Prolactin and the shared regulation of parental care and cooperative helping in white-browed sparrow weaver societies

Lindsay A. Walker[1], Linda Tschirren[2], Jennifer E. York[1,3], Peter J. Sharp[4], Simone L. Meddle[4], Andrew J. Young[1*]

[1] Centre for Ecology and Conservation, University of Exeter Cornwall Campus, Penryn, Cornwall TR10 9EZ, UK

[2] ZHAW School of Life Sciences and Facility Management, Institute of Natural Resource Sciences, Grüental, 8820 Wädenswil

[3] Department of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ, UK

[4] The Roslin Institute, The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian EH25 9RG, Scotland, UK

[*] Corresponding author

A.J.Young@exeter.ac.uk

Centre for Ecology and Conservation

University of Exeter, Cornwall Campus

TR10 9EZ

UK

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ABSTRACT

In many cooperatively breeding species 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. Accordingly, 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 species, 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 alloparental '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; Capilla-Lasheras et al., 2021). 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; Vullioud et al., 2021), despite a surge of interest in the origins of consistent individual differences in both cooperative behavior and endocrine traits (English et al., 2010; Sanderson et al., 2015; Dantzer et al., 2019; Houslay et al., 2019; 2022). As cooperatively breeding species typically evolved from species in which parental care was already well developed in both sexes (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 can disrupt incubation behavior (e.g. Thierry et al., 2013) and the expression of post-natal nestling care (e.g. Smiley and Adkins-Regan, 2018), suggest that these elevated prolactin levels are necessary for the onset and/or maintenance of both pre- and post-natal 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; Buntin et al., 1991). 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, 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 could 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 (Angelier et al., 2016).

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

98 and non-breeding helpers during the transition to incubation and nestling care in at least three species
99 of cooperatively breeding bird (Schoech et al., 1996; Brown and Vleck, 1998; Khan et al., 2001; see
100 also Vleck et al., 1991). While few studies have investigated specifically whether variation in circulating
101 prolactin levels predicts variation in cooperative contributions to helping, studies of at least two
102 cooperative breeders have yielded compelling evidence in this regard. In Florida scrub jays
103 (*Aphelocoma coerulescens*), breeders fed offspring at higher rates than non-breeders and showed
104 higher circulating prolactin levels (Schoech et al., 1996; see also Vleck et al., 1991), and those non-
105 breeders that helped to feed offspring showed higher prolactin levels than those that did not (Schoech
106 et al., 1996). Continuous variation in circulating prolactin levels also predicted continuous variation in
107 feeding contributions, both among all birds combined and specifically among non-breeders (Schoech et
108 al., 1996). Similarly, in meerkat (*Suricata suricatta*) societies, continuous variation in the prolactin levels
109 of helpers positively predicted their cooperative contributions to both babysitting and pup-feeding
110 (Carlson et al., 2006a; 2006b). In the pup-feeding study, prolactin levels only predicted the cooperative
111 pup-feeding rates of helpers in statistical models that did not allow for an independent positive effect of
112 circulating cortisol levels on the helper's pup-feeding rates (Carlson et al., 2006a). Experimental work
113 since highlights that this putative positive effect of cortisol on pup-feeding rates may not have been
114 causal, however, as glucocorticoid receptor blockade increased rather than decreased pup-feeding
115 rates among meerkat helpers (Dantzer et al., 2017). Such relationships between circulating prolactin
116 levels and helping behavior are not always apparent. For example, prolactin levels did not predict the
117 offspring provisioning rates of helpers in red-cockaded woodpecker (*Picoides borealis*) groups (Khan et
118 al., 2001), and the pituitary gland prolactin mRNA levels of a cooperatively breeding fish were not
119 evidently related to care-giving behavior (Bender et al., 2008); though the relevant sample sizes in both
120 studies were modest. Attempts to identify correlations between natural continuous variation in prolactin
121 levels and care-giving behavior are expected to be complicated, however, by the existence of other
122 neuroendocrine pathways that may also modulate the expression of care-giving (e.g. glucocorticoids
123 may play a role in its state-dependent modulation; Sanderson et al., 2014; Dantzer et al., 2017; see
124 also Angelier and Chastel, 2009; Angelier et al., 2016), potentially independent of circulating prolactin

levels (Schoech et al., 1998; Ziegler, 2000; Young et al., 2005; Carlson et al., 2006a). Nevertheless, the promising findings to date highlight the need for further studies to investigate whether natural circulating levels of prolactin predict variation in contributions to both parental care and helping behavior in cooperatively breeding species, and, ultimately, the use of experimental manipulations to test the causality and nature of any hormone-behavior 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; 2023). 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; 2023). Helping behavior by subordinates in this species appears to have positive effects on both the helped offspring being fed and their parents. First, helping behavior has a causal positive effect on the overall rate at which nestlings are fed (Capilla-Lasheras et al., 2021), and, accordingly, helper numbers positively predict nestling survival to fledging during dry periods, reducing the environmentally-induced variance in the reproductive success of the dominant pair (Capilla-Lasheras et al., 2021). Evidence suggestive of positive helper effects on offspring telomere lengths suggest that helpers may also

151 improve the downstream performance of surviving young (Wood, 2017; Wood and Young, 2019).
152 Second, helping behavior appears to lighten the post-natal provisioning workload of the dominant female
153 (Capilla-Lasheras et al., in press), which may explain why mothers with more help increase their pre-
154 natal investment in the egg (Capilla-Lasheras et al., in press) and show higher overwinter survival
155 (O'Callaghan, 2021). While the neuroendocrine correlates of white-browed sparrow weaver
156 reproduction, aggression and song production have been investigated (e.g. Wingfield and Lewis, 1993;
157 Voigt et al., 2007; York, 2012; York et al., 2016), the regulation of parental and helper contributions to
158 offspring provisioning remains unexplored.

159

160 We test three predictions of the hypothesis that prolactin promotes the expression of both parental care
161 (among dominants) and cooperative helping behavior (among non-breeding subordinates) in
162 cooperatively breeding societies. First, with regard to parental care, we predict that differences between
163 the nestling provisioning rates of dominant females and dominant males will be mirrored by parallel
164 differences in their mean circulating prolactin levels. Dominant females are expected to provision
165 nestlings at higher rates (and to have higher prolactin levels) than dominant males, as the 12-18%
166 incidence of extra-group paternity in this population leaves dominant females more closely related than
167 dominant males, on average, to the offspring that they rear (Harrison et al., 2013a). Second, with regard
168 to alloparental helping behavior, we predict that differences between the nestling provisioning rates of
169 subordinates still residing within their natal group (hereafter 'natal subordinates') and immigrant
170 subordinates, will also be mirrored by parallel differences in their mean circulating prolactin levels. Natal
171 subordinates are expected to provision nestlings at higher rates (and to have higher prolactin levels)
172 than immigrant subordinates, as while the former are typically rearing future generations of siblings born
173 to their parents, the latter are typically unrelated to the nestlings in their group (Harrison et al., 2013a).
174 Finally, we predict that continuous variation in circulating prolactin levels will positively predict
175 continuous variation in the nestling provisioning rates of birds, and that this relationship will be apparent

(i) at the population level (when all four of the bird classes above are combined), and (ii) within bird classes, having factored out the among-class differences in prolactin levels and provisioning rates.

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), and 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 (Wood et al., 2021; Capilla-Lasheras 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

202 detected, the active nest was visited daily in the afternoon until no new eggs were detected (the birds
203 lay one egg per day in the morning, and typically lay clutches of 2 eggs (range 1-4); Harrison et al.,
204 2013a). To determine hatch dates, daily monitoring of the active nest resumed 14 days after the
205 detection of the first egg (as incubation lasts 14-19 days; Harrison et al., 2013a). This method yielded
206 accurate information on the day on which the first nestling in each clutch hatched, which was termed
207 'Day 1' of the nestling provisioning period for the focal breeding attempt. All protocols were approved by
208 the Ethics Committees of the Universities of Exeter and Pretoria and complied with regulations stipulated
209 in The Association for the Study of Animal Behaviour (ASAB) Guidelines for Use of Animals in Research.

210

211 *Monitoring provisioning behavior*

212 To identify individuals during the recording of nestling provisioning events, group members were
213 captured from their roost chambers (details below) during the incubation period and marked on the vent
214 with a unique dye-mark. The dominant female was left unmarked to minimize disturbance during
215 incubation, but could still be distinguished from other group members by being the only unmarked bird
216 within her group (only resident group members provision offspring). To record provisioning events, a
217 Panasonic SDR-S50 camcorder attached to a tripod (approximately 0.5 meters in height) was placed
218 on the ground beneath the entrance to the active nest two days before recording commenced (to allow
219 the birds to habituate to it). On the days of provisioning monitoring, the recordings (approximately 3
220 hours in the duration) were started between 06:15 and 07:54, with this start time being adjusted through
221 the season to maintain an approximately constant time offset from sunrise. Provisioning videos were
222 collected in this way for all focal breeding attempts (n = 37 broods across 30 social groups) on two
223 mornings between Days 6 and 9 inclusive of the nestling provisioning period (typically for the two
224 consecutive mornings of Days 7 and 8; nestlings fledged from day 20). This approach yielded a mean
225 total duration of provisioning video of 6.08 hours (range 4.05 – 8.12 hours) per breeding attempt.

226

Video recordings were transcribed using VLC Media Player version 2.2, with the observer recording, for each provisioning visit, the identity of the bird visiting the nest (determined via their distinct dye mark and bill color, which reveals their sex) and the duration of time that they spent within the nest (the time elapsed between passing in and out of the enclosed nest structure; hereafter 'Provisioning visit duration'). Prior work on this study population using within-nest cameras has shown that all nest visits during the nestling age window studied here entail the delivery of a single prey item to the brood, unless the visiting bird is carrying a feather or grass in which case no food is delivered (Walker, 2016). We therefore excluded such feather- or grass-carrying nest visits from our provisioning visit records. From the transcribed data for each focal brood we then calculated two provisioning trait values for each adult group member: (i) 'Provisioning rate' (feeds / hr) was calculated as the total number of provisioning visits that the bird conducted over the two monitored mornings divided by the total duration of video collected over those two mornings, and (ii) 'Mean Visit Duration' (minutes) was calculated as the mean duration of all provisioning visits conducted by the focal bird over the two monitored mornings.

Bird capture and blood sampling

To obtain a matched blood sample for prolactin measurement, we attempted to capture and blood sample all adult (> 1 year old at the time of sampling) birds within the monitored brood's social group on the evening of the second day of provisioning behavior recording. Birds were captured individually at night from the woven roost chambers within their group's territory (Cram et al., 2015a) by flushing individuals into a custom-made capture bag. All captures, dye-marking and blood sampling were conducted by a single investigator (LW). Birds were then immediately returned to a roost chamber within their territory to pass the remainder of the night.

Upon capture, a blood sample (*c*. 140 μL) was taken from the brachial vein of the bird using a 26g needle and heparinized capillary tubes. Captures occurred soon after dusk, once the birds were roosting in their woven chambers. Time of capture was recorded (to allow us to fit the time lag from sunset to

253 capture as a covariate predictor in our prolactin analyses, in case of diel variation and/or effects of the
254 time elapsed since roosting on a bird's prolactin levels), along with the time lag between capture and
255 the completion of blood sampling (mean ± standard deviation [S.D.] = 3.12 ± 0.73 minutes [range 1.65
256 – 4.78 minutes]; to allow us to control for potential effects of capture stress on prolactin levels in our
257 statistical models). Blood samples were immediately centrifuged in the field (12,000 g for 3 minutes;
258 Haematospin 1400; Hawksley Medical and Laboratory Equipment, Lancing, UK) and the plasma was
259 drawn off and stored in a cryovial on ice until it could be transferred to liquid nitrogen on return from the
260 field (mean ± S.D. time lag from sample collection to storage on liquid nitrogen = 148 ± 63 min). At the
261 end of the field season, samples were transferred to the UK on dry ice and then stored at -80 degrees
262 Celsius until analysis for prolactin.

263

264 *Prolactin Radioimmunoassay*

265 The prolactin assay was carried out in July 2014 at the Roslin Institute (University of Edinburgh, Easter
266 Bush, Midlothian, Scotland, UK). Plasma prolactin levels were measured using a highly specific
267 heterologous micro-radioimmunoassay of donkey anti-rabbit serum to European starling (*Sturnus*
268 *vulgaris*) prolactin (Sharp antibody code 44/2). Prolactin was radiolabeled with iodine^{125} using
269 chloramine-T. 168 (out of a total of 208) samples were assayed in duplicate, and the remaining 40
270 samples were assayed as singletons (not all samples assayed were for use in this study). All samples
271 were measured in a single assay, in which the intra-assay coefficient of variation for the duplicate
272 samples was 3.31%.

273

274 *Statistical methods*

275 The above methods yielded a final data set of matched provisioning trait data (estimated from the focal
276 bird's average performance over two mornings of provisioning recordings; see above) and circulating
277 prolactin levels (when sampled on the evening of the second day of provisioning monitoring) for 70

different adult birds, each sampled once (for all traits), while feeding a total of 37 broods across 30 social groups. 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 engaged in parental care or subordinates engaged in cooperative helping behavior), 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 (see results).

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) We conducted our statistical modelling using a full model approach, in which we (i) specified a full model containing both the primary fixed effect predictors of interest and covariates whose potential effects we also wished to control for, and then (ii) tested the effects of these predictors in that context, without any model selection or simplification. The statistical significance of a given predictor was assessed by using a likelihood ratio test to determine the significance of the change in the explanatory power of the full model when the focal predictor was dropped from the full model. This conservative approach ensures that the significance of all predictors is assessed while controlling for the potential effects of the other predictors specified in the full model, regardless of whether those other predictors themselves have significant effects. The specific modelling exercises conducted for each results section are described below.

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

We used two separate mixed effects models with Gaussian error structure to model the causes of variation in (i) the provisioning rates and (ii) the mean provisioning visit durations of dominant birds (i.e. engaged in parental care). The two modeling exercises began with an identical full model structure. In addition to the primary predictor of interest, 'parent class' (dominant female or dominant male), we fitted

304 the following terms as fixed effect predictors: brood size (the brood size being fed), adult group size (the
305 number of adult group members during the focal nestling provisioning period) and year (a two-level
306 factor capturing the year in which sampling occurred; 2013 or 2014). We fitted both social group ID and
307 brood ID (the identity of the brood being fed) as random effects, retaining them in the model structure
308 regardless of the degree of variance that they explained.
309
310 We then used a third mixed effects model with Gaussian error structure to model the causes of variation
311 in the circulating prolactin levels of these same dominant birds, starting with a full model structure
312 containing the same fixed and random effect predictors as the provisioning trait models just described,
313 but with the addition of two further fixed effects to account for potential methodological effects on
314 prolactin concentrations: (i) the time lag from sunset to the bird's capture for blood sampling (to allow
315 for the possibility of diel variation in prolactin levels and/or changes in prolactin levels once the birds
316 entered their roosts) and (ii) the time lag from capture to the completion of blood sampling (to allow for
317 possible effects of capture stress on circulating prolactin levels).
318
319 **2. Are differences in the provisioning behavior of natal and immigrant subordinates (engaged in**
320 **alloparental helping behavior) mirrored by differences in their circulating prolactin levels?**
321 We then used two mixed effects models with Gaussian error structure to model the causes of variation
322 in the provisioning rates and circulating prolactin levels of subordinate birds engaged in alloparental
323 helping behavior. We fitted the same set of fixed and random effect predictors to these models as were
324 fitted to the corresponding provisioning rate and prolactin level models conducted for dominant birds
325 (see above) with two exceptions: (i) in place of 'parent class' we fitted 'helper class', reflecting whether
326 the focal bird was a natal subordinate or an immigrant subordinate, and (ii) here only brood ID was fitted
327 as a random effect (social group ID was not, as all subordinate birds from any given social group were
328 sampled while feeding the same single brood, leaving brood ID and social group ID with identical
329 structure). We did not model the causes of variation in the mean provisioning visit durations of

330 subordinates as too few immigrant subordinates actually provisioned the focal broods, leaving us with
331 an insufficient sample size of measures of the provisioning visit durations of this bird class.
332
333 **3. Does continuous variation in prolactin levels predict variation in provisioning behaviour?**
334 To investigate whether natural variation in prolactin levels predicted continuous variation in the birds'
335 nestling provisioning rates and mean provisioning visit durations at the population level (i.e. when all
336 bird classes were combined) we conducted two mixed effect models (one for each provisioning trait
337 response term), with circulating prolactin concentration as the sole fixed effect predictor (as we have
338 not hypothesized specific mechanisms by which other variables might impact provisioning traits
339 independent of prolactin levels) and social group ID and brood ID as random effects. Mean provisioning
340 visit duration was logarithm transformed for analysis, to normalize model residuals.
341
342 Inspection of the patterns of the mean prolactin levels and provisioning trait values of the four different
343 bird classes (i.e. dominant females, dominant males, natal subordinates and immigrant subordinates)
344 suggested that any such continuous relationship between prolactin levels and provisioning trait values
345 at the population level across all bird classes could be driven principally by the variation in these traits
346 *among* the bird classes (Figures 3a & 3c). In order to then investigate whether the more limited variation
347 in circulating prolactin levels *within* bird classes predicted the *within*-class variation in provisioning trait
348 values, we first mean-centered each birds' prolactin level and provisioning trait values (the log
349 transformed values in the case of mean provisioning visit duration) around the mean value of the focal
350 trait for birds of their class (by subtracting from it the mean value of the focal trait for their bird class).
351 We then conducted two mixed effects models (one for each mean-centered provisioning trait response
352 term), with mean-centered circulating prolactin concentration as the sole fixed effect predictor and social
353 group ID and clutch ID as random effects.

354 RESULTS

355 **1. Are differences in the provisioning behavior of dominant females and males (engaged in**
356 **parental care) mirrored by differences in their circulating prolactin levels?**

357 Analyzing the provisioning behavior of dominant birds engaged in parental care (n = 46 dominants, 20
358 females and 26 males, feeding 31 broods at 28 social groups) revealed that dominant females feed
359 offspring at significantly higher rates than dominant males (Figure 1a; parent class effect, for dominant
360 male relative to dominant female, ± S.E. = +5.11 ± 0.57; $P < 0.001$; Table 1) and also show significantly
361 longer mean provisioning visit durations (Figure 1b; parent class effect, for dominant male relative to
362 dominant female, ± S.E. = +1.29 ± 0.31; $P < 0.001$; Table 2). There was also evidence that dominant
363 birds fed larger broods at significantly higher rates (brood size effect ± S.E. = +1.08 ± 0.50; $P = 0.035$;
364 Table 1). There was no compelling evidence that the provisioning rates or mean visit durations of
365 dominant birds were associated with either the year of study or adult group size (Tables 1 & 2).

366

367 Analyzing the circulating prolactin levels of dominant birds during the provisioning periods analyzed
368 above (again, n = 46 dominants, 20 females and 26 males, feeding 31 broods at 28 social groups)
369 revealed evidence that dominant females also have significantly higher circulating prolactin levels than
370 dominant males (Figure 1c; parent class effect, for dominant male relative to dominant female, ± S.E. =
371 +1.69 ± 0.26; $P < 0.001$; Table 3). There was no compelling evidence that the prolactin levels of
372 dominants were associated with group size, brood size, the time lag from sunset to capture or the time
373 lag from capture to blood sampling (Table 3). Note that our full model approach ensured that any effects
374 of these latter predictors were controlled (regardless of their significance) when assessing the effects of
375 other terms.

376

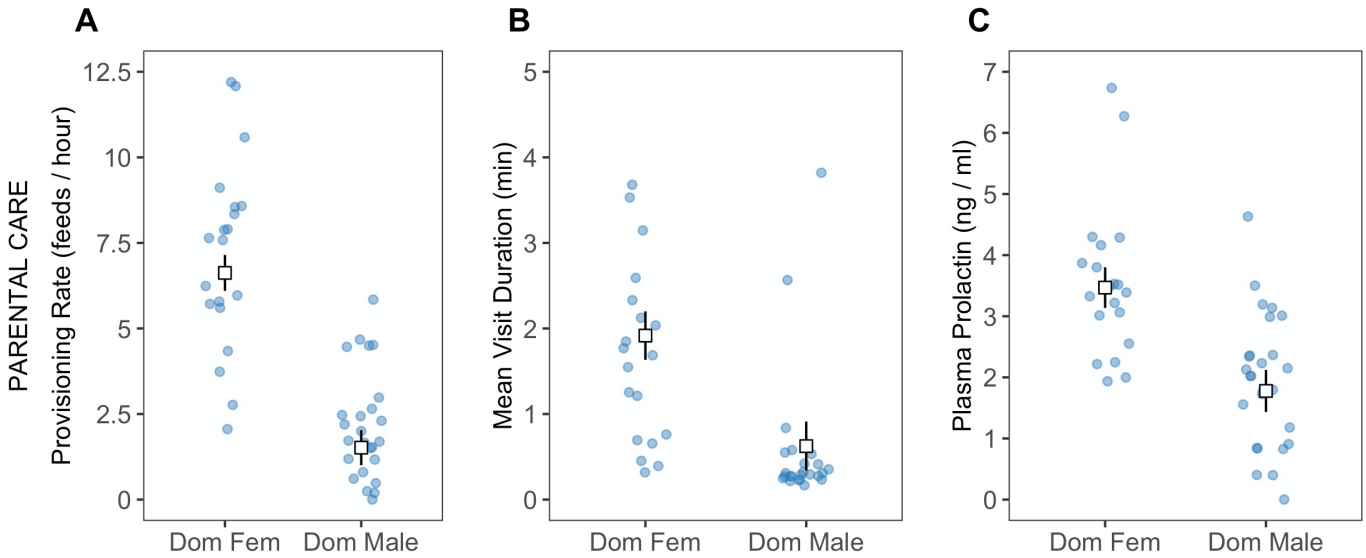

Figure 1. The (a) provisioning rates, (b) mean provisioning visit durations, and (c) circulating prolactin concentrations of dominant females (Dom Fem) and dominant males (Dom Male) engaged in parental care. Squares present the predicted means (± S.E.) from the full model for the relevant trait (Tables 1, 2 and 3 respectively), while controlling for the effects of all other variables in the full model. The predicted means were calculated with all continuous predictors set to their mean values and for the 2013 category of the Season factor. The points show the raw data points.

Table 1: Modelling the effects of **Parental Sex on Nestling Provisioning Rate during Parental care**. N = 46 dominant birds, 20 female and 26 male, feeding 31 broods at 28 social groups. The 'Parent class' effect size is for the dominant female relative to the dominant male. The 'Year' effect size is for the 2014 field season relative to the 2013 field season. Significant effect sizes (on the basis of likelihood ratio tests when comparing the full model to the full model without the focal term) are highlighted in bold. SE = Standard Error; LRT = Likelihood ratio test and associated P value.

	Estimate	SE	t	LRT	P
(Intercept)	-0.461	1.252	-0.368		
Parent class (female > male)	**5.106**	**0.568**	**8.984**	**46.611**	**<0.001**
Year	1.027	0.638	1.608	2.516	0.113
Group size	0.050	0.276	0.181	0.033	0.856
Brood size	**1.084**	**0.501**	**2.164**	**4.461**	**0.035**

Table 2: Modelling the effects of **Parental Sex on Mean Nestling Provisioning Visit Duration during Parental care**. N = 44 dominant birds, 20 female and 24 male, feeding 31 broods at 28 social groups; the sample size for this analysis was slightly lower than that for the Provisioning Rate analysis (Table 1) as 2 dominant males did not provision their brood, leaving us without a measure of their mean visit duration. The 'Parent class' effect size is for the dominant female relative to the dominant male. The 'Year' effect size is for the 2014 field season relative to the 2013 field season. Significant effect sizes (on the basis of likelihood ratio tests when comparing the full model to the full model without the focal term) are highlighted in bold. SE = Standard Error; LRT = Likelihood ratio test and associated P value.

	Estimate	**SE**	**t**	**LRT**	**P**
Intercept	0.960	0.720	1.333		
Parent class (female > male)	**1.292**	**0.314**	**4.119**	**14.351**	**<0.001**
Year	-0.091	0.348	-0.262	0.069	0.793
Group size	0.032	0.153	0.210	0.044	0.834
Brood size	-0.253	0.285	-0.887	0.780	0.377

Table 3: Modelling the effects of **Parental Sex on Circulating Prolactin level during Parental care**. N = 46 dominant birds, 20 female and 26 male, feeding 31 broods at 28 social groups (identical to the nestling provisioning rate analysis in Table 1, as all birds were sampled for both traits in the same contexts). The 'Parent class' effect size is for the dominant female relative to the dominant male. The 'Year' effect size is for the 2014 field season relative to the 2013 field season. Significant effect sizes (on the basis of likelihood ratio tests when comparing the full model to the full model without the focal term) are highlighted in bold. SE = Standard Error; LRT = Likelihood ratio test and associated P value. Sunset to capture lag = time elapsed between sunset and capture (to account for potential circadian variation in prolactin levels). Capture to bleed lag = time elapsed between first contact with the roost chamber and blood sample (to allow for the possibility of a prolactin stress response).

	Estimate	**SE**	**t**	**LRT**	**P**
(Intercept)	0.731	1.199	0.610		
Parent class (female > male)	**1.691**	**0.261**	**6.487**	**23.196**	**<0.001**
Year	0.328	0.446	0.735	0.378	0.539
Group size	0.271	0.180	1.509	2.217	0.137
Brood size	0.298	0.320	0.931	0.783	0.376
Sunset to capture lag	0.000	0.003	-0.002	0.000	0.998
Capture to bleed lag	-0.001	0.004	-0.295	0.087	0.768

2. Are differences in the provisioning behavior of natal and immigrant subordinates (engaged in alloparental 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; helper class effect, for natal subordinates relative to immigrant subordinates, ± S.E. = +1.61 ± 0.33; $P < 0.001$; Table 4). There was also evidence that helpers fed larger broods at significantly higher rates (brood size effect ± S.E. = +1.06 ± 0.35; $P = 0.007$; Table 4). There was no compelling evidence that helper provisioning rates were associated with either group size or the year of study (Table 4). 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 significantly higher prolactin levels than immigrant subordinates (Figure 2b; helper class effect, for natal subordinates relative to immigrant subordinates, ± S.E. = +0.86 ± 0.28; $P = 0.005$; Table 5). There was also evidence that subordinate prolactin levels were significantly higher in the second year of study (year effect, for 2014 relative to 2013, ± S.E. = +0.85 ± 0.37; $P = 0.029$; Table 5) and in smaller groups (Group size effect ± S.E. = -0.37 ± 0.13; $P = 0.007$; Table 5). 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 (Table 5). Note that our full model approach ensured that any effects of these latter predictors were controlled (regardless of their significance) when assessing the effects of other terms.

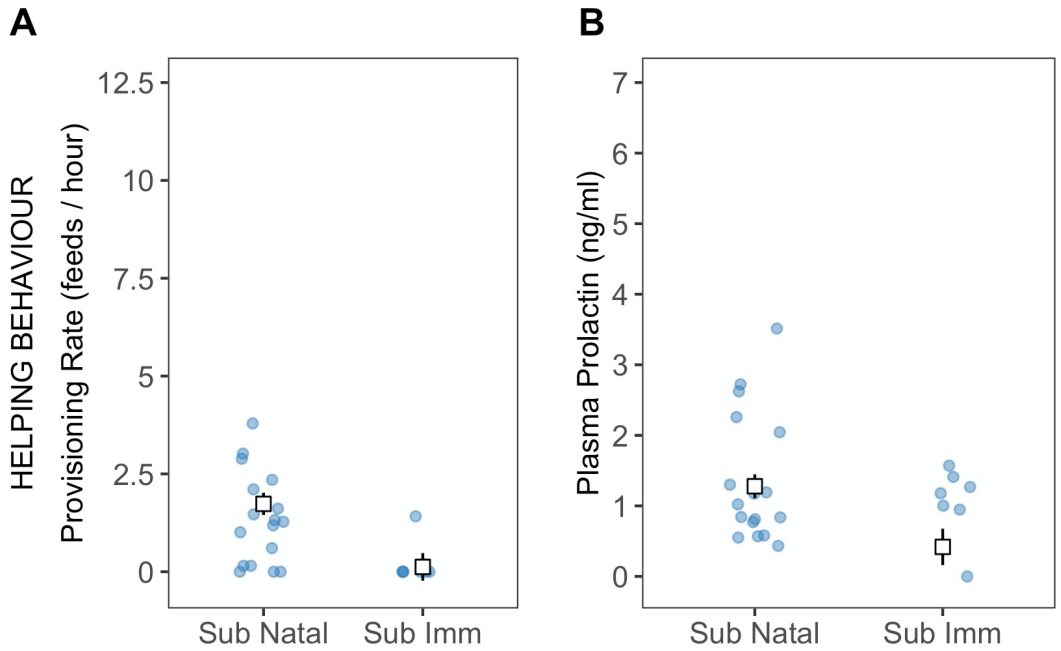

Figure 2. The (a) provisioning rates, and (b) circulating prolactin levels of natal subordinates (Sub Natal) and immigrant subordinates (Sub Imm) engaged in cooperative helping behavior, feeding the broods of the dominant male and female. Y axis scales match those in Figure 1 to facilitate comparison. Squares present the predicted means (± S.E.) from the full model for the relevant trait (Tables 4 and 5 respectively), while controlling for the effects of all other variables in the full model. The predicted means were calculated with all continuous predictors set to their mean values and for the 2013 category of the Season factor. 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).

462 **Table 4:** Modelling the effects of **Helper Class on Nestling Provisioning Rate during Alloparental**
463 **Helping Behaviour**. N = 24 subordinate birds, 17 natal and 7 immigrant, feeding 16 broods at 16 social
464 groups. The 'Helper class' effect size is for natal subordinates (those residing within their natal groups)
465 relative to immigrant subordinates (those that have dispersed to another group). The 'year' effect size
466 is for the 2014 field season relative to the 2013 field season. Significant effect sizes (on the basis of
467 likelihood ratio tests when comparing the full model to the full model without the focal term) are
468 highlighted in bold. SE = Standard Error; LRT = Likelihood ratio test and associated P value.
469

	Estimate	SE	t	LRT	P
Intercept	-0.297	1.097	-0.271		
Helper class (natal > immigrant)	**1.610**	**0.334**	**4.826**	**15.858**	**<0.001**
Year	-0.810	0.435	-1.861	3.173	0.075
Group size	-0.274	0.226	-1.212	1.415	0.234
Brood size	**1.057**	**0.354**	**2.986**	**7.166**	**0.007**

470

471 **Table 5:** Modelling the effects of **Helper Class on Circulating Prolactin level during Alloparental**
472 **Helping Behaviour**. N = 24 subordinate birds, 17 natal and 7 immigrant, feeding 16 broods at 16 social
473 groups (identical to the nestling provisioning rate analysis in Table 4, as all birds were sampled for both
474 traits in the same contexts). The 'Helper class' effect size is for natal subordinates (those residing within
475 their natal groups) relative to immigrant subordinates (those that have dispersed to another group). The
476 'year' effect size is for the 2014 field season relative to the 2013 field season. Significant effect sizes
477 (on the basis of likelihood ratio tests when comparing the full model to the full model without the focal
478 term) are highlighted in bold. SE = Standard Error; LRT = Likelihood ratio test and associated P value.
479 Sunset to capture lag = time elapsed between sunset and capture (to account for potential circadian
480 variation in prolactin levels). Capture to bleed lag = time elapsed between first contact with the roost
481 chamber and blood sample (to allow for the possibility of a prolactin stress response).
482

	Estimate	SE	t	LRT	P
Intercept	1.255	0.910	1.378		
Helper class (natal > immigrant)	**0.860**	**0.281**	**3.054**	**7.881**	**0.005**
Year (2014 > 2013)	**0.848**	**0.370**	**2.294**	**4.757**	**0.029**
Group size	**-0.365**	**0.126**	**-2.901**	**7.152**	**0.007**
Brood size	-0.175	0.227	-0.770	0.586	0.444
Sunset to capture lag	0.002	0.003	0.709	0.498	0.480
Capture to bleed lag	0.004	0.003	1.561	2.320	0.128

483 **3. Does continuous variation in prolactin levels predict variation in provisioning behaviour?**

484 Our analysis at the population level, including birds of all classes, revealed that a bird's circulating
485 prolactin level strongly and significantly positively predicts both (i) its provisioning rate (Figure 3a; effect
486 size ± S.E. = 1.20 ± 0.23; $P < 0.001$; n = 70 birds feeding 37 broods at 30 social groups) and (ii) its mean
487 provisioning visit duration (Figure 3c; effect size ± S.E. = 0.32 ± 0.076; $P < 0.001$; n = 59 birds feeding
488 36 broods at 30 social groups). Plotting out the mean prolactin levels and provisioning trait values of the
489 different bird classes (Figure 3a & 3c), reveals that both of these population-level relationships between
490 prolactin and provisioning traits are driven in large part by the among-bird-class differences in prolactin
491 levels being mirrored by parallel among-bird-class differences in mean provisioning rate (Figure 3a) and
492 mean provisioning visit duration (Figure 3c). Indeed, after mean-centering each bird's prolactin level and
493 provisioning trait values around the focal trait's mean value for their bird class, we found no evidence
494 that within-bird-class variation in prolactin levels predicted within-bird-class variation in either
495 provisioning rate (Figure 3b; effect size ± S.E. = -0.15 ± 0.21; P = 0.50) or mean provisioning visit
496 duration (Figure 3d; effect size ± S.E. = 0.049 ± 0.081; P = 0.55).

497

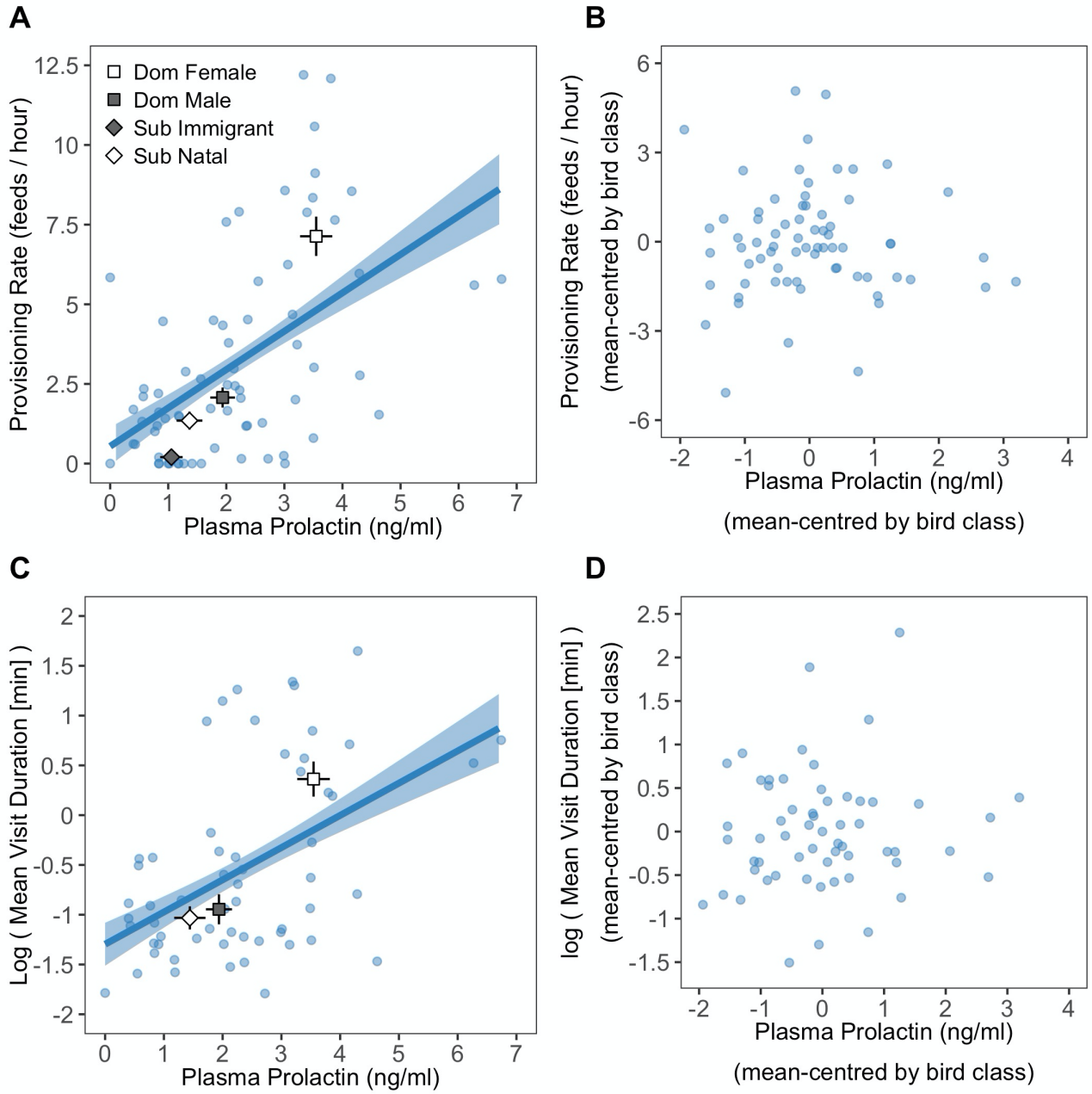

Figure 3. At the population level, considering all bird classes together, natural variation in circulating prolactin levels predicts variation in both (**a**) provisioning rate and (**c**) mean provisioning visit duration. These relationships are driven principally by differences among the mean trait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

525 DISCUSSION

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

545

546 While our findings are consistent with the hypothesis that parental care and helping behavior are
547 regulated by a common mechanism in which prolactin plays a role, the lack of a relationship between
548 within-class variation in prolactin levels and provisioning traits, coupled with the correlative nature of our
549 findings, leave it important to consider the range of possible roles that prolactin could play in the
550 regulation of provisioning behavior in this species. At least three main possibilities exist, which will

551 require careful experimentation to tease apart. First, it is possible that circulating prolactin is one key
552 regulator of continuous variation in individual contributions to offspring provisioning, both among parents
553 and helpers. While most of our findings are consistent with this hypothesis, the absence of evident
554 relationships between within-class variation in prolactin levels and provisioning traits complicates this
555 view. However, the lack of evident *within*-class relationships could be attributable simply to a major
556 source of variation in both traits (*among*-class variation) having been factored out at this stage of the
557 analysis, leaving these within-class analyses seeking relationships between the more limited *within*-
558 class variation in both traits, which could be readily obscured by a number of mechanisms. First,
559 difficulties with the synchronous and accurate assessment of both prolactin levels and provisioning rates
560 could have yielded noise in the data set that precluded the detection of these more subtle prolactin-
561 provisioning relationships. While we sampled birds for prolactin on the evening following the morning
562 provisioning-monitoring session (a time lag comparable to, or shorter than, those of similar studies; e.g.
563 Duckworth et al., 2003; Ouyang et al., 2011), individuals may have differed in the way that their prolactin
564 levels changed during the day, leaving their evening prolactin levels only a modest proxy for those while
565 provisioning. The focal birds also varied in the timing of blood sampling, and while our analyses did not
566 detect any overall effects on prolactin levels of the time lag from capture to sampling, any individual
567 variation in the prolactin stress response (if this species shows one; Krause et al., 2015) could have
568 further decoupled the assessed prolactin levels from those during provisioning. Second, even if prolactin
569 levels were a key regulator of continuous variation in provisioning rates, alternative mechanisms are
570 also expected to impact provisioning rates potentially independent of circulating prolactin levels, leaving
571 the relationship between natural variation in prolactin levels and provisioning behavior potentially weak
572 in the first place (Schoech et al., 1998; Angelier et al., 2016). Key among these could be (i) variation in
573 other components of a prolactin-mediated pathway (such as inter-individual and temporal variation in
574 the density of prolactin receptors; Zhou et al., 1996; Ohkubo et al., 1998; Angelier et al., 2016), as well
575 as (ii) mechanisms that may impact provisioning behavior via prolactin-independent pathways (e.g. the
576 effects of circulating testosterone; Schoech et al., 1998; Angelier et al., 2016). Third, even if prolactin
577 levels alone determined provisioning 'motivation', the extent to which variation in provisioning motivation

578 was reflected in provisioning rates would depend upon the prey capture skills of the focal bird and the
579 environmental availability of prey. Indeed, all points considered, it is arguably mechanistically naïve to
580 expect particularly fine-grained associations between the levels of a single hormone and behavior to be
581 evident in natural populations even where a causal link exists between the two. To now robustly test the
582 hypothesis that prolactin regulates continuous variation in the magnitude of both parental and helper
583 contributions to offspring provisioning, there is a need to experimentally elevate the circulating prolactin
584 levels of actively provisioning birds whose natural prolactin levels are not at the upper end of the
585 physiological range (dominant males and natal subordinates may serve this purpose well; Figure 3a).
586 This manipulation would allow one to test the key prediction that an increase in the prolactin levels of
587 an actively provisioning bird will cause it to increase its provisioning rate; a prediction that to our
588 knowledge has yet to be tested in either a parenting or helping context (the few experimental elevations
589 of endogenous prolactin secretion in a provisioning context to date have focussed on the establishment
590 of provisioning in non-provisioning birds rather than its quantitative variation within actively provisioning
591 birds; e.g. Badyaev and Duckworth, 2005).

592

593 A second potential explanation for the balance of our findings is that prolactin could instead play a causal
594 role in the onset and maintenance of provisioning behavior among parents and helpers, without playing
595 a role in the quantitative regulation of contributions to provisioning among actively provisioning birds
596 (Angelier et al., 2016). For example, a threshold level of prolactin may be required for the onset and/or
597 maintenance of provisioning behavior (Angelier et al., 2006; Boos et al., 2007). Under this scenario, the
598 higher prolactin levels of natal subordinates and dominant birds, relative to immigrant subordinates,
599 could be causally responsible for the former bird classes engaging in provisioning while the latter
600 typically does not. This could be the case without prolactin playing any causal role in regulating
601 continuous variation in the provisioning rates of actively provisioning birds; a scenario that could account
602 for the lack of within-class correlations between prolactin levels and provisioning behavior. The elevated
603 prolactin levels of dominant females (relative to dominant males and natal subordinates) could

604 conceivably be a downstream consequence of either a role for prolactin in incubation (Buntin, 1996;
605 Sharp et al., 1998; Khan et al., 2001; as dominant females are the sole incubator in this species) and/or
606 their differential exposure to offspring cues during the nestling period (which can increase prolactin
607 secretion; Hall, 1987; Sharp et al., 1998), given their markedly higher provisioning rates and mean visit
608 durations than other classes. The hypothesis that prolactin maintains provisioning behavior but does
609 not quantitatively regulate contributions to it could now be tested by (i) experimentally elevating the
610 prolactin levels of subordinate immigrants, to test the prediction that this would cause these typically
611 non-provisioning birds to commence provisioning behavior (e.g. see Badyaev and Duckworth (2005) for
612 a demonstration of this transition in the context of parental nestling feeding), (ii) experimentally reducing
613 the prolactin levels of the actively-provisioning classes to test whether this eliminates provisioning
614 behavior (e.g. Smiley and Adkins-Regan, 2018), and (iii) experimentally elevating the prolactin levels of
615 actively provisioning dominant males and/or natal subordinates (the manipulation proposed in the
616 previous paragraph), as doing so should *not* increase their provisioning rates if prolactin merely
617 maintains provisioning behavior without regulating contributions to it.

618

619 Given the correlative nature of our findings, it is also conceivable that prolactin plays no causal role in
620 the onset, maintenance or quantitative regulation of parenting and/or cooperative helping in this species
621 (despite experimental evidence of causal effects on parenting in other species; see Introduction;
622 Angelier et al., 2016). In this scenario, one might attribute the evident associations between prolactin
623 and provisioning to a 'reverse causal' relationship, in which provisioning interactions with offspring
624 stimulate prolactin release (Hall, 1987; Sharp et al., 1998). However, such a reverse causal argument
625 alone cannot readily account for our findings in their entirety, as within-class variation in provisioning
626 rates and mean provisioning visit durations were not evidently associated with prolactin levels (though,
627 again, the lack of such an association could be attributable to challenges with accurately and
628 simultaneously quantifying hormone and behavior; see above). When considering whether our findings
629 could be attributable solely to effects of provisioning on prolactin levels (i.e. in the absence of any effect

630 of prolactin on provisioning), it is worth considering why selection would have left prolactin levels
631 sensitive to offspring interactions in the first place. Arguably the most plausible explanation is that this
632 mechanism plays a role in a feedback loop in which a causal relationship exists in both directions: if
633 prolactin did establish, maintain and/or regulate care, selection may have favored regulating prolactin
634 secretion according to offspring interactions in order to maintain care as long as offspring survive and/or
635 regulate care according to offspring viability or need (Hall, 1987; Sharp et al., 1998; Angelier et al.,
636 2016). As such, where offspring cues do stimulate prolactin release, such a relationship might generally
637 be expected to occur alongside causal effects of prolactin on care. While the experiments outlined above
638 would shed light on the causality of the prolactin-provisioning associations detected here, wider
639 investigations are also needed to probe the role, if any, that such a feedback loop (with causal
640 relationships in both directions) may play in the maintenance and/or regulation of cooperative care.

641

642 While experimental tests of causality are needed, our findings are broadly consistent with the hypothesis
643 that pre-existing mechanisms that regulated parental care in ancestral bi-parental species were co-
644 opted for the regulation of cooperative helping behavior on the evolution of cooperative breeding. The
645 often-overlooked possibility that parenting and cooperative helping are indeed regulated by a common
646 mechanism has important evolutionary implications. Explanations for the evolution, maintenance and
647 optimization of cooperative behavior typically focus on the roles of the fitness benefits and costs of
648 cooperation *per se* (Hamilton, 1964; Cockburn, 1998; West et al., 2007; Capilla-Lasheras et al., 2021).
649 However, if cooperation and parenting are regulated by a common underlying mechanism, it is
650 conceivable that this shared regulatory architecture for care giving is shaped as much by the payoffs
651 from its outcomes in a parental context as by the payoffs from its outcomes in a cooperative helping
652 context. While selection might independently optimize parental and non-breeding helper caring
653 strategies (e.g. via the evolution of an entirely context-dependent caring strategy), it is conceivable that
654 mechanistic constraints preclude their independent optimization. For example, genetic variants that
655 modified sensitivity to begging could conceivably impact the expression of both parental care and

656 cooperative helping, yielding scope for intra-locus genetic conflict to constrain the independent
657 optimization of both parental care and cooperative helping (Pennell et al., 2018; see also the conceptual
658 parallels with sexual conflict: Stewart et al., 2010; Pennell and Morrow, 2013). Where this is the case,
659 attempts to understand the evolutionary origins, maintenance and optimization of cooperative behavior
660 may require attention to the extent to which genetic correlations exist between parental and cooperative
661 behavior. Notably, our findings suggest that cooperative helping behavior in sparrow-weaver societies
662 is not maintained by selection *solely* because a genetic correlation with parenting has precluded the
663 evolution of 'non-helping' (see Brown and Vleck, 1998 for a similar debate), because a context-
664 dependent helping strategy does appear to have evolved. Subordinates routinely help while within their
665 natal groups (where they are closely related to the broods that they help to rear; Harrison et al., 2013a),
666 but typically cease to do so following immigration into another group (where they are typically unrelated
667 to broods, reducing the potential indirect fitness payoff from helping; Harrison et al., 2013a). While
668 endocrine research on cooperative breeders has historically focused principally on the proximate causes
669 of the rank-related reproductive disparities that typify such societies (Schoech et al., 2004; Young et al.,
670 2006; 2008), a renewed focus on the endocrinology of care in cooperative breeders (Schoech et al.,
671 2004; Soares et al., 2010; Dantzer et al., 2017; 2019) would now help to shed light on the extent to
672 which parenting and cooperation are indeed regulated by shared underlying pathways.

673

674 CONCLUSION

675 Our findings lend new support to the hypotheses that helping behavior in cooperatively breeding
676 societies has shared mechanistic underpinnings with parental care, and that prolactin plays a key role
677 in this pathway (see also Vleck et al., 1991; Schoech et al., 1996; Khan et al., 2001; Carlson et al.,
678 2006). Our findings and their complexity highlight the need for experimental studies to investigate both
679 the causality and nature of the relationship between prolactin and provisioning in this species, in both
680 parental and cooperative helping contexts. Our findings also highlight that attempts to understand the
681 evolution of cooperative helping may benefit from attention to the possibility of constraints on the

682 independent optimization of cooperative helping and parenting. Our study has implications too for the
683 growing interest in the mechanistic origins of consistent individual differences in cooperative helping
684 behavior (Sanderson et al., 2015; Dantzer et al., 2019). Specifically, our findings highlight that such
685 differences could arise from consistent individual differences within the pathway by which prolactin acts
686 (e.g. via differences in prolactin secretion and/or reception; Ohkubo et al., 1998; Zhou et al., 1996).

687

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