by a brood parasite host. Our

346   correlational data set and model averaging approach showed that, in decreasing order of

347   importance, among the most likely factors that may explain variation in model egg rejection by

348   American Robins were the number of eggs in the nest at the time of the experimental

349   parasitism, adult mass, the baseline corticosterone level, and the incubation stage relative to

350   clutch completion: females with smaller clutches, lower mass, lower corticosterone, and those

351   parasitized sooner after clutch completion were more likely to reject the model eggs. These

352   relationships suggest that egg rejection is a complex behavior that is regulated by a diversity of

353   factors.

354   The strongest and most consistent predictor of egg rejection in our models was clutch

355   size, indicating that females with smaller clutches at the time of experimental parasitism were

356   more likely to reject the model egg within a day compared to females with larger clutches.

357   Because all of the females that were included in the analysis had completed laying their

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358   clutches at the time of experimental parasitism, this suggests that this relationship is not driven

359   by variation in whether or not the clutch was completed, but by the final clutch size.

360   The effect of clutch size (and, thus, the parasite-to-host egg ratio) on egg rejection

361   appears to vary between studies and species. On the one hand, multiple parasitism (higher

362   ratio) has been shown to reduce the rejection of parasitic eggs (Bán et al., 2013; Manna et al.,

363   2019; Moskát et al., 2009; M. Stevens et al., 2013) supporting the hypothesis that the ability of

364   hosts to reject eggs is compromised because multiple eggs can be perceived as the most

365   different when using a discordancy-based egg rejection mechanism (but see Lang, Bollinger, &

366   Peer, 2014). On the other hand, irrespective of the extent of parasitism, greater absolute

367   variation in egg coloration, associated with larger host clutches, has also been suggested to

368   decrease the host's ability to detect the foreign egg (Øein, Moksnes, & Røskaft, 1995). In

369   support of this hypothesis, higher within-clutch variation due to more variable host eggs, or a

370   bigger difference between the host and parasite egg colors, is associated with lower probability

371   of egg rejection in mimetic parasite-host systems (Moskát et al., 2008; Stokke, Moksnes,

372   Roskaft, Rudolfsen, & Honza, 1999), although it causes no change in egg rejection in non-

373   mimetic parasite-host systems (Abernathy & Peer, 2014; Rebecca Croston & Hauber, 2015;

374   Lang et al., 2014).

375   Yet another hypothesis that has not so far been explicitly addressed in host-brood

376   parasite systems is that the proportion of parasite-to-host eggs in the nest affects the ability of

377   hosts to perceive the parasitic egg due to the way animals respond to proportional differences in

378   stimuli (Weber's law; Akre, Farris, Lea, Page, & Ryan, 2011; Akre & Johnsen, 2014; Weber,

379   1978). Akre & Johnsen (2014) specifically proposed that in avian host-brood parasite systems,

380   hosts may be better able to detect a parasitic egg if it is laid in a smaller clutch as opposed to a

381   larger clutch. This is because in a smaller clutch, the addition of a parasitic egg causes a larger

382   proportional increase compared to a large clutch. This, in turn, makes foreign eggs in smaller

383   clutches easier to perceive and reject. The relationship between model egg rejection and clutch

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384   size in our study is consistent with the predictions of Weber's law of proportional processing as

385   applied to avian host-parasite systems (Akre & Johnsen, 2014). Importantly, however, because

386   robins do not reject mimetic eggs (Luro et al., 2018; Rothstein, 1982), the relationship between

387   clutch size and egg rejection in robins cannot be explained by a proportional increase in the

388   clutch size alone (irrespective of color). Instead, a larger relative increase in the clutch size may

389   affect the color threshold at which eggs are rejected (Abolins-Abols et al., 2019; Hanley et al.,

390   2017, 2019). We suggest that proportional processing may therefore constitute an important

391   aspect of the cognitive and perceptual processes, which, in concert, regulate egg rejection.

392   Female mass was negatively related to the rejection of model eggs in our study,

393   indicating that heavier females either had higher thresholds for rejection of model eggs or

394   showed, on average, higher maternal motivation to respond affiliatively towards (all) eggs. Mass

395   has been associated with egg rejection in inter-specific comparisons (Peer & Sealy, 2004),

396   where larger species are more likely to reject parasitic eggs. At an intraspecific level, heavier

397   females may have higher expected future reproductive success (Abolins-Abols & Ketterson,

398   2017; Blums, Clark, & Mednis, 2002), possibly leading to a reduced sensitivity to parasitic

399   stimuli. More focal experimental work will be needed to assess the mechanisms behind this

400   association.

401   We also found evidence that females with lower corticosterone levels rejected the model

402   eggs more rapidly, although the support for this relationship was statistically weak. This finding

403   contradicts our prediction that, because higher corticosterone levels may lead to suppression of

404   maternal attachment to eggs, robin females with higher baseline corticosterone levels should be

405   more likely to reject the model eggs (Abolins-Abols & Hauber, 2018). However, we consider the

406   alternative, namely that corticosterone may increase the motivation to care for eggs, unlikely.

407   Instead, we argue that corticosterone levels may covary with or influence other phenotypic,

408   sensory, and/or cognitive traits that are linked to egg perception, attention and, indirectly, to egg

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409   rejection propensity. Experimental tests with manipulated circulating hormone levels will be

410   needed to assess these alternatives fully.

411   Finally, we found support for the pattern that females that experienced experimental

412   parasitism earlier into the incubation stage were more likely to reject the model eggs. This

413   agrees with similar findings in other avian host-parasite systems, where females are more likely

414   to reject eggs earlier into the incubation stage of nesting (Moskát, 2005). This finding is

415   consistent with at least two alternative hypotheses. First, because brood parasites target hosts

416   nests during laying to ensure that the parasitic chicks hatch earlier or at the same time as host

417   chicks, hosts should be selected to respond more selectively towards brood parasite eggs

418   earlier in laying/incubation (Moskát, 2005). Alternatively, hosts may be less willing to eject any

419   eggs later in incubation as a consequence of increasing general maternal motivation to care for

420   advanced eggs or nestlings (Knight & Temple, 1986; Montgomerie & Weatherhead, 1988).

421   Proximally, both of these alternative motivations – decrease in agonistic responses against

422   brood parasitic stimuli, and an increase in general maternal motivation – may be mediated by

423   hormones that regulate maternal care and motivation in other organisms, such as prolactin,

424   estradiol, and progesterone (Abolins-Abols & Hauber, 2018; Bridges, Numan, Ronsheim, Mann,

425   & Lupini, 1990; Cheng & Silver, 1975). Future studies should therefore also address these

426   potential endocrine mediators of egg rejection observationally and experimentally.

427   In summary, we show that predicting egg rejection by American Robins requires

428   complex models, and that this behavior is likely regulated by the perceptual environment, life

429   history, as well as physiological factors. These factors may contribute to both plastic as well as

430   individually consistent variation in egg rejection, although further studies are needed to

431   experimentally establish the precise causal links between these variables and hot behavior.

432   Despite our extensive efforts to include a diversity of proximate factors to understand variation

433   in egg rejection, our best models were able to explain only 28% of variation in the rejection

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434   behavior. Research to assess what other factors underlie and cause variation in this ecologically

435   and evolutionarily indispensable behavior therefore remains a potentially fruitful field.

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706
707 *Figure 1.* Model egg (dark blue, right) alongside three natural American Robin eggs (light blue).
708 Photo by MAA.
709
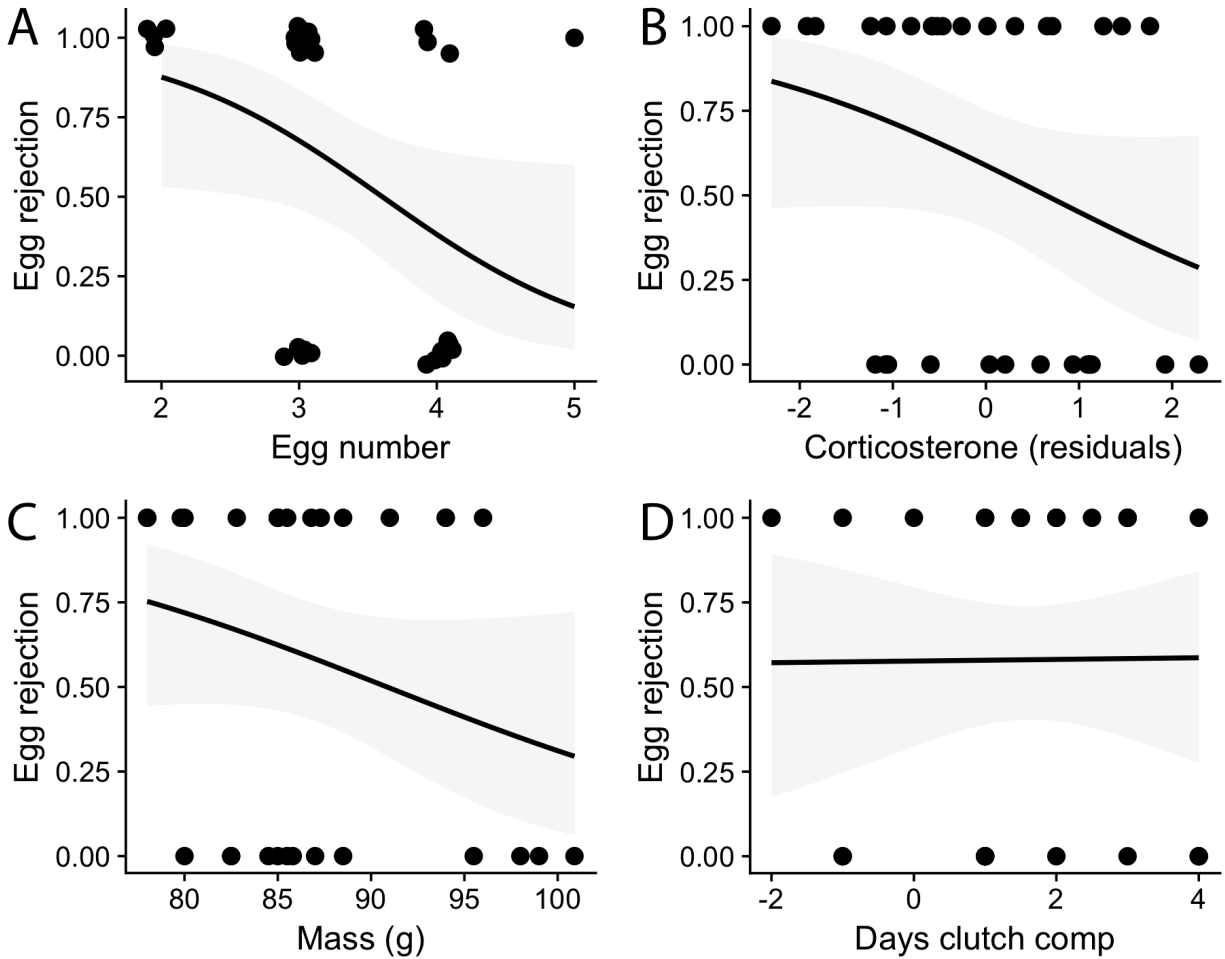
Figure 2. Relationship between the probability of egg rejection at day one (egg rejection) and (A) egg number at the time of parasitism, (B) baseline corticosterone levels (time-corrected residual levels), (C) mass, and (D) days between clutch completion and parasitism ("days clutch comp"). Grey shading indicates the 95% confidence intervals from the logistic regressions. Plots show relationship between raw data points. Data points at discrete x-values are jittered for visibility.

719 Table 1

720 *Generalized linear models predicting egg rejection at day 1 with AICc higher than the null*

721 *model. k = number of fixed terms in the model, AICc = Akaike information criterion, ΔAICc =*

722 *delta AICc between the focal model and the best model, w_i = Akaike weights, p = probability that*

723 *the model explains more variation than the null model, r^2 = McFadden's pseudo r^2 of model fit.*

724 *Egg num: number of eggs at the time of parasitism; cort: baseline corticosterone adjusted for*

725 *bleeding time, days clutch comp: number of days elapsed between clutch completion and*

726 *parasitism, mass: female mass in g; age: second year or after second year age groups. Top*

727 *seven models with ΔAICc < 2.0 are in italic font.*

model	k	AICc	ΔAICc	w_i	p	r^2
egg num	*1*	*41.80*	*0.00*	*0.11*	*0.03*	*0.11*
egg num + cort + days clutch comp + mass	*4*	*42.75*	*0.95*	*0.07*	*0.02*	*0.28*
egg num + cort	*2*	*42.93*	*1.13*	*0.06*	*0.05*	*0.15*
egg num + mass	*2*	*42.97*	*1.17*	*0.06*	*0.05*	*0.14*
egg num + days clutch com + mass	*3*	*43.12*	*1.33*	*0.05*	*0.04*	*0.20*
egg num + days clutch comp	*2*	*43.52*	*1.73*	*0.04*	*0.06*	*0.13*
cort	*1*	*43.72*	*1.93*	*0.04*	*0.09*	*0.07*
egg num + cort + days clutch comp	3	43.92	2.12	0.04	0.05	0.18
egg num + date	2	44.09	2.29	0.03	0.08	0.12
egg num+ age	2	44.25	2.45	0.03	0.09	0.11
null model	0	44.30	2.51	0.03	NA	NA
full model: egg num + cort + days clutch comp + date + mass + age	6	49.06	7.27	0	0.06	0.28

728
729

730 Table 2
731 *GLM model predicting the probability of egg rejection at day 1, including all of the covariates*
732 *present in the 7 top models; s.e. = standard error. Significant variables and p-values are*
733 *italicized.*

covariate	estimate	s.e.	z	p
(Intercept)	0.61	0.48	1.25	0.21
egg number	*-1.18*	*0.56*	*-2.11*	*0.04*
mass	-1.12	0.64	-1.76	0.08
corticosterone	-0.86	0.53	-1.62	0.11
days since clutch completion	-1.22	0.64	-1.92	0.06

734
735

736 Table 3
737 *Averaged parameter estimates and the importance of covariates in explaining the probability of*
738 *egg rejection on day 1; s.e. = adjusted standard errors, importance = sum of Akaike weights in*
739 *all models that include the variable; CI = confidence interval.*

covariate	estimate (95% CIs)	s.e.	z	importance
(Intercept)	0.42 (-0.47, 1.31)	0.45	0.93	NA
egg number	-0.96 (-2.04, 0.11)	0.55	1.76	0.72
mass	-0.79 (-2.04, 0.46)	0.64	1.24	0.46
corticosterone	-0.60 (-1.53, 0.32)	0.47	1.27	0.44
days since clutch completion	-0.73 (-2.01, 0.55)	0.65	1.12	0.37
date	-0.09 (-1.36, 1.18)	0.65	0.14	0.21
age (older)	-0.17 (-2.06, 1.73)	0.97	0.17	0.18

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