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

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ABSTRACT

In many cooperatively breeding societies non-breeding individuals help to rear the offspring of breeders. The physiological mechanisms that regulate such cooperative helping behavior are poorly understood, but may have been co-opted, during the evolution of cooperative breeding, from pre-existing mechanisms that regulated parental care. Key among these may be a role for prolactin. Here we investigate whether natural variation in circulating prolactin levels predicts both parental and helper contributions to nestling provisioning in cooperatively breeding white-browed sparrow weavers, *Plocepasser mahali*. In sparrow weaver groups, a single dominant pair monopolize reproduction and non-breeding subordinates help with nestling feeding. We show that: (i) among parents, dominant females feed nestlings at higher rates, make longer provisioning visits and have higher prolactin levels than dominant males; and (ii) among subordinates, engaged in cooperative helping behavior, those within their natal groups feed nestlings at higher rates and have higher prolactin levels than immigrants. Moreover, continuous variation in prolactin levels positively predicts nestling-provisioning rates and mean provisioning visit durations when all bird classes are combined. These relationships are principally driven by differences among bird classes in both circulating prolactin levels and provisioning traits; the more limited within-class variation in prolactin and provisioning traits were not evidently correlated, highlighting a likely role for additional mechanisms in the fine-scale regulation of care. Our findings broadly support the hypothesis that parental care and cooperative helping behavior are regulated by a common underlying mechanism and highlight the need for experimentation to now establish the causality of any role for prolactin.

[250 words]

INTRODUCTION

In many cooperatively breeding societies, non-breeding helpers assist with the rearing of parents' young, via cooperative contributions to diverse forms of care (e.g. incubation, babysitting and offspring provisioning; Solomon and French 1997, Koenig and Dickinson 2004, 2016). The majority of research on such 'helping behavior' has sought to explain its evolution, by identifying the effects of helping on recipients and the means by which these yield fitness benefits to helpers (Cockburn 1998, Dickinson and Hatchwell 2004, Koenig and Dickinson 2016). By contrast, our understanding of the proximate physiological mechanisms that regulate the expression of cooperative behavior is less advanced (Schoech et al. 2004, Soares et al. 2010, Sanderson et al. 2014, Dantzer et al. 2017), despite a surge of interest in the origins of consistent individual differences in both cooperative behavior and endocrine traits (Sanderson et al. 2015, English et al. 2010, Dantzer et al. 2019, Houslay et al. 2019). As cooperatively breeding species commonly evolved from monogamous pair-breeding species in which parental care was well developed (Cornwallis et al. 2010, Lukas and Clutton-Brock 2012), it seems likely that the physiological mechanisms that regulate cooperative helping behavior among non-breeders were co-opted from the pre-existing mechanisms that regulated parental care among breeders. Attempts to identify the proximate mechanisms that regulate helping behavior may therefore be well served by testing candidate mechanisms already identified for the regulation of parental care in non-cooperative species (Ziegler 2000, Schoech et al. 2004, Carlson et al. 2006a, 2006b). One such mechanism is the neuroendocrine pathway involving the anterior pituitary gland hormone prolactin (Buntin 1996, Sharp et al. 1998, Ziegler 2000, Carlson et al. 2006a, 2006b, Angelier et al. 2016).

Numerous studies suggest that prolactin can play a causal role in the expression of parental care, though its precise role is not clear and seems likely to vary across taxa (Buntin 1996, Sharp et al. 1998, Angelier et al. 2016). In birds, prolactin is thought to play a causal role in the onset and maintenance of parental care, but it is less clear whether variation in circulating prolactin levels is also involved in the quantitative regulation of contributions to care once caring behavior has begun (Boos et al. 2007,

Angelier et al. 2016, Smiley and Adkins-Regan 2018). The transition from sexual activity to parenting is typically associated with an increase in circulating prolactin levels, which peak during the care period (Buntin 1996, Sharp et al. 1998, Angelier et al. 2016, e.g. Schoech et al. 1996, Badyaev and Duckworth 2005). Evidence that naturally low prolactin levels are commonly associated with breeding attempt abandonment and/or failure (e.g. Chastel and Lormee 2002, Chastel et al. 2005), and that experimental reductions in circulating prolactin levels during late incubation can disrupt post-natal care (e.g. Smiley and Adkins-Regan 2018), suggest that these elevated prolactin levels are necessary for the onset and/or maintenance of parental care. Indeed, experimental elevations of circulating prolactin suggest that elevated prolactin levels can promote the onset of both incubation behavior (e.g. Sockman et al. 2000) and nestling provisioning behavior (e.g. Badyaev and Duckworth 2005). Positive associations between continuous variation in circulating prolactin levels and the rates at which parents provision their offspring (e.g. Duckworth et al. 2003, Ouyang et al. 2011) highlight the possibility that prolactin levels also regulate the *amount* of care that an actively caring parent provides to its offspring. However, this hypothesis has yet to be experimentally tested, and such positive associations could arise instead via effects of provisioning activity on a bird's circulating prolactin levels, as parental contact with offspring cues can increase prolactin secretion (Hall 1987, Sharp et al. 1998). Causal links between prolactin and provisioning rates may therefore exist in both directions. Indeed, such a feedback loop (in which offspring cues stimulate prolactin secretion that in turn maintains and/or elevates the expression of parental care) could conceivably both maintain parental care while offspring survive, and regulate its expression according to offspring vigor and need.

A number of studies of cooperatively breeding species have now begun to investigate the relationships between prolactin and care-giving behavior, both among parents and non-breeding helpers (Ziegler 2000, Schoech et al. 2004, Soares et al. 2010). Prolactin levels have been shown to rise in parents and non-breeding helpers during the transition to incubation and nestling care in at least three species of 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

et al. 2006a), highlights the potential complexity of seeking relationships between continuous variation in prolactin levels and care. Notably too, given the potential for contact with offspring to raise circulating prolactin levels (Sharp et al. 1998, Hall 1987), it is possible that the prolactin-helping associations documented above arose not via causal effects of prolactin on care, but via either the reverse causal relationship or in the absence of any causal link between the two. Nevertheless, the promising findings to date highlight the need for further studies to investigate whether natural circulating levels of prolactin predict variation in individual contributions to both parental care and helping behavior in cooperatively breeding species, and ultimately the use of experimental manipulations of prolactin levels to test the causality and nature of any relationships detected (Sockman et al. 2000, Carlson et al. 2003, Badyaev and Duckworth 2005, Smiley and Adkins-Regan 2018).

Here we investigate whether natural variation in circulating prolactin levels positively predicts the nestling provisioning behavior of both parents and non-breeding helpers in a wild cooperatively breeding bird, the white-browed sparrow-weaver (*Plocepasser mahali*). White-browed sparrow weavers are rain-dependent breeders that live in year-round territorial groups throughout the semi-arid regions of sub-Saharan Africa (Lewis 1982, Wood et al. 2021). Within each social group, a single dominant male and female completely monopolize within-group reproduction and up to 10 non-breeding subordinates of both sexes help to feed their nestlings (Harrison et al. 2013a, 2013b, Capilla-Lasheras et al. 2021). Subordinates are typically offspring from previous broods that have delayed dispersal from their natal group (and so are helping to rear their parents' young), but subordinate immigrants of both sexes do also occur (Harrison et al. 2013a, 2013b, Harrison et al. 2014). Subordinates contribute to several cooperative activities year-round, including territorial defense, roost construction and anti-predator vigilance (Lewis 1982, Walker et al. 2016, York et al. 2019), and during breeding periods they contribute substantially to nestling provisioning (Cram et al. 2015a, Capilla-Lasheras et al. 2021). Helping behavior by subordinates has a causal positive effect on the overall rate at which nestlings are fed (Capilla-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

METHODS

General field methods

Data were collected in the context of a long-term research project that monitors ~40 cooperative groups of white-browed sparrow weavers at Tswalu Kalahari Reserve, South Africa (27°160S, 22°250E). Data were collected at a similar time in two separate breeding seasons (January to February 2013, and January to March 2014). White-browed sparrow weavers in this population may breed at any time from September through to May (the Southern summer), depending on the timing of unpredictable summer rainfall (Capilla-Lasheras 2019, Wood et al. 2021). Each bird within our study population is fitted with a metal ring and three color rings, providing a unique ring combination for identification in the field (SAFRING license 1444). From around six months of age, males and females of the focal subspecies (*Plocepasser mahali mahali*) can be distinguished by their bill color; males have a dark brown bill while females have a paler grey-to-pink bill (Leitner et al. 2009). Dominance status and social group compositions were determined via regular (at least twice weekly) group visits. Social dominance was assigned based on the monitoring of key dominance-related behaviors: the dominant pair routinely displace other group members and produce synchronized duet song, the dominant female is the sole incubator, and the dominant male consistently produces dawn song during breeding periods (Harrison et al. 2013a, Cram et al. 2015b, York et al. 2016). The dispersal status (natal or immigrant) of subordinate birds was determined via the continuous monitoring of the study population since 2007. Based on this information, four classes of birds were assigned: Dominant Females; Dominant Males; Natal Subordinates and Immigrant Subordinates. Social group size was defined as the number of adult (> 1 year of age) birds consistently seen foraging and roosting together at the time of the focal breeding attempt. The breeding status of each group was determined by monitoring the contents of all woven nest structures within each group's territory, at least every other day throughout the two study periods. When one or more eggs were newly detected, the active nest was visited daily in the afternoon until no new eggs were detected (the birds lay one egg per day in the morning, and typically lay clutches of 2 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

available). All analyses therefore utilized the data from only the first sampling event per bird, obviating the need to include bird ID as a random effect within our mixed effects models (see below). While some analyses utilized the whole data set (i.e. n = 70 adults birds each sampled once for all traits), others used subsets of it (e.g. when focusing only on dominants or subordinates), and so the sample sizes for each analysis are reported within the relevant results section and model output table. As mean provisioning visit duration data were only available for birds that had a non-zero provisioning rate, the sample sizes for mean visit duration analyses were sometimes smaller than those for provisioning rate.

All statistical models and visualizations were carried out in R (version 4.1.0; R Core Team). Mixed effects modelling was conducted using the R package 'lme4' (Bates et al. 2015), and the importance of a given combination of model predictors was assessed using an information-theoretic (IT) approach. Each modelling exercise started with the specification of a 'global model' containing all predictors of interest. This global model and all possible simpler models containing subsets of the global model's fixed effect predictors (including the intercept-only model) were then fitted to the data using maximum likelihood and ranked based on AICc (Akaike's Information Criterion corrected for small sample size; Burnham and Anderson 2002). This 'all subsets' approach was appropriate as all simpler models were biologically plausible. ΔAICc values (i.e. the difference in AICc between the focal model and the best supported 'top' model) were then calculated for every model fitted. As lower AICc values are indicative of stronger statistical support for a given model, the ΔAICc for the best-supported 'top' model = 0 and models that attracted less statistical support had progressively more positive ΔAICc values. We gave consideration to models within a ΔAICc value of six (Richards 2008, Richards et al. 2011) and subsequently reduced this Δ6 'top model set' by applying the 'nesting rule' described in (Richards 2008). This rule aims to avoid the retention of overly complex models that do not improve model fit, by discarding models that are more complex versions of simpler (nested) models with weaker AIC support (Richards 2008, Arnold 2010). Adding variables with little or no explanatory power to a top model can weaken AIC by less than 6 points, leading to the retention within the Δ6 top model set of more complex versions of better 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

possibility of diel variation in prolactin levels and/or changes in prolactin levels once the birds entered their roosts) and (ii) the time lag from capture to the completion of blood sampling (to allow for possible effects of capture stress on circulating prolactin levels). While neither of these two time-lag variables were present within the best-supported model that arose from model comparisons (suggesting no appreciable effect of these variables on circulating prolactin levels; Table 1), we nevertheless verified that our inferences regarding the effect of bird class on prolactin levels on the basis of the best-supported model overall were also upheld by the best-supported model that contained both of these time lag terms (see results text).

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

We then used two mixed effects models with Gaussian error structure to model the causes of variation in the provisioning rates and prolactin levels of subordinate birds engaged in helping behavior. We fitted the same set of fixed and random effect predictors to these models as were fitted to the corresponding provisioning rate and prolactin level models conducted for dominant birds (see above) with two exceptions: (i) here 'bird class' reflected whether the bird was a natal subordinate or an immigrant subordinate, and (ii) here only brood ID was fitted as a random effect (social group ID was not, as all subordinate birds from any given social group were sampled while feeding the same single brood, leaving brood ID and social group ID with identical structure in this case). Again, we verified that our inferences regarding the effect of bird class on prolactin levels on the basis of the best-supported model overall were also upheld by the best-supported model that contained both of the methodological time lag terms (see results text). We did not model the causes of variation in the mean provisioning visit durations of subordinates as too few immigrant subordinates actually provisioned the focal broods, leaving us with an insufficient sample size of measures of the provisioning visit durations of this bird class.

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.

RESULTS

1. Are differences in the nestling provisioning rates and nest visit durations of dominant females and males (engaged in parental care) mirrored by differences in their circulating prolactin levels?

Analyzing the provisioning behavior of dominant birds engaged in parental care (n = 46 dominants, 20 females and 26 males, feeding 31 broods at 28 social groups) revealed strong evidence that dominant females feed offspring at higher rates than dominant males (Figure 1a; bird class was present in all models down to ΔAICc = 42.36; Table 1) and that the mean provisioning visit durations of dominant females are also longer than those of dominant males (Figure 1b; ΔAICc = 11.49; Table 1). There was also evidence that brood size positively predicted the provisioning rates of dominant birds (ΔAICc = 1.92; Table 1), but not their mean visit durations (the best-supported visit duration model containing brood size scored 1.42 AICc points below the top model and was rejected under the nesting rule; Table 1; Table A1). There was no compelling evidence that the provisioning rates or mean visit durations of dominant birds were associated with either the year of study or adult group size (which were absent from the best-supported model for both traits; Table 1).

Analyzing the circulating prolactin levels of dominant birds during the provisioning periods analyzed above (again, n = 46 dominants, 20 females and 26 males, feeding 31 broods at 28 social groups) revealed strong evidence that dominant females also have higher circulating prolactin levels than dominant males (Figure 1c; bird class was present in all models down to ΔAICc = 19.93; Table 1). There was no compelling evidence that the prolactin levels of dominants were associated with group size, brood size, the time lag from sunset to capture or the time lag from capture to blood sampling (none of which were present within the best-supported model; Table 1). 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 A1; model number 22).

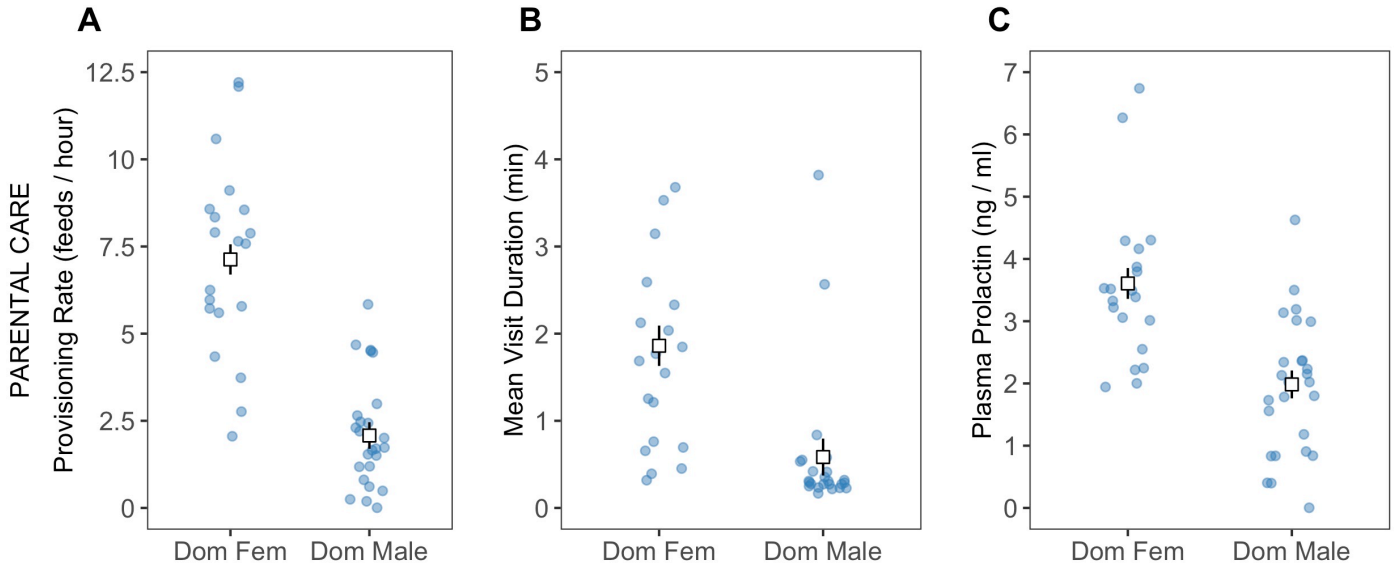

Figure 1. The (a) provisioning rates, (b) mean provisioning visit durations, and (c) circulating prolactin concentrations of dominant females (Dom Fem) and dominant males (Dom Male) engaged in parental care. Squares and error bars present the predicted means ± S.E. from the top model for the relevant trait (Table 2) while controlling for the effects of the other variables present in that model. The points show the raw data points.

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).

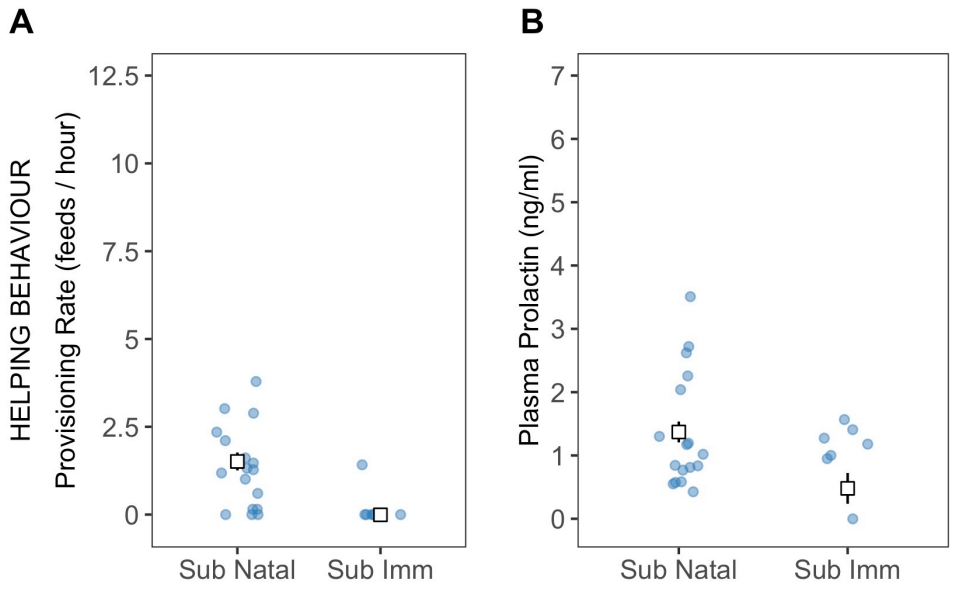

Figure 2. The (a) provisioning rates, and (b) circulating prolactin levels of natal subordinates (Sub Natal) and immigrant subordinates (Sub Imm) engaged in cooperative helping behavior, feeding the broods of the dominant male and female. Y axis scales match those in Figure 1 to facilitate comparison. 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).

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

Model selection of the factors affecting the (i) nestling provisioning rates, and (ii) prolactin levels of natal subordinates ('Sub Natal') and immigrant subordinates ('Sub Imm') engaged in cooperative helping behavior. The table shows all models within <6 ΔAIC of the top model, after applying the model nesting rule (Richards et al., 2011; see methods). The grey lines indicate the response term for the set of models beneath it. The numbers indicate coefficients (± standard errors; S.E.) and df is the degrees of freedom. The 'Year' effect is the change from 2013 to 2014. Bird Class was present within all models down to ΔAICc = 10.64 in the provisioning rate analysis. N = 24 subordinate birds, 17 natal and 7 immigrant, feeding 16 broods at 16 social groups. Table A2 presents these top model sets prior to application of the model nestling rule.

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

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

489 Our analysis at the population level, including birds of all classes, revealed strong evidence that a bird's
490 circulating prolactin level positively predicts both its provisioning rate (Figure 3a; effect size ± S.E. =
491 1.20 ± 0.23; the provisioning rate model containing the prolactin predictor scored 20.36 AICc points
492 stronger than the intercept-only model; n = 70 birds feeding 37 broods at 30 social groups) and its mean
493 provisioning visit duration (Figure 3c; effect size ± S.E. = 0.32 ± 0.076; the mean visit duration model
494 containing the prolactin predictor scored 12.59 AICc points stronger than the intercept-only model; n =
495 59 birds feeding 36 broods at 30 social groups). Plotting out the mean prolactin levels and provisioning
496 trait values of the different bird classes (Figure 3a & 3c), reveals that both of these population-level
497 relationships between prolactin and provisioning traits are driven in large part by the among-bird-class
498 differences in prolactin levels being mirrored by parallel among-bird-class differences in mean
499 provisioning rate (Figure 3a) and mean provisioning visit duration (Figure 3c). Indeed, after mean-
500 centering each bird's prolactin level and provisioning trait values around the focal trait's mean value for
501 their bird class, we found no evidence that within-bird-class variation in prolactin levels predicted within-
502 bird-class variation in either provisioning rate (Figure 3b; effect size ± S.E. = -0.15 ± 0.21; the model
503 containing the mean-centered-prolactin predictor scored 1.87 AICc points weaker than an intercept-only
504 model) or mean provisioning visit duration (Figure 3d; effect size ± S.E. = -0.049 ± 0.081; the model
505 containing the mean-centered prolactin predictor scored 2.03 AICc points weaker than an intercept-only
506 model).

507

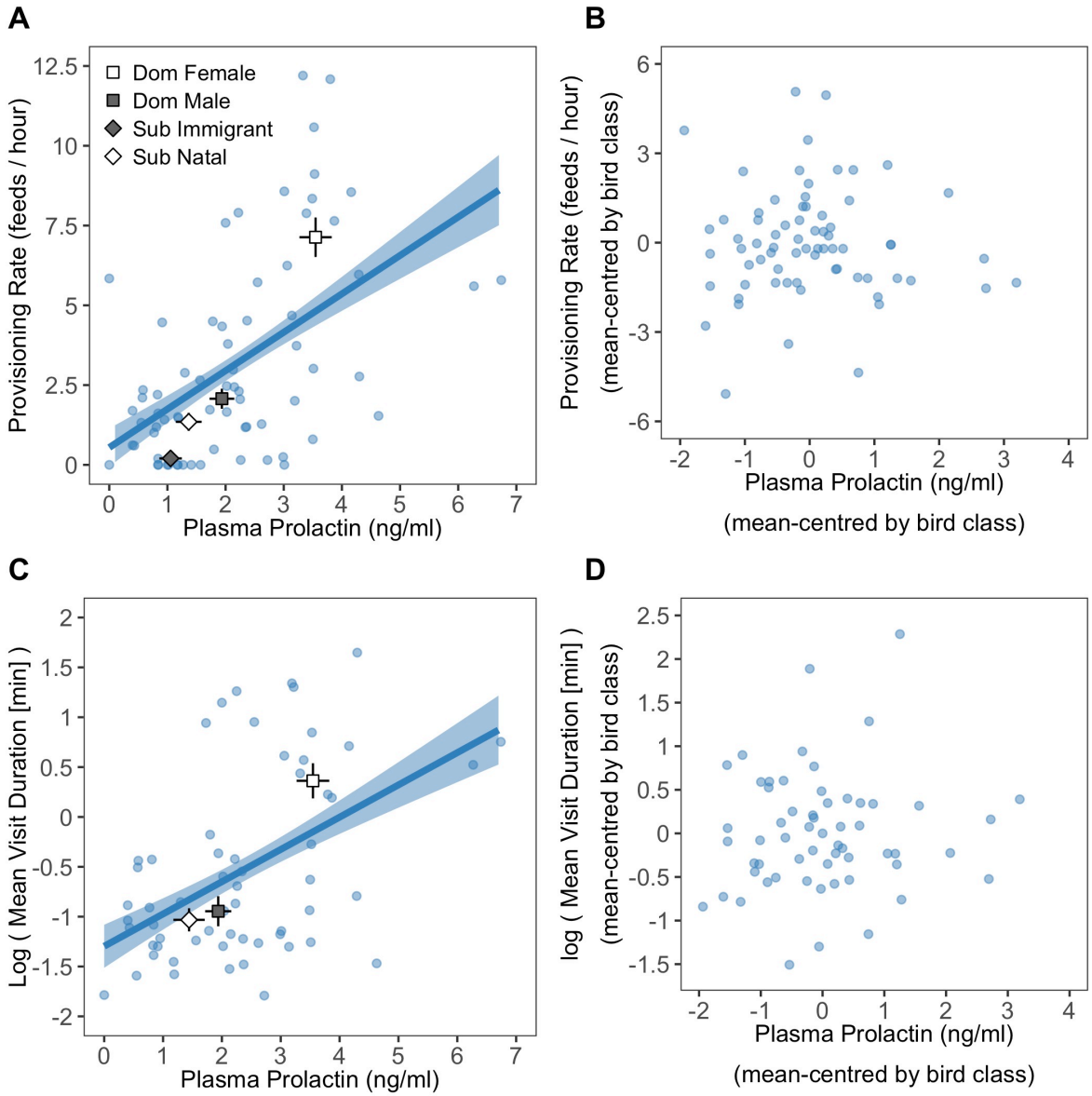

Figure 3. At the population level, considering all bird classes together, natural variation in circulating prolactin levels predicts variation in both (**a**) provisioning rate and (**c**) mean provisioning visit duration. These relationships are driven principally by differences among the mean trait 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.

DISCUSSION

This study investigated the hypothesis that prolactin plays a role in the regulation of nestling provisioning, both among dominant birds (engaged in parental care) and subordinate birds (that engage in cooperative helping behavior), in cooperatively breeding white-browed sparrow weaver societies. Among dominants engaged in parental care, we found that the dominant female (the mother) fed offspring at higher rates, made longer provisioning visits and had higher circulating prolactin levels than the dominant male (typically the father). Among subordinates, we found that natal subordinates helped to feed offspring at higher rates and had higher circulating prolactin levels than immigrant subordinates. Indeed, when all bird classes were combined, we found that continuous variation in the circulating prolactin levels of the birds predicted continuous variation in their provisioning rates and mean provisioning visit durations. These patterns appear to be driven principally by correlated differences among the four different bird classes in their prolactin levels and provisioning traits. We found no evidence that the more limited variation in circulating prolactin levels within the different bird classes predicted the more limited within-class variation in their provisioning traits. Together, these findings are broadly consistent with the hypothesis that parental care and cooperative helping behavior are regulated by a common underlying mechanism, and the hypothesis that prolactin plays a role in that pathway, but also highlight the need for experimental studies to now probe the causality and nature of any role for prolactin. Below, we discuss potential explanations for these findings, the different roles that prolactin could conceivably play in the regulation of parenting and cooperative helping in this species, and the wider implications of our findings for mechanistic and evolutionary research on cooperative behavior.

While our findings are broadly consistent with the hypothesis that parental care and helping behavior are regulated by a common mechanism in which prolactin plays a role, the lack of a relationship between within-class variation in prolactin levels and provisioning traits, coupled with the correlative nature of our findings, leave it important to consider the range of possible roles that prolactin could play in the 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).

708

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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	

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	