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Early differences in maternal food allocation precede later aggression toward dependent offspring in domestic canaries

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Abstract

Maternal aggression (MA) toward offspring is typically observed near nutritional independence and interpreted as conflict over care duration. In domestic canaries, however, MA arises while offspring still depend on provisioning, raising the question of whether it is associated with pre-existing differences in maternal reproductive strategies or in interactions between females and individual offspring. We tested whether females that later express MA differ in reproductive investment and food allocation rules before aggression begins, and whether nestlings later exposed to MA already differ from their siblings. Midway through the nestling period, we experimentally manipulated hunger and recorded begging and maternal food allocation. MA and noMA females did not differ significantly in initial reproductive investment, overall provisioning rates or within-brood inequality in food allocation. However, they integrated offspring traits differently, with the association between begging duration and food allocation depending on begging order in opposite ways in the two phenotypes. Within MA nests, we found no clear evidence that nestlings later exposed to aggression differed from their siblings in food received, body mass or begging behaviour. Together, these findings indicate that differences in maternal food allocation rules precede MA, while the processes linking them to its onset remain unresolved.

INTRODUCTION

Parents and their offspring share overlapping but not identical fitness interests, resulting in different optima for the allocation of parental resources, as parents are expected to balance investment among all offspring, including future progeny (Trivers, 1974; Mock & Parker, 1997). The resolution of this parent-offspring conflict is often an intermediate outcome, with parental investment representing a compromise between the optima of parents and offspring (Parker & Macnair, 1979). Yet, offspring may attempt to over-demand, and parents may develop counterstrategies to resist exploitation. Thus, the conflict remains a dynamic continuum both in evolutionary and behavioural timescales, with varying alignments of parental and offspring interests (Dawkins & Krebs, 1978; Godfray, 1991; Maynard-Smith & Harper, 2003).

When parental capacity is sufficient and environmental conditions are favourable, parent-offspring conflict may be partly ameliorated, allowing parents to sustain investment across the entire brood and distribute care relatively evenly among offspring, which can ultimately benefit the neediest young (Caro et al., 2016; Davis et al., 1999; Lack, 1947; Pijanowski, 1992; Royle et al., 2002). Conversely, brood reduction may arise when parental rearing capacity becomes constrained relative to initial reproductive investment (Clutton-Brock, 1991). For example, when parents make an initially optimistic investment decision, at laying or birth, but fail to accurately predict future environmental conditions (e.g., food availability), or experience unexpected changes in their own state that reduce the amount of care they can sustain without compromising future reproduction (Clutton-Brock, 1991; Lack, 1947, 1966; Mock & Parker, 1997). Thus, parental strategies are diverse and context dependent, varying among others with parental sex, individual condition, environmental conditions (e.g., Caro et al., 2016; Lessells, 2002;

Smiseth et al., 1998). These strategies can range from promoting brood maintenance and the survival of all offspring to promoting brood reduction and the survival of only a subset of offspring.

These parental strategies can be implemented through specific rules of resource allocation among offspring, which may either favour particular offspring over others or promote a more even distribution of resources across the brood (Caro et al., 2016; Forbes & Mock, 2000; Magrath, 1989; Mock & Parker, 1997; O'Connor, 1978; Zieliński, 2002). Parents may adjust the allocation of resources in response to offspring cues, such as size, and signals, such as tegument coloration or begging displays (Parejo-Pulido & Redondo, 2026). These traits can convey multiple, partially decoupled dimensions of information about offspring: while begging intensity is strongly influenced by short-term hunger and therefore provides parents with information about offspring need (Parejo-Pulido & Redondo, 2026), traits such as body size or colour signals may provide information related to offspring quality, competitive ability or survival prospects (Caro et al., 2016; Dugas & McGraw, 2011; Mock et al., 2011). However, food allocation does not depend solely on parental decisions but is also influenced by offspring behaviour. Offspring may compete for feeding opportunities, and signalling effort can escalate to gain a competitive advantage and increase the likelihood of being selected by parents (Parker et al., 2002; Royle et al., 2002). Offspring may further influence parental investment by exploiting differences in size or competitive performance (e.g., by monopolizing the favourable position within a nest cup) arising from asynchronous hatching (Kilner & Johnstone, 1997; Mock & Parker, 1997). Thus, sibling competition and parental biases act jointly rather than exclusively: by preferentially feeding larger or competitively advantaged nestlings, parents can reinforce small initial asymmetries, amplifying within-brood hierarchies and increasing the likelihood that disadvantaged offspring fail without necessarily reducing overall provisioning effort (Forbes & Mock, 2000; Magrath, 1989; Mock & Parker, 1997).

However, gradual adjustment through biased allocation and sibling competition may not always regulate brood-level demand effectively (Drummond et al., 2025; Moreno, 2012). In such cases, more direct forms of parent–offspring interaction may emerge, ranging from mechanisms that directly alter brood composition, such as facultative siblicide or parental infanticide (O'Connor, 1978; Zielinski, 2002), to parental aggression towards offspring. Within this framework, parental aggression toward dependent offspring could represent a parental allocation rule, through which parents further influence resource distribution among offspring and brood composition (Mock & Parker, 1997; O'Connor, 1978; Zieliński, 2002). Alternatively, parental aggression could arise as a reactive response to offspring behaviour, for example in response to persistently elevated or strategically exaggerated begging, potentially as a way to limit or suppress excessive solicitation (Godfray, 1991; Kilner & Johnstone, 1997; Lessells & McNamara, 2012; Magrath, 1989; Magrath et al., 2010). In birds, parental aggression has also been proposed as a mechanism by which parents encourage offspring to fledge or disperse (Caro, 2005; Leonard et al., 1988; Shizuka & Lyon, 2013). However, when parental aggression occurs while offspring are still dependent on care, rather than near independence, it raises the question of whether it is associated with pre-existing differences in parental allocation strategies or with differences in the interactions between parents and individual offspring.

In this study, we investigated whether maternal aggression (hereafter MA) in the domestic canary (*Serinus canaria*) is preceded by differences in maternal investment and in the rules by which mothers allocate resources among offspring, including their responsiveness to offspring traits (i.e., body size and begging behaviour). In this species, MA is expressed by only a subset of females; this naturally occurring variation in expression of maternal aggression is central to our design, as it allows direct comparison between females that do and do not later become aggressive. MA emerges in the second half of the nestling period, when offspring are still dependent on care (Garcia-Co et al., 2025), allowing us to test whether differences between females, or among their offspring, are already apparent before aggression begins. We tested two alternative, non-exclusive hypotheses: under the maternal strategy hypothesis, MA is associated with pre-existing differences between females in how they invest resources in their offspring. From this hypothesis, we derived two predictions. (i) First, we predicted that MA and noMA females would differ in their overall reproductive investment, considering initial reproductive investment (i.e., clutch size and brood size) and total post-hatching provisioning, and that these differences could also be reflected in maternal body mass trajectories across the breeding attempt. (ii) Second, we predicted that MA and noMA females would differ in their rules of post-hatching resource allocation among offspring, that is, in how they select which nestlings to feed according to their individual traits. Specifically, we predicted differences in how females weight and integrate experimentally manipulated short-term hunger, body mass, and begging behaviour when distributing food within the brood. Thus, these predictions distinguish between how much females invest at the brood level and how they distribute that investment among individual offspring. Alternatively, we hypothesized that MA arises during parent-offspring interactions, so here we predicted that MA is associated with pre-existing differences in the interactions between females and particular offspring that later become targets of aggression. (iii) We therefore predicted that nestlings that later experience MA would already differ from their non-exposed siblings before the onset of aggression, either in the amount of food they received, their body mass, or their begging behaviour.

MATERIALS AND METHODS

Study Species and Breeding

In this study, we used 394 first-year captive Fife fancy canaries over two breeding seasons (2023 and 2024). Birds originated from a local population maintained at the University of Antwerp, supplemented each autumn with new birds from local breeders to ensure genetic diversity. In both years, experiments were conducted under identical conditions and following the same procedures. In February, all individuals selected for breeding were housed for five weeks in single-sex aviaries at 20-24°C with long artificial daylight (14h light: 10h dark; Estramil et al., 2014) before pair formation. We then formed 80 couples in 2023 and 117 in 2024 by placing the male a few days before the female into a breeding cage (50 x 64 x 40 cm^3, GEHU cages, The Netherlands). Mating pairs were created randomly, but pair composition was subsequently checked to make sure that the partners were unrelated. Breeding cages were equipped with two perches, a nest cup, nesting material, cuttlefish bones, shell sand, canary seed

mixture (Van Camp, Belgium), egg food (provided twice a week; Van Camp, Belgium), and water *ad libitum*. After the breeding pairs completed nest-building, we monitored egg laying daily, weighed the freshly laid eggs, and marked them using a non-toxic coloured marker. All clutches were reciprocally cross-fostered two or three days after clutch completion as part of a parallel experiment to assess the inheritance of MA. Foster nests were selected based on mean egg weight (<0.2 g difference), clutch size (±1 egg), and day when the last egg was laid (± 1 day). Because whole clutches were exchanged, every nestling in a foster brood originated from a single biological mother, and each female received only one clutch. MA as measured here is therefore expressed by a female towards a foster brood and not towards her genetic offspring, and the identity of the rearing female cannot be statistically separated from the origin of the brood she received. This applies equally to all females in this study, so that no female reared her own young and differences between MA and noMA females cannot be attributed to the fostering itself. MA also occurs in this population in females rearing their own offspring (Garcia-Co et al., 2025; Garcia-Co et al., in review), so it is not a consequence of cross-fostering.

From day 14 after the first egg of the clutch was laid (i.e., the minimum incubation period), we checked daily for newly hatched nestlings. Upon hatching, nestlings were marked with a non-toxic colour marker for individual identification, and hatching order was recorded. From this point onward, parents were provided with egg food daily and supplemented regularly with freshly germinated seeds. Nestlings were weighed every other day until fledging (≈ 25 days old) with an electronic balance to the nearest 0.01 g. When nestlings reached 7 g, they were ringed with a unique number combination for further individual identification. All nestlings were molecularly sexed at the end of the breeding season using blood samples (50 µl) collected from the brachial vein at fledging, as part of a wider study; sex was not included in the analyses reported here, as it does not form part of the predictions tested.

Experimental hunger-level manipulation and female provisioning responsiveness

We performed an experimental manipulation to test whether MA mothers were equally responsive to variation in offspring hunger (a proxy of short-term need), or whether they ignored such differences. Behavioural testing was conducted when the first-hatched nestling in a nest was between 8 and 11 days old. This testing window was chosen to allow quantification of both male and female provisioning rates. Females are primarily responsible for brooding and provisioning during the first days post-hatching, but from around day 10 onwards, both parents contribute to feeding (Estramil et al., 2014). From the total of 197 breeding pairs, we selected a subsample of nests with at least two nestlings for the experimental hunger-level manipulation (N = 39; 25 nests in 2023 and 14 in 2024). Canaries hatch asynchronously, but as usually 1–2 nestlings hatch per day, age differences among siblings during the tests were minimal.

Before testing, video cameras (Vizu Extreme X6S) were installed at the side of the nest. To standardize hunger levels, we followed a two-step process. First, food was removed from the cages 30 minutes before testing in 2023 and 45 minutes before testing in 2024. The difference in deprivation duration

reflected minor procedural adjustments between field seasons. Both deprivation periods fall within the range commonly used to induce short-term variation in nestling hunger in passerines and were sufficient to elicit measurable changes in begging behaviour (e.g., Parejo-Pulido et al., 2024, 2025). Because the duration differed slightly between years, deprivation duration is completely confounded with year, and the two cannot be separated statistically. Year was retained as a fixed effect in all models.

After the food deprivation period, the entire nest cup (with nestlings inside) was removed from the cage. For identification on the video, nestlings were specifically marked using non-toxic coloured pens (applied to the head and beak tip) and weighed to the nearest 0.01g. Then, all nestlings were hand-fed a mixture of *Orlux Handmix* (1-part *Handmix* to 3 parts water) via syringe until they stopped begging, indicating satiation, and the nest was returned to the cage (Fresneau & Müller, 2016). In addition, to create differences in hunger levels between siblings of the same nest before the start of the experiment, all nestlings remained without food for another 30 minutes, and after this period, a subset of the nestlings were hand-fed again to satiation inside the cage, out of sight of the parents, while their siblings remained unfed. Per nest, the selection of these hand-fed nestlings was based on nestling relative weight: either the heaviest and lightest (nestlings 1 and 3 in broods of ≥3) or the middle-weighted nestling (nestling 2), which provided variation in hunger among nestlings of differing relative size. In nests with only two nestlings, only the heaviest nestling was chosen for the additional feeding whenever the “1-and-3” condition was applied, as no third-ranked nestling was available. The relative weight of the nestlings selected for additional feeding (nestlings 1 and 3 vs. nestling 2) was randomly determined in the first nest and then alternated systematically across subsequent nests. Thus, across the dataset, both heavy and light nestlings were represented in both satiated and hungry treatment. In two-nestling broods this alternation meant that the heaviest nestling received the additional feeding under the “1-and-3” condition and the lightest nestling under the “nestling 2” condition, so that the additional feeding was not systematically assigned to the heaviest nestling. The resulting distribution across within-brood weight rank and brood size is given in Table S2. After this second feeding, a plate with fresh egg food and germinated seeds was provided, as these food items are highly palatable to canaries and reliably elicit parental provisioning. Video recording then started and continued for 1 hour. At the end of the recording period, all nestlings were hand-fed until satiation by the experimenters, and the standard seed feeder was reinstalled.

Determining Nestling Begging Behaviour and Maternal Provisioning

Nestling begging behaviour and maternal provisioning during provisioning trials were scored from recordings using *Solomon Coder* (v19.08.02; Péter, 2019). Before annotation, clear definitions of all behaviours were established, and inter-rater reliability was assessed using randomly selected videos to ensure consistency between observers (FM, BT, and CG). Agreement between CG and FM and CG and BT was very high, indicating strong consistency across observers (ICC = 0.981, $F_{11,11.5} = 114$, $p < 0.001$; and ICC = 0.927, $F_{26,26.2} = 25.5$, $p < 0.001$, respectively).

Maternal provisioning was quantified as the number of food transfers (dips) delivered to each nestling. A dip was defined as a single insertion of the female's beak into a nestling's gape, accompanied by a swallowing response by the chick, thereby excluding exploratory or food-testing contacts (Müller et al., 2010). A provisioning bout was defined as the period beginning when the female landed on the nest cup and ending when she stopped feeding the nestlings for more than 10s, left the nest, or switched to brooding behaviour. For each trial, only the first provisioning bout was analysed. This corresponds to the first uninterrupted provisioning sequence after food presentation and reflects the period during which experimentally induced hunger differences among nestlings were still present (see 'Experimental hunger-level manipulation and female responsiveness' section). Nests in which the male fed first were excluded from the analysis. This was the case in two nests, one of each maternal phenotype, so exclusions were not associated with MA phenotype.

Nestling begging was scored using postural displays, since canaries do not produce begging calls until ~14 days of age. Postural begging intensity was assessed on a five-level ordinal scale adapted from Redondo & Parejo-Pulido (2026): 0 = no begging; 1 = gaping with neck flexed, body horizontal; 2 = gaping with neck extended, body horizontal; 3 = gaping with neck extended, body off the nest floor, back reclining; 4 = fully stretched, vertical back. For each nestling, the duration of time spent at each postural level was first measured separately. Total begging duration was then calculated as the sum of durations across all postural categories. Begging order was determined within each brood by ranking nestlings according to the sequence in which they initiated begging. To account for brood size differences, this rank was standardized to a relative scale ranging from 0 (first to beg) to 1 (last to beg; Smith & Montgomerie, 1991).

Exposure to Maternal Aggression

The criterion for classifying a nestling as exposed to MA was the presence of feather plucking (Garcia-Co et al., 2025). Nestlings were monitored daily for signs of feather plucking from day 14 post-hatching until approximately day 25, when they reached nutritional independence. Importantly, not all nestlings in a nest with a MA female necessarily showed evidence of aggression. For statistical analyses using individual nestling data, MA exposure was therefore coded at the individual level (yes/no) based on visible feather damage. The MA phenotype of the females was instead defined at the nest level, classifying nests according to whether at least one nestling showed signs of feather plucking or not. Of the 39 nests used in the provisioning responsiveness tests, we were able to determine the MA phenotype for 37 nests. In the remaining two nests, either one of the parents died, or all nestlings died before MA typically starts (around day 14). Among the 37 nests for which MA could be assessed, 15 females exhibited MA towards at least one of their nestlings, while the remaining 22 females did not. Across MA nests, between 1 and 3 nestlings per brood showed signs of aggression (median = 2). In total, 27 individual nestlings were classified as MA-exposed and 11 non-exposed within MA broods.

Female Body Mass Trajectories Across the Breeding Season

For a subset of the breeding females in 2024 chosen at random, we measured the body mass using a precision scale to the nearest 0.01g at four relevant time points during the breeding cycle: *Before Breeding* (right before being paired; N=121), at *Clutch Completion* (the day the last egg was laid; N=101), when the *First Nestling Hatched* (N=71), and at *Day 16* (in the last tercile of the nestling period corresponding with high offspring demand and with the onset of MA, which usually starts around day 14; N=60). The decrease in sample size across time points is due to breeding failures (either the female did not mate, or the clutch/brood failed) or the death of either the breeding male or female.

Statistical analysis

All statistical analyses were conducted in R version 4.3.0 (R Core Team, 2025). For each research question, we first specified a full model including all biologically relevant fixed effects and interaction terms (see below for details). All main effects were retained in the models, whereas interaction terms were simplified by backward elimination, starting with the highest-order interactions and sequentially removing non-significant terms. We used linear models and linear mixed models (lme4 package, v1.1-35.2; Bates et al., 2015) to analyse nestling behaviours and generalised linear models and generalised linear mixed models (GLMMs, glmmTMB package, v1.1.7; McGillycuddy et al., 2025) for models analysing maternal provisioning. Provisioning models were fitted using a negative binomial distribution (either nbinom1 or nbinom2), selected based on dispersion diagnostics and model fit. For the provisioning models reported here, nbinom1 was preferred over nbinom2 on the basis of AIC (549.4 versus 561.6). Analyses at the level of the individual nestling (Predictions ii and iii) were restricted to the nestlings whose exposure to aggression could be determined, since nestlings that died during the nestling period may beg atypically as their condition deteriorates. Brood-level quantities, including total provisioning per nest and the within-brood coefficient of variation (Prediction i), necessarily include every nestling present, as does the denominator of each nestling's share of brood total. Sample sizes for each analysis are given in Table S1. Nest identity was included as a random effect in mixed models with repeated measures per brood. P-values for fixed effects were obtained using Wald z-tests. For the three-way interaction under Prediction (ii) we additionally report a likelihood ratio test against the model without that term, as Wald statistics can be unreliable for higher-order interactions at moderate sample sizes (Bolker et al., 2009). Effect sizes are reported with 95% confidence intervals throughout, and interactions are interpreted using model-predicted marginal slopes. Collinearity among offspring traits was assessed using variance inflation factors (VIFs) computed from the corresponding main-effects model; all values were below 2.4. Spearman correlations among begging order, begging duration, maximum begging intensity and nestling relative weight were all ≤ 0.52. Note that maternal phenotype is a nest-level factor, whereas begging traits and relative weight vary within broods; interactions between them are therefore estimated from within-brood variation across all nests rather than from a contrast

between the numbers of MA and noMA females. Model assumptions and dispersion were checked using the DHARMa package (v0.4.6; Hartig, F., 2016). The significance threshold was set at $\alpha = 0.05$. Analyses were structured as follows to test three main predictions:

Prediction (i): Overall maternal investment (full dataset; brood level)

To test whether MA and noMA females differed in initial reproductive investment or overall provisioning effort, we compared clutch size and brood size using Welch's t-tests. Differences in total female provisioning per nest were analysed using a negative binomial GLM. Within-brood variation in food allocation (coefficient of variation of dips per nestling) was analysed using a linear model. Both models included MA phenotype (nest level), brood size, and year as fixed effects. The within-brood variation in provisioning (CV) model was conducted to examine asymmetries in food allocation among nestlings, specifically whether feedings were distributed evenly across offspring or instead biased towards certain individuals. Because these were brood-level analyses, the experimental hunger manipulation was not included as a predictor. We additionally quantified the duration of provisioning bout from the original behavioural coding files, defined as the interval between the female's arrival at the nest and her final food transfer. All provisioning models were repeated including time at the nest as an exposure term (offset).

To test whether MA and noMA females differed in their body mass trajectories across the breeding attempt, we analysed changes in female body mass using an LMM including breeding stage, MA phenotype, total clutch mass, and clutch size as fixed effects, with female identity as a random effect. The interaction between breeding stage and MA phenotype constitutes the hypothesis tested and was therefore retained a priori and not subject to model simplification. The model contains a single interaction term. We report the omnibus test for this interaction, which has three degrees of freedom, together with contrasts that decompose it at each breeding stage relative to the pre-breeding measurement. These contrasts were specified a priori, since the onset of MA around day 16 was the stage of interest. Female body mass was square-root transformed to meet model assumptions, and this transformation is applied consistently in the corresponding table and figure.

Prediction (ii): Post-hatching food allocation among offspring (full dataset; nestling level)

To test whether MA and noMA females differed in how they weighted and integrated different offspring traits when allocating food, we modelled the number of dips received per nestling using negative binomial GLMMs following two different approaches.

1. Responsiveness to hunger and body mass

First, we fitted a model including MA phenotype (nest level), experimental hunger manipulation (hungry vs satiated), nestling relative weight, brood size, and year as fixed effects, plus the interactions MA × hunger manipulation and MA × relative weight. Nest identity was included as a random effect. Final

models retained all main effects and the MA × relative weight interaction. This model tested whether females that later expressed MA differed in their responsiveness to short-term hunger and/or body mass (i.e., whether they differed in the extent to which they favoured offspring according to immediate need or traits associated with offspring quality; Caro et al., 2016).

2. Integration of multiple begging traits in food allocation decisions

To test whether females varied in their food allocation rules more subtly, regarding the way they integrated multiple offspring traits and behaviours, we fitted a second negative binomial GLMM including MA phenotype (nest level), begging order, begging duration, maximum begging intensity, nestling relative weight, brood size, and year as fixed effects. We included interactions between MA and each behavioural predictor and tested three-way interactions in which MA was always included as one of the interacting terms. These interactions were included because food allocation decisions arise from complex dynamics, and we aimed to assess how females integrate multiple offspring traits simultaneously, as well as how these traits interact with one another to influence feeding outcomes (e.g., Parejo-Pulido et al., 2023; Parejo-Pulido & Redondo, 2026). The final model retained all main effects, the three-way interaction begging order × begging duration × MA, and its component two-way interactions. Nest identity was included as a random effect.

Prediction (iii): Offspring differences prior to aggression (MA nests only; nestling level)

To test the additional possibility that aggression reflects early differences in offspring behaviour rather than maternal food allocation rules, we examined whether nestlings that later experienced MA already differed from their non-exposed siblings in the amount of food they received, body mass, or begging behaviour prior to the onset of aggression.

First, we repeated the provisioning models, restricting the dataset to MA nests only, to test whether nestlings later exposed to MA already received differential treatment before aggression occurred. The main predictor was MA exposure at the individual level (yes/no) instead of MA phenotype at the nest level. Models included MA exposure, experimental hunger manipulation, nestling relative weight, brood size, and year as fixed effects. Because this comparison is made between siblings within the same brood, nest identity was included as a random effect. Nestlings for which exposure to MA could not be determined were retained in brood-level totals, since they were present during the trial and received food. The estimated between-brood variance was negligible (standard deviation below 0.001), indicating that variation among broods in number of food transfers received per nestling was already accounted for by the dispersion parameter of the negative binomial distribution. Retaining the term therefore makes little difference to the estimates, but represents the nested structure of the data more explicitly. Because a variance component estimated from a small number of broods is imprecise, we additionally compared broods directly. Aggression was directed at all nestlings in 7 of the 15 MA nests, so 8 nests contained both exposed and non-exposed nestlings. For each of these, we calculated the difference between the

mean share of the brood's food received by exposed nestlings and by their siblings, and tested these values across broods. This treats the brood as the unit of replication and does not require estimating variance components.

Second, we fitted a model to test whether nestlings exposed to MA differed in their size or begging behaviour. Begging order, total begging duration, and maximum begging intensity were modelled as response variables in LMMs including individual MA exposure (yes/no), experimental hunger manipulation, nestling relative weight, brood size, and year as fixed effects, and nest identity as a random effect. Experimental hunger manipulation was also included to test whether MA nestlings exhibited exaggerated begging independently of their short-term hunger level (i.e., at a comparable level of short-term hunger and after accounting for other offspring traits, such as relative body mass). Begging duration was square-root transformed to improve model fit.

RESULTS

Prediction (i): Overall maternal investment

Initial reproductive investment did not differ between MA and noMA females. Clutch size was similar between females that later expressed MA and those that did not (noMA: 3.1 ± 0.7 eggs; MA: 3.2 ± 0.8 eggs; $t_{34} = -0.32$, $p = 0.75$), as was brood size (noMA: 2.7 ± 0.8 chicks; MA: 2.9 ± 0.8 chicks; $t_{23} = -0.87$, $p = 0.39$).

At the nest level, total maternal provisioning did not differ between MA and noMA nests ($p = 0.52$; Table 1; Fig. 1). Within-brood variation in food allocation likewise did not differ between MA and noMA nests ($p = 0.409$; estimate -0.151 ± 0.181, 95% CI -0.519 to 0.217; Table 1). This provides no evidence for a difference in the overall degree of within-brood inequality, but does not establish that food allocation rules were similar between the two groups. Neither brood size nor year significantly predicted total maternal provisioning or its within-brood coefficient of variation. Females did not differ in the time they spent at the nest during the provisioning trial (MA: 42.8 s; noMA: 36.0 s; $p = 0.41$), nor in the latency between arrival and the first food transfer ($p = 0.92$). Food transfers scaled with time at the nest with a log-log slope of 0.76 (SE 0.21). Including time at the nest as an exposure term did not alter any of the results reported below.

	Estimate (SE)	*95% CI*	*p*
Total food transfers			
Intercept	2.794 (±0.666)	1.428 to 4.047	**<0.001**
Maternal aggression	0.144 (±0.222)	-0.297 to 0.577	0.521
Brood size	0.084 (±0.192)	-0.286 to 0.471	0.664
Year	0.155 (±0.271)	-0.361 to 0.705	0.571
Within-brood variation (CV)			
Intercept	0.992 (±0.516)	-0.060 to 2.044	0.064
Maternal aggression	-0.151 (±0.181)	-0.519 to 0.217	0.409
Brood size	0.093 (±0.153)	-0.218 to 0.404	0.546
Year	-0.189 (±0.212)	-0.621 to 0.243	0.379

Table 1. Effects of maternal aggression phenotype on measures of overall maternal investment, fitted at the level of the nest (N = 37 nests: 15 MA, 22 noMA). Total food provisioning was analysed using a negative binomial generalised linear model and the within-brood coefficient of variation using a linear model. Brood-level quantities include every nestling present. Estimates are given with standard errors, 95% confidence intervals and exact p-values.

Female body mass trajectories across breeding stages

Female body mass changed significantly across the breeding period. The interaction between breeding stage and MA phenotype was not statistically significant (F = 2.30, p = 0.07). The a priori contrast at day 16 indicated that MA females were on average 1.41 g heavier than noMA females at this late, high-demand stage (95% CI 0.09 to 2.72, p = 0.036; Table 2; Fig. 2), with no differences detected at earlier stages. Laying a heavier clutch did not have a negative effect on female body mass; on the contrary, total clutch mass had a significant positive effect on female body mass (p = 0.006; see Table 2), whereas clutch size had no effect.

	Estimate (SE)	*dF*	*F value*	*p*
Intercept	4.256 (±0.156)	-	-	**<0.001**
Total clutch mass	0.059 (±0.030)	(1, 87.64)	3.790	0.055
Clutch size	-0.035 (±0.066)	(1, 86.70)	0.285	0.595
Maternal aggression	-	(1, 86.94)	1.113	0.294
Maternal aggression (yes vs. no)	0.031 (±0.066)	(1, 128.80)	0.221	0.637
Time point	-	(3, 213.58)	54.192	**<0.001**
Clutch Completion	0.229 (±0.028)	(1, 212.10)	67.733	**<0.001**
Hatching First Nestling	0.299 (±0.031)	(1, 215.30)	92.930	**<0.001**
Day 16	0.048 (±0.033)	(1, 215.70)	2.190	0.140
MA: Time point	-	(3, 213.63)	2.371	0.071
MA: Clutch Completion	0.031 (±0.049)	(1, 212.20)	0.397	0.529
MA: Hatching First Nestling	-0.024 (±0.051)	(1, 213.63)	0.212	0.644
MA: Day 16	0.119 (±0.054)	(1, 214.70)	4.796	**0.030**

Table 2. Female body mass across four breeding stages in relation to maternal aggression phenotype. Linear mixed model with female identity as a random effect, fitted to square-root transformed body mass (N = 91 females, each measured at four stages: 30 MA, 61 noMA). The interaction between maternal aggression phenotype and breeding stage was specified a priori as the hypothesis under test and was not subject to model simplification. Unshaded rows give the omnibus test for each term, together with the covariates clutch size and total clutch mass; because breeding stage and its interaction with maternal aggression have three degrees of freedom, no single estimate is given for these rows. Shaded rows give the contrasts that decompose those terms: the effect of maternal aggression phenotype, the effect of each breeding stage relative to the pre-breeding measurement, and the phenotype by stage interaction at each stage. The omnibus test for maternal aggression averages over breeding stages, whereas the corresponding shaded row gives the phenotype difference at the pre-breeding measurement, which is why the two differ. Estimates are on the square-root scale, with standard errors, F tests with Kenward–Roger denominator degrees of freedom, and exact p-values. Predicted values back-transformed to grams are shown in Figure 2. Bold values indicate $p < 0.05$.

Prediction (ii): Post-hatching food allocation among offspring

1. Responsiveness to experimentally manipulated hunger and body mass

Across all nests, there was a significant negative effect of the experimentalhunger-level manipulationon food allocation, with satiated nestlings receiving fewer food transfers than hungry nestlings (*hunger-level manipulation*: p < 0.001; see Table 3; Fig. 3). The number of food transfers received also increased with nestling relative weight (p = 0.045; see Table 3), with heavier nestlings receiving more food. The interaction between MA phenotype and nestling relative weight provided no evidence that this relationship differed between phenotypes (estimate 0.204, 95% CI −0.036 to 0.443, p = 0.096; see Table 3). *Brood size* and *year* had no significant effect on food allocation.

	Estimate (SE)	***Z value***	***95% CI***	***p***
Intercept	2.990 (±0.667)	4.48	1.683 to 4.297	**<0.001**
Maternal aggression (MA)	0.313 (±0.251)	1.25	-0.180 to 0.805	0.213
Nestling relative weight	0.144 (±0.072)	2.01	0.003 to 0.285	**0.045**
Brood size	-0.332 (±0.196)	-1.70	-0.715 to 0.052	0.090
Hunger-level manipulation	-0.767 (±0.222)	-3.45	-1.202 to -0.331	**<0.001**
Year	0.079 (±0.276)	0.29	-0.462 to 0.620	0.774
MA: Nestling relative weight	0.204 (±0.122)	1.67	-0.036 to 0.443	0.096

Table 3. Effects of the experimental hunger manipulation and nestling relative weight on the number of food transfers received, in relation to maternal aggression phenotype. Negative binomial generalised linear mixed model (nbinom1, log link) with nest identity as a random effect, fitted at the level of the individual nestling (N = 91 nestlings in 37 nests: 15 MA, 22 noMA). Nestling relative weight is the deviation of a nestling's mass from the mean of its brood. Estimates are on the log scale, with standard errors, Wald Z statistics, 95% confidence intervals and exact p-values. Non-significant interactions were removed during model simplification. Bold values indicate p < 0.05.

2. Integration of multiple offspring traits in food allocation decisions

Maternal provisioning was associated with multiple offspring traits, and these relationships differed between MA and noMA females (see Table 4). There was a significant three-way interaction between begging order, begging duration, and MA phenotype on the number of food transfers received (*begging order* x *begging duration* x *MA*: p = 0.001; see Table 4; Fig. 4), indicating that the combined effect of begging order and duration on provisioning differed between MA and noMA females. The interaction was also supported by a likelihood ratio test comparing the model with and without the three-way interaction (χ^2 = 10.18, df = 1, p = 0.001). Model-predicted marginal slopes (Table S3) showed that the association between begging duration and provisioning varied with begging order in opposite ways in the two

maternal phenotypes. Among nestlings that began begging first, the positive association between begging duration and provisioning was stronger in MA females (0.062, 95% CI 0.035 to 0.089) than in noMA females (0.024, 95% CI 0.001 to 0.047). Among nestlings that began begging last, the pattern was reversed, with a stronger association in noMA females (0.108, 95% CI 0.070 to 0.146) than in MA females (0.040, 95% CI 0.007 to 0.073). Thus, begging order and duration were integrated differently by the two maternal phenotypes: in noMA females, sustained begging had its strongest positive association with provisioning among nestlings that began begging late, suggesting a compensatory relationship between these two begging traits. In MA females, sustained begging had its strongest positive association among nestlings that began begging early, indicating that early and sustained begging reinforced one another in their association with food allocation. Across all females, provisioning increased with nestling relative weight (*nestling relative weight:* p<0.001; see Table 4). Maximum begging intensity showed a marginal positive association with provisioning (p = 0.052; see Table 4). Brood size had a significant negative effect on provisioning (p < 0.001; see Table 4), whereas year had no significant effect.

	Estimate (SE)	***Z value***	***95% CI***	***p***
Intercept	2.900 (±0.604)	4.80	1.716 to 4.084	**<0.001**
Begging order	-2.12 (±0.630)	-3.37	-3.357 to -0.887	**<0.001**
Maximum Begging Intensity	0.249 (±0.128)	1.94	-0.002 to 0.501	0.052
Begging duration	0.024 (±0.012)	2.07	0.001 to 0.047	**0.038**
Nestling relative weight	0.204 (±0.046)	4.41	0.113 to 0.294	**<0.001**
Maternal aggression (MA)	-0.631 (±0.542)	-1.17	-1.693 to 0.430	0.244
Brood size	-0.754 (±0.158)	-4.76	-1.064 to -0.444	**<0.001**
Year	-0.363 (±0.245)	-1.48	-0.844 to 0.117	0.138
Begging order: MA	2.432 (±0.924)	2.63	0.621 to 4.244	**0.008**
Begging duration: MA	0.038 (±0.017)	2.21	0.004 to 0.071	**0.027**
Begging order: Begging duration	0.083 (±0.024)	3.51	0.037 to 0.130	**<0.001**
Begging order: Begging duration: MA	-0.105 (±0.033)	-3.20	-0.170 to -0.041	**0.001**

Table 4. Effects of nestling begging traits and relative weight on the number of food transfers received, in relation to maternal aggression phenotype. Negative binomial generalised linear mixed model (nbinom1, log link) with nest identity as a random effect, fitted at the level of the individual nestling (N = 91 nestlings in 37 nests: 15 MA, 22 noMA). Begging order is the standardised within-brood rank of the latency to begin begging, from 0 (first to beg) to 1 (last to beg). Nestling relative weight is the deviation of a nestling's mass from the mean of its brood. Estimates are on the log scale, with standard errors, Wald Z statistics, 95% confidence intervals and exact p-values. The three-way interaction was

additionally supported by a likelihood ratio test against the model excluding it ($\chi^2 = 10.18$, df = 1, P = 0.001). Marginal slopes derived from this model are given in Table S3 and are the basis for the interpretation in the text**.** Bold values indicate p < 0.05.

Prediction (iii): Offspring differences prior to aggression (MA nests only)

1. Early provisioning differences within MA nests

Aggression was directed at all nestlings in 7 of the 15 MA nests and at a subset of nestlings in the remaining 8. Across all MA nests, nestlings later exposed to MA tended to receive fewer food transfers than their non-exposed siblings, although this difference was not statistically significant (p = 0.063; see Table 5). A similar descriptive pattern emerged from the brood-level comparison restricted to the 8 nests containing both exposed and non-exposed nestlings: exposed nestlings received a smaller share of the brood's food in 7 of the 8 broods, but the overall difference was not statistically significant (mean difference in share −0.29, 95% CI −0.77 to 0.19, p = 0.19; Fig. 5). Food allocation increased with nestling relative weight (p < 0.001; see Table 5). The experimentalhunger-level manipulationalso significantly affected food allocation, with satiated nestlings receiving fewer food transfers than hungry nestlings (p = 0.018; see Table 5). Brood size had a significant negative effect on food allocation per nestling (p = 0.019; see Table 5), while year had no significant effect.

	Estimate (SE)	***Z value***	***95% CI***	***p***
Intercept	4.251 (±0.795)	5.35	2.694 to 5.809	<0.001
Maternal aggression (yes vs. no)	-0.557 (±0.299)	-1.86	-1.144 to 0.030	0.063
Exposure not determined	-0.191 (±0.712)	-0.27	-1.587 to 1.205	0.788
Nestling relative weight	0.329 (±0.096)	3.41	0.140 to 0.518	**<0.001**
Hunger-level manipulation	-0.660 (±0.280)	-2.36	-1.208 to -0.112	**0.018**
Brood size	-0.521 (±0.221)	-2.35	-0.954 to -0.087	**0.019**
Year	-0.033 (±0.343)	-0.10	-0.705 to 0.638	0.923

Table 5. Food transfers received by nestlings later exposed to maternal aggression compared with their non-exposed siblings. Negative binomial generalised linear mixed model (nbinom1, log link) with nest identity as a random effect, fitted at the level of the individual nestling within nests where maternal aggression occurred (N = 44 nestlings in 15 nests). Nestlings whose exposure could not be determined are retained as a separate category, since they were present during the provisioning trial and received food. Nestling relative weight is the deviation of a nestling's mass from the mean of its brood. Estimates are on the log scale, with standard errors, Wald Z statistics, 95% confidence intervals and exact p-values.

The estimated between-brood variance was negligible (standard deviation below 0.001), indicating that variation among broods was already accounted for by the dispersion parameter. Bold values indicate $p < 0.05$.

2. Early differences in offspring traits and begging behaviour within MA nests

To assess whether nestlings later exposed to MA already differed from their non-exposed siblings before aggression began, we compared their relative body mass and begging behaviour within MA nests. Begging order, begging duration, maximum begging intensity, and nestling relative weight did not differ between nestlings that were later exposed to MA and their non-exposed siblings (see Table 6). Thus, we found no evidence that future targets of MA differed in their begging behaviour before aggression, after accounting for experimentally manipulated short-term hunger and other offspring traits. The experimental hunger-level manipulation only had a significant effect on maximum begging intensity, with satiated nestlings showing reduced maximum begging intensity compared to hungry nestlings ($p = 0.009$; see Table 6). Nestling relative weight also had a significant positive effect on maximum begging intensity, with heavier nestlings displaying more intense begging ($p = 0.047$; see Table 6). In contrast, neither begging order nor begging duration was significantly affected by the experimental hunger manipulation or by nestling relative weight. No significant effects of brood size or year were detected for any begging variable (see Table 6).

	Estimate (SE)	95% CI	dF	F value	p
Begging Order					
Intercept	0.557 (±0.443)	-0.345 to 1.459	-	-	-
Maternal aggression	0.037 (±0.161)	-0.291 to 0.364	(1, 28.95)	0.04	0.837
Nestling relative weight	-0.080 (±0.047)	-0.177 to 0.016	(1, 30.43)	2.63	0.115
Hunger-level manipulation	0.224 (±0.144)	-0.070 to 0.518	(1, 24.78)	2.33	0.140
Brood size	-0.052 (±0.120)	-0.296 to 0.192	(1, 13.64)	0.18	0.678
Year	-0.040 (±0.190)	-0.428 to 0.348	(1, 13.69)	0.04	0.840
Begging Duration					
Intercept	6.583 (±2.552)	1.148 to 12.018	-	-	-
Maternal aggression	-1.140 (±0.802)	-2.775 to 0.494	(1, 31.36)	1.78	0.191
Nestling relative weight	0.252 (±0.224)	-0.208 to 0.711	(1, 27.34)	1.17	0.288
Hunger-level manipulation	-1.140 (±0.660)	-2.500 to 0.220	(1, 23.15)	2.90	0.102
Brood size	-0.298 (±0.695)	-1.784 to 1.187	(1, 12.92)	0.18	0.677
Year	-0.319 (±1.104)	-2.673 to 2.036	(1, 13.11)	0.08	0.779
Maximum Begging Intensity					
Intercept	2.555 (±1.077)	0.267 to 4.843	-	-	-
Maternal aggression	-0.045 (±0.383)	-0.827 to 0.738	(1, 30.56)	0.01	0.916
Nestling relative weight	0.242 (±0.112)	0.014 to 0.470	(1, 29.92)	4.30	**0.047**
Hunger-level manipulation	-0.975 (±0.338)	-1.670 to -0.280	(1, 24.44)	8.08	**0.009**
Brood size	-0.042 (±0.292)	-0.665 to 0.581	(1, 13.49)	0.02	0.889
Year	0.937 (±0.464)	-0.053 to 1.926	(1, 13.58)	3.96	0.067
Nestling Relative Weight					
Intercept	-0.927(±1.617)	-4.216 to 2.363	-	-	-
Maternal aggression	-0.899 (±0.569)	-2.057 to 0.259	(1, 30.35)	2.07	0.160
Hunger-level manipulation	0.815 (±0.511)	-0.224 to 1.854	(1, 24.65)	2.48	0.128
Brood size	0.330 (±0.436)	-0.557 to 1.217	(1, 14.03)	0.56	0.467
Year	1.018 (±0.676)	-0.358 to 2.393	(1, 13.57)	2.22	0.159

Table 6. Offspring traits of nestlings later exposed to maternal aggression compared with their non-exposed siblings. Separate linear mixed models with nest identity as a random effect, fitted at the level of the individual nestling within nests where maternal aggression occurred (N = 38 nestlings in 15 nests). Begging duration was square-root transformed; the remaining responses are untransformed. Estimates for maternal aggression refer to the contrast between exposed and non-exposed nestlings, with standard errors, 95% confidence intervals, and F tests with Kenward–Roger denominator degrees of freedom. The estimated between-brood variance was negligible in the models for begging order and nestling relative weight. Bold values indicate $p < 0.05$.

DISCUSSION

In this study, we investigated whether females that later express MA towards dependent offspring differ in their reproductive investment and food allocation rules before aggression emerges, and whether nestlings subsequently exposed to MA already differ from their non-aggressed siblings. Our results provided no evidence that MA females invested less in reproduction or parental care: MA and noMA females laid clutches of comparable size, delivered similar amounts of food, and distributed food no more unevenly among their nestlings than noMA females did. However, we found that MA females lost less body mass during the breeding attempt than noMA females. Importantly, the two maternal phenotypes differed in how they integrated offspring traits when allocating food: the association between begging duration and provisioning depended on begging order in opposite ways in MA and noMA females. Within MA broods, we found no clear evidence that nestlings later targeted had already been treated differently, or that they differed in body mass or begging behaviour. Together, these findings indicate that differences in maternal food allocation rules are already detectable before the onset of aggression, although whether and how these differences contribute to the subsequent expression of MA remains unresolved.

We found no evidence that MA and noMA females differed in initial reproductive investment or in the total amount of food delivered to their offspring before aggression emerged. However, comparable overall investment does not necessarily imply that mothers allocate resources in the same way among individual nestlings. Indeed, despite similar overall provisioning, MA and noMA females differed in how they integrated offspring traits and behaviours when deciding which nestling to feed. By maintaining comparable overall provisioning while biasing food allocation among offspring, females can shape brood structure through differential post-hatching investment, effectively adjusting brood size without altering total provisioning (Forbes & Mock, 2000; Lack, 1947, 1966; Mock & Parker, 1997; Royle et al., 2012). In the model examining food allocation in relation to experimental hunger, hungry nestlings received more food transfers than satiated nestlings, and relatively heavier nestlings also received more food. Preferences for hungrier and larger nestlings have been widely documented in the literature (reviewed in Parejo-Pulido & Redondo, 2026).

When examining maternal responses to offspring traits and behaviours, the clearest difference between phenotypes concerned how begging duration was associated with food received depending on begging

order. In MA females, nestlings that began begging early benefited most from sustaining their begging, suggesting that these two traits reinforced each other in determining food allocation. In noMA females, by contrast, sustained begging appeared to compensate for a late start, allowing nestlings that began begging later to receive more food when they begged for longer. These contrasting patterns resemble those predicted by passive and active models of parental food allocation (Parejo-Pulido et al., 2023; Parker et al., 2002; Royle et al., 2002). Maximum begging intensity, by contrast, was not significantly associated with the amount of food received after accounting for the other offspring traits included in the model. This is noteworthy in light of our experimental manipulation of hunger, which allowed us to examine how different components of begging behaviour were associated with short-term hunger and relative body mass at this developmental stage. Maximum begging intensity was higher in hungry nestlings and was also positively associated with relative body mass, suggesting that it conveys information about both short-term hunger and size-related differences among siblings (Parejo-Pulido & Redondo, 2026). In contrast, neither begging order nor begging duration was significantly affected by the hunger manipulation or relative body mass. Thus, although hungry nestlings received more food and showed greater maximum begging intensity, the begging trait most clearly associated with hunger (i.e., maximum postural intensity) was not significantly associated with amount of food received. Conversely, begging order and duration were associated with food allocation through their interaction, despite neither trait showing a significant response to the hunger manipulation. Similarly, relatively heavier nestlings begged more intensely and received more food, but these results do not establish that their greater postural intensity explains the association between body mass and provisioning. Together, these findings indicate that maternal food allocation involves the integration of multiple offspring traits and behaviours, which MA and noMA females weight differently.

Importantly, this difference does not indicate that mothers were insensitive to offspring signals; rather, it indicates that MA and noMA females differed in how they integrated the available cues (Parejo-Pulido et al., 2023). One possible interpretation is that females later exhibiting MA follow a qualitatively different provisioning strategy before aggression emerges. Such a strategy could result in a more consistently biased allocation process, in which early and persistent beggars are repeatedly rewarded. If these allocation rules persist across feeding visits, this may create strong incentives for nestlings to escalate these behaviours to obtain food, potentially reinforcing within-brood asymmetries over time (Johnstone & Kilner, 2011; Mock & Parker, 1997; Parejo-Pulido et al., 2023; Royle et al., 2002). Under this scenario, such differences could contribute to increasing asymmetries among siblings, but we did not measure whether the allocation patterns observed early in development persisted or affected subsequent growth or survival. We therefore cannot establish whether they contributed to brood reduction or to the later onset of aggression, so we raise this only as a hypothesis for future work. One possibility is that aggression emerges when females eventually become unable or unwilling to maintain provisioning at the required level demanded by offspring (Grodzinski & Johnstone, 2011; Trivers, 1974). Aggression towards dependent offspring has been interpreted in other species as a means of reducing offspring demand (Leonard et al., 1988; Trivers, 1974). However, neither a decline in maternal provisioning capacity nor a change in offspring demand preceding aggression was demonstrated here. The presence of differences

in food allocation rules before aggression suggests that MA is associated with earlier variation in maternal behaviour, rather than representing a random dysregulation. This contrasts with some taxa in which aggression towards dependent offspring has been associated with social instability or pathological responses, and shows weak integration with parental investment patterns (Maestripieri, 2005), but our study was not designed to evaluate those explanations directly. A useful comparison can also be drawn with weaning conflict in mammals, where offspring remain nutritionally dependent while maternal investment declines and maternal rejection or aggression emerges (Bateson, 1994; Trivers, 1974). However, weaning is a species-typical developmental process in mammals, whereas MA in canaries is expressed by only a subset of females, suggesting that the occurrence of aggression in this system depends on factors that vary among females, broods or their interactions.

Intriguingly, MA and noMA females did not differ significantly in body mass before breeding, indicating that females that later expressed MA were not initially in poorer condition. Furthermore, MA females lost less body mass across the breeding period, with differences becoming most apparent around day 16 of the nestling period, when offspring demand peaks. We note that the overall interaction between breeding stage and MA phenotype was not statistically significant, and that this difference was confined to day 16. This pattern suggests that the emergence of MA was not preceded by an obvious decline in maternal condition. One possible explanation is that females expressing MA did not increase their total provisioning effort in response to increasing offspring demands later in development (see Parejo-Pulido & Redondo, 2026, and references therein), potentially allowing them to retain a higher body mass. However, because we did not measure provisioning behaviour at this later stage, we cannot determine whether the difference in body mass was associated with contemporaneous differences in feeding effort, food allocation or other aspects of maternal energy balance. If overall provisioning rates were similar between female phenotypes, reduced mass loss in MA females would more likely reflect differences in the structure and distribution of feeding effort rather than reduced investment per se (Roff, 2002; Tinbergen & Dietz, 1994; Williams, 1996). Alternatively, reduced mass loss in MA females could reflect life-history trade-offs, with females strategically allocating resources toward future reproduction at the expense of current reproduction, consistent with parent-offspring conflict dynamics (Lessells, 1991; Stearns, 1998).

Within MA broods, nestlings that later experienced MA tended to receive fewer food transfers prior to aggression, although this difference was not statistically significant and we therefore treat it as suggestive. Aggression was not preferentially directed toward the smallest nestlings, which does not support the idea that aggression functions to eliminate the lowest-quality offspring (O'Connor, 1978). We also found no evidence that nestlings later exposed to MA exhibited exaggerated begging prior to aggression, providing no support for the idea that MA is triggered as a corrective response to persistent overdemand at the stage we measured (Godfray, 1991; Kilner & Johnstone, 1997; Lessells & McNamara, 2012; Magrath et al., 2010). Thus, the traits and behaviours examined here did not identify which nestlings would subsequently be exposed to MA. However, this does not exclude the possibility that other offspring characteristics or interactions with their mothers contribute to the onset of aggression. Moreover, parent-offspring interactions are dynamic, and offspring signaling strategies may change later

over development (Khayutin, 1985; Khayutin et al., 1988; Leonard et al., 2005; Slagsvold & Rohwer, 2000). Currently, the temporal gap between our experimental tests and the onset of aggression limits our ability to assess whether differences in offspring behaviour or maternal responses emerged later. The specific cues triggering aggressive interactions therefore remain unclear.

Two features of our design affect how these results should be read. Because whole clutches were reciprocally cross-fostered, the aggression we measured is directed at foster offspring, and each female reared a single clutch from a single biological mother, so female identity and brood origin cannot be separated statistically. However, MA also occurs in this population in females rearing their own offspring (Garcia-Co et al., 2025; Garcia-Co et al., in review) and thus, it is not an artefact of fostering. Moreover, in our wider cross-fostering dataset a female's aggression was unrelated to the aggression phenotype of the biological mother of her foster brood (Table S4). We cannot distinguish whether this reflects an absence of brood-related effects or limited power to detect them. Separating maternal from brood contributions would nonetheless require females to rear more than one brood of differing origin, which the present design does not provide.

Taken together, our findings show that, in the canary, females that later express MA already differ in their food allocation rules before aggression emerges, despite no detectable differences in initial reproductive investment or overall provisioning. This pattern is compatible with the hypothesis that MA forms part of a broader, context-dependent pattern of maternal care, but it does not establish a progressive transition from biased allocation to brood reduction or aggression. The mechanisms linking early allocation rules to later aggression, as well as the cues associated with its onset, remain to be identified.

Declarations

The experiments described in this study were carried out with the approval of the Ethical Committee for Animal Experimentation (ECD) at the University of Antwerp under licence ECD-2022-87. They were performed in accordance with Belgian and Flemish legislation regarding animal welfare, adhered to the ASAB/ABS guidelines for the use of animals in behavioural research and teaching, and complied with the ARRIVE guidelines. A constant effort was made to keep the birds in semi-natural conditions and in good health. To this end, we frequently sought advice from breeders and veterinarians to further optimise the housing conditions. The invasiveness of all procedures described was minimised and handling time was kept under two minutes per bird. Birds were handled exclusively by trained personnel (CG, WM: FELASA C; caretakers: FELASA B). Body mass and behaviour of adults and nestlings were monitored throughout the experiment, as they reflect general well-being (Paul-Murphy & Hawkins, 2015). Nestlings were temporarily marked with non-toxic coloured markers until they reached approximately 7 g, when they were fitted with standard metal leg rings. These methods have negligible effects in passerines (Eldegard et al., 2024). Blood sampling for molecular sexing was conducted once, at fledging (50 µl from the brachial vein, < 2 min handling time per nestling). During breeding, pairs were housed in standard canary breeding cages with ad libitum water and, outside the provisioning trials, ad libitum food. During the hunger manipulation, food was removed from the cage for 30 or 45 min. All nestlings were then hand-fed

to satiation, and a subset was hand-fed again after a further 30 min while their siblings remained unfed. Nestlings in the hungry treatment were therefore without food for approximately 30 min after satiation before parental provisioning resumed. The nest cup was removed from the cage only briefly for marking, weighing and hand-feeding. At the end of each one-hour recording, all nestlings were hand-fed to satiation and the standard food supply was restored. Maternal aggression occurred spontaneously and was not induced experimentally. MA phenotypes were defined through daily nest checks from day 14 onwards, which minimised disturbance at the nest. At the end of the experiment, all canaries were kept in the lab-bred population at the University of Antwerp, as they are part of a transgenerational transmission experiment, and were allowed to breed in the following years.

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References

1. Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*, 1–48. https://doi.org/10.18637/jss.v067.i01
2. Caro, S. M., Griffin, A. S., Hinde, C. A., & West, S. A. (2016). Unpredictable environments lead to the evolution of parental neglect in birds. *Nature Communications*, *7*(1), 10985. https://doi.org/10.1038/ncomms10985
3. Caro, T. M. (2005). *Antipredator Defenses in Birds and Mammals*. University of Chicago Press.
4. Clutton-Brock, T. H. (1991). *The Evolution of Parental Care*. Princeton University Press.
5. Davis, J. N., Todd, P. M., & Bullock, S. (1999). Environment quality predicts parental provisioning decisions. *Proceedings of the Royal Society B: Biological Sciences*, *266*(1430), 1791–1797. https://doi.org/10.1098/rspb.1999.0848
6. Dawkins, R., & Krebs, J. R. (1978). Animal Signals: Information or Manipulation? In J. R. Krebs & N. B. Davies (Eds), *Behavioural Ecology: An Evolutionary Approach* (pp. 282–309). Blackwell Scientific.
7. Drummond, H., Rodríguez, C., & Ortega, S. (2025). Long-term insights into who benefits from brood reduction. *Behavioral Ecology*, *36*(4), araf050. https://doi.org/10.1093/beheco/araf050
8. Dugas, M. B., & McGraw, K. J. (2011). Proximate Correlates of Carotenoid-Based Mouth Coloration in Nestling House Sparrows. *The Condor: Ornithological Applications*, *113*(3), 691–700. https://doi.org/10.1525/cond.2011.100204

9. Estramil, N., Eens, M., & Müller, W. (2014). On the coadaptation of offspring begging and parental supply—A within-individual approach across life stages. *Behavioral Ecology and Sociobiology*, *68*(9), 1481–1491. https://doi.org/10.1007/s00265-014-1756-1
10. Forbes, S., & Mock, D. W. (2000). A Tale of two Strategies: Life-History Aspects of Family Strife. *The Condor: Ornithological Applications*, *102*(1), 23–34. https://doi.org/10.1093/condor/102.1.23
11. Fresneau, N., & Müller, W. (2016). Information warfare in avian families: Sex-specific begging responses to need and social environment in canary nestlings. *Animal Behaviour*, *115*, 137–143. https://doi.org/10.1016/j.anbehav.2016.03.015
12. Garcia-Co, C., Verbruggen, F., Morales, J., & Müller, W. (2025). The balance between care and conflict may be key to understanding maternal aggression in canaries. *Scientific Reports*, *15*(1), 26278. https://doi.org/10.1038/s41598-025-10698-4
13. Garcia-Co, C., Verbruggen, F., Morales, J., & Müller, W. (2026). Transmission of maternal aggression in canaries requires direct mother-daughter interaction. *Behavioural Ecology and Sociobiology*, in review.
14. Godfray, H. C. J. (1991). Signalling of need by offspring to their parents. *Nature*, *352*(6333), 328–330. https://doi.org/10.1038/352328a0
15. Grodzinski, U., & Johnstone, R. A. (2011). Parents and offspring in an evolutionary game: The effect of supply on demand when costs of care vary. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1726), 109–115. https://doi.org/10.1098/rspb.2011.0776
16. Hartig, F. (2016). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. *CRAN: Contributed Packages*. https://doi.org/10.32614/cran.package.dharma
17. Johnstone, R. A., & Kilner, R. M. (2011). New labels for old whines. *Behavioral Ecology*, *22*(5), 918–919. https://doi.org/10.1093/beheco/arr095
18. Khayutin, S. N. (1985). Sensory Factors in the Behavioral Ontogeny of Altricial Birds. In J. S. Rosenblatt, C. Beer, M.-C. Busnel, & P. J. B. Slater (Eds), *Advances in the Study of Behavior* (Vol. 15, pp. 105–152). Academic Press. https://doi.org/10.1016/S0065-3454(08)60488-X
19. Khayutin, S. N., Dmitrieva, L. P., & Alexandrov, L. I. (1988). Psychobiological Aspects of the Acceleration of Postembryonic Development in the Asynchronous Breeder, Pied Flycatcher (Ficedula Hypoleuca). *International Journal of Comparative Psychology*, *1*(3). https://doi.org/10.46867/C4501F
20. Kilner, R., & Johnstone, R. A. (1997). Begging the question: Are offspring solicitation behaviours signals of need? *Trends in Ecology & Evolution*, *12*(1), 11–15. https://doi.org/10.1016/S0169-5347(96)10061-6
21. Lack, D. (1947). The Significance of Clutch-size. *Ibis*, *89*(2), 302–352. https://doi.org/10.1111/j.1474-919X.1947.tb04155.x
22. Lack, D. (1966). *Population Studies of Birds*. Clarendon Press.
23. Leonard, M. L., Horn, A. G., & Eden, S. F. (1988). Parent-offspring aggression in moorhens. *Behavioral Ecology and Sociobiology*, *23*(4), 265–270. https://doi.org/10.1007/BF00302949

24. Leonard, M. L., Horn, A. G., & Mukhida, A. (2005). False alarms and begging in nestling birds. *Animal Behaviour*, *69*(3), 701–708. https://doi.org/10.1016/j.anbehav.2004.05.022

25. Lessells, C. M. (1991). The evolution of life histories. In J. R. Krebs & N. B. Davies (Eds), *Behavioural Ecology: An Evolutionary Approach* (3rd edn, pp. 32–68). Blackwell Scientific Publications.

26. Lessells, C. M. (2002). Parentally biased favouritism: Why should parents specialize in caring for different offspring? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *357*(1419), 381–403. https://doi.org/10.1098/rstb.2001.0928

27. Lessells, C. M., & McNamara, J. M. (2012). Sexual conflict over parental investment in repeated bouts: Negotiation reduces overall care. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1733), 1506–1514. https://doi.org/10.1098/rspb.2011.1690

28. Maestripieri, D. (2005). Early experience affects the intergenerational transmission of infant abuse in rhesus monkeys. *Proceedings of the National Academy of Sciences*, *102*(27), 9726–9729. https://doi.org/10.1073/pnas.0504122102

29. Magrath, R. D. (1989). Hatching asynchrony and reproductive success in the blackbird. *Nature*, *339*(6225), 536–538. https://doi.org/10.1038/339536a0

30. Magrath, R. D., Haff, T. M., Horn, A. G., & Leonard, M. L. (2010). Calling in the Face of Danger. In *Advances in the Study of Behavior* (Vol. 41, pp. 187–253). Elsevier. https://doi.org/10.1016/S0065-3454(10)41006-2

31. McGillycuddy, M., Popovic, G., Bolker, B. M., & Warton, D. I. (2025). Parsimoniously Fitting Large Multivariate Random Effects in glmmTMB. *Journal of Statistical Software*, *112*, 1–19. https://doi.org/10.18637/jss.v112.i01

32. Mock, D. W., Dugas, M. B., & Strickler, S. A. (2011). Honest begging: Expanding from Signal of Need. *Behavioral Ecology*, *22*(5), 909–917. https://doi.org/10.1093/beheco/arr091

33. Mock, D. W., & Parker, G. A. (1997). Parent-offspring conflict I: Introduction to theory. In D. W. Mock & G. A. Parker (Eds), *The Evolution of Sibling Rivalry* (p. 0). Oxford University Press. https://doi.org/10.1093/oso/9780198577430.003.0007

34. Moreno, J. (2012). Parental infanticide in birds through early eviction from the nest: Rare or under-reported? *Journal of Avian Biology*, *43*(1), 43–49. https://doi.org/10.1111/j.1600-048X.2011.05608.x

35. Müller, W., Boonen, S., Groothuis, T. G. G., & Eens, M. (2010). Maternal yolk testosterone in canary eggs: Toward a better understanding of mechanisms and function. *Behavioral Ecology*, *21*(3), 493–500. https://doi.org/10.1093/beheco/arq010

36. Neuenschwander, S., Brinkhof, M. W. G., Kölliker, M., & Richner, H. (2003). Brood size, sibling competition, and the cost of begging in great tits (Parus major). *Behavioral Ecology*, *14*(4), 457–462. https://doi.org/10.1093/beheco/arg025

37. O'Connor, R. J. (1978). Brood reduction in birds: Selection for fratricide, infanticide and suicide? *Animal Behaviour*, *26*, 79–96. https://doi.org/10.1016/0003-3472(78)90008-8

38. Parejo-Pulido, D., Pérez-Rodríguez, L., Abril-Colón, I., Potti, J., & Redondo, T. (2023). Passive and active parental food allocation in a songbird. *Behavioral Ecology*, *34*(5), 729–740.

https://doi.org/10.1093/beheco/arad043

39. Parejo-Pulido, D., & Redondo, T. (2026). Food-solicitation displays in nestling birds: Still begging for answers. *Biological Reviews*, *n/a*(n/a). https://doi.org/10.1002/brv.70210
40. Parejo-Pulido, D., Redondo, T., Casquero, S., & Pérez-Rodríguez, L. (2025). Escalated begging does not compromise nestling health. *Behavioral Ecology*, *36*(2), araf003. https://doi.org/10.1093/beheco/araf003
41. Parejo-Pulido, D., Redondo, T., & Pérez-Rodríguez, L. (2024). Immune challenge reduces begging effort and modifies begging call structure in spotless starling nestlings. *Behavioral Ecology and Sociobiology*, *78*(7), 81. https://doi.org/10.1007/s00265-024-03497-w
42. Parker, G. A., & Macnair, M. R. (1979). Models of parent-offspring conflict. IV. Suppression: Evolutionary retaliation by the parent. *Animal Behaviour*, *27*, 1210–1235. https://doi.org/10.1016/0003-3472(79)90068-X
43. Parker, G. A., Royle, N. J., & Hartley, I. R. (2002). Begging scrambles with unequal chicks: Interactions between need and competitive ability. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *357*(1419). https://onlinelibrary.wiley.com/doi/full/10.1046/j.1461-0248.2002.00301.x
44. Pijanowski, B. C. (1992). A Revision of Lack's Brood Reduction Hypothesis. *The American Naturalist*, *139*(6), 1270–1292. https://doi.org/10.1086/285386
45. Redondo, T. & Parejo-Pulido, D. (2026). Effectiveness of multicomponent, dynamic begging displays as honest signals of nestling hunger. Royal Society Open Science 13, 260413.
46. Roff, D. A. (2002). *Life History Evolution*. Sinauer.
47. Royle, N. J., Hartley, I. R., & Parker, G. A. (2002a). Begging for control: When are offspring solicitation behaviours honest? *Trends in Ecology & Evolution*, *17*(9), 434–440. https://doi.org/10.1016/S0169-5347(02)02565-X
48. Royle, N. J., Hartley, I. R., & Parker, G. A. (2002b). Begging for control: When are offspring solicitation behaviours honest? *Trends in Ecology & Evolution*, *17*(9), 434–440. https://doi.org/10.1016/S0169-5347(02)02565-X
49. Royle, N. J., Smiseth, P. T., & Kölliker, M. (2012). *The Evolution of Parental Care*. Oxford University Press.
50. Shizuka, D., & Lyon, B. E. (2013). Family dynamics through time: Brood reduction followed by parental compensation with aggression and favouritism. *Ecology Letters*, *16*(3). https://onlinelibrary.wiley.com/doi/full/10.1111/ele.12040
51. Slagsvold, T., & Rohwer, S. (2000). Behavioral Domination of Food Delivery by Tree Swallow Nestlings. *The Wilson Bulletin*, *112*(2), 278–281. https://doi.org/10.1676/0043-5643(2000)112%5B0278:BDOFDB%5D2.0.CO;2
52. Smiseth, P. T., Amundsen, T., & Hansen, L. T. T. (1998). Do males and females differ in the feeding of large and small siblings? An experiment with the bluethroat. *Behavioral Ecology and Sociobiology*, *42*(5), 321–328. https://doi.org/10.1007/s002650050444

53. Smith, H. G., & Montgomerie, R. (1991). Nestling American robins compete with siblings by begging. *Behavioral Ecology and Sociobiology*, *29*(4), 307–312. https://doi.org/10.1007/BF00163989
54. Smith, J. M., & Harper, D. D. (2003). *Animal Signals*. OUP Oxford.
55. Stearns, S. C. (1998). *The Evolution Of Life Histories*. Oxford University Press. https://doi.org/10.1093/oso/9780198577416.001.0001
56. Tinbergen, J. M., & Dietz, M. W. (1994). Parental Energy Expenditure During Brood Rearing in the Great Tit (Parus major) in Relation to Body Mass, Temperature, Food Availability and Clutch Size. *Functional Ecology*, *8*(5), 563–572. https://doi.org/10.2307/2389916
57. Trivers, R. (1974). Parent-Offspring Conflict. *American Zoologist*, *14*, 249–264.
58. Williams, G. C. (1996). *Adaptation and natural selection: A critique of some current evolutionary thought*. Princeton University Press.
59. Zielinski, P. (2002). Brood Reduction and Parental Infanticide—Are the White Stork Ciconia ciconia and the Black Stork C. nigra exceptional? *Acta Ornithologica*, *37*, 113–119. https://doi.org/10.3161/068.037.0207

Figures

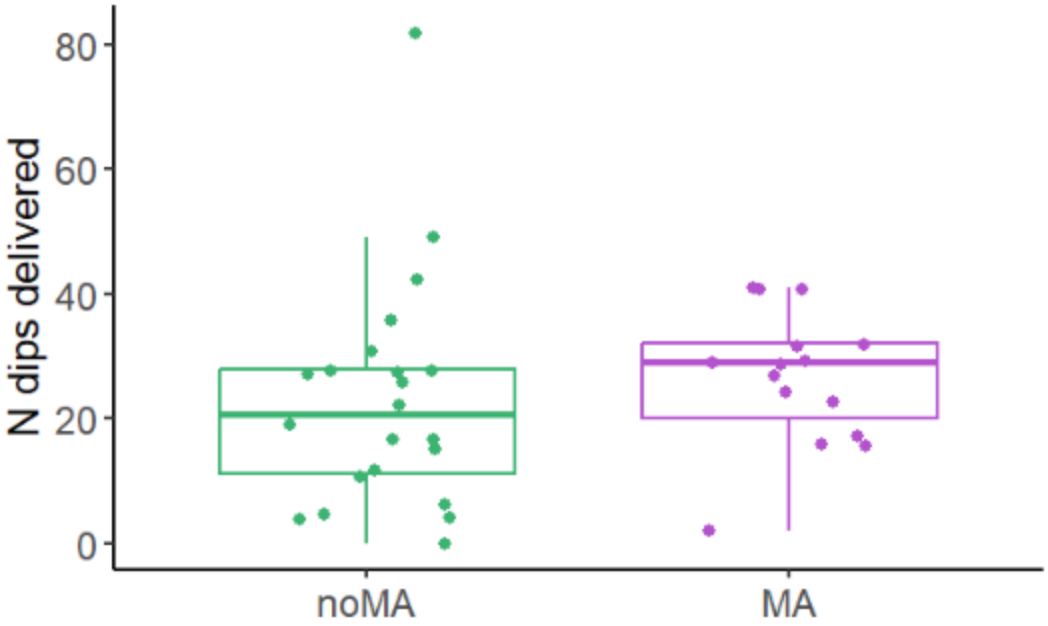


Figure 1

Total number of food transfers (dips) delivered by females during the observation trial in nests where mothers later showed maternal aggression (MA) and nests where they did not (noMA). Boxplots show median, interquartile range, and range; points represent individual nests and are jittered to display raw values. The unit of analysis is the nest (N = 37 nests).

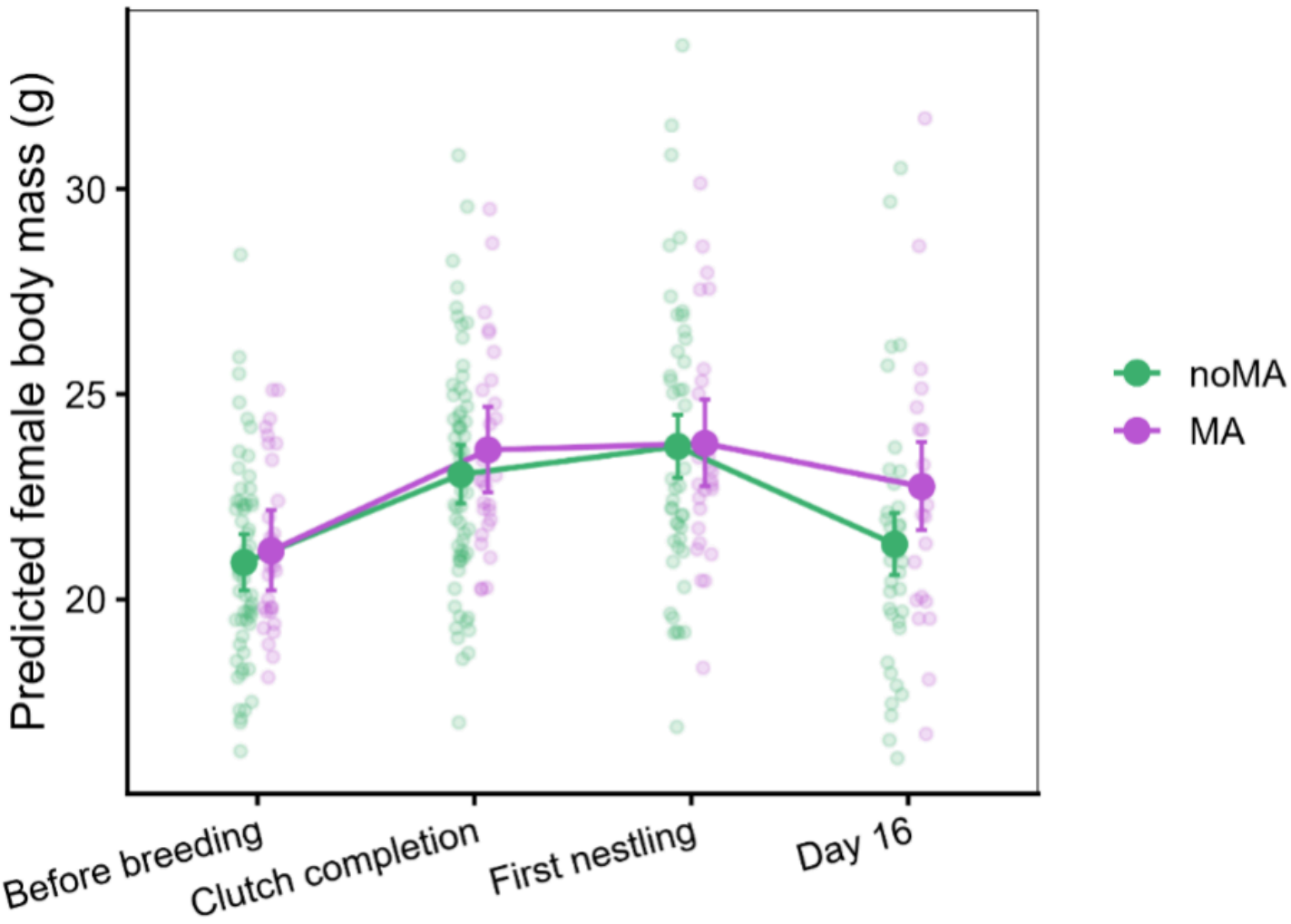


Figure 2

Predicted female body mass across four breeding stages for females that later expressed maternal aggression (MA, purple) and those that did not (noMA, green). Values are predictions from the linear mixed models reported in Table 2, fitted on the square-root scale and back-transformed to grams; error bars are 95% confidence intervals. The unit of analysis is the female (N = 91 females, four measurements each). Faded background points are raw body mass values, jittered for visibility.

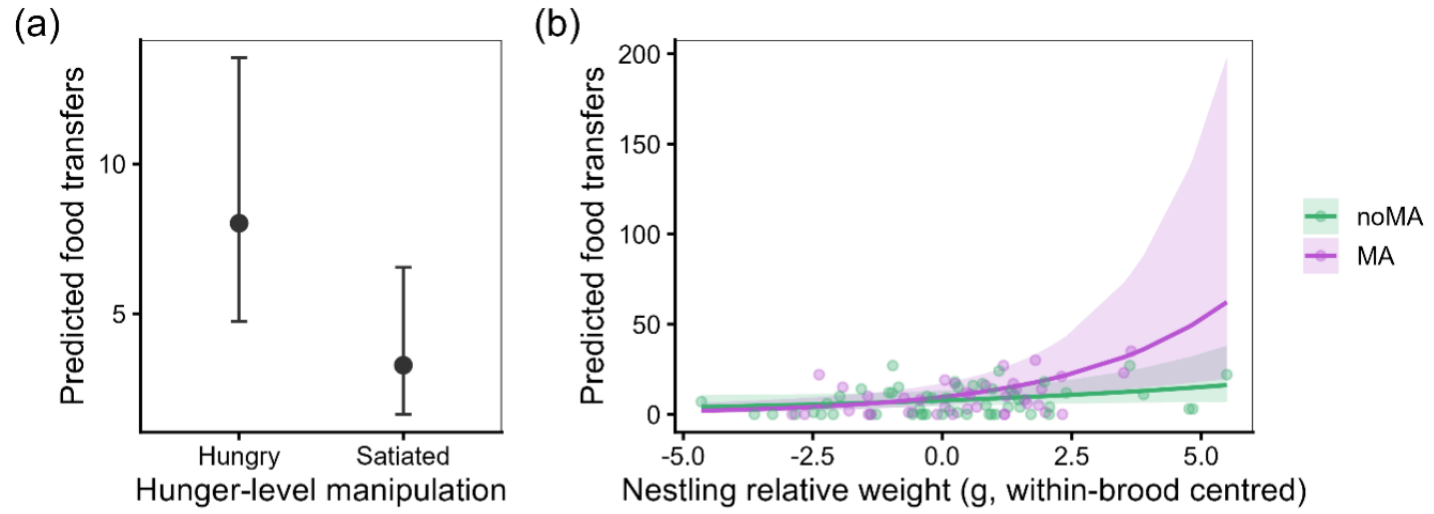


Figure 3

Maternal provisioning (number of food transfers/dips by the female) in relation to **(a)** and the experimental hunger level manipulation, and **(b)** nestling relative weight. Values are predictions from the negative binomial GLMM reported in Table 3, with 95% confidence intervals. The unit of analysis is the individual nestling (N = 91 nestlings in 37 nests). Panel (a) shows the retained main effect of the hunger manipulation. Panel (b) shows fitted relationships for noMA (green) and MA (purple) females, with raw values in the background.

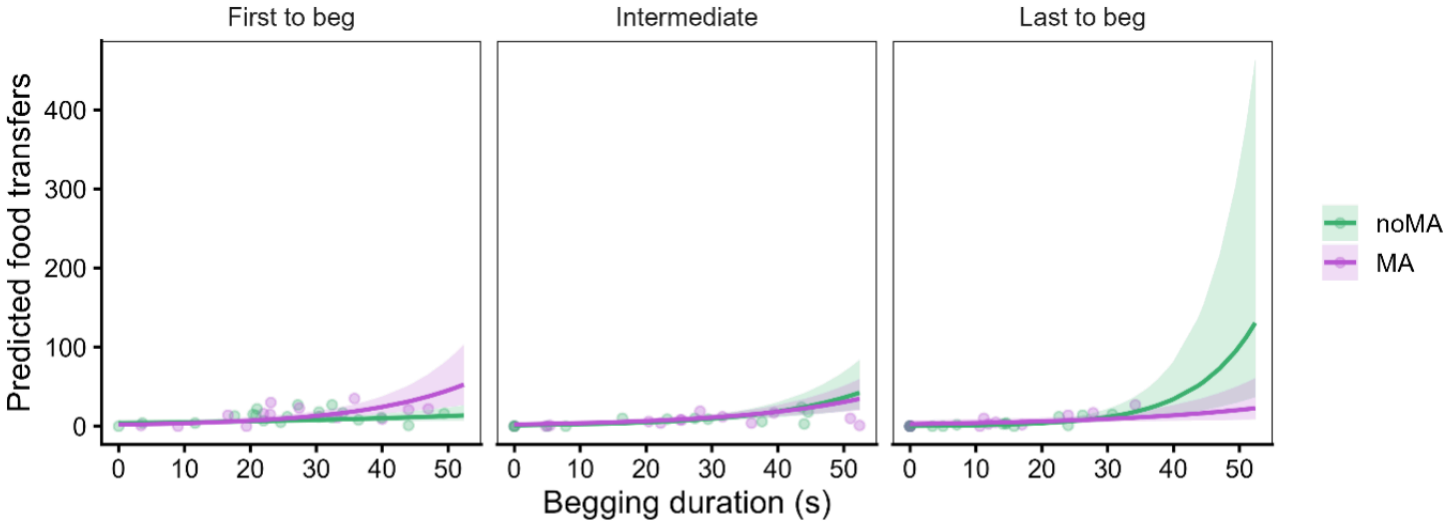


Figure 4

Predicted maternal provisioning (total number of food transfers) as a function of nestling begging duration, for noMA (green) and MA (purple) females, shown at three positions in the within-brood begging sequence. Values are predictions from a negative binomial GLMM reported in Table 4, with shaded ribbons indicating 95% confidence intervals; semi-transparent points in the background

represent raw values in the corresponding colours. The unit of analysis is the individual nestling (N = 91 nestlings in 37 nests). Begging order was modelled as a continuous variable; the three panels are model predictions evaluated at earliest, intermediate and latest positions in the begging sequence and are shown for visualisation only.

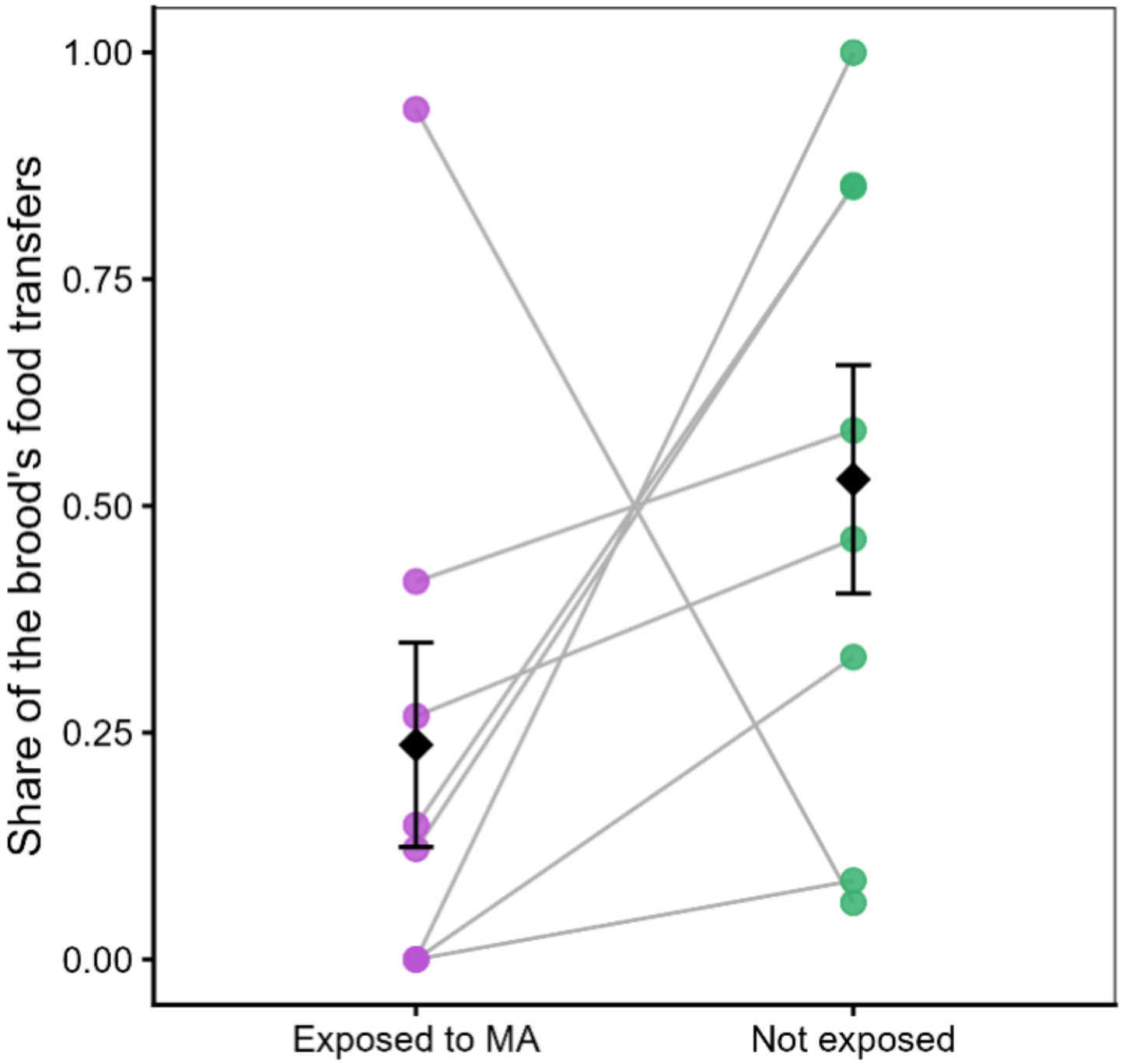


Figure 5

Share of the brood total food transfers received by nestlings later exposed to maternal aggression and their non-exposed siblings, within the eight broods in which aggression was directed at a subset of nestlings. Each grey line joins the two groups within one brood; coloured points are brood means for

each group. Black diamonds and bars show the overall mean and standard error across broods. The unit of analysis is the brood (N = 8). Labels refer to nestling-level exposure, not to the maternal phenotype used elsewhere.

Supplementary Files

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