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Prolactin and the regulation of parental care and helping behavior in cooperatively breeding white-browed sparrow weaver societies

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25   ABSTRACT

26   In many cooperatively breeding societies non-breeding individuals help to rear the offspring of breeders.

27   The physiological mechanisms that regulate such cooperative helping behavior are poorly understood,

28   but may have been co-opted, during the evolution of cooperative breeding, from pre-existing

29   mechanisms that regulated parental care. Key among these may be a role for prolactin. Here we

30   investigate whether natural variation in circulating prolactin levels predicts both parental and helper

31   contributions to nestling provisioning in cooperatively breeding white-browed sparrow weavers,

32   *Plocepasser mahali*. In sparrow weaver groups, a single dominant pair monopolize reproduction and

33   non-breeding subordinates help with nestling feeding. We show that: (i) among parents, dominant

34   females feed nestlings at higher rates, make longer provisioning visits and have higher prolactin levels

35   than dominant males; and (ii) among subordinates, engaged in cooperative helping behavior, those

36   within their natal groups feed nestlings at higher rates and have higher prolactin levels than immigrants.

37   Moreover, continuous variation in prolactin levels positively predicts nestling-provisioning rates and

38   mean provisioning visit durations when all bird classes are combined. These relationships are principally

39   driven by differences among bird classes in both circulating prolactin levels and provisioning traits; the

40   more limited within-class variation in prolactin and provisioning traits were not evidently correlated,

41   highlighting a likely role for additional mechanisms in the fine-scale regulation of care. Our findings

42   broadly support the hypothesis that parental care and cooperative helping behavior are regulated by a

43   common underlying mechanism and highlight the need for experimentation to now establish the

44   causality of any role for prolactin.

45   [250 words]

46   INTRODUCTION

47   In many cooperatively breeding societies, non-breeding helpers assist with the rearing of parents'

48   young, via cooperative contributions to diverse forms of care (e.g. incubation, babysitting and offspring

49   provisioning; Solomon and French 1997, Koenig and Dickinson 2004, 2016). The majority of research

50   on such 'helping behavior' has sought to explain its evolution, by identifying the effects of helping on

51   recipients and the means by which these yield fitness benefits to helpers (Cockburn 1998, Dickinson

52   and Hatchwell 2004, Koenig and Dickinson 2016). By contrast, our understanding of the proximate

53   physiological mechanisms that regulate the expression of cooperative behavior is less advanced

54   (Schoech et al. 2004, Soares et al. 2010, Sanderson et al. 2014, Dantzer et al. 2017), despite a surge

55   of interest in the origins of consistent individual differences in both cooperative behavior and endocrine

56   traits (Sanderson et al. 2015, English et al. 2010, Dantzer et al. 2019, Houslay et al. 2019). As

57   cooperatively breeding species commonly evolved from monogamous pair-breeding species in which

58   parental care was well developed (Cornwallis et al. 2010, Lukas and Clutton-Brock 2012), it seems likely

59   that the physiological mechanisms that regulate cooperative helping behavior among non-breeders

60   were co-opted from the pre-existing mechanisms that regulated parental care among breeders.

61   Attempts to identify the proximate mechanisms that regulate helping behavior may therefore be well

62   served by testing candidate mechanisms already identified for the regulation of parental care in non-

63   cooperative species (Ziegler 2000, Schoech et al. 2004, Carlson et al. 2006a, 2006b). One such

64   mechanism is the neuroendocrine pathway involving the anterior pituitary gland hormone prolactin

65   (Buntin 1996, Sharp et al. 1998, Ziegler 2000, Carlson et al. 2006a, 2006b, Angelier et al. 2016).

66

67   Numerous studies suggest that prolactin can play a causal role in the expression of parental care,

68   though its precise role is not clear and seems likely to vary across taxa (Buntin 1996, Sharp et al. 1998,

69   Angelier et al. 2016). In birds, prolactin is thought to play a causal role in the onset and maintenance of

70   parental care, but it is less clear whether variation in circulating prolactin levels is also involved in the

71   quantitative regulation of contributions to care once caring behavior has begun (Boos et al. 2007,

72   Angelier et al. 2016, Smiley and Adkins-Regan 2018). The transition from sexual activity to parenting is

73   typically associated with an increase in circulating prolactin levels, which peak during the care period

74   (Buntin 1996, Sharp et al. 1998, Angelier et al. 2016, e.g. Schoech et al. 1996, Badyaev and Duckworth

75   2005). Evidence that naturally low prolactin levels are commonly associated with breeding attempt

76   abandonment and/or failure (e.g. Chastel and Lormee 2002, Chastel et al. 2005), and that experimental

77   reductions in circulating prolactin levels during late incubation can disrupt post-natal care (e.g. Smiley

78   and Adkins-Regan 2018), suggest that these elevated prolactin levels are necessary for the onset and/or

79   maintenance of parental care. Indeed, experimental elevations of circulating prolactin suggest that

80   elevated prolactin levels can promote the onset of both incubation behavior (e.g. Sockman et al. 2000)

81   and nestling provisioning behavior (e.g. Badyaev and Duckworth 2005). Positive associations between

82   continuous variation in circulating prolactin levels and the rates at which parents provision their offspring

83   (e.g. Duckworth et al. 2003, Ouyang et al. 2011) highlight the possibility that prolactin levels also regulate

84   the *amount* of care that an actively caring parent provides to its offspring. However, this hypothesis has

85   yet to be experimentally tested, and such positive associations could arise instead via effects of

86   provisioning activity on a bird's circulating prolactin levels, as parental contact with offspring cues can

87   increase prolactin secretion (Hall 1987, Sharp et al. 1998). Causal links between prolactin and

88   provisioning rates may therefore exist in both directions. Indeed, such a feedback loop (in which

89   offspring cues stimulate prolactin secretion that in turn maintains and/or elevates the expression of

90   parental care) could conceivably both maintain parental care while offspring survive, and regulate its

91   expression according to offspring vigor and need.

92

93   A number of studies of cooperatively breeding species have now begun to investigate the relationships

94   between prolactin and care-giving behavior, both among parents and non-breeding helpers (Ziegler

95   2000, Schoech et al. 2004, Soares et al. 2010). Prolactin levels have been shown to rise in parents and

96   non-breeding helpers during the transition to incubation and nestling care in at least three species of

97   cooperatively breeding bird (Schoech et al. 1996, Brown and Vleck 1998, Khan et al. 2001, see also

98   Vleck et al. 1991). While few studies have investigated specifically whether variation in circulating

99   prolactin levels predicts variation in cooperative contributions to helping, studies of at least two

100   cooperative breeders have yielded compelling evidence in this regard. In Florida scrub jays

101   (*Aphelocoma coerulescens*), breeders fed offspring at higher rates than non-breeders and showed

102   higher circulating prolactin levels (Schoech et al. 1996, see also Vleck et al. 1991), and those non-

103   breeders that helped to feed offspring showed higher prolactin levels than those that did not (Schoech

104   et al. 1996). Indeed, continuous variation in circulating prolactin levels predicted continuous variation in

105   feeding contributions, both among all birds combined and specifically among non-breeders (Schoech et

106   al. 1996). Similarly, in cooperatively breeding meerkat (*Suricata suricatta*) societies, continuous variation

107   in the prolactin levels of helpers positively predicted their cooperative contributions to both babysitting

108   and pup-feeding (Carlson et al. 2006a, 2006b). In the pup-feeding study, prolactin levels only predicted

109   the pup-feeding rates of helpers in statistical models that did not allow for an independent positive effect

110   of circulating cortisol levels on the animal's pup-feeding rates (Carlson et al. 2006a). Experimental work

111   since highlights that this putative positive effect of cortisol on pup-feeding rates may not have been

112   causal, however, as glucocorticoid receptor blockade increased rather than decreased pup-feeding

113   rates among meerkat helpers (Dantzer et al. 2017). Such relationships between circulating prolactin

114   levels and helping behavior are not always apparent. For example, prolactin levels did not predict the

115   offspring provisioning rates of helpers in red-cockaded woodpecker (*Picoides borealis*) groups (Khan et

116   al. 2001), and the pituitary gland prolactin mRNA levels of a cooperatively breeding fish were not

117   evidently related to care-giving behavior (Bender et al. 2008); though the relevant sample sizes in both

118   studies were modest.

119

120   The likelihood that glucocorticoids play a role in mediating the state-dependence of cooperative helping

121   (Sanderson et al. 2014, Dantzer et al. 2017, see also Angelier and Chastel 2009, Angelier et al. 2016),

122   coupled with the expectation that other neuroendocrine pathways also modulate helping potentially

123   independent of circulating prolactin levels (Schoech et al. 1998, Ziegler 2000, Young et al. 2005, Carlson

124 et al. 2006a), highlights the potential complexity of seeking relationships between continuous variation

125 in prolactin levels and care. Notably too, given the potential for contact with offspring to raise circulating

126 prolactin levels (Sharp et al. 1998, Hall 1987), it is possible that the prolactin-helping associations

127 documented above arose not via causal effects of prolactin on care, but via either the reverse causal

128 relationship or in the absence of any causal link between the two. Nevertheless, the promising findings

129 to date highlight the need for further studies to investigate whether natural circulating levels of prolactin

130 predict variation in individual contributions to both parental care and helping behavior in cooperatively

131 breeding species, and ultimately the use of experimental manipulations of prolactin levels to test the

132 causality and nature of any relationships detected (Sockman et al. 2000, Carlson et al. 2003, Badyaev

133 and Duckworth 2005, Smiley and Adkins-Regan 2018).

134

135 Here we investigate whether natural variation in circulating prolactin levels positively predicts the

136 nestling provisioning behavior of both parents and non-breeding helpers in a wild cooperatively breeding

137 bird, the white-browed sparrow-weaver (*Plocepasser mahali*). White-browed sparrow weavers are rain-

138 dependent breeders that live in year-round territorial groups throughout the semi-arid regions of sub-

139 Saharan Africa (Lewis 1982, Wood et al. 2021). Within each social group, a single dominant male and

140 female completely monopolize within-group reproduction and up to 10 non-breeding subordinates of

141 both sexes help to feed their nestlings (Harrison et al. 2013a, 2013b, Capilla-Lasheras et al. 2021).

142 Subordinates are typically offspring from previous broods that have delayed dispersal from their natal

143 group (and so are helping to rear their parents' young), but subordinate immigrants of both sexes do

144 also occur (Harrison et al. 2013a, 2013b, Harrison et al. 2014). Subordinates contribute to several

145 cooperative activities year-round, including territorial defense, roost construction and anti-predator

146 vigilance (Lewis 1982, Walker et al. 2016, York et al. 2019), and during breeding periods they contribute

147 substantially to nestling provisioning (Cram et al. 2015a, Capilla-Lasheras et al. 2021). Helping behavior

148 by subordinates has a causal positive effect on the overall rate at which nestlings are fed (Capilla-

149 Lasheras et al. 2021), and appears to reduce environmentally-induced variance in the reproductive

150   success of the dominant pair (Capilla-Lasheras et al. 2021) and lighten the post-natal provisioning

151   workload of the dominant female (Capilla-Lasheras 2019). While the neuroendocrine correlates of white-

152   browed sparrow weaver reproduction, aggression and song production have been investigated (e.g.

153   Wingfield and Lewis 1993, Voigt et al. 2007, York et al. 2016), the regulation of parental and helper

154   contributions to offspring provisioning remains unexplored.

155

156   We test three predictions of the hypothesis that prolactin plays a role in regulating the expression of

157   both parental care (among dominants) and cooperative helping behavior (among non-breeding

158   subordinates) in cooperatively breeding societies. First, with regard to parental care, we predict that

159   differences between the nestling provisioning rates of dominant females and dominant males will be

160   mirrored by parallel differences in their mean circulating prolactin levels. Dominant females are expected

161   to provision nestlings at higher rates and to have higher prolactin levels than dominant males (as the

162   12-18% incidence of extra-group paternity in this population leaves dominant females more closely

163   related than dominant males, on average, to the offspring that they rear; Harrison et al. 2013a). Second,

164   with regard to helping behavior, we predict that differences between the nestling provisioning rates of

165   subordinates still residing within their natal group (hereafter 'natal subordinates') and immigrant

166   subordinates, will also be mirrored by parallel differences in their mean circulating prolactin levels. Natal

167   subordinates are expected to provision nestlings at higher rates than immigrants, as while the former

168   are typically rearing future generations of siblings born to their parents, the latter will typically be

169   unrelated to the nestlings in their group (Harrison et al. 2013a). Finally, we predict that continuous

170   variation in circulating prolactin levels will positively predict continuous variation in the provisioning rates

171   of birds, and that this relationship will be apparent (i) at the population level (when all four of the bird

172   classes above are combined), and (ii) within bird classes, having factored out the among-class

173   differences in prolactin levels and provisioning rates.

174

175

176   METHODS

177   *General field methods*

178   Data were collected in the context of a long-term research project that monitors ~40 cooperative groups

179   of white-browed sparrow weavers at Tswalu Kalahari Reserve, South Africa (27°160S, 22°250E). Data

180   were collected at a similar time in two separate breeding seasons (January to February 2013, and

181   January to March 2014). White-browed sparrow weavers in this population may breed at any time from

182   September through to May (the Southern summer), depending on the timing of unpredictable summer

183   rainfall (Capilla-Lasheras 2019, Wood et al. 2021). Each bird within our study population is fitted with a

184   metal ring and three color rings, providing a unique ring combination for identification in the field

185   (SAFRING license 1444). From around six months of age, males and females of the focal subspecies

186   (*Plocepasser mahali mahali*) can be distinguished by their bill color; males have a dark brown bill while

187   females have a paler grey-to-pink bill (Leitner et al. 2009). Dominance status and social group

188   compositions were determined via regular (at least twice weekly) group visits. Social dominance was

189   assigned based on the monitoring of key dominance-related behaviors: the dominant pair routinely

190   displace other group members and produce synchronized duet song, the dominant female is the sole

191   incubator, and the dominant male consistently produces dawn song during breeding periods (Harrison

192   et al. 2013a, Cram et al. 2015b, York et al. 2016). The dispersal status (natal or immigrant) of

193   subordinate birds was determined via the continuous monitoring of the study population since 2007.

194   Based on this information, four classes of birds were assigned: Dominant Females; Dominant Males;

195   Natal Subordinates and Immigrant Subordinates. Social group size was defined as the number of adult

196   (> 1 year of age) birds consistently seen foraging and roosting together at the time of the focal breeding

197   attempt. The breeding status of each group was determined by monitoring the contents of all woven

198   nest structures within each group's territory, at least every other day throughout the two study periods.

199   When one or more eggs were newly detected, the active nest was visited daily in the afternoon until no

200   new eggs were detected (the birds lay one egg per day in the morning, and typically lay clutches of 2

201   eggs (range 1-4); Harrison et al. 2013a). To determine hatch dates, daily monitoring of the active nest

202   resumed 14 days after the detection of the first egg (as incubation lasts 14-19 days; Harrison et al.

203   2013a). This method yielded accurate information on the day on which the first nestling in each clutch

204   hatched, which was termed 'Day 1' of the nestling provisioning period for the focal breeding attempt. All

205   protocols were approved by the Ethics Committees of the Universities of Exeter and Pretoria and

206   complied with regulations stipulated in The Association for the Study of Animal Behaviour (ASAB)

207   Guidelines for Use of Animals in Research.

208

209   *Monitoring provisioning behavior*

210   To identify individuals during the recording of nestling provisioning events, group members were

211   captured from their roost chambers during the incubation period (details below) and marked on the vent

212   with a unique dye-mark. The dominant female was left unmarked to minimize disturbance during

213   incubation, but could still be distinguished from other group members by being the only unmarked bird

214   within her group (only resident group members provision offspring). To record provisioning events, a

215   Panasonic SDR-S50 camcorder attached to a tripod (approximately 0.5 meters in height) was placed

216   on the ground beneath the entrance to the active nest two days before recording commenced (to allow

217   the birds to habituate to it). On the days of provisioning monitoring, the recordings were started between

218   06:15 and 07:54, with this start time being adjusted through the season to maintain an approximately

219   constant time offset from sunrise, and an expected video duration of approximately 3 hours. Provisioning

220   videos were collected in this way for all focal breeding attempts (n = 37 broods across 30 social groups)

221   on two mornings between Days 6 and 9 inclusive of the nestling provisioning period (typically for the

222   two consecutive mornings of Days 7 and 8; nestlings fledged from day 20). This approach yielded a

223   mean total duration of provisioning video of 6.08 hours (range 4.05 – 8.12 hours) per breeding attempt.

224

225   Video recordings were transcribed using VLC Media Player version 2.2, with the observer recording, for

226   each provisioning visit, the identity of the bird visiting the nest (determined via their distinct dye mark

227   and bill color, which reveals their sex) and the duration of time that they spent within the nest (the time

228   elapsed between passing in and out of the enclosed nest structure; hereafter 'Provisioning visit

229   duration'). Prior work on this study population using within-nest cameras has shown that all nest visits

230   during the nestling age window studied here entail the delivery of a single prey item to the brood, unless

231   the visiting bird is carrying a feather or grass in which case no food is delivered (Walker 2016). We

232   therefore excluded such feather- or grass-carrying nest visits from our provisioning visit records. From

233   the transcribed data for each focal brood we then calculated two provisioning trait values for each adult

234   group member: (i) 'Provisioning rate' (feeds / hr) was calculated as the total number of provisioning visits

235   that the bird conducted over the two monitored mornings divided by the total duration of video collected

236   over those two mornings, and (ii) 'Mean Visit Duration' (minutes) was calculated as the mean duration

237   of all provisioning visits conducted by the focal bird over the two monitored mornings.

238

239   *Bird capture and blood sampling*

240   To obtain a matched blood sample for prolactin measurement, we attempted to capture and blood

241   sample all adult (> 1 year old at the time of sampling) birds within the monitored brood's social group on

242   the evening of the second day of provisioning behavior recording. Birds were captured individually at

243   night from the woven roost chambers within their group's territory (Cram et al. 2015a) by flushing

244   individuals into a custom-made capture bag. All captures, leg ring fitting, dye-marking and blood

245   sampling were conducted by a single investigator. Birds were then immediately returned to a roost

246   chamber within their territory to pass the remainder of the night. Upon capture, a blood sample (*c.* 140

247   µL) was taken from the brachial vein of the bird using a 26-g needle and heparinized capillary tubes.

248   Captures occurred soon after dusk, once the birds were roosting in their woven chambers. Time of

249   capture was recorded (to allow us to fit the time lag from sunset to capture as a covariate predictor in

250   our prolactin analyses, in case of diel variation and/or effects of the time elapsed since roosting on a

251   bird's prolactin levels), along with the time lag between capture and the completion of blood sampling

252   (mean ± standard deviation [S.D.]: 3.12 ± 0.73 minutes [range 1.65 – 4.78 minutes]; to allow us to control

253   for potential mean effects of capture stress on prolactin levels in our statistical models). Blood samples

254 were immediately centrifuged in the field (12,000 g for 3 minutes; Haematospin 1400; Hawksley Medical

255 and Laboratory Equipment, Lancing, UK) and the plasma was drawn off and stored in a cryovial on ice

256 until it could be transferred to liquid nitrogen on return from the field (mean ± S.D. time lag from sample

257 collection to storage on liquid nitrogen = 148 ± 63 min). At the end of the field season, samples were

258 transferred to the UK on dry ice and then stored at -80 degrees Celsius until analysis for prolactin.

259

260 *Prolactin Radioimmunoassay*

261 The prolactin assay was carried out at the Roslin Institute (University of Edinburgh, Easter Bush,

262 Midlothian, Scotland, UK). Plasma prolactin levels were measured using a highly specific heterologous

263 micro-radioimmunoassay of donkey anti-rabbit serum to European starling (*Sturnus vulgaris*) prolactin

264 (Sharp antibody code 44/2). Prolactin was radiolabeled with iodine^{125} using chloramine-T. 168 (out of a

265 total of 208) samples were assayed in duplicate, and the remaining 40 samples were assayed as

266 singletons (not all samples assayed were for use in this study). All samples were measured in a single

267 assay, in which the intra-assay coefficient of variation for the duplicate samples was 3.31%.

268

269 *Statistical methods*

270 The above methods yielded a final data set of matched provisioning trait data (estimated from the focal

271 bird's average performance over two mornings of provisioning recordings; see above) and circulating

272 prolactin levels (when sampled on the evening of the second day of provisioning monitoring) for 70

273 different adult birds, each sampled once (for all traits), while feeding a total of 37 broods across 30 social

274 groups. For a small number of these birds, a second matched measure of the provisioning traits and

275 prolactin levels was also available from a subsequent breeding attempt. However, these few repeat

276 measures were not included in our final data set for analysis, as attempts to conduct mixed effects

277 models using bird ID to account for the presence of these repeated measures of individuals typically

278 failed to estimate non-zero variance for bird ID (quite possibly because too few repeated measures were

279   available). All analyses therefore utilized the data from only the first sampling event per bird, obviating

280   the need to include bird ID as a random effect within our mixed effects models (see below). While some

281   analyses utilized the whole data set (i.e. n = 70 adults birds each sampled once for all traits), others

282   used subsets of it (e.g. when focusing only on dominants or subordinates), and so the sample sizes for

283   each analysis are reported within the relevant results section and model output table. As mean

284   provisioning visit duration data were only available for birds that had a non-zero provisioning rate, the

285   sample sizes for mean visit duration analyses were sometimes smaller than those for provisioning rate.

286

287   All statistical models and visualizations were carried out in R (version 4.1.0; R Core Team). Mixed effects

288   modelling was conducted using the R package 'lme4' (Bates et al. 2015), and the importance of a given

289   combination of model predictors was assessed using an information-theoretic (IT) approach. Each

290   modelling exercise started with the specification of a 'global model' containing all predictors of interest.

291   This global model and all possible simpler models containing subsets of the global model's fixed effect

292   predictors (including the intercept-only model) were then fitted to the data using maximum likelihood

293   and ranked based on AICc (Akaike's Information Criterion corrected for small sample size; Burnham

294   and Anderson 2002). This 'all subsets' approach was appropriate as all simpler models were biologically

295   plausible. ΔAICc values (i.e. the difference in AICc between the focal model and the best supported 'top'

296   model) were then calculated for every model fitted. As lower AICc values are indicative of stronger

297   statistical support for a given model, the ΔAICc for the best-supported 'top' model = 0 and models that

298   attracted less statistical support had progressively more positive ΔAICc values. We gave consideration

299   to models within a ΔAICc value of six (Richards 2008, Richards et al. 2011) and subsequently reduced

300   this Δ6 'top model set' by applying the 'nesting rule' described in (Richards 2008). This rule aims to

301   avoid the retention of overly complex models that do not improve model fit, by discarding models that

302   are more complex versions of simpler (nested) models with weaker AIC support (Richards 2008, Arnold

303   2010). Adding variables with little or no explanatory power to a top model can weaken AIC by less than

304   6 points, leading to the retention within the Δ6 top model set of more complex versions of better

305   supported models, containing such uninformative variables (Arnold 2010). The nesting rule thus reduces

306   the chance of considering such overly complex models containing uninformative variables (Richards

307   2008). In the model selection tables presented within the main paper (Tables 1 & 2) each line presents

308   a model from the top model set (i.e. those models within ΔAICc = 6 of the best-supported model) after

309   the application of the nesting rule, while each of the fixed effect (predictor) columns presents the

310   estimated effect size for that fixed effect predictor within that model (or is blank if the focal fixed effect

311   was absent from that model). The corresponding full top model sets *prior* to the application of the nesting

312   rule are presented in the Appendices (Tables A1 & A2) in case of interest. The specific modelling

313   exercises conducted for each results section are described below.

314

315   1. Are differences in the nestling provisioning rates and nest visit durations of dominant females

316   and males (engaged in parental care) mirrored by differences in their circulating prolactin levels?

317   We used two separate mixed effects models with Gaussian error structure to model the causes of

318   variation in (i) the provisioning rates and (ii) the mean provisioning visit durations of dominant birds. The

319   two modeling exercises began with an identical global model structure. In addition to the primary

320   predictor of interest, 'bird class' (dominant female or dominant male), we fitted the following terms as

321   fixed effect predictors: brood size (the brood size being fed), adult group size (the number of adult group

322   members during the focal nestling provisioning period) and year (a two-level factor capturing the year in

323   which sampling occurred; 2013 or 2014). We fitted both social group ID and brood ID (the identity of the

324   brood being fed) as random effects, retaining them in the model structure regardless of the degree of

325   variance that they explained.

326

327   We then used a third mixed effects model with Gaussian error structure to model the causes of variation

328   in the circulating prolactin levels of dominant birds, starting with a global model structure containing the

329   same fixed and random effect predictors as the provisioning trait models just described, but with the

330   addition of two further fixed effects to account for potential methodological effects on prolactin

331   concentrations: (i) the time lag from sunset to the bird's capture for blood sampling (to allow for the

332   possibility of diel variation in prolactin levels and/or changes in prolactin levels once the birds entered

333   their roosts) and (ii) the time lag from capture to the completion of blood sampling (to allow for possible

334   effects of capture stress on circulating prolactin levels). While neither of these two time-lag variables

335   were present within the best-supported model that arose from model comparisons (suggesting no

336   appreciable effect of these variables on circulating prolactin levels; Table 1), we nevertheless verified

337   that our inferences regarding the effect of bird class on prolactin levels on the basis of the best-supported

338   model overall were also upheld by the best-supported model that contained both of these time lag terms

339   (see results text).

340

341   **2. Are differences in the provisioning rates of natal and immigrant subordinates (engaged in**

342   **cooperative helping behavior) mirrored by differences in their circulating prolactin levels?**

343   We then used two mixed effects models with Gaussian error structure to model the causes of variation

344   in the provisioning rates and prolactin levels of subordinate birds engaged in helping behavior. We fitted

345   the same set of fixed and random effect predictors to these models as were fitted to the corresponding

346   provisioning rate and prolactin level models conducted for dominant birds (see above) with two

347   exceptions: (i) here 'bird class' reflected whether the bird was a natal subordinate or an immigrant

348   subordinate, and (ii) here only brood ID was fitted as a random effect (social group ID was not, as all

349   subordinate birds from any given social group were sampled while feeding the same single brood,

350   leaving brood ID and social group ID with identical structure in this case). Again, we verified that our

351   inferences regarding the effect of bird class on prolactin levels on the basis of the best-supported model

352   overall were also upheld by the best-supported model that contained both of the methodological time

353   lag terms (see results text). We did not model the causes of variation in the mean provisioning visit

354   durations of subordinates as too few immigrant subordinates actually provisioned the focal broods,

355   leaving us with an insufficient sample size of measures of the provisioning visit durations of this bird

356   class.

357

358 **3. Does continuous variation in prolactin levels predict continuous variation in provisioning**

359 **rates?**

360 To investigate whether natural variation in prolactin levels predicted continuous variation in the birds'

361 nestling provisioning rates and mean provisioning visit durations at the population level (i.e. when all

362 bird classes were combined) we conducted two mixed effect models (one for each provisioning trait

363 response term), with circulating prolactin concentration as the sole fixed effect predictor (as we have

364 not hypothesized specific mechanisms by which other variables might impact provisioning traits

365 independent of prolactin levels) and social group ID and brood ID as random effects. Mean provisioning

366 visit duration was logarithm transformed for analysis, to normalize model residuals. Inspection of the

367 patterns of the mean prolactin levels and provisioning trait values of the different bird classes (i.e.

368 dominant females, dominant males, natal subordinates and immigrant subordinates) suggested that any

369 such continuous relationship between prolactin levels and provisioning trait values at the population

370 level could be driven principally by the variation in these traits *among* the bird classes (Figures 3a & 3c).

371 In order to then investigate whether the more limited variation in circulating prolactin levels *within* bird

372 classes predicted the *within*-class variation in provisioning trait values, we first mean-centered each

373 birds' prolactin level and provisioning trait values (the log transformed values in the case of mean

374 provisioning visit duration) around the mean value of the focal trait for birds of their class (by subtracting

375 from it the mean value of the focal trait for their bird class). We then conducted two mixed effects models

376 (one for each mean-centered provisioning trait response term), with mean-centered circulating prolactin

377 concentration as the sole fixed effect predictor and social group ID and clutch ID as random effects.

378   RESULTS

379   **1. Are differences in the nestling provisioning rates and nest visit durations of dominant females**

380   **and males (engaged in parental care) mirrored by differences in their circulating prolactin levels?**

381   Analyzing the provisioning behavior of dominant birds engaged in parental care (n = 46 dominants, 20

382   females and 26 males, feeding 31 broods at 28 social groups) revealed strong evidence that dominant

383   females feed offspring at higher rates than dominant males (Figure 1a; bird class was present in all

384   models down to ΔAICc = 42.36; Table 1) and that the mean provisioning visit durations of dominant

385   females are also longer than those of dominant males (Figure 1b; ΔAICc = 11.49; Table 1). There was

386   also evidence that brood size positively predicted the provisioning rates of dominant birds (ΔAICc =

387   1.92; Table 1), but not their mean visit durations (the best-supported visit duration model containing

388   brood size scored 1.42 AICc points below the top model and was rejected under the nesting rule; Table

389   1; Table A1). There was no compelling evidence that the provisioning rates or mean visit durations of

390   dominant birds were associated with either the year of study or adult group size (which were absent

391   from the best-supported model for both traits; Table 1).

392

393   Analyzing the circulating prolactin levels of dominant birds during the provisioning periods analyzed

394   above (again, n = 46 dominants, 20 females and 26 males, feeding 31 broods at 28 social groups)

395   revealed strong evidence that dominant females also have higher circulating prolactin levels than

396   dominant males (Figure 1c; bird class was present in all models down to ΔAICc = 19.93; Table 1). There

397   was no compelling evidence that the prolactin levels of dominants were associated with group size,

398   brood size, the time lag from sunset to capture or the time lag from capture to blood sampling (none of

399   which were present within the best-supported model; Table 1). Among the considered models that

400   included both of the methodological time lag terms (i.e. those models forced to account for any effects

401   of these terms, regardless of their importance), the best-supported model again contained an effect of

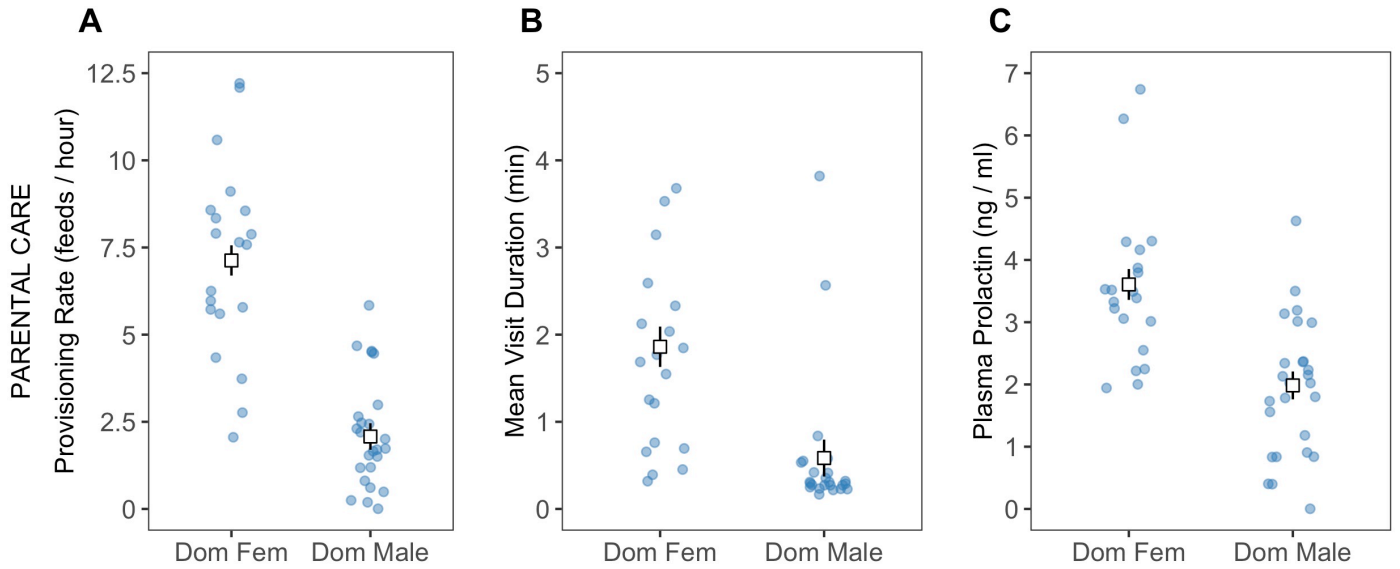402   bird class (see Table A1; model number 22).

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413 **Figure 1**. The (a) provisioning rates, (b) mean provisioning visit durations, and (c) circulating prolactin

414 concentrations of dominant females (Dom Fem) and dominant males (Dom Male) engaged in parental

415 care. Squares and error bars present the predicted means ± S.E. from the top model for the relevant

416 trait (Table 2) while controlling for the effects of the other variables present in that model. The points

417 show the raw data points.

418 **Table 1. Modelling the Provisioning Behavior and Prolactin Levels of DOMINANTS**

419 Model selection of the factors affecting the (i) nestling provisioning rates, (ii) mean provisioning visit

420 durations and (iii) prolactin levels of dominant females ('Dom Fem') and dominant males ('Dom Male')

421 engaged in parental care. The table shows all models within <6 ΔAICc of the top model, after applying

422 the model nesting rule (Richards et al., 2011; see methods). The grey lines indicate the response term

423 for the set of models beneath it. The numbers indicate coefficients (± standard errors; S.E.) and df is

424 the degrees of freedom. The 'Year' effect is the change from 2013 to 2014. Bird Class was present

425 within all models down to ΔAICc = 42.36 in the provisioning rate analysis, all models down to ΔAICc =

426 11.49 in the mean visit duration analysis, and all models down to ΔAICc = 19.93 in the prolactin analysis.

427 N = 46 dominant birds, 20 female and 26 male, feeding 31 broods at 28 social groups for the provisioning

428 rate and prolactin level analyses. The sample size for the mean provisioning visit duration analysis was

429 slightly smaller (n = 44 dominant birds), as 2 dominant males did not provision their brood, leaving us

430 without a measure of their mean visit duration. Table A1 presents these top model sets prior to

431 application of the model nestling rule.

Intercept	Bird Class (Dom Fem vs Dom Male)	Brood Size	Group Size	Year	df	AICc	ΔAICc
(i) **Provisioning Rate (feeds / hr)**							
-0.36 (0.87)	5.05 (0.58)	1.44 (0.46)			6	205.8	0.00
1.21 (0.50)	5.12 (0.59)			1.59 (0.59)	6	207.7	1.92
2.06 (0.42)	5.12 (0.61)				5	211.8	5.98
(ii) **Mean Provisioning Visit Duration (minutes)**							
0.58 (0.21)	1.28 (0.31)				5	139.7	0.00
(iii) **Plasma Prolactin (ng / ml)**							
1.98 (0.22)	1.62 (0.25)				5	149.6	0.00

2. Are differences in the provisioning rates of natal and immigrant subordinates (engaged in helping behavior) mirrored by differences in their circulating prolactin levels?

Analyzing the provisioning behavior of subordinate birds engaged in cooperative helping behavior (n = 24 subordinates, 17 natal and 7 immigrant, feeding 16 broods at 16 social groups) revealed evidence that subordinates within their natal groups feed offspring at higher rates than immigrant subordinates (Figure 2a; bird class was present in all models down to ΔAICc = 10.64; Table 2). There was also evidence that brood size positively predicted subordinate provisioning rates (ΔAICc = 2.13; Table 2). There was no compelling evidence that helper provisioning rates were associated with either group size or the year of study (neither term was present within the best-supported model; Table 2). No analysis of the provisioning visit durations of subordinates was conducted as an insufficient number of subordinate immigrants ever provisioned the broods (see Figure 2a).

Analyzing the circulating prolactin levels of subordinate birds during the provisioning periods analyzed above (again, n = 24 subordinates, 17 natal and 7 immigrant, feeding 16 broods at 16 social groups) revealed that natal subordinates also have higher prolactin levels than immigrant subordinates (Figure 2b; bird class was present in all models down to ΔAICc = 4.15; Table 2). There was also evidence that subordinate prolactin levels were negatively associated with group size (ΔAICc = 4.15) and very weak evidence that they were higher in the second year of study (ΔAICc = 0.02). There was no compelling evidence that subordinate prolactin levels were associated with brood size or the time lags from sunset to capture and from capture to blood sampling (none of which appeared in the best-supported model; Table 2). Among the considered models that included both of the methodological time lag terms (i.e. those models forced to account for any effects of these terms, regardless of their importance), the best-supported model again contained an effect of bird class (see Table A2; model number 30).
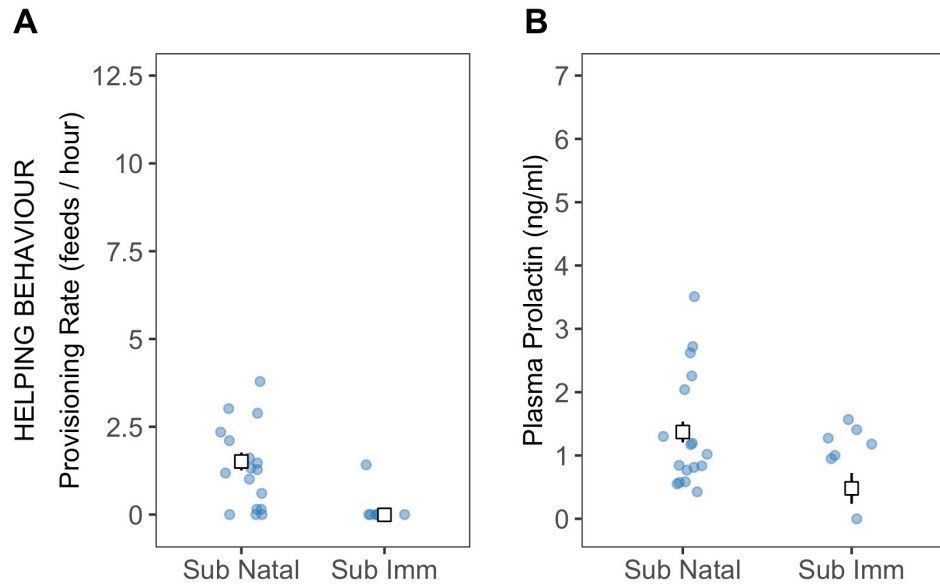
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Figure 2. The (a) provisioning rates, and (b) circulating prolactin levels of natal subordinates (Sub Natal) and immigrant subordinates (Sub Imm) engaged in cooperative helping behavior, feeding the broods of the dominant male and female. Y axis scales match those in Figure 1 to facilitate comparison. Squares and error bars (which do not always extend beyond the square) present the predicted means ± S.E. from the top model for the relevant trait (Table 2) while controlling for the effects of the other variables present in that model. The points show the raw data points. No analysis of provisioning visit durations was conducted as an insufficient number of subordinate immigrants ever provisioned the broods (see panel 2a).

475 **Table 2. Modelling the Provisioning Behavior and Prolactin Levels of SUBORDINATES**

476 Model selection of the factors affecting the (i) nestling provisioning rates, and (ii) prolactin levels of natal

477 subordinates ('Sub Natal') and immigrant subordinates ('Sub Imm') engaged in cooperative helping

478 behavior. The table shows all models within <6 ΔAIC of the top model, after applying the model nesting

479 rule (Richards et al., 2011; see methods). The grey lines indicate the response term for the set of models

480 beneath it. The numbers indicate coefficients (± standard errors; S.E.) and df is the degrees of freedom.

481 The 'Year' effect is the change from 2013 to 2014. Bird Class was present within all models down to

482 ΔAICc = 10.64 in the provisioning rate analysis. N = 24 subordinate birds, 17 natal and 7 immigrant,

483 feeding 16 broods at 16 social groups. Table A2 presents these top model sets prior to application of

484 the model nestling rule.

485

Intercept	Bird Class (Sub Natal vs Sub Imm)	Brood Size	Group Size	Year	df	AICc	ΔAICc
(i) **Provisioning Rate (feeds / hr)**							
-1.39 (0.68)	1.52 (0.34)	0.92 (0.37)			5	66.6	0.00
0.12 (0.34)	1.49 (0.37)				4	68.8	2.13
(ii) **Plasma Prolactin (ng/ml)**							
2.20 (0.54)	0.89 (0.28)		-0.41 (0.13)	0.56 (0.28)	6	57.13	0.00
2.82 (0.48)	0.91 (0.31)		-0.51 (0.12)		5	57.15	0.02
1.02 (0.17)				0.86 (0.32)	4	61.28	4.15
2.74 (0.56)			-0.34 (0.13)		4	61.42	4.29

486

3. Does continuous variation in prolactin levels predict continuous variation in provisioning rates?

Our analysis at the population level, including birds of all classes, revealed strong evidence that a bird's circulating prolactin level positively predicts both its provisioning rate (Figure 3a; effect size ± S.E. = 1.20 ± 0.23; the provisioning rate model containing the prolactin predictor scored 20.36 AICc points stronger than the intercept-only model; n = 70 birds feeding 37 broods at 30 social groups) and its mean provisioning visit duration (Figure 3c; effect size ± S.E. = 0.32 ± 0.076; the mean visit duration model containing the prolactin predictor scored 12.59 AICc points stronger than the intercept-only model; n = 59 birds feeding 36 broods at 30 social groups). Plotting out the mean prolactin levels and provisioning trait values of the different bird classes (Figure 3a & 3c), reveals that both of these population-level relationships between prolactin and provisioning traits are driven in large part by the among-bird-class differences in prolactin levels being mirrored by parallel among-bird-class differences in mean provisioning rate (Figure 3a) and mean provisioning visit duration (Figure 3c). Indeed, after mean-centering each bird's prolactin level and provisioning trait values around the focal trait's mean value for their bird class, we found no evidence that within-bird-class variation in prolactin levels predicted within-bird-class variation in either provisioning rate (Figure 3b; effect size ± S.E. = -0.15 ± 0.21; the model containing the mean-centered-prolactin predictor scored 1.87 AICc points weaker than an intercept-only model) or mean provisioning visit duration (Figure 3d; effect size ± S.E. = -0.049 ± 0.081; the model containing the mean-centered prolactin predictor scored 2.03 AICc points weaker than an intercept-only model).
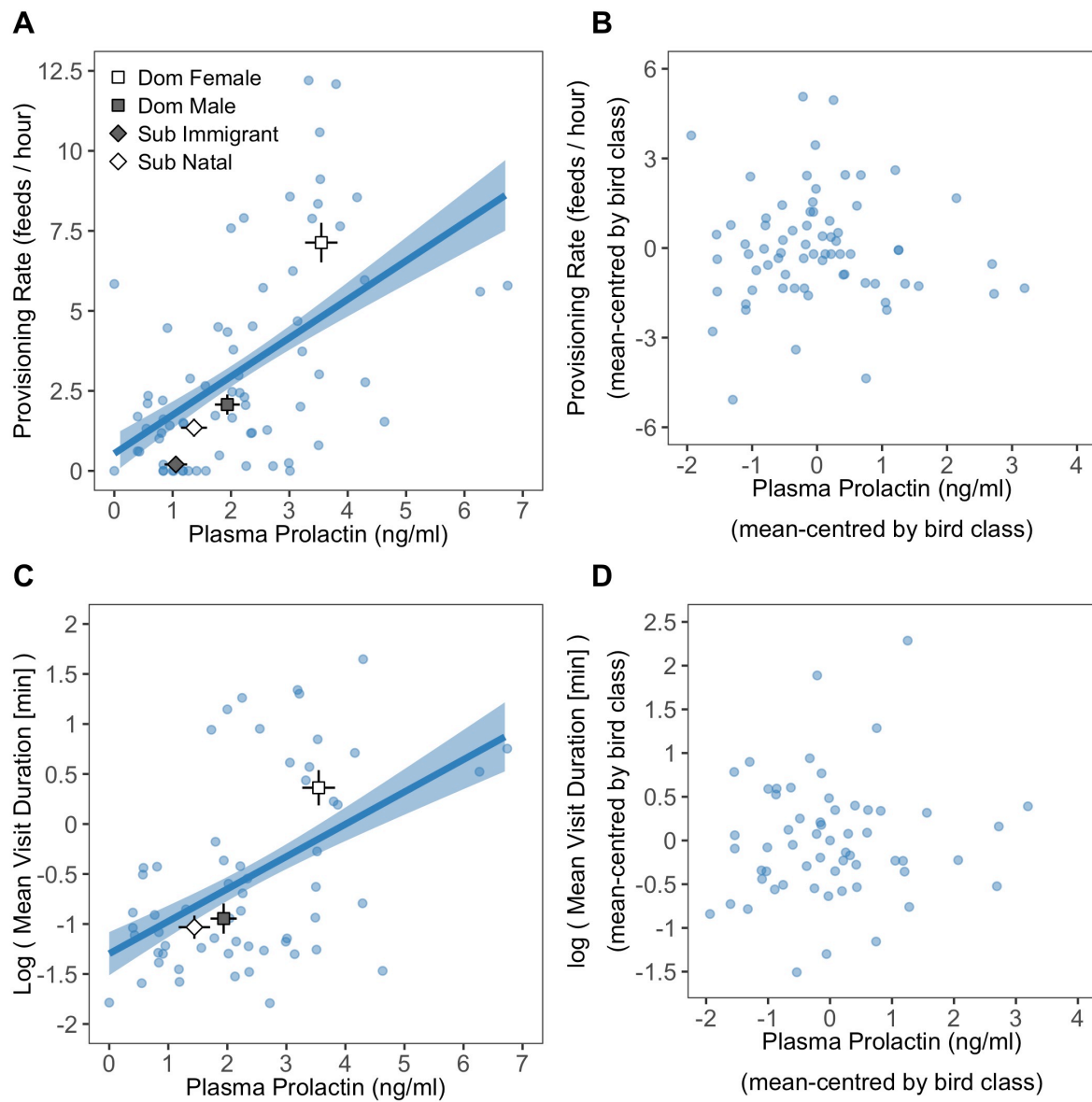
Figure 3. At the population level, considering all bird classes together, natural variation in circulating prolactin levels predicts variation in both (**a**) provisioning rate and (**c**) mean provisioning visit duration. These relationships are driven principally by differences among the mean trait values of the different focal bird classes (presented ± S.E. by the squares and diamonds within panels **a** and **c**; see legend within panel **a**). Follow-up analyses revealed no evidence that variation in prolactin levels *within* these bird classes predicted within-bird-class variation in either (**b**) provisioning rate or (**d**) mean provisioning visit duration. In panels **a** and **c** the line and shaded ribbon present the predicted mean relationship and its standard error, while the shaded circular points within all panels present the raw data points.

535   DISCUSSION

536   This study investigated the hypothesis that prolactin plays a role in the regulation of nestling

537   provisioning, both among dominant birds (engaged in parental care) and subordinate birds (that engage

538   in cooperative helping behavior), in cooperatively breeding white-browed sparrow weaver societies.

539   Among dominants engaged in parental care, we found that the dominant female (the mother) fed

540   offspring at higher rates, made longer provisioning visits and had higher circulating prolactin levels than

541   the dominant male (typically the father). Among subordinates, we found that natal subordinates helped

542   to feed offspring at higher rates and had higher circulating prolactin levels than immigrant subordinates.

543   Indeed, when all bird classes were combined, we found that continuous variation in the circulating

544   prolactin levels of the birds predicted continuous variation in their provisioning rates and mean

545   provisioning visit durations. These patterns appear to be driven principally by correlated differences

546   among the four different bird classes in their prolactin levels and provisioning traits. We found no

547   evidence that the more limited variation in circulating prolactin levels within the different bird classes

548   predicted the more limited within-class variation in their provisioning traits. Together, these findings are

549   broadly consistent with the hypothesis that parental care and cooperative helping behavior are regulated

550   by a common underlying mechanism, and the hypothesis that prolactin plays a role in that pathway, but

551   also highlight the need for experimental studies to now probe the causality and nature of any role for

552   prolactin. Below, we discuss potential explanations for these findings, the different roles that prolactin

553   could conceivably play in the regulation of parenting and cooperative helping in this species, and the

554   wider implications of our findings for mechanistic and evolutionary research on cooperative behavior.

555

556   While our findings are broadly consistent with the hypothesis that parental care and helping behavior

557   are regulated by a common mechanism in which prolactin plays a role, the lack of a relationship between

558   within-class variation in prolactin levels and provisioning traits, coupled with the correlative nature of our

559   findings, leave it important to consider the range of possible roles that prolactin could play in the

560   regulation of provisioning behavior in this species. At least three main possibilities exist, which will now

561   require careful experimentation to tease apart. First, it is possible that circulating prolactin is one key

562   regulator of continuous variation in individual contributions to offspring provisioning, both among parents

563   and helpers. While most of our findings are consistent with this hypothesis, the absence of evident

564   relationships between within-class variation in prolactin levels and provisioning traits complicates this

565   view. However, the lack of *within*-class relationships could be attributable simply to a major source of

566   variation in both traits (*among*-class variation) having been factored out at this stage, leaving these

567   within-class analyses seeking more subtle prolactin-provisioning relationships (than those at the

568   population level) that could be obscured by a number of mechanisms. For example, difficulties with the

569   synchronous and accurate assessment of both prolactin levels and provisioning rates could have yielded

570   noise in the data set that precluded the detection of these more subtle prolactin-provisioning

571   relationships. While we sampled birds for prolactin on the evening following the morning provisioning-

572   monitoring session (a time lag comparable to, or shorter than, those of similar studies; e.g. Duckworth

573   et al. 2003, Ouyang et al. 2011), individuals may have differed in the way that their prolactin levels

574   changed during the day, leaving their evening prolactin levels only a modest proxy for those while

575   provisioning. The focal birds also varied in the timing of blood sampling, and while our analyses did not

576   detect any overall effects on prolactin levels of the time lags either from sunset to capture or from capture

577   to sampling, any individual variation in the circadian rhythm of prolactin secretion or in the prolactin

578   stress response (if this species shows one; Krause et al. 2015) could have further decoupled the

579   assessed prolactin levels from those during provisioning. Ultimately though, even if prolactin levels were

580   a key regulator of provisioning behavior, alternative mechanisms are also expected to impact

581   provisioning rates potentially independent of circulating prolactin levels, leaving the relationship between

582   natural variation in prolactin levels and provisioning behavior potentially modest in the first place

583   (Schoech et al. 1998, Angelier et al. 2016). Key among these could be (i) variation in other components

584   of a prolactin-mediated pathway (such as inter-individual and temporal variation in the density of

585   prolactin receptors; Zhou et al. 1996, Ohkubo et al. 1998), as well as (ii) mechanisms that may impact

586   provisioning behavior via prolactin-independent pathways (e.g. the effects of circulating testosterone;

587   Schoech et al. 1998). Moreover, even if prolactin levels determined provisioning 'motivation', the extent

588   to which variation in provisioning motivation was reflected in provisioning rates would depend upon the

589   prey capture skills of the focal bird and the environmental availability of prey. Indeed, all points

590   considered, it is arguably mechanistically naïve to expect particularly fine-grained associations between

591   the levels of a single hormone and behavior to be evident in natural populations even where a causal

592   link exists. To now robustly test the hypothesis that prolactin regulates continuous variation in the

593   magnitude of individual contributions to offspring provisioning, there is a need to experimentally elevate

594   the circulating prolactin levels of actively provisioning birds whose natural prolactin levels are not at the

595   upper end of the physiological range (dominant males and natal subordinates may serve this purpose

596   well; Figure 3a). This manipulation would allow one to test the key prediction that an increase in the

597   prolactin levels of an actively provisioning bird will cause it to increase its provisioning rate; a prediction

598   that to our knowledge has yet to best tested in either a parenting or helping context (the few experimental

599   elevations of endogenous prolactin secretion in a provisioning context to date have focussed on the

600   establishment of provisioning in non-provisioning birds rather than its quantitative variation within

601   actively provisioning birds; e.g. Badyaev and Duckworth 2005).

602

603   A second potential explanation for the balance of our findings is that prolactin could instead play a causal

604   role in the onset and maintenance of provisioning behavior among parents and helpers, without playing

605   a role in the quantitative regulation of contributions to provisioning among actively provisioning birds.

606   For example, a threshold level of prolactin may be required for the onset and/or maintenance of

607   provisioning behavior (Angelier et al. 2006, Boos et al. 2007). Under this scenario, the higher prolactin

608   levels of natal subordinates and dominant birds, relative to immigrant subordinates, could be causally

609   responsible for the former bird classes engaging in provisioning while the latter typically did not. This

610   could be the case without prolactin playing any causal role in regulating continuous variation in the

611   provisioning rates of actively provisioning birds; a scenario that could account for the lack of within-class

612   correlations between prolactin levels and provisioning behavior. The elevated prolactin levels of

613   dominant females (relative to dominant males and natal subordinates) could conceivably be a

614   downstream consequence of either a role for prolactin in incubation (Buntin 1996, Sharp et al. 1998,

615   Khan et al. 2001; as dominant females are the sole incubator in this species) and/or their differential

616   exposure to offspring cues during the nestling period (which can increase prolactin secretion; Hall 1987,

617   Sharp et al. 1998), given their markedly higher provisioning rates and mean visit durations than other

618   classes. The hypothesis that prolactin maintains provisioning behavior but does not quantitatively

619   regulate contributions to it could now be tested by (i) experimentally elevating the prolactin levels of

620   subordinate immigrants, to test the key prediction that this would cause these typically non-provisioning

621   birds to commence provisioning behavior (e.g. see Badyaev and Duckworth (2005) for a rare

622   demonstration of this transition in the context of parental nestling feeding), (ii) experimentally reducing

623   the prolactin levels of the actively-provisioning classes to test whether this eliminates provisioning

624   behavior (e.g. Smiley and Adkins-Regan 2018), and (iii) experimentally elevating the prolactin levels of

625   actively provisioning dominant males and/or natal subordinates (the manipulation proposed in the

626   previous paragraph), as doing so should *not* increase their provisioning rates if prolactin merely

627   maintains provisioning behavior without regulating contributions to it.

628

629   Given the correlative nature of our findings, it is also conceivable that prolactin plays no role in the onset,

630   maintenance or quantitative regulation of parenting and cooperative helping behavior. Under this

631   scenario, one might attribute the evident associations between prolactin and provisioning to a 'reverse

632   causal' relationship, in which provisioning interactions with offspring stimulate prolactin release (Hall

633   1987, Sharp et al. 1998). However, such a reverse causal argument alone cannot readily account for

634   our findings in their entirety, as within-class variation in provisioning rates and mean provisioning visit

635   durations were not evidently associated with circulating prolactin levels (though, again, the lack of such

636   an association could be attributable to challenges with accurately and simultaneously quantifying both

637   hormone and behavior; see above). When considering whether our findings could be attributable solely

638   to effects of provisioning behavior on prolactin levels (i.e. in the absence of any effect of prolactin on

639   provisioning), it is worth considering why selection would have left prolactin levels sensitive to offspring

640 interactions in the first place. Arguably the most plausible explanation is that such a mechanism plays

641 a role in a feedback loop in which a causal relationship exists in both directions: if prolactin did establish,

642 maintain and/or quantitatively regulate care, selection may have favored regulating prolactin secretion

643 according to offspring interactions in order to maintain care as long as offspring survive and/or regulate

644 care according to offspring viability or need (Hall 1987, Sharp et al. 1998, Angelier et al. 2016). As such,

645 where offspring cues do stimulate prolactin release, such a relationship might generally be expected to

646 occur alongside causal effects of prolactin on care. While the experiments outlined above would shed

647 light on the causality of the prolactin-provisioning associations detected here, wider investigations are

648 also needed to probe the role, if any, that such a feedback loop (with causal relationships in both

649 directions) may play in the maintenance and/or regulation of cooperative care.

650

651 Our analyses revealed evidence that the circulating prolactin levels of subordinates were lower in larger

652 social groups, but that the same relationship was not apparent among dominants. This finding is notable

653 in that it parallels the apparent effects of group size on survival in this study population: subordinate

654 birds are markedly less likely to survive in larger groups, but the same is not true for dominants

655 (O'Callaghan & Young *unpublished data*). This survival relationship may be driven by enhanced foraging

656 competition in larger groups (which subordinates may suffer from to a greater extent than dominants,

657 given their lower rank), as territory size does not increase proportionally with group size (Martin-Taylor

658 2018; likely leaving resources more strongly contested in larger groups). Accordingly, subordinates in

659 larger groups show significantly lower body condition (O'Callaghan & Young *unpublished data*); a

660 relationship that could conceivably account for their reduced prolactin levels in larger groups too.

661 Chronic exposure to nutritionally stressful conditions is often associated with reduced prolactin levels; a

662 relationship that could be due in part to negative effects of circulating corticosterone on prolactin release

663 (Delehanty et al. 1997, Criscuolo et al. 2005, Angelier and Chastel 2009, Riechert et al. 2014, Angelier

664 et al. 2016). Indeed, as the Kalahari experienced drought conditions during the two years of this study,

665 such negative effects of environmental stress on prolactin release might also explain why the plasma

666   prolactin concentrations detected here were lower than those generally reported in studies of other

667   passerines engaged in care (e.g. Vleck et al. 1991, Schoech et al. 1996, Khan et al. 2001, Duckworth

668   et al. 2003, Ouyang et al. 2011).

669

670   While experimental tests of causality are needed (see above), our findings are broadly consistent with

671   the hypothesis that pre-existing mechanisms that regulated parental care in ancestral bi-parental

672   species were co-opted for the regulation of cooperative helping behavior on the evolution of cooperative

673   breeding. The possibility that parenting and cooperative helping are indeed regulated by a common

674   mechanism has important evolutionary implications. Explanations for the evolution, maintenance and

675   optimization of cooperative behavior typically focus on the roles of the fitness benefits and costs of

676   cooperation *per se*. However, if cooperation and parenting *are* regulated by a common underlying

677   mechanism it is conceivable that this shared architecture for the regulation of care-giving is shaped as

678   much by the payoffs from its expression in a parental context as by the payoffs from its expression in a

679   cooperative helping context. While selection might independently optimize parental and helper caring

680   strategies (e.g. via the evolution of an entirely context-dependent caring strategy), it is conceivable that

681   mechanistic constraints may preclude their independent optimization. For example, genetic variants that

682   modified a bird's sensitivity to begging could conceivably impact both its parental and cooperative care,

683   yielding scope for intra-locus genetic conflict to constrain the independent optimization of both parental

684   and cooperative care (Pennell et al. 2018, see also the conceptual parallels with sexual conflict: Stewart

685   et al. 2010, Pennell and Morrow 2013). Where this is the case, attempts to understand the evolutionary

686   origins, maintenance and optimization of cooperative behavior may require attention to the extent to

687   which genetic correlations exist between parental and cooperative behavior. Notably, our findings

688   suggest that helping behavior in sparrow-weaver societies is not maintained by selection solely because

689   a genetic correlation with parenting has precluded the evolution of 'non-helping' (see Brown and Vleck

690   1998 for a similar debate), because a context-dependent helping strategy appears to have evolved.

691   Subordinates routinely help while within their natal groups but typically cease to do so as immigrants

692   (when their low relatedness to the brood would markedly reduce their indirect fitness payoff from helping;

693   Harrison et al. 2013a).

694

695   **Conclusion**

696   Our findings lend new support to the hypotheses that helping behavior in cooperatively breeding

697   societies has shared mechanistic underpinnings with parental care, and that prolactin plays a key role

698   in this pathway (see also Vleck et al., 1991; Schoech et al., 1996; Khan et al., 2001; Carlson et al.,

699   2006). Our findings and their complexity highlight the need for experimental studies to investigate both

700   the causality and nature of the relationship between prolactin and provisioning in this species, in both

701   parental and cooperative contexts. Our findings also highlight that attempts to understand the evolution

702   of cooperative helping may benefit from attention to the possibility of genetic constraints on the

703   independent optimization of cooperation and parenting. Our study has implications too for the growing

704   interest in the mechanistic origins of consistent individual differences in cooperative behavior

705   (Sanderson et al. 2015, Dantzer et al. 2019). Specifically, our findings highlight that such differences

706   could arise from consistent individual differences within the pathway by which prolactin acts (e.g. via

707   differences in prolactin secretion and/or reception; Ohkubo et al. 1998, Zhou et al. 1996).

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912

913 APPENDIX A

914 **Table A1. Top model sets prior to applying the model nesting rule when modelling the**

915 **Provisioning Behavior and Prolactin Levels of DOMINANTS**

916 Model selection of the factors affecting the (i) nestling provisioning rates, (ii) mean provisioning visit

917 durations and (iii) prolactin levels of dominant females ('Dom Fem') and dominant males ('Dom Male')

918 engaged in parental care. This table shows all models within <6 ΔAIC of the top model, *prior to* applying

919 the model nesting rule (Richards et al., 2011; see methods). Table 1 in the main paper presents the

920 subset of these models that were retained *after* applying the model nesting rule (which are flagged here

921 with 'YES' in the 'Retained' column). The grey lines indicate the response term for the set of models

922 beneath it. The presence of a continuous variable within a focal model is indicated by the presence of

923 its effect size estimate, while the presence of factorial variable is indicated with a '+'. The 'Year' effect

924 is the change from 2013 to 2014. 'Int' = Intercept. 'df' = degrees of freedom.

925

926

Model Number	Int	Bird Class (Sub Natal vs Sub Imm)	Brood Size	Group Size	Year	Sunset to Capture Lag	Capture to Bleed lag	df	AICc	ΔAICc	Retain
Provisioning Rate (feeds / hr)											
4	-0.36	+	1.44			N/A	N/A	6	205.78	0.00	YES
12	-0.29	+	1.08		+	N/A	N/A	7	206.06	0.28	
11	1.21	+			+	N/A	N/A	6	207.70	1.92	YES
8	-0.19	+	1.42	-0.05		N/A	N/A	7	208.54	2.76	
16	-0.46	+	1.08	0.05	+	N/A	N/A	8	208.97	3.19	
15	1.14	+		0.02	+	N/A	N/A	7	210.49	4.71	
3	2.06	+				N/A	N/A	5	211.76	5.98	YES
Mean Provisioning Visit Duration (minutes)											
3	0.58	+				N/A	N/A	5	139.66	0.00	YES
4	1.08	+	-0.30			N/A	N/A	6	141.08	1.42	
11	0.71	+			+	N/A	N/A	6	141.79	2.13	
7	0.37	+		0.07		N/A	N/A	6	142.11	2.45	
12	1.07	+	-0.26		+	N/A	N/A	7	143.81	4.15	
8	0.93	+	-0.28	0.04		N/A	N/A	7	143.84	4.18	

15	0.57	+		0.04	+	N/A	N/A	7	144.55	4.89	
Plasma Prolactin (ng/ml)											
5	1.98	+						5	149.62	0.00	YES
7	1.32	+	0.39					6	150.56	0.94	
13	1.31	+		0.23				6	150.68	1.07	
15	0.50	+	0.43	0.25				7	151.36	1.74	
37	1.77	+			+			6	151.41	1.79	
45	0.82	+		0.29	+			7	151.68	2.07	
6	2.51	+					-0.0028	6	151.79	2.17	
21	2.03	+				-0.0005		6	152.24	2.62	
14	1.81	+		0.22			-0.0025	7	153.06	3.44	
8	1.76	+	0.37				-0.0021	7	153.07	3.45	
39	1.32	+	0.33		+			7	153.17	3.55	
23	1.32	+	0.39			-0.0001		7	153.35	3.73	
29	1.29	+		0.23		0.0001		7	153.48	3.86	
47	0.47	+	0.30	0.28	+			8	153.76	4.14	
38	2.17	+			+		-0.0020	7	153.97	4.35	
53	1.86	+			+	-0.0013		7	153.99	4.37	
16	0.89	+	0.41	0.25			-0.0017	8	154.11	4.49	
31	0.42	+	0.44	0.26		0.0006		8	154.26	4.64	
61	0.90	+		0.29	+	-0.0009		8	154.53	4.91	
46	1.09	+		0.29	+		-0.0012	8	154.54	4.92	
22	2.54	+				-0.0003	-0.0027	7	154.57	4.95	

927

928 **Table A2. Top model sets prior to applying the model nesting rule when modelling the**

929 **Provisioning Behavior and Prolactin Levels of SUBORDINATES**

930 Model selection of the factors affecting the (i) nestling provisioning rates and (ii) prolactin levels of natal

931 subordinates ('Sub Nat') and immigrant subordinates ('Sub Imm') engaged in cooperative helping

932 behavior. This table shows all models within <6 ΔAIC of the top model, *prior to* applying the model

933 nesting rule (Richards et al., 2011; see methods). Table 2 in the main paper presents the subset of

934 these models that were retained *after* applying the model nesting rule (which are flagged here with 'YES'

935 in the 'Retained' column). The grey lines indicate the response term for the set of models beneath it.

936 The presence of a continuous variable within a focal model is indicated by the presence of its effect size

937 estimate, while the presence of factorial variable is indicated with a '+'. The 'Year' effect is the change

938 from 2013 to 2014. 'Int' = Intercept. 'df' = degrees of freedom.

939

Model Number	Int	Bird Class (Sub Nat vs Sub Imm)	Brood Size	Group Size	Year	Sunset to Capture Lag	Capture to Bleed lag	df	AICc	ΔAICc	Retain
Provisioning Rate (feeds / hr)											
4	-1.39	+	0.92			N/A	N/A	5	66.64	0.00	YES
12	-1.40	+	1.12		+	N/A	N/A	6	67.92	1.28	
3	0.12	+				N/A	N/A	4	68.77	2.13	YES
8	-0.65	+	0.86	-0.18		N/A	N/A	6	69.68	3.04	
16	-0.30	+	1.06	-0.27	+	N/A	N/A	7	70.56	3.93	
7	1.22	+		-0.31		N/A	N/A	5	70.73	4.09	
11	0.23	+			+	N/A	N/A	5	71.79	5.15	
Plasma Prolactin (ng / ml)											
45	2.20	+		-0.41	+			6	57.13	0.00	YES
13	2.82	+		-0.51				5	57.15	0.02	YES
29	2.47	+		-0.48		0.0038		6	59.10	1.97	
46	1.15	+		-0.35	+		0.0038	7	59.22	2.09	
15	2.62	+	0.08	-0.50				6	60.62	3.49	
14	2.83	+		-0.51			0.0000	6	60.76	3.62	
61	2.11	+		-0.40	+	0.0019		7	60.78	3.65	
47	2.27	+	-0.03	-0.41	+			7	61.17	4.04	
33	1.02				+			4	61.28	4.15	YES

9	2.74			-0.34				4	61.42	4.29	YES
25	2.22			-0.32		0.0057		5	61.62	4.49	
38	-0.86	+			+		0.0068	6	61.68	4.55	
41	2.09			-0.23	+			5	61.76	4.63	
37	0.64	+			+			5	62.00	4.87	
34	0.06				+		0.0046	5	62.57	5.43	
30	2.37	+		-0.48		0.0039	0.0004	7	63.12	5.99	

940