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

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25 ABSTRACT

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

45 [250 words]

46 INTRODUCTION

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

66

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

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

Vleck et al. 1991). While few studies have investigated specifically whether variation in circulating prolactin levels predicts variation in cooperative contributions to helping, studies of at least two cooperative breeders have yielded compelling evidence in this regard. In Florida scrub jays (*Aphelocoma coerulescens*), breeders fed offspring at higher rates than non-breeders and showed higher circulating prolactin levels (Schoech et al. 1996, see also Vleck et al. 1991), and those non-breeders that helped to feed offspring showed higher prolactin levels than those that did not (Schoech et al. 1996). Indeed, continuous variation in circulating prolactin levels predicted continuous variation in feeding contributions, both among all birds combined and specifically among non-breeders (Schoech et al. 1996). Similarly, in cooperatively breeding meerkat (*Suricata suricatta*) societies, continuous variation in the prolactin levels of helpers positively predicted their cooperative contributions to both babysitting and pup-feeding (Carlson et al. 2006a, 2006b). In the pup-feeding study, prolactin levels only predicted the pup-feeding rates of helpers in statistical models that did not allow for an independent positive effect of circulating cortisol levels on the animal's pup-feeding rates (Carlson et al. 2006a). Experimental work since highlights that this putative positive effect of cortisol on pup-feeding rates may not have been causal, however, as glucocorticoid receptor blockade increased rather than decreased pup-feeding rates among meerkat helpers (Dantzer et al. 2017). Such relationships between circulating prolactin levels and helping behavior are not always apparent. For example, prolactin levels did not predict the offspring provisioning rates of helpers in red-cockaded woodpecker (*Picoides borealis*) groups (Khan et al. 2001), and the pituitary gland prolactin mRNA levels of a cooperatively breeding fish were not evidently related to care-giving behavior (Bender et al. 2008); though the relevant sample sizes in both studies were modest.

The likelihood that glucocorticoids play a role in mediating the state-dependence of cooperative helping (Sanderson et al. 2014, Dantzer et al. 2017, see also Angelier and Chastel 2009, Angelier et al. 2016), coupled with the expectation that other neuroendocrine pathways also modulate helping potentially 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

success of the dominant pair (Capilla-Lasheras et al. 2021) and lighten the post-natal provisioning workload of the dominant female (Capilla-Lasheras 2019). While the neuroendocrine correlates of white-browed sparrow weaver reproduction, aggression and song production have been investigated (e.g. Wingfield and Lewis 1993, Voigt et al. 2007, York et al. 2016), the regulation of parental and helper contributions to offspring provisioning remains unexplored.

We test three predictions of the hypothesis that prolactin plays a role in regulating the expression of both parental care (among dominants) and cooperative helping behavior (among non-breeding subordinates) in cooperatively breeding societies. First, with regard to parental care, we predict that differences between the nestling provisioning rates of dominant females and dominant males will be mirrored by parallel differences in their mean circulating prolactin levels. Dominant females are expected to provision nestlings at higher rates and to have higher prolactin levels than dominant males (as the 12-18% incidence of extra-group paternity in this population leaves dominant females more closely related than dominant males, on average, to the offspring that they rear; Harrison et al. 2013a). Second, with regard to helping behavior, we predict that differences between the nestling provisioning rates of subordinates still residing within their natal group (hereafter ‘natal subordinates’) and immigrant subordinates, will also be mirrored by parallel differences in their mean circulating prolactin levels. Natal subordinates are expected to provision nestlings at higher rates than immigrants, as while the former are typically rearing future generations of siblings born to their parents, the latter will typically be unrelated to the nestlings in their group (Harrison et al. 2013a). Finally, we predict that continuous variation in circulating prolactin levels will positively predict continuous variation in the 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.

176 METHODS

177 *General field methods*

178 Data were collected in the context of a long-term research project that monitors ~40 cooperative groups
 179 of white-browed sparrow weavers at Tswalu Kalahari Reserve, South Africa (27°160S, 22°250E). Data
 180 were collected at a similar time in two separate breeding seasons (January to February 2013, and
 181 January to March 2014). White-browed sparrow weavers in this population may breed at any time from
 182 September through to May (the Southern summer), depending on the timing of unpredictable summer
 183 rainfall (Capilla-Lasheras 2019, Wood et al. 2021). Each bird within our study population is fitted with a
 184 metal ring and three color rings, providing a unique ring combination for identification in the field
 185 (SAFRING license 1444). From around six months of age, males and females of the focal subspecies
 186 (*Plocepasser mahali mahali*) can be distinguished by their bill color; males have a dark brown bill while
 187 females have a paler grey-to-pink bill (Leitner et al. 2009). Dominance status and social group
 188 compositions were determined via regular (at least twice weekly) group visits. Social dominance was
 189 assigned based on the monitoring of key dominance-related behaviors: the dominant pair routinely
 190 displace other group members and produce synchronized duet song, the dominant female is the sole
 191 incubator, and the dominant male consistently produces dawn song during breeding periods (Harrison
 192 et al. 2013a, Cram et al. 2015b, York et al. 2016). The dispersal status (natal or immigrant) of
 193 subordinate birds was determined via the continuous monitoring of the study population since 2007.
 194 Based on this information, four classes of birds were assigned: Dominant Females; Dominant Males;
 195 Natal Subordinates and Immigrant Subordinates. Social group size was defined as the number of adult
 196 (> 1 year of age) birds consistently seen foraging and roosting together at the time of the focal breeding
 197 attempt. The breeding status of each group was determined by monitoring the contents of all woven
 198 nest structures within each group's territory, at least every other day throughout the two study periods.
 199 When one or more eggs were newly detected, the active nest was visited daily in the afternoon until no
 200 new eggs were detected (the birds lay one egg per day in the morning, and typically lay clutches of 2
 201 eggs (range 1-4); Harrison et al. 2013a). To determine hatch dates, daily monitoring of the active nest

resumed 14 days after the detection of the first egg (as incubation lasts 14-19 days; Harrison et al. 2013a). This method yielded accurate information on the day on which the first nestling in each clutch hatched, which was termed 'Day 1' of the nestling provisioning period for the focal breeding attempt. All protocols were approved by the Ethics Committees of the Universities of Exeter and Pretoria and complied with regulations stipulated in The Association for the Study of Animal Behaviour (ASAB) Guidelines for Use of Animals in Research.

Monitoring provisioning behavior

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

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, leg ring fitting, dye-marking and blood sampling were conducted by a single investigator. 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 μ L) was taken from the brachial vein of the bird using a 26-g 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 capture as a covariate predictor in our prolactin analyses, in case of diel variation and/or effects of the time elapsed since roosting on a bird's prolactin levels), along with the time lag between capture and the completion of blood sampling (mean $\pm$ standard deviation [S.D.]: 3.12 ± 0.73 minutes [range 1.65 – 4.78 minutes]; to allow us to control for potential mean effects of capture stress on prolactin levels in our statistical models). Blood samples

were immediately centrifuged in the field (12,000 g for 3 minutes; Haematospin 1400; Hawksley Medical and Laboratory Equipment, Lancing, UK) and the plasma was drawn off and stored in a cryovial on ice until it could be transferred to liquid nitrogen on return from the field (mean $\pm$ S.D. time lag from sample collection to storage on liquid nitrogen = 148 ± 63 min). At the end of the field season, samples were transferred to the UK on dry ice and then stored at -80 degrees Celsius until analysis for prolactin.

Prolactin Radioimmunoassay

The prolactin assay was carried out at the Roslin Institute (University of Edinburgh, Easter Bush, Midlothian, Scotland, UK). Plasma prolactin levels were measured using a highly specific heterologous micro-radioimmunoassay of donkey anti-rabbit serum to European starling (*Sturnus vulgaris*) prolactin (Sharp antibody code 44/2). Prolactin was radiolabeled with iodine¹²⁵ using chloramine-T. 168 (out of a total of 208) samples were assayed in duplicate, and the remaining 40 samples were assayed as singletons (not all samples assayed were for use in this study). All samples were measured in a single assay, in which the intra-assay coefficient of variation for the duplicate samples was 3.31%.

Statistical methods

The above methods yielded a final data set of matched provisioning trait data (estimated from the focal bird's average performance over two mornings of provisioning recordings; see above) and circulating 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. For a small number of these birds, a second matched measure of the provisioning traits and prolactin levels was also available from a subsequent breeding attempt. However, these few repeat measures were not included in our final data set for analysis, as attempts to conduct mixed effects models using bird ID to account for the presence of these repeated measures of individuals typically failed to estimate non-zero variance for bird ID (quite possibly because too few repeated measures were

279 available). All analyses therefore utilized the data from only the first sampling event per bird, obviating
 280 the need to include bird ID as a random effect within our mixed effects models (see below). While some
 281 analyses utilized the whole data set (i.e. $n = 70$ adults birds each sampled once for all traits), others
 282 used subsets of it (e.g. when focusing only on dominants or subordinates), and so the sample sizes for
 283 each analysis are reported within the relevant results section and model output table. As mean
 284 provisioning visit duration data were only available for birds that had a non-zero provisioning rate, the
 285 sample sizes for mean visit duration analyses were sometimes smaller than those for provisioning rate.
 286

287 All statistical models and visualizations were carried out in R (version 4.1.0; R Core Team). Mixed effects
 288 modelling was conducted using the R package 'lme4' (Bates et al. 2015), and the importance of a given
 289 combination of model predictors was assessed using an information-theoretic (IT) approach. Each
 290 modelling exercise started with the specification of a 'global model' containing all predictors of interest.
 291 This global model and all possible simpler models containing subsets of the global model's fixed effect
 292 predictors (including the intercept-only model) were then fitted to the data using maximum likelihood
 293 and ranked based on AICc (Akaike's Information Criterion corrected for small sample size; Burnham
 294 and Anderson 2002). This 'all subsets' approach was appropriate as all simpler models were biologically
 295 plausible. $\Delta AICc$ values (i.e. the difference in AICc between the focal model and the best supported 'top'
 296 model) were then calculated for every model fitted. As lower AICc values are indicative of stronger
 297 statistical support for a given model, the $\Delta AICc$ for the best-supported 'top' model = 0 and models that
 298 attracted less statistical support had progressively more positive $\Delta AICc$ values. We gave consideration
 299 to models within a $\Delta AICc$ value of six (Richards 2008, Richards et al. 2011) and subsequently reduced
 300 this $\Delta 6$ 'top model set' by applying the 'nesting rule' described in (Richards 2008). This rule aims to
 301 avoid the retention of overly complex models that do not improve model fit, by discarding models that
 302 are more complex versions of simpler (nested) models with weaker AIC support (Richards 2008, Arnold
 303 2010). Adding variables with little or no explanatory power to a top model can weaken AIC by less than
 304 6 points, leading to the retention within the $\Delta 6$ top model set of more complex versions of better
 305 supported models, containing such uninformative variables (Arnold 2010). The nesting rule thus reduces

the chance of considering such overly complex models containing uninformative variables (Richards 2008). In the model selection tables presented within the main paper (Tables 1 & 2) each line presents a model from the top model set (i.e. those models within $\Delta AIC_c = 6$ of the best-supported model) after the application of the nesting rule, while each of the fixed effect (predictor) columns presents the estimated effect size for that fixed effect predictor within that model (or is blank if the focal fixed effect was absent from that model). The corresponding full top model sets *prior* to the application of the nesting rule are presented in the Appendices (Tables A1 & A2) in case of interest. The specific modelling exercises conducted for each results section are described below.

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?

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. The two modeling exercises began with an identical global model structure. In addition to the primary predictor of interest, ‘bird class’ (dominant female or dominant male), we fitted the following terms as fixed effect predictors: brood size (the brood size being fed), adult group size (the number of adult group members during the focal nestling provisioning period) and year (a two-level factor capturing the year in which sampling occurred; 2013 or 2014). We fitted both social group ID and brood ID (the identity of the brood being fed) as random effects, retaining them in the model structure regardless of the degree of variance that they explained.

We then used a third mixed effects model with Gaussian error structure to model the causes of variation in the circulating prolactin levels of dominant birds, starting with a global model structure containing the same fixed and random effect predictors as the provisioning trait models just described, but with the addition of two further fixed effects to account for potential methodological effects on prolactin 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.

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

To investigate whether natural variation in prolactin levels predicted continuous variation in the birds' nestling provisioning rates and mean provisioning visit durations at the population level (i.e. when all bird classes were combined) we conducted two mixed effect models (one for each provisioning trait response term), with circulating prolactin concentration as the sole fixed effect predictor (as we have not hypothesized specific mechanisms by which other variables might impact provisioning traits independent of prolactin levels) and social group ID and brood ID as random effects. Mean provisioning visit duration was logarithm transformed for analysis, to normalize model residuals. Inspection of the patterns of the mean prolactin levels and provisioning trait values of the different bird classes (i.e. dominant females, dominant males, natal subordinates and immigrant subordinates) suggested that any such continuous relationship between prolactin levels and provisioning trait values at the population level could be driven principally by the variation in these traits *among* the bird classes (Figures 3a & 3c). In order to then investigate whether the more limited variation in circulating prolactin levels *within* bird classes predicted the *within*-class variation in provisioning trait values, we first mean-centered each birds' prolactin level and provisioning trait values (the log transformed values in the case of mean provisioning visit duration) around the mean value of the focal trait for birds of their class (by subtracting from it the mean value of the focal trait for their bird class). We then conducted two mixed effects models (one for each mean-centered provisioning trait response term), with mean-centered circulating prolactin concentration as the sole fixed effect predictor and social group ID and clutch ID as random effects.

378 RESULTS

379 **1. Are differences in the nestling provisioning rates and nest visit durations of dominant females** 380 **and males (engaged in parental care) mirrored by differences in their circulating prolactin levels?**

381 Analyzing the provisioning behavior of dominant birds engaged in parental care (n = 46 dominants, 20
382 females and 26 males, feeding 31 broods at 28 social groups) revealed strong evidence that dominant
383 females feed offspring at higher rates than dominant males (Figure 1a; bird class was present in all
384 models down to $\Delta\text{AICc} = 42.36$; Table 1) and that the mean provisioning visit durations of dominant
385 females are also longer than those of dominant males (Figure 1b; $\Delta\text{AICc} = 11.49$; Table 1). There was
386 also evidence that brood size positively predicted the provisioning rates of dominant birds ($\Delta\text{AICc} =$
387 1.92; Table 1), but not their mean visit durations (the best-supported visit duration model containing
388 brood size scored 1.42 AICc points below the top model and was rejected under the nesting rule; Table
389 1; Table A1). There was no compelling evidence that the provisioning rates or mean visit durations of
390 dominant birds were associated with either the year of study or adult group size (which were absent
391 from the best-supported model for both traits; Table 1).

392

393 Analyzing the circulating prolactin levels of dominant birds during the provisioning periods analyzed
394 above (again, n = 46 dominants, 20 females and 26 males, feeding 31 broods at 28 social groups)
395 revealed strong evidence that dominant females also have higher circulating prolactin levels than
396 dominant males (Figure 1c; bird class was present in all models down to $\Delta\text{AICc} = 19.93$; Table 1). There
397 was no compelling evidence that the prolactin levels of dominants were associated with group size,
398 brood size, the time lag from sunset to capture or the time lag from capture to blood sampling (none of
399 which were present within the best-supported model; Table 1). Among the considered models that
400 included both of the methodological time lag terms (i.e. those models forced to account for any effects
401 of these terms, regardless of their importance), the best-supported model again contained an effect of
402 bird class (see Table A1; model number 22).

403

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 $\pm$ 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.

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

Model selection of the factors affecting the (i) nestling provisioning rates, (ii) mean provisioning visit durations and (iii) prolactin levels of dominant females ('Dom Fem') and dominant males ('Dom Male') engaged in parental care. The table shows all models within $<6 \Delta AICc$ 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 ($\pm$ 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 $\Delta AICc = 42.36$ in the provisioning rate analysis, all models down to $\Delta AICc = 11.49$ in the mean visit duration analysis, and all models down to $\Delta AICc = 19.93$ in the prolactin analysis. N = 46 dominant birds, 20 female and 26 male, feeding 31 broods at 28 social groups for the provisioning rate and prolactin level analyses. The sample size for the mean provisioning visit duration analysis was slightly smaller (n = 44 dominant birds), as 2 dominant males did not provision their brood, leaving us without a measure of their mean visit duration. Table A1 presents these top model sets prior to application of the model nestling rule.

Intercept	Bird Class (Dom Fem vs Dom Male)	Brood Size	Group Size	Year	df	AICc	$\Delta AICc$
(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 $\Delta AICc = 10.64$; Table 2). There was also evidence that brood size positively predicted subordinate provisioning rates ($\Delta AICc = 2.13$; 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 $\Delta AICc = 4.15$; Table 2). There was also evidence that subordinate prolactin levels were negatively associated with group size ($\Delta AICc = 4.15$) and very weak evidence that they were higher in the second year of study ($\Delta AICc = 0.02$). 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 $\pm$ 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 $<6 \Delta AIC$ 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 ($\pm$ 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 $\Delta AICc = 10.64$ 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	$\Delta AICc$
(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

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

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

Figure 3. At the population level, considering all bird classes together, natural variation in circulating prolactin levels predicts variation in both (a) provisioning rate and (c) mean provisioning visit duration. These relationships are driven principally by differences among the mean trait values of the different focal bird classes (presented $\pm$ S.E. by the squares and diamonds within panels a and c; see legend within panel a). Follow-up analyses revealed no evidence that variation in prolactin levels *within* these bird classes predicted within-bird-class variation in either (b) provisioning rate or (d) mean provisioning visit duration. In panels a and c the line and shaded ribbon present the predicted mean relationship and its standard error, while the shaded circular points within all panels present the raw data points.

535 DISCUSSION

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

555

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

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

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

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

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

Our analyses revealed evidence that the circulating prolactin levels of subordinates were lower in larger social groups, but that the same relationship was not apparent among dominants. This finding is notable in that it parallels the apparent effects of group size on survival in this study population: subordinate birds are markedly less likely to survive in larger groups, but the same is not true for dominants (O'Callaghan & Young *unpublished data*). This survival relationship may be driven by enhanced foraging competition in larger groups (which subordinates may suffer from to a greater extent than dominants, given their lower rank), as territory size does not increase proportionally with group size (Martin-Taylor 2018; likely leaving resources more strongly contested in larger groups). Accordingly, subordinates in larger groups show significantly lower body condition (O'Callaghan & Young *unpublished data*); a relationship that could conceivably account for their reduced prolactin levels in larger groups too. Chronic exposure to nutritionally stressful conditions is often associated with reduced prolactin levels; a relationship that could be due in part to negative effects of circulating corticosterone on prolactin release (Delehanty et al. 1997, Criscuolo et al. 2005, Angelier and Chastel 2009, Riechert et al. 2014, Angelier et al. 2016). Indeed, as the Kalahari experienced drought conditions during the two years of this study, 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

(when their low relatedness to the brood would markedly reduce their indirect fitness payoff from helping; Harrison et al. 2013a).

Conclusion

Our findings lend new support to the hypotheses that helping behavior in cooperatively breeding societies has shared mechanistic underpinnings with parental care, and that prolactin plays a key role in this pathway (see also Vleck et al., 1991; Schoech et al., 1996; Khan et al., 2001; Carlson et al., 2006). Our findings and their complexity highlight the need for experimental studies to investigate both the causality and nature of the relationship between prolactin and provisioning in this species, in both parental and cooperative contexts. Our findings also highlight that attempts to understand the evolution of cooperative helping may benefit from attention to the possibility of genetic constraints on the independent optimization of cooperation and parenting. Our study has implications too for the growing interest in the mechanistic origins of consistent individual differences in cooperative behavior (Sanderson et al. 2015, Dantzer et al. 2019). Specifically, our findings highlight that such differences could arise from consistent individual differences within the pathway by which prolactin acts (e.g. via differences in prolactin secretion and/or reception; Ohkubo et al. 1998, Zhou et al. 1996).

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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 Δ AIC 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.

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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	Δ AICc	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	

Table A2. Top model sets prior to applying the model nesting rule when 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 Nat') and immigrant subordinates ('Sub Imm') engaged in cooperative helping behavior. This table shows all models within $<6 \Delta AIC$ of the top model, *prior to* applying the model nesting rule (Richards et al., 2011; see methods). Table 2 in the main paper presents the subset of these models that were retained *after* applying the model nesting rule (which are flagged here with 'YES' in the 'Retained' column). The grey lines indicate the response term for the set of models beneath it. The presence of a continuous variable within a focal model is indicated by the presence of its effect size estimate, while the presence of factorial variable is indicated with a '+'. The 'Year' effect is the change from 2013 to 2014. 'Int' = Intercept. 'df' = degrees of freedom.

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	$\Delta AICc$	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	