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Drivers of variation in egg size in a cooperative breeder with a redirected helping system

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Abstract

Females are expected to balance the benefits of current reproductive investment against the costs of that investment for future reproduction. Egg size may be subject to this trade-off, the outcome of which may depend on the intrinsic characteristics of the laying female or the environmental conditions that she encounters, such as weather and food supply. In addition, a female's social environment may affect egg investment: in some cooperatively breeding species, females adjust egg investment according to the availability of help at the nest. In this study, we used long-term data and a field experiment to investigate the factors influencing egg size in the long-tailed tit *Aegithalos caudatus*, a cooperative breeder with a redirected helping system and relatively variable egg size. We show that females laid eggs of a consistent size within and across clutches and that skeletally larger females laid larger eggs. However, we found no evidence that environmental conditions or social environment influenced egg investment. Therefore, egg size appears largely to be an intrinsic characteristic of individual females. We discuss the importance of the predictability of future conditions for females when making investment decisions during egg-laying and stress the need for further studies of pre-laying investment in a wider range of cooperative breeding systems.

Keywords: Egg size, reproductive investment, load-lightening, life history, long-tailed tit

Introduction

Life-history theory proposes that increases in parental investment are beneficial to offspring fitness but detrimental to parental survival, resulting in a trade-off between parents' current and future reproductive investment (Stearns, 1992). Egg size, representing the resources invested in each offspring prior to hatching, can affect both offspring and maternal fitness, and therefore may be subject to such a trade-off (Bernardo, 1996). In birds, larger eggs are more likely to hatch and produce offspring with improved growth and survival rates (Krist, 2011). However, egg production is costly to mothers (Nager, 2006; Pick et al., 2016), as it requires investment of finite resources. Accordingly, laying females may need to redirect resources away from self-maintenance or increase their rate of foraging, placing themselves at a greater risk of predation (Bernardo, 1996; Nager, 2006). To maximize their lifetime reproductive success, mothers are expected to navigate these fitness costs and benefits, which may vary across environmental and social contexts, and invest optimally in each egg (Bernardo, 1996; Stearns, 1992).

Egg size often varies dramatically within bird populations, but individual repeatability and heritability estimates are generally high relative to other reproductive traits, suggesting that egg size is intrinsic to laying females (Christians, 2002). Indeed, some of the individual variation in egg size can often be attributed to characteristics such as body size and age, the largest eggs being laid by larger (Fortuna et al., 2021; Verhoeven et al., 2019) and older females (Ardia et al., 2006; Kontiainen et al., 2008). These trends may result from constraints imposed by the size or maturation of individuals' reproductive systems (Pick et al., 2016), or by the attainment of breeding and foraging experience, facilitating more efficient resource acquisition (Daunt et al., 2007; Verhoeven et al., 2019). In some species, however, per-egg investment decreases as individuals reach the oldest age classes, perhaps as a symptom of senescence (Oro et al., 2023; Sydeman & Emslie, 1992; Vedder et al., 2022).

Although most of the variation in egg size within populations tends to be found between individuals, females may adjust their investment in eggs. This within-individual variation may exist because external environmental conditions influence breeding females' ability or incentive to produce large eggs. For example, many studies report positive associations between environmental food availability and egg size (Ardia et al., 2006; Kontiainen et al., 2008; Martin, 1987; Sorensen et al., 2009). In corroboration, breeding females often respond to experimental nutrient supplementation by laying larger eggs (De Neve et al., 2004; Hargitai et al., 2013), although other studies find no such effect (Christians, 2002). Climatic factors can also drive variation in egg size, favourable conditions being associated with the production of larger eggs (Langmore et al., 2016; Saino et al., 2004). This association may be confounded by the tendency for favourable conditions to increase food availability, e.g., insect biomass (Recher et al., 1996). Alternatively, elevated thermoregulatory costs in unfavourable conditions may leave breeding females with fewer resources for egg investment (Salvante et al., 2007; Stevenson & Bryant, 2000).

The social environment of breeding females has also been proposed to influence their egg investment, by altering the

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costs and benefits of laying relatively large or small eggs (Russell et al., 2007; Savage et al., 2015; Taborsky et al., 2007). Cooperative breeders, in which helpers supplement the parental care of a breeding pair's brood, are ideal for testing this hypothesis because the number of helpers often varies between nests. Russell et al. (2007) proposed that female superb fairy-wrens Malurus cyaneus should lay smaller eggs in the presence of helpers because helpers will compensate for this reduced prenatal investment by increasing the overall provisioning of hatched broods. Thus, breeding females can reduce investment costs without impairing offspring fitnessan example of load-lightening (Crick, 1992) at the egg-laying stage. While Russell et al.'s (2007) hypothesis was supported by their own study and some others (Canestrari et al., 2011; Santos & Macedo, 2011), evidence that cooperative breeders adjust egg investment depending on levels of help is currently equivocal. Other studies have found that females increase investment in eggs when more helpers are present or social groups are larger (Valencia et al. 2016; Capilla-Lasheras et al. 2023; Van de Loock et al. 2023), which may maximize offspring fitness when post-hatching conditions are likely to be good. However, a meta-analysis, first performed by Dixit et al. (2017) and updated by Fortuna et al. (2021), suggests that there is no general trend for cooperative breeders to adjust egg investment with increasing helper number. The social organization of cooperative breeding systems is diverse, with variations in the types and timing of help that breeders receive (Rubenstein & Abbott 2017); to understand the social effects on egg investment across species, it is necessary to study species across that broad range of social systems.

Here, we investigate the drivers of variation in egg size in long-tailed tits *Aegithalos caudatus* using a long-term dataset spanning 13 years of egg mass measurements and a supplementary feeding experiment. The study represents the first exploration of per-egg investment in a cooperatively breeding species with a redirected helping system, in which helpers are failed breeders that redirect their care towards the offspring of other individuals, rather than attempting to breed again (MacColl & Hatchwell, 2002).

We test three hypotheses, the first being that the intrinsic characteristics of breeding females predict the size of eggs that they lay. Specifically, we investigate the influence of female body size and age on egg investment, predicting that egg size increases with body size and that older, more experienced breeders produce larger eggs. We also evaluate the repeatability of egg size within individuals and assess whether egg size is associated with two other measures of a female's reproductive performance—clutch size and lay date.

The second hypothesis we test, using long-term data and a field experiment, is that environmental conditions influence mothers' per-egg investment. Long-tailed tits are small (7-8g) and lose significant proportions of their body mass overnight, especially at lower temperatures (Hatchwell et al., 2009). Consequently, unfavourable periods of weather may diminish the resources breeding females can allocate towards egg production. The summed mass of eggs in a long-tailed tit clutch typically exceeds the body mass of laying females (see Results), so the availability of nutrients may determine their capacity to invest in eggs. Here, we test for effects of natural variation in temperature and rainfall, and experimental variation in food availability, on egg size.

Finally, we test the hypothesis that the social environment of breeding females influences their per-egg investment. In typical cooperative breeders, helpers are offspring that have delayed dispersal from their natal territory (Brown, 1987) and are therefore present during their mother's egg-laying period, making help highly predictable. In contrast, the redirected helping system of long-tailed tits means that helpers almost always arrive after eggs have hatched (Hatchwell et al., 2004). Therefore, females must anticipate the help they will receive before it has arrived if they are to adjust egg investment. Two findings suggest this might be possible. First, long-tailed tits have active kin discrimination and helpers are generally close relatives of one of the breeding pair (Leedale et al., 2020; Sharp et al., 2005). Second, social associations during the non-breeding season predict future helping (Napper & Hatchwell, 2016). Egg-laying females may therefore be aware of any potentially helpful relatives living nearby. We assess whether the size of eggs laid by females depends on whether they are subsequently helped in raising their offspring.

Methods

Study system

Long-tailed tits are small, cooperatively breeding passerines in which helpers are failed breeders (MacColl & Hatchwell, 2002). The high predation rate of long-tailed tit nests (~72%) means that approximately half of all successful breeding attempts are completed with 1-8 helpers present at the nest (Hatchwell et al., 2013), most of which are males (82%; Leedale et al. 2018). The variation among nests in help received makes long-tailed tits an ideal system for investigating the effect of helping on life-history traits such as egg size.

This study was conducted in the Rivelin Valley, Sheffield, UK (53°23'N, 1°34' W). The site is ~2.5km², comprising farmland and areas of deciduous woodland and scrub. Individuals were ringed with unique colour combinations under British Trust for Ornithology licence, allowing them to be identified and their reproductive attempts monitored. We used the mass of eggs as a measure of breeding females' peregg reproductive investment. While this may overlook any differential investment in specific macro- and micronutrients that may have consequences for parental and offspring fitness (Bourgault et al., 2007), overall egg size is generally considered a good estimate of the total energy stored within an egg (Williams, 1994).

Long-term dataset

Egg masses were recorded in clutches laid in most years between 2011 and 2024 (n = 153 clutches in 10 years). During the breeding season (late February-early June), nests were located by following adult birds and monitored every 1-3 days. Once lined, nests were checked manually for eggs and the laying date of the first egg was recorded. Approximately eleven days later (mean \pm SD = 11.03 \pm 0.95 days after clutch initiation), following clutch completion, the size of the clutch was recorded, and all eggs in those nests were temporarily extracted using a spoon and weighed to the nearest 0.001g with a Tanita 1230 digital scale. The mass of avian eggs declines during incubation through water loss (Rahn & Ar, 1974). However, all eggs were measured soon after incubation had started (mean \pm SD = 2.59 \pm 1.34 days following initiation of incubation, assumed to be the day the last egg was laid), and the estimated number of days spent incubating before eggs were weighed did not significantly influence the mass of eggs recorded in this study (LRT: $\chi^2 = 0.355$, df = 1, p = 0.551). We were unable to record the order in which eggs were laid in each nest, even though laying order has been shown to impact egg size and composition in other systems (Fortuna et al. 2023). However, because we sampled complete clutches, we obtained a representative measure of the variation present within each clutch.

In addition to egg masses, other characteristics of females and their breeding attempts were recorded. Relative lay date for each clutch was calculated as the number of days after 1 March of that year that the first egg in that clutch was laid. The tarsus length of breeding females was measured to the nearest 0.1 mm using callipers, to index their skeletal body size (Garnett, 1981). Females' ages were known precisely if they had hatched within the study area and been ringed as chicks. Immigrant adult females were assumed to be one year old at the time of ringing because juveniles disperse during their first winter (Hatchwell 2016). Finally, the number of helpers at each nest was counted during routine nest monitoring and 1 hr provisioning watches at two-day intervals throughout the nestling stage from day 2 (hatch date = day 0) to either fledging (typically days 16-18) or nest failure (Halliwell et al. 2022).

To investigate the weather's influence on egg size, daily minimum temperatures, and rainfall measurements were obtained from Weston Park Weather Station, ~5 km from the study site (53°38'N, 1°49'W). Temperatures recorded here strongly correlate with temperatures recorded on-site (Gullett, 2014). Minimum temperatures were used because, in temperate environments, the coolest temperatures are likely to have the most impact on the energy expenditure of small passerines (Hatchwell et al., 2009). These daily temperature and rainfall measurements were averaged across the prelaying period for each clutch, arbitrarily judged to begin 20 days before the first egg was laid and to end the day before the last egg was laid. This is because weather conditions in the weeks leading up to egg-laying influence female body condition and egg investment in other species (Langmore et al., 2016; Lejeune et al., 2016), and because weather influences other breeding traits in long-tailed tits several weeks before breeding starts (MacColl & Hatchwell, 2002). Allowing the pre-laying period to begin 10 or 5 days prior to laying, rather than 20 days, produced qualitatively identical results.

Supplementary feeding experiment

During the breeding seasons of 2022 and 2023, experiments were conducted to test whether supplementing female longtailed tits with food influenced the size of eggs they lay. As nests were discovered, they were assigned to one of two groups: (i) a fed treatment, in which pairs received supplementary food in the form of suet blocks; or (ii) a control treatment, in which pairs were not provided with feeders. Nests were numbered in the order in which they were discovered and assigned to each treatment alternately. This controlled for any variation in egg mass with laying date or location. Inaccessible nests, e.g., those built high in trees, were skipped when assigning treatments. If two or more nests were built in close proximity (within 75 m), they were assigned the same treatment because long-tailed tits are weakly territorial so pairs may have used feeders positioned at closely neighbouring nests.

Suet blocks containing insects were used as an energy-rich food source. Feeders were hung \sim 1–2 m above the ground, \sim 25 m from the nests of the pairs to which they were assigned. This was close enough that focal females could easily locate

and travel to and from their respective feeders, but far enough that their nests would not be disturbed by other animals attracted by the food. Feeders were provided as soon as nests were discovered, checked every 1-3 days, and replenished once emptied. Feeding continued throughout egg-laving and incubation, until eggs hatched. If nests were predated during incubation, females continued to be fed so eggs from any renesting attempts could be included in analyses. There was unavoidable variation in the timing of feeder placement relative to the start of laying because the stage at which nests were found varied and the date of laying is unpredictable. The mean interval between placement of a feeder, or the failure of their previous nest, and laying of the first egg was 17.73 ± 5.05 SD days (*n* = 26 attempts), with just one nest having an interval<11 days; exclusion of this nest from the dataset did not qualitatively influence the result. Nests were monitored and egg masses were measured as described above. Some nests were depredated before clutch completion; here we report only on the experimental and control nests that survived long enough for eggs to be counted and weighed.

In 2022, feeders at 14 nests were filmed for 1 hr while each pair was lining their nest (i.e., before laying) and for one hour during egg-laying. This footage was reviewed to verify that exclusively the focal pairs at each nest (identified from their colour rings) were using their assigned feeders. At 10/14 feeders, the focal pair were indeed the only individuals observed feeding, with focal females visiting feeders an average of 1.25 ± 1.2 SD times per hour. However, at two feeders, the focal females were not observed feeding and, at three feeders, members of non-focal pairs were observed feeding. If these non-focal individuals had been originally assigned the control treatment, they were instead assigned to the experimental group in analyses because they were using a supplementary food source. Accordingly, two pairs originally assigned the control treatment were reassigned to the experimental treatment group in analyses. In 2023, direct observation of feeders using binoculars was used to confirm that all 10 focal females in the fed treatment used the supplementary food; no control females were seen at feeders. In total, 26 pairs were analysed as belonging to the fed treatment, and 29 to the control treatment.

Statistical analyses

All analyses were performed using R v. 4.4.1 (R Core Team, 2021). To test for intrinsic, environmental, and social effects on egg mass in long-tailed tits, linear mixed-effects models (LMMs) were fitted using the lme4 package (Bates et al., 2015). In all mixed models, egg mass was the response variable, and female ID and year were included as random effects. This was to account for the non-independence of eggs laid by the same female and laid in the same year. Nest ID was not included as a random effect as most females had only a single clutch in the dataset, and including nest ID did not improve model fit. All models assumed a normal distribution of residuals and diagnostic plots were used to assess the linearity and normality of residuals. P-values were generated using likelihood ratio tests (LRTs), comparing the final model with a reduced model that lacked the explanatory term in question. Parametric bootstrapping was used to verify significant results. Additionally, R² was calculated for final models using the rsq package (Zhang, 2022). For Figures 3 and 4, estimated marginal means were calculated using the emmeans package (Lenth 2024).

The adjusted repeatability of egg mass within females was estimated by fitting our final model using the *rptR* package (Stoffel et al., 2017). This package calculates the uncertainty in its estimates using parametric bootstrapping. As a test of the repeatability of egg size across individuals' breeding attempts, a linear regression was used to compare the mean mass of eggs from females' first breeding attempt in the dataset with the mean mass of eggs in their second attempt (n = 32) attempts by 16 females). In addition, a linear regression of the mean egg mass in a clutch against the mean mass and tarsus length of nestlings in the corresponding brood (n = 66)clutches/broods) was used to test whether larger eggs produce larger offspring (Garnett, 1981). Nestling mass (± 0.1g) and tarsus length (± 0.1 mm) were measured 11 days (± 1 d) after hatching. The mean nestling tarsus length was normalized relative to the maximum value in the dataset and logarithmically transformed to ensure model assumptions were met. These assumptions were checked by producing residuals vs fitted values, Q-Q and scale-location plots. Helpers increase the total brood provisioning rate (Hatchwell et al., 2004), so to control for any effect of this difference on nestling growth, helper presence/absence was included in the model.

To identify the factors driving egg mass in the long-term dataset, a model was fitted that included the following fixed effects, based on a priori predictions: female tarsus length, estimated female age, clutch size, relative lay date, average daily minimum temperature during the pre-laying period, average daily rainfall during the pre-laying period, and whether helpers were present or absent during the nestling period; we included only those clutches for which all information was available (n = 571 eggs from 60 clutches laid by 57 females; Supplementary Table S1). We included supplementary fed nests in this long-term analysis, as excluding them had no qualitative effect on the results. All breeding attempts that did not reach fledging were excluded because helpers can arrive late on during a nesting attempt, so it cannot be assumed that nests that failed earlier would not have received help. An alternative model was run using the number of helpers at the nest, rather than a simple absence vs presence variable, in case there was an additive effect. Interactions between temperature and rainfall, and between both weather variables and helper presence were also included because weather variables can act on egg size synergistically, and helper effects can modulate weather effects (Langmore et al., 2016). However, as none of these interactions were significant, they were not included in the final model. To account for the possibility of a non-linear effect of age on egg size, we included both linear and quadratic terms (i.e., age and age²). Age² was nonsignificant so we removed it from the final model, though we acknowledge that the underrepresentation of older females in the dataset may have reduced the detectability of any quadratic relationship (Supplementary Table S2). p-Values for terms not included in the final model were obtained by individually reintroducing them into the model.

To analyse data collected in the supplementary feeding experiment (n = 487 eggs from 55 clutches; Supplementary Table S3), a mixed model was constructed with treatment group (fed vs. control) as the only fixed effect. This analysis included all breeding attempts that reached egg-laying, including those that later failed, as helper presence was not included in the model. To verify that no other predictors were confounded with treatment group, we confirmed that there were no significant differences in clutch size (Supplementary Table S4), female tarsus length, female age, lay date, and pre-laying weather conditions between fed and control nests.

Results

Egg mass ranged from 0.640 g to 1.144 g (mean = 0.891 g \pm 0.068 SD, n = 1,369 eggs from 153 clutches), making the largest egg in the population 79% larger than the smallest. An average egg was 11.5% of mean female body mass (7.66g \pm 0.56 SD) in this population, and an average clutch of 9.75 eggs therefore constitutes 112% of a female's body mass.

The final model explained 52% (conditional R^2) of variation in egg mass, with random effects (female ID and year) explaining 49% and fixed effects (female tarsus length) explaining only 4% (marginal R^2) of the overall variation. The mean mass of eggs in a clutch did not significantly predict the mean body mass (LM: F = 2.07, df = 1,63, p = 0.155) of the chicks in broods that hatched from those eggs by the time they were 11 days old. Helper presence was included as a covariate but did not have a significant effect (LM: F = 2.43, df = 1,63, p = 0.124). The within-clutch mean egg mass also failed to predict mean within-brood tarsus length (LM: F = 1.22, df = 1,63, p = 0.272) and again helper presence had no significant effect (LM: F = 0.289, df = 1,63, p = 0.593).

Intrinsic female characteristics

For females with repeat clutches, the mean mass of eggs from their first breeding attempt predicted the mean mass of eggs from their second attempt (LM: F = 14.68, df = 1,14, p = 0.002; Figure 1). Adjusted repeatability of egg size across all breeding attempts in the dataset was 0.407 (SE = 0.075, CI = [0.255, 0.547], p = 0.001).

Female tarsus length was significantly positively associated with egg mass (table 1; Figure 2), indicating that egg size scaled with the skeletal body size of females. No significant relationship was detected between egg mass and either female age, clutch size or lay date (table 1; Supplementary Figures S1–S3).

Environmental conditions

No significant relationship was detected between egg size and either mean rainfall or mean minimum temperature during the pre-laying period, nor for the interaction between these weather variables (Table 1; Supplementary Figures S4 and S5). Furthermore, food supplementation had no effect on egg size, as females provided with suet did not lay significantly larger eggs than control females that received no supplementary food (LRT: $\chi^2 = 0.37$, df = 1, p = 0.543; Figure 3).

Social conditions

Females that were helped laid eggs that were no different in size from those laid by females that received no help (Table 1; Figure 4). Considering the number of helpers assisting breeding females, rather than the presence or absence of helpers, also revealed no significant effect on egg size (LRT: $\chi^2 = 6.99$, df = 5, p = 0.221; Supplementary Figure S6). The availability of helpers did not significantly interact with temperature or rainfall in affecting egg mass (Table 1). Additionally, we confirmed that clutch size adjustment was not employed as an alternative strategy for modifying brood investment based on helper availability (Supplementary Table S5).

Discussion

We investigated whether intrinsic female characteristics, environmental factors, and social conditions influenced per-egg investment in the long-tailed tit, a cooperative breeder with a redirected system of helping. Egg size varied considerably within the study population, the largest egg being 79% larger than the smallest. Individual females laid similarly sized eggs within and across breeding attempts, a small but significant portion of this variation is explained by skeletally larger females laying larger eggs. However, egg size was not predicted by female age, lay date, or clutch size. Natural variation in weather conditions was not significantly related to egg investment, nor was experimental supplementation of some



Figure 1. Association between the mean mass of eggs in females' first vs. second recorded breeding attempts. Line (\pm 95% CI) represents predicted values.

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pairs with food. Finally, the social environment of breeding females was not related to the size of eggs they laid.

Life-history theory suggests that larger eggs produce fitter offspring (Bernardo, 1996; Krist, 2011; Stearns, 1992) by providing developing chicks with greater nutrition, thus improving their body condition and growth. However, we found no relationship between the mean size of eggs in a clutch and the mean body mass or size in the broods of 11-day-old nestlings that hatched from them. This could be because variation in the amount of post-hatching care received by nestlings quickly obscured any relationship between egg and nestling size at hatching. We accounted for variation in helper availability, which influences the provisioning rate (Hatchwell et al., 2004), but numerous other factors also affect the provisioning rate (Adams et al. 2015) making it hard to isolate the effect of egg size on the mass of 11-day-old chicks across nests.

Repeatability of egg size in our population was 0.407, suggesting a large amount of variation in this trait is explained by among-individual differences, though this score is lower than that found in many other bird species (Christians, 2002). Mean within-clutch egg mass was also repeatable across females' breeding attempts, suggesting females lay eggs of relatively consistent size between seasons. These results may be explained if egg size is a highly heritable trait, as has been evidenced in many other species (Christians, 2002). The tarsus length of laying females was positively associated with egg mass, indicating that skeletally smaller females tend to be more physically constrained in their egg-laving than larger females. However, less than 4% of the variation was explained by this effect. The size of more specific anatomical features may more directly determine egg size. For example, Pick et al. (2016) found that, while egg size could be selected independently of body size in Japanese quail (Coturnix japonica), it was strongly correlated with the size of individuals' reproductive systems. In long-tailed tits, tarsus length may be loosely correlated with the size of more critical aspects of their reproductive anatomy, explaining the positive relationship observed. It is likely that had we measured reproductive tract size, it would have explained a greater proportion of the variance in egg size than tarsus length did.

 Table 1. LMM analysis of long-term egg mass data (n = 571 eggs from 60 clutches). Estimates and standard errors are provided only for explanatory terms included in the final model (see methods).

Explanatory term	χ^2	df	þ	Estimate	SE
Intercept				0.579	0.197
Female tarsus length	4.96	1	0.026	0.019	0.009
Female age	0.25	1	0.617	-0.002	0.004
Female age ²	2.15	1	0.143		
Clutch size	0.19	1	0.667	-0.003	0.007
Lay date	0.89	1	0.369	-0.001	0.001
Temperature	0.50	1	0.478	-0.007	0.008
Rainfall	1.08	1	0.300	-0.007	0.005
Helper presence	0.10	1	0.747	0.003	0.011
Temperature × Rainfall	0.29	1	0.593		
Temperature × Helper presence	2.46	1	0.117		
Rainfall × Helper presence	2.80	1	0.094		
Temperature × Rainfall × helper presence	0.64	1	0.424		

Figure 2. Association between egg mass and the tarsus length of laying females. Line (\pm 95% CI) represents prediction drawn from the final LMM

Egg size was unrelated to the age of breeding females, suggesting that the youngest breeders in the population are capable of laying eggs that are as large as those laid by the most experienced breeders. The relatively short lifespan of long-tailed tits (Roper et al. 2022) may explain why egg size is much more consistent across ages in this species than it is in species with longer lifespans (Verhoeven et al., 2019). The absence of a decline in egg investment is consistent with Roper et al. (2022), who found no evidence of senescent decline in other aspects of reproductive performance in long-tailed tits. While egg size is a trait that is intrinsic to laying females, there is still considerable variation found within females. To resolve whether any of this variation can be attributed to plastic adjustments by individuals across contexts, a larger number of clutches laid by the same individuals should be sampled as their intrinsic, environmental, and social circumstances change (Fortuna et al., 2021).

We hypothesized that environmental conditions would significantly influence breeding females' per-egg investment by altering thermoregulatory costs and the availability of food, and thus the resources females could allocate to each egg. However, neither minimum temperature nor mean rainfall prior to laying had a significant influence on egg size. In a prior study of long-tailed tits, weather variables were associated with recruitment rates, but not clutch size, brood size, or nestling mass (Gullett et al., 2015). Thus, it appears that environmental conditions are more important to individuals after they fledge than they are to levels of parental investment prior to fledging.

Consistent with this conclusion, experimentally supplementing females with food did not cause them to produce larger eggs than control females (Figure 3), providing a strong indication that food availability does not alter long-tailed tits' investment in eggs. This aligns with a meta-analysis suggesting that other reproductive traits respond to food supplementation more often than egg size does (Ruffino et al., 2014). We cannot discount the possibility that some birds assigned to

Figure 3. The masses of eggs laid by females that received no supplementary food (n = 251 eggs in 29 clutches) compared with those laid by females that were supplemented with suet (n = 236 eggs in 26 clutches). Small points represent individual eggs, with the width of point placement being allowed to vary so that densely plotted points can be more easily visualized. Large dots represent estimated marginal means, based on the LMM. Error bars represent 95% confidence intervals.

Treatment

Control

1.1

1.0

0.8

0.7

Egg mass (g) 6'0

Figure 4. The masses of eggs laid by females that received no help (n = 306 eggs in 32 clutches) compared to those laid by females that later received help (n = 265 eggs in 28 clutches). Small points represent individual eggs, with the width of point placement being allowed to vary so that densely plotted points can be more easily visualized. Large dots represent estimated marginal means, based on the final LMM with the helper presence variable also included. Error bars represent 95% confidence intervals.

the control treatment regularly used feeders at fed nests, but video recordings and direct observation of feeders suggested such behaviour occurred only rarely. Another possibility is





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that egg size may be constrained by specific nutrients that were not available in the suet and insect food source used in this study—e.g., calcium, required for eggshell production (Hargitai et al., 2013; Mänd & Tilgar, 2003). Alternatively, nutrients may have been abundant enough in the natural environment that the supplemental food did not significantly increase individuals' capacity to lay large eggs (Bernardo, 1996; Ruffino et al., 2014).

Combined, the weather, lay date, and food supplementation results suggest that females invest in eggs regardless of the thermoregulatory costs they are facing or the availability of food in their environment. These costs may manifest in other ways, e.g., by impairing the condition of females (Gaston & Hipfner 2006; Langmore et al., 2016).

The presence or number of helpers during the nestling stage was not related to female long-tailed tits' per-egg investment. This result contrasts with helper-related egg size adjustments observed in some other cooperative breeding systems (Canestrari et al., 2011; Santos & Macedo, 2011; Capilla-Lasheras et al. 2023; Van de Loock et al. 2023), though it is consistent with the general lack of a relationship found across studies (Dixit et al. 2017; Fortuna et al., 2021). Our result may be attributable to the unusual redirected helping system in long-tailed tits. Laying females would have to reliably predict help before they receive it. This is relatively straightforward for females living in stable social groups, but not in a system where helpers are failed breeders, and the failure of breeding attempts is driven primarily by the stochastic process of nest predation (Hatchwell et al., 2013). Furthermore, some failed breeders do not help, even when they have a relative with whom they are familiar breeding nearby (Leedale et al., 2018; Napper & Hatchwell, 2016). Additionally, as most helpers are relatives of breeding males (Leedale et al. 2018), females would require knowledge of the presence of relatives that are socially associated with their male partner. This combination of factors means that help is probably not sufficiently predictable for female long-tailed tits to adjust egg investment in the way that some other cooperatively breeding species do. Furthermore, even if females were aware that many potential helpers were nesting nearby, reducing egg size would be a risky strategy; all those potential helpers may breed successfully, leaving the focal female with no help and offspring that would be disadvantaged.

In conclusion, egg size in long-tailed tits appears to be primarily an intrinsic characteristic of laying females, with this study finding no evidence that females respond to variations in environmental or social conditions by differentially investing in eggs. This individual repeatability is consistent with what is broadly found across bird species (Christians, 2002). However, substantial variation does exist in the size of different eggs laid by the same females. Repeated sampling of breeding attempts made by the same individuals, across a range of contexts, is required to understand where this variation arises from and whether any of it can be explained by plastic adjustments to ecological circumstances. Finally, adaptive adjustment of egg investment based on helper availability may only be a viable strategy in systems of cooperative breeding with highly predictable levels of help, such as in species with delayed dispersal and stable group membership. Expanding research to include a wider range of cooperative breeding systems will facilitate a comparative analysis of how adjustments in egg investment relate to the predictability of help.

Supplementary material

Supplementary material is available at *Journal of Evolutionary Biology* online.

Author contributions

Joey Baxter (Conceptualization [Supporting], Data curation [Lead], Formal analysis [Lead], Investigation [Equal], Methodology [Equal], Visualization [Lead], Writing - original draft [Lead]), Arleya Baxter (Data curation [Supporting], Formal analysis [Supporting], Investigation [Equal], Methodology [Supporting], Writing - review & editing [Supporting]), and Ben Hatchwell (Conceptualization [Lead], Funding acquisition [Lead], Methodology [Supporting], Project administration [Lead], Supervision [Lead], Writing review & editing [Lead])

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Data Availability

The data underlying this article and the code used to analyse it are available in the Dryad Digital Repository, at https://doi.org/10.5061/dryad.nvx0k6f31.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical approval

Field techniques for the study of long-tailed tits were conducted with permission from the British Trust for Ornithology (Permit C3770).

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