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RESEARCH ARTICLE

Position in the laying order has sex-specific consequences for reproductive success in adult black-headed gulls

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Abstract

Mothers who produce multiple offspring within one reproductive attempt often allocate resources differentially; some maternally derived substances are preferentially allocated to last-produced offspring and others to first-produced offspring. The combined effect of these different allocation regimes on the overall fitness of offspring produced early or late in the sequence is not well understood, partly because production order is often coupled with birth order, making it difficult to separate effects of pre-natal maternal allocation from those of post-natal social environments. In addition, very little is known about the influence of laying order on fitness in later life. In this study, we used a semi-natural captive colony of black-headed gulls to test whether an offspring's position in the laying order affected its early-life survival and later-life reproductive success, independent of its hatching order. Later-laid eggs were less likely to hatch, but among those that did, survival to adulthood was greater than that of first-laid eggs. In adulthood, the laying order of females did not affect their likelihood of breeding in the colony, but male offspring hatched from last-laid eggs were significantly less likely to gain a breeding position than earlier-laid males. In contrast, later-laid female parents hatched lower proportions of their clutches than first-laid females, but hatching success was unrelated to the laying order of male parents. Our results indicate that gull mothers induce complex and sex-specific effects on both the early survival of their offspring and on long-term reproductive success through laying order effects among eggs of the same breeding attempt.

KEYWORDS

birth order, black-headed gull, later-life fitness, laying order, maternal effects, reproductive success, survival

1 | INTRODUCTION

An individual's position in the sequence in which offspring are produced in a clutch or brood plays a crucial role in determining its phenotype (Groothuis et al., 2005; Ryan & Vandenberg, 2002).

Such production order effects on phenotype are widespread in many animal taxa, including humans (Courtiol et al., 2009; Damian & Roberts, 2015) and other mammals (Altmann & Alberts, 2005; Cabrera et al., 2012), birds (Groothuis et al., 2005), fish (Schradler & Travis, 2012) and invertebrates (Mousseau & Dingle, 1991; Schroeder

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& Gilbert, 2009). Over recent years, impressive advances have been made in our understanding of the maternally derived substances that underlie such lay-order effects. In birds, maternal allocation of specific hormones, micronutrients and immunofactors often increases or decreases across the sequence of eggs produced in a clutch. Such allocation patterns can be adaptive mechanisms to compensate for lower concentrations of other substances (Groothuis et al., 2006; Royle et al., 2001) or a lower position in the social hierarchy (Müller, Eising, et al., 2004), and/or may simply result from maternal resource depletion (Karell et al., 2008; Montesana et al., 2019). However, we know comparatively little about the combined effect that all these different allocation regimes have on offspring fitness across the lifespan. The combined phenotypic outcome of lay-order-dependent maternal effects later in life is especially important because traits that are beneficial in young offspring, such as competitive ability or investment in rapid growth (Muller & Groothuis, 2013), might not be as beneficial for adulthood fitness (Ruuskanen et al., 2012). Given that selection pressure on traits linked to survival is likely to be strongest in early life, selection on mothers to produce offspring phenotypes that are able to successfully deal with the competitive environment of the nest may have varied and potentially even opposing consequences for offspring fitness later in life.

There are two main reasons why studies on the overall effect of laying order on later-life fitness are scarce. First, earlier-produced embryos tend to be born or hatch earlier than later ones (Magrath, 1990; Slagsvold, 1985), meaning that laying order is difficult to separate from hatching order and its subsequent influence on social position in the family's age or size hierarchy. This distinction is crucial for understanding maternal effects, since effects of laying order are purely intrinsic to the embryo and its exposure to maternally derived developmental conditions, while effects of hatching order are also a product of the offspring's social environment after it emerges (Diaz-Real et al., 2016; Gilby et al., 2012). Second, quantifying effects of laying order across the entire lifespan requires detailed monitoring of individual eggs from the moment of laying until far into adulthood, which is often difficult to achieve in most vertebrates.

We investigated the relationship between laying order and several fitness components in a captive population of black-headed gulls (*Chroicocephalus ridibundus*). We were able to use several useful properties of the study system to disentangle pre-natal effects of laying order from post-hatching effects of competition on later-life performance. Firstly, offspring do not always hatch in the order they are laid in this species, and one third of the focal individuals in the current study shared the nest with at least one other nest mate that survived the whole nestling period; this means that laying and hatching order are only moderately correlated. The situation is similar in nearby wild populations, where pairs usually hatch an average of 1.5 nestlings per clutch (Thyen & Becker, 2006). Secondly, the colony has *ad libitum* access to food, meaning that offspring have little motivation to compete for parental resources. Thirdly, in the early post-hatching phase (until around Day 4), nestlings are very rarely fed—feeding events only occur on average 1.4 times per hour—and have not yet developed their begging displays, living mostly on

their yolk reserves (unpublished data *in prep.*). In the later nestling phase, parents regurgitate a pile of food onto the floor in the vicinity of the nestlings; all nestlings can access this food, and we have never witnessed any begging behaviours or any other evidence of potential scramble competition, in contrast to nestlings from different broods that compete heavily over food and territory boundaries (Groothuis, 1989). Even if competition does occur in the later nestling phase, size differences between nest mates are minimal by this point and so there is unlikely to be any physical disadvantage to last-hatched offspring. Previous work in this species has demonstrated a strong pattern of increasing yolk androgens (Groothuis & Schwabl, 2002) and antibodies (Müller, Groothuis, et al., 2004), but decreasing antioxidants and immunofactors (Groothuis et al., 2006) across the lay sequence, suggesting a complex interplay of different maternal effects on offspring phenotype that have difficult-to-predict effects on offspring fitness.

We first determined whether laying order affected offspring survival to adulthood. We then tested whether the likelihood of gaining a breeding position in the colony and the speed at which individuals were able to find a mate were related to laying order. Lastly, we tested whether laying order affected an individual's reproductive success across the whole study period.

2 | METHODS

Between 2012 and 2021, we studied a captive colony of ca. 100 wild-type black-headed gulls at the University of Groningen, the Netherlands. The study population has been housed at the Groningen Institute of Evolutionary Life Sciences since 2010 in a large aviary (10m×35m) with a transparent roof, netted sides and a sand-covered floor. The aviary is large enough to allow flying and contains two ponds for swimming, as well as numerous natural and semi-natural shelters around which the birds construct their nests. The aviary receives natural light and temperature variation according to the climate of the north of the Netherlands. The colony is fed *ad libitum* with pelleted trout food (E-3P Stella, Trouw Nutrition Nederland), and three times a week with cat food (Huismerk 3-mix kattenbrok, Arie Blok) soaked in water. During the nestling period, cat food is provided daily to support nestling feeding and growth. Data collection for this study was largely observational and was performed from an observation tunnel outside the aviary, minimizing disturbance to the animals. The research was performed with permission of the Animal Welfare Body of the University of Groningen and under a permit issued by the Dutch Competency Authority (permit number AVD1050020209349).

The population was established using adults, nestlings and eggs that were taken from wild black-headed colonies that breed in the north of the Netherlands. All birds in the colony can be individually identified through a unique combination of coloured leg rings. Since the first birds were brought into captivity in the 1980s, the genetic composition of the colony has been kept as close to a wild population as possible by periodically releasing adult individuals and introducing

new eggs and adults from the wild (most recently in 2010). Although the colony is in captivity, these periodical mixing with wild colonies ensures that the captive population is sufficiently outbred and exhibits natural behaviours. While predators are excluded from the colony, intraspecific predation of both nests and eggs is common in our population, as is also the case in the wild (Patterson, 1965). Hatching and fledging success is comparable to that reported in wild colonies (Patterson, 1965; Thyen & Becker, 2006): (means per nest: N hatched eggs = 1.5 in wild vs. 1.9 in captivity, N fledglings = 0.7 in wild vs. 0.62 in captivity).

Not all individuals in the colony find a breeding partner and among those that do, the age of first breeding is variable. Each breeding season, all nesting attempts were monitored to determine the identity of the breeding pair. During daily nest checks, freshly-laid eggs in each nest were marked with a non-toxic marker (A, B and C for first-, second- and third-laid eggs). Each nest was enclosed by ca. 10 cm high plastic fences, which prevented eggs and nestlings (but not adults) from being accidentally moved around between neighbouring nests. Eggs were checked every 1–2 days to determine hatching and fledging success. Around the hatching period, nests were checked more often to accurately assign each nestling to the egg from which it hatched. From hatching until Day 6 (when nestlings are large enough to be marked with coloured leg rings), individual nestlings were identified and monitored based on size differences. Nestlings that hatched within 48 h of each other are more difficult to distinguish from each other because of the relatively small size difference between them; these nestlings were excluded from our analyses. In total, approximately 60% of the hatchlings produced over the course of the study period could be assigned with high confidence to the correct egg, either because they were the only nestling in the brood to hatch (unhatched eggs remain in the nest, so the laying order of the hatched offspring can be easily deduced), or because they hatched at least 48 h apart from other nest mates, and were therefore of sufficiently different size to enable us to consistently distinguish them from other nestlings. In order to disentangle effects of laying order and those arising in the post-hatching environment, each nestling in the data set was assigned a hatch order that described its position in the post-hatching hierarchy: those who were alone in the nest were given a hatching order of 0, and those hatched first, second or third in nests with multiple offspring were assigned hatching orders of 1, 2 or 3, respectively. On ca. Day 6 after hatching, all nestlings were caught in order to fit them with uniquely identifiable colour rings and to take a small (ca. 20 μ L) blood sample for molecular sexing. DNA was extracted using Chelex-100 following standard protocol (Walsh et al., 1991), and sex was determined using a standard PCR method for sexing birds (Griffiths et al., 1998).

Our data set included a total of 702 eggs of known lay order that were laid between 2012 and 2021. Of the 98 nestlings that survived to adulthood (1 year), 59 individuals could be reliably traced back to the egg from which they hatched (20, 24 and 14 nestlings hatched from A, B and C eggs). One nestling hatched from a D egg and was lumped together with C eggs for further analysis. The fate of all individuals was then followed until the end of 2021. We determined

whether an individual recruited into the breeding population by recording the identities of all breeding birds in every year, and monitored all nesting attempts in order to quantify their subsequent reproductive success.

2.1 | Statistical analyses

All analyses were performed in R (v. 3.6.0) (R Core Team, 2020), using the 'lme4' package to fit generalized linear mixed models (GLMM) (Bates et al., 2015). For all analyses, we assessed data distribution and model fit using the 'DHARMA' package (Hartig & Hartig, 2017) and the 'performance' package (Lüdtke et al., 2021). Tests for outliers, collinearity, residual uniformity and overdispersion were non-significant in all cases (Appendix S1). First, across all 702 eggs in the data set, we fitted a binomial mixed model to test whether laying order affected hatching probability. In this data set, some eggs and offspring shared a common parentage. In addition to the fact that one breeding pair's offspring may differ from those of another pair, the slope of laying order effects (i.e. the degree of difference between A, B and C offspring) might also vary between pairs. In order to control for both of these sources of non-independence deriving from common parentage, we included a random intercept of parental identity and a random slope for each level of laying order (following (Schielzeth & Forstmeier, 2009)). We included an additional random effect of lay year (intercept only). We fitted a second binomial mixed model to test whether laying order affected survival to adulthood, again including the random intercept of parental identity with a random slope for laying order and a random intercept for lay year. This second analysis included 592 of the eggs in our data set; we excluded hatchlings that were still less than 1 year at time of analysis and also those for which egg order could not be reliably determined (some nest mates hatched very synchronously and were too similar in size to reliably assign each back to the correct egg, see above).

Next, we fitted a binomial mixed model to test whether the likelihood of gaining a breeding position was associated with laying order across all individuals that survived to adulthood and could be reliably traced back to their egg ($N=59$). We also tested whether the influence of laying order differed between males and females (egg order \times sex interaction). We included hatching order as a fixed effect to account for post-hatching effects on reproductive success; although moderately correlated with laying order (0.41), this was not sufficient to cause problems in our analyses (all variance inflation factors < 2 (Zuur et al., 2010)). We fitted a random intercept for parental identity to account for common parentage but since the data set contained very few individuals of different laying order with the same parents, we did not fit a random slope for laying order within parental identity. We also included the random effect of birth year to account for the fact that birds born later in the study period had less time to acquire a partner; since mean age at first reproduction in this population is 2 years old, all birds in the data set had the opportunity to gain a breeding position by the end of the study period. Among the 38 individuals that gained a breeding position, we used

a Poisson mixed model to test whether the age of first reproduction was related to laying order, again including hatching order, sex and the interaction between laying order and sex. We included random intercepts for birth year to account for cohort differences in mate availability and natal nest (to account for common parentage).

Lastly, we tested whether laying order was associated with reproductive success across the entire study period. Twenty-two of the 38 individuals in the data set were the only one of two parents sampled from a given pair, but the other 16 individuals were part of a breeding pair for which we had laying order information for both parents. In order to avoid duplicating identical reproductive parameters from the same nest in the analysis, we randomly selected one of the two parents in these cases. In some years of the study, some eggs were removed from certain nests for use in other experiments. We excluded all nests from which eggs were removed, resulting in a data set of 69 unmanipulated nests belonging to 30 individuals from 30 different pairs. We fitted two binomial mixed models with the proportion of eggs that hatched and the proportion of nestlings that fledged as two separate dependent variables with binomial error structure (Zuur et al., 2009). In both models, we included laying order, sex, hatching order and the interaction between laying order and sex. We included random intercepts for parental identity and also for individual identify (to account for repeat breeding measures within individuals).

In all analyses, we removed non-significant interactions from the final model but retained all fixed effects and significant interactions. We performed several extra tests to assist in the interpretation of our results: (1) We report overall effects of both laying order and significant interactions between laying order and sex, where they arose; (2) we further tested the robustness of interaction effects by comparing models with and without interactions using likelihood ratio tests; (3) we used the package 'emmeans' (Lenth et al., 2018) to obtain contrasts between different laying orders; (4) For models where we did not detect any effects of laying order, we used the package 'SIMR' (Green & MacLeod, 2016) to perform post hoc power analyses over 1000 simulations where $\alpha=0.05$. We estimated the power required to detect moderate (0.5) and large (0.7) effect sizes; this corresponds to the magnitude of effect sizes we report (see Section 3).

3 | RESULTS

Hatching probability was significantly related to laying order (Wald $\chi^2=43.67$, $p<0.01$, Table 1). While hatching success of A and B eggs did not differ (GLMM: $\beta \pm SE=0.38 \pm 0.22$, $p=0.19$), C eggs were less likely to hatch than both A eggs (GLMM: $\beta \pm SE=1.52 \pm 0.24$, $p<0.01$) and B eggs (GLMM: $\beta \pm SE=1.15 \pm 0.24$, $p<0.01$) (Figure 1a). Survival to adulthood did not significantly vary with laying order (Wald $\chi^2=1.43$, $p=0.49$, Table 1). Power analysis suggests that there was sufficient power to detect a large effect of laying order (97.2% (CI=96.0, 98.1)) but marginal power to detect a moderate effect (59.7% (CI=56.6, 62.8)). However, post hoc analysis showed that the

lack of overall survival effect was likely due to the fact that, among the 330 eggs that did hatch, there was a significant effect of laying order (Wald $\chi^2=6.76$, $p=0.03$). C eggs were more likely to survive than B eggs (GLMM: $\beta \pm SE=-1.12 \pm 0.48$, $p=0.05$) and also tended to have higher survival than A eggs (GLMM: $\beta \pm SE=-1.01 \pm 0.45$, $p=0.06$) (Figure 1b); this survival advantage of C eggs after hatching likely counter-acted their hatching disadvantage.

There was no overall effect of either laying order (Wald $\chi^2=1.90$, $p=0.39$) or sex (Table 2) on the likelihood of becoming a breeder in the colony, but the interaction between these terms was significant (Wald $\chi^2=5.54$, $p=0.02$). Males that hatched from last-laid eggs were less likely to become breeders (Table 2, Figure 2a). The model including this interaction term was also a significantly better fit than one only containing fixed effects (likelihood ratio test: $\chi^2=6.34$, $p=0.04$). Hatching order was not linked to likelihood of breeding (Table 2). However, our power to detect this effects was marginal: (46.5% (CI=43.4, 49.7) and 31.2% (CI=28.3, 34.2) to detect large and moderate hatching order effects, respectively). Among individuals who gained a breeding position, age at first reproduction did not differ according to hatching order, laying order, sex or the interaction between them (Table 2); the model containing the interaction also did not perform better than the one without (likelihood ratio test: $\chi^2=0.84$, $p=0.66$). We had sufficient power to detect large (100% (CI=99.6, 100.0)) and moderate (98.5%, (CI=97.5, 99.2)) effects of laying order, and also for hatching order large: (100% (CI=99.6, 100.0)); moderate: (100% (CI=99.6, 100.0)).

Across the whole study period there was a significant interaction between sex and laying order ($\chi^2=7.16$, $p=0.03$) on the proportion of a parent's eggs that successfully hatched. First-laid female parents hatched a significantly larger portion of their clutches than those from B and C eggs, but laying order had no effect on hatching success for male parents (significant laying order \times sex interactions, Table 3, Figure 2b). The model including this interaction term was a significantly better fit than the model with only fixed effects (likelihood ratio test: $\chi^2=6.34$, $p=0.04$). There were no overall effects of parental laying order ($\chi^2=5.11$, $p=0.08$), although parents hatched from C eggs hatched a lower proportion of their eggs than those from A eggs (Table 3). There was no effect of sex or hatching order (Table 3). We would have been able to detect both large and moderate effects of hatching order with 100% power (CI=99.6, 100.0). Fledgling success was very low in general (only 26% of nests successfully fledged any nestlings) and was not related to any of the predictors (Table 3). However, power analyses suggest that we would have had sufficient power to detect effects of both laying order (large effects: 99.9% (CI=99.4, 100.0); moderate effects: 99.3% (CI=98.6, 99.7)) and hatching order (large effects: 100% (CI=99.6, 100.0), moderate effects: 100% (CI=99.6, 100.0)) had they been present.

4 | DISCUSSION

In this study, we showed that in spite of suffering lower hatching success, later-laid offspring do not experience a reduction in survival

TABLE 1 Effects of laying order on hatching success and early life survival in black-headed gulls.

	Fixed effects	Z	$\beta \pm SE$	p
Hatch success N = 702 eggs	Intercept	5.18	1.12 ± 0.22	<0.01
	Laying order B eggs	-1.74	-0.38 ± 0.22	0.08
	Laying order C eggs	-6.45	-1.52 ± 0.24	<0.01
	Random effects	SD		
	Birth year (intercept)	0.25		
	Parental ID (intercept)	0.98		
	Laying order B eggs (slope)	0.13		
Laying order C eggs (slope)	0.21			
First year survival (N = 592 eggs)	Intercept	-5.20	-2.52 ± 0.49	<0.01
	Laying order B eggs	-0.33	-0.18 ± 0.54	0.74
	Laying order C eggs	0.78	0.39 ± 0.50	0.43
	Random effects	SD		
	Birth year (intercept)	0.59		
	Parental ID (intercept)	1.01		
	Laying order B eggs (slope)	0.03		
Laying order C eggs (slope)	0.95			

Note: Reference groups for laying order and sex are A eggs and females, significant terms in bold font.

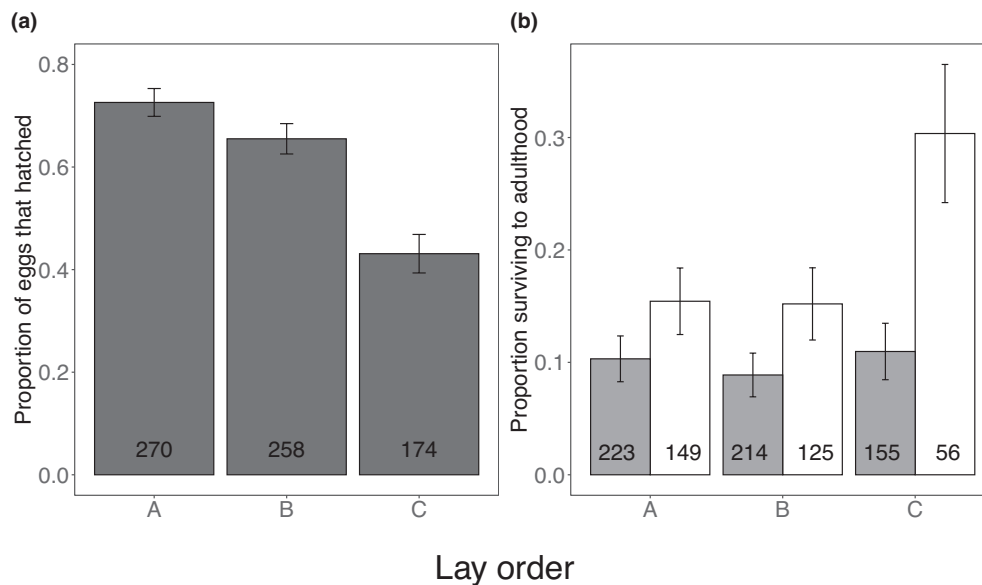


FIGURE 1 Laying order effects on early life survival in black-headed gulls in terms of (a) hatching success and (b) survival to one year. In (b), survival is shown separately for all eggs (grey bars) and only for those eggs that hatched (white bars). Numbers represent sample sizes per group, bars represent mean proportions per group and their standard errors calculated following (Brayer, 1957).

to adulthood. Adult mortality in our population is in general very rare, but both males and females from later-laid eggs suffered reduced reproductive success. Interestingly, this fitness cost was found in different components of reproduction: males hatched from last-laid eggs were less likely to become breeders, whereas females from second- and third-laid eggs had lower offspring hatching success. These sex-dependent fitness effects of laying order in adulthood likely result from the complex interplay between competing

maternal effects that vary across the lay sequence and incur different costs for males and females.

Hatching order often influences survival (Spear & Nur, 1994; Ferrari et al., 2006), but it is often unclear to what extent such survival differences are the result of pre-natal lay-order effects or post-natal social effects. In black-headed gulls, last-laid eggs were significantly less likely to hatch, which supports findings elsewhere that telomere loss, a predictor of lifespan, is also greater

TABLE 2 Effects of laying order and sex on success in obtaining a breeding position in black-headed gulls.

	Fixed effects	Z	$\beta \pm SE$	p
Likelihood of breeding (N = 59 individuals)	Intercept	0.48	0.23 ± 0.48	0.63
	Laying order B eggs	0.62	0.44 ± 0.70	0.53
	Laying order C eggs	-0.86	-0.69 ± 0.80	0.39
	Sex	-0.02	-0.01 ± 0.59	0.98
	Hatching order	1.91	0.83 ± 0.43	0.06
	Laying order × sex: B eggs	-0.89	-1.30 ± 1.47	0.37
	Laying order × sex: C eggs	-2.28	-4.02 ± 1.76	0.02
	Random effects	SD		
	Birth year (intercept)	<0.01		
	Parental ID (intercept)	<0.01		
Age of first breeding (N = 38 individuals)	Intercept	4.50	0.93 ± 0.21	<0.01
	Laying order B eggs	-0.45	-0.11 ± 0.25	0.65
	Laying order C eggs	-0.71	-0.24 ± 0.33	0.48
	Sex	0.42	0.09 ± 0.22	0.67
	Hatching order	-0.16	-0.02 ± 0.13	0.88
	Random effects	SD		
	Birth year (intercept)	<0.01		
	Parental ID (intercept)	<0.01		

Note: Reference groups for laying order and sex are A eggs and females, significant terms in bold font.

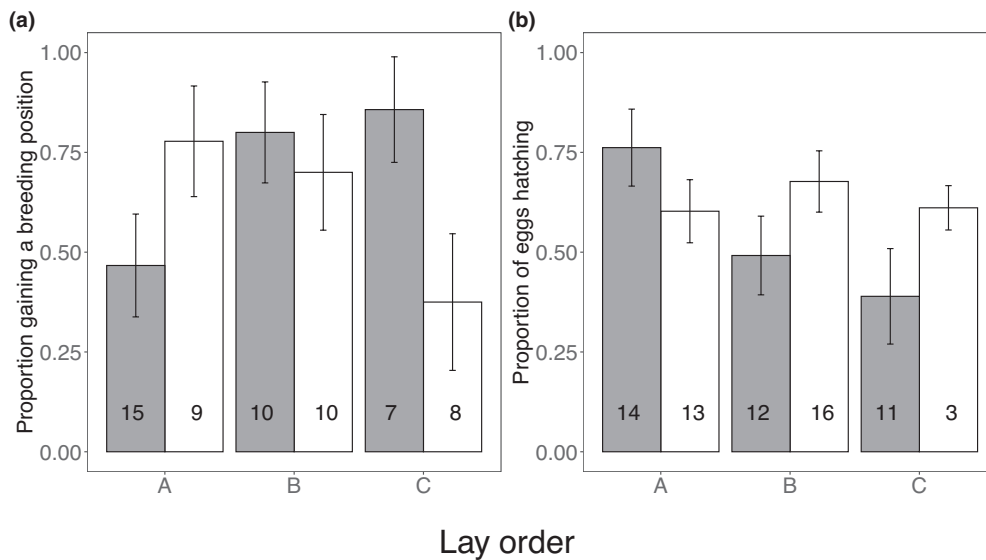


FIGURE 2 Laying order effects on offspring's later-life (a) likelihood of gaining a breeding position and (b) hatching success in female (grey bars) and male (white bars) black-headed gulls. Bars show mean proportions per group and their standard errors (following (Brayer, 1957)), numbers denote sample sizes per group.

for last-laid offspring (Noguera et al., 2016). However, there was no overall difference between early and later-laid eggs in survival to 1 year of age; this pattern likely results from the fact that, among eggs that did hatch, last-laid eggs had the highest survival (Figure 1b). We suggest that selection on phenotypic quality during

embryo development may drive this pattern; thanks to selective pre-hatching mortality of embryos in last-laid eggs, the proportion of C eggs that do hatch are not of different quality to A eggs. However, functional differences in maternal effects across the egg order many also play a role. In black-headed gulls, last-laid eggs

TABLE 3 Effects of laying order and sex on reproductive success in black-headed gulls.

	Fixed effects	Z	$\beta \pm SE$	p
Hatching success N = 69 nests	Intercept	2.08	0.74 ± 0.35	0.04
	Laying order B eggs	-0.92	-0.35 ± 0.38	0.36
	Laying order C eggs	-2.19	-1.26 ± 0.57	0.03
	Sex	0.40	0.13 ± 0.34	0.69
	Hatching order	0.11	0.02 ± 0.22	0.91
	Laying order × sex: B eggs	2.33	1.61 ± 0.69	0.02
	Laying order × sex: C eggs	2.09	1.91 ± 0.92	0.04
	Random effects	SD		
Fledging success (N = 69 nests)	Intercept	-1.70	-0.90 ± 0.53	0.09
	Laying order B eggs	0.60	0.37 ± 0.62	0.55
	Laying order C eggs	0.49	0.48 ± 0.96	0.62
	Sex	0.15	0.08 ± 0.55	0.88
	Hatching order	-0.44	-0.17 ± 0.39	0.66
	Random effects	SD		
	Individual ID (intercept)	<0.01		
	Parental ID (intercept)	0.83		

Note: Reference groups for laying order and sex are A eggs and females, significant terms in bold font.

have higher levels of maternal androgens (Groothuis et al., 2006) that increase competitive behaviours (Eising et al., 2006; Müller et al., 2009); perhaps the reduced hatching success and subsequently increased nestling success of last-laid offspring results from contrasting influences of such maternal androgens pre- and post-hatching. At least among avian species, effects of laying order on hatching success are mixed. In contrast to what we report here, last-laid eggs are more likely to hatch in Lincoln's sparrows (*Melospiza lincolnii*) (Sockman, 2008). Moreover, hatching success across the lay sequence can be context-dependent, varying with both how synchronously eggs were laid (Veiga & Viñuela, 1993) and with the total clutch size (Beissinger & Waltman, 1991). Clearly, the species- and context-specific fitness of offspring produced at different points in the laying sequence can play an important role in determining how maternal effects evolve.

In our study population, where not all individuals find a breeding partner, males hatched from last-laid eggs were least likely to become breeders. We might have predicted that offspring from later-laid eggs should have an advantage when competing for mates, since offspring with higher pre-natal androgens moult earlier into nuptial plumage (Eising et al., 2006) and earlier-moulting individuals produce larger clutches (van Rhijn & Groothuis, 1987). The fact that the opposite is true for male offspring suggests that other maternal effects have a stronger influence on male reproductive success. Reduced concentration of seemingly beneficial maternal substances across the laying order are common, but this is not usually sex-dependent (carotenoids: (Groothuis et al., 2006; Royle et al., 2001, 2003; Rubolini et al., 2011; Saino et al., 2002); immunoglobins:

(Groothuis et al., 2006), but see (Hargitai et al., 2006); vitamin E: (Ardia et al., 2006; Badyaev et al., 2005; Barbraud & Chastel, 1999; Glutz von Blotzheim & Bauer, 1982; Groothuis et al., 2006; Houston et al., 1983; Parolini et al., 2015; Partecke & Schwabl, 2008; Rubolini et al., 2011; Soma et al., 2007)). In black-headed gulls, testosterone allocation across the clutch does not differ according to sex (although this is the case in other species, e.g. (Badyaev et al., 2005)). Even in the absence of sex differences in the concentration of maternal substances, male and female embryos may experience different fitness consequences of reduced maternal allocation. Black-headed gull males are larger than females (Glutz von Blotzheim & Bauer, 1982), suggesting that males body size is sexually selected; perhaps the small size of last-laid embryos caused by limited maternal resources (Hargitai et al., 2006; Soma et al., 2007) is costly in outcomes of mate choice.

In female offspring, laying order did not influence the chance of finding a breeding partner but did influence reproductive output. Embryos in last-laid eggs of both sexes receive lower concentration of antioxidants and immunoglobins in this species (Groothuis et al., 2006), but the high costs of egg production means that this is likely to have a disproportionately large impact on the reproduction of females. Both egg mass and egg quality are known to be linked to female condition (Ardia et al., 2006; Houston et al., 1983), and in another seabird species females in better condition have greater hatching success (Barbraud & Chastel, 1999). We suggest that the sex-dependent effect of laying order on hatching success is due to the fact that females have a disproportionately large influence on the size, resources and survival of the eggs they produce. While we

did not find evidence that laying order affects the ability of parents to successfully produce fledglings, this is perhaps not surprising given the very low fledging rate in our population (pairs fledge on average 0.41 offspring per nest and only 26% of nests produce at least one fledgling). It is possible that, with greater variation in fledging success, such effects would be detectable.

Our results suggest that differential exposure to maternal effects and conditions across the production order can have consequences on offspring phenotype that extend well past the juvenile stage. Although we can currently only speculate about the mechanisms underlying these long-lasting effects, we suggest that organizational effects of maternal hormones may have a strong role to play. In both house sparrows (*Passer domesticus*) and rock pigeons (*Columba livia*), expression of increased dominance and aggression in adults hatched from eggs with elevated testosterone have been suggested to arise through organizational changes to brain structure and function (Hsu et al., 2016; Partecke & Schwabl, 2008); given the complex and nuanced nature of black-headed gull mate choice and courtship displays (Bebbington & Groothuis, 2022), it is possible that differential development of brain tissue in last-laid eggs is responsible for their reduced ability to secure a mate.

Since we tested our hypotheses about the effect of laying order on offspring fitness in a captive study population, we were able to exclude the effects of predation and food limitation on survival and therefore reveal more subtle differences in fitness that would potentially be difficult to detect in wild populations. However, it is important to note that selection on laying order effects may be weaker in wild populations due to relatively large effects of stochastic external factors such as nest predation. Based on our findings, we argue that directional selection on laying order effects is in any case unlikely. Positive selection on the phenotypes of last-laid offspring (that allow them to survive the first year of life) appear to be traded off against the disadvantages (in terms of reproductive success) that those phenotypes confer in adulthood (Schluter et al., 1991). Such opposing selection pressures across life may constrain the evolution of maternal effects, at least with respect to laying order. Our results demonstrate that pre-natal position in the laying sequence, rather than post-natal position in the brood hierarchy, drives sex-specific effects in this species. While hatching order was not related to any of the measures of adult reproductive success, it is important to note that the majority (ca. 70%) of individuals for which we had data on reproductive success were the only offspring that hatched in their nest, leaving little variation to detect hatching order effects on reproductive success. At the same time, the relatively high proportion of nestlings raised without nest mates allowed us to disentangle pre- and post-hatching effects; this is often difficult in other systems because earlier-laid eggs tend to hatch first. In the few studies that have successfully distinguished between laying order and hatching order effects, conclusions are mixed: first-hatched common kestrel (*Falco tinnunculus*) nestlings have higher fitness than later-hatched siblings regardless of laying order (Martínez-Padilla et al., 2017), while in zebra finches (*Taeniopygia guttata*), ovulation order affects offspring telomere length before the eggs have even

been laid (Noguera et al., 2016), ruling out any influence of post-hatching conditions on the effect of laying order. Whether pre-natal maternal effects, post-natal effects of the social environment or a combination of the two drives variation in offspring fitness likely depends on species-specific ecology. Distinguishing between these two components of offspring hierarchies and determining the potential interplay between them will be crucial in advancing our understanding of the evolution of maternal effects, sibling competition and reproductive strategies more broadly.

AUTHOR CONTRIBUTIONS

Kat Bebbington: Conceptualization (equal); data curation (equal); formal analysis (lead); funding acquisition (equal); investigation (lead); methodology (lead); validation (equal); visualization (lead); writing – original draft (lead); writing – review and editing (equal).

Ton G.G. Groothuis: Conceptualization (equal); data curation (equal); formal analysis (supporting); funding acquisition (equal); investigation (supporting); methodology (supporting); project administration (lead); resources (lead); supervision (lead); validation (equal); visualization (supporting); writing – original draft (supporting); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at <https://doi.org/10.5061/dryad.gtht76hrq>.

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