

Nanette Verboven · Neil P. Evans · Liliana D'Alba ·
Ruedi G. Nager · Jonathan D. Blount · Peter F. Surai ·
Pat Monaghan

Intra-specific interactions influence egg composition in the lesser black-backed gull (*Larus fuscus*)

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Abstract Egg composition, which is under maternal control, can have a profound effect on offspring fitness. The presence of maternal testosterone and carotenoids in avian egg yolk, for example, is thought to enhance the development and competitive ability of the offspring and protect the hatching and growing chick against oxidative stress. Egg quality often differs between females and such variation can be due to differences in maternal social environment, e.g. breeding density. However, this is confounded by the possibility that the quality of individuals breeding in high- or low-density areas may vary. We tested if maternal social environment influences egg composition in a colonial seabird, the lesser black-backed gull (*Larus fuscus*). To control for confounding effects of female quality, we experimentally manipulated maternal social environment during egg formation. We increased the frequency of intra-specific interactions (i.e. aggressive encounters with conspecifics other than nest mates) in which the females were involved, by placing an elevated platform in their territory. Females that took part in more intra-specific interactions produced a heavier last egg, but the yolk testosterone concentration in eggs laid by control and experimental females did not differ. Differences in

yolk testosterone concentration in relation to embryo sex were found neither in the control nor in the experimental group. In contrast, within the control group, eggs with a male embryo contained more carotenoids than eggs with a female embryo. Moreover, experimental females that had been involved in more intra-specific interactions produced female eggs with higher carotenoid levels compared to female eggs of control birds. An experimental increase in carotenoid levels was not observed in eggs containing a male embryo. Our results suggest that intra-specific interactions experienced by female birds during egg formation can influence conditions for embryonic development.

Keywords Egg composition · Embryo sex · Intra-specific interactions · Yolk carotenoids · Yolk testosterone

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N. Verboven (✉) · L. D'Alba · R. G. Nager · J. D. Blount ·
P. Monaghan

Division of Environmental and Evolutionary Biology,
Institute of Biomedical and Life Sciences, Graham Kerr Building,
University of Glasgow,
Glasgow, G12 8QQ, UK
e-mail: nv6r@udcf.gla.ac.uk
Tel.: +44-141-3302428
Fax: +44-141-3305971

N. P. Evans
Institute of Comparative Medicine, Veterinary School,
University of Glasgow,
Bearsden Road, Glasgow, G61 1QH, UK

P. F. Surai
Avian Science Research Centre,
Scottish Agricultural College,
Auchincruive, Ayr, KA6 5HW, UK

Introduction

The phenotype of an individual is determined not only by its genotype but also by the environmental conditions it experiences during development. The latter can vary as a consequence of effects operating via maternal state. Such maternal effects can have a profound impact on an individual's fitness (Mousseau and Fox 1998), as conditions during early life may have far-reaching and long-lasting consequences for offspring development and adult performance (Lindström 1999; Metcalfe and Monaghan 2001, 2003). In birds, it has been proposed that offspring performance is influenced by maternal resource allocation during egg formation. For example, eggs can vary in size (Williams 1994; Christians 2002), water and lipid content (Royle et al. 1999; Nager et al. 2000). In addition, females may deposit specific components in the egg, such as antibodies (Gasparini et al. 2001; Saino et al. 2003a), carotenoids (Royle et al. 1999, 2001; Blount et al. 2002a) and steroid hormones (Schwabl 1993), all of which may influence offspring growth and survival.

Exposure to steroids via the egg yolk may increase offspring fitness as it can enhance growth and competitive

ability of the chick. Indeed, yolk testosterone has been found to accelerate embryonic development (Eising et al. 2001), strengthen the hatching muscle (Lipar and Ketterson 2000), enhance nestling growth (Eising et al. 2001), begging behaviour (Schwabl 1993; Eising and Groothuis 2003) and dominance rank of fledglings (Schwabl 1996). However, exposure to maternal androgens during development may only be warranted under certain conditions, as yolk androgens may also carry costs for the mother and/or offspring (Eising 2004). The ability of the female to differentially allocate androgens, in response to prevailing environmental conditions, may provide parents with a means by which they may influence the performance of their offspring so as to maximise their fitness.

Carotenoids are responsible for yellow coloration of avian egg yolk. They can influence offspring performance, because they deactivate reactive oxidative metabolites and free radicals produced during metabolism that can cause extensive DNA, protein and lipid damage (Surai 2002). As such, the presence of carotenoids in egg yolk can act to reduce the susceptibility of embryonic tissue to oxidative damage and enhance nestling immune function (Haq et al. 1996; Surai 2002).

It is possible that the maternal deposition of steroid hormones and carotenoids into the egg yolk are not independent processes, because steroid hormones increase metabolic rate (Råberg et al. 1998) and are involved in carotenoid partitioning between different body components (Bjerkeng et al. 1999). Therefore, high steroid concentrations in the adult female may reduce the carotenoids available for deposition within the egg. Indeed, within a clutch, the testosterone concentration and carotenoid content were found to be inversely correlated, suggesting that the deposition of these egg components may indeed be linked (Royle et al. 2001).

Maternal social environment has been identified as a key factor influencing steroid production in adult birds. Interaction between conspecifics stimulates testosterone secretion in both sexes (Nelson 1995), and elevated concentrations of testosterone are associated with aggressive behaviours that characterise the early stages of reproductive activity, such as territory establishment, the formation of dominance relationships and challenges by conspecifics for access to mates (Wingfield et al. 1990). Although females usually have lower concentrations of circulating testosterone than males (e.g. Feder et al. 1977; Hannon and Wingfield 1990), testosterone levels are also increased in females exposed to social stress (Schwabl 1992). In mating systems with intense female-female competition, these periods of increased conspecific interaction and consequent elevated testosterone concentrations often, but not always, coincide with the pre-laying and egg formation period (Donham 1979; Cristol and Johnsen 1994; Elekonich and Wingfield 2000; Eens and Pinxten 2000; Langmore et al. 2002). Indeed, it has been shown that androgen concentrations are elevated in eggs found in areas of high versus low breeding density (Schwabl 1997a; Reed and Vleck 2001; Groothuis and

Schwabl 2002; Pilz and Smith 2004) and where territorial intrusions are frequent versus infrequent (Whittingham and Schwabl 2002; Mazuc et al. 2003).

Most field studies that have investigated the effects of social environment on yolk hormone concentrations have been based on correlations between characteristics of the nesting environment and hormone content of the eggs (Schwabl 1997a; Reed and Vleck 2001; Groothuis and Schwabl 2002; Whittingham and Schwabl 2002; Pilz and Smith 2004). However, females may not be distributed randomly over the available nest sites, and factors that influence nest site choice, such as age and body condition, are also known to influence the deposition of androgens in egg yolk (Pilz et al. 2003; Verboven et al. 2003). To investigate a causal relationship between the social environment and yolk composition, while controlling for such confounding factors, it is necessary to change the social environment experimentally. This is often difficult in the field, but has been attempted by the presentation of nesting females with caged intruders (Mazuc et al. 2003). While this treatment may increase exposure to social stress, the inability of the nest owners and the intruder to display normal behaviour (e.g. the intruder cannot be chased away) may also influence the hormonal state of the female.

Lesser black-backed gulls (*Larus fuscus*) nest in dense colonies and, during the pre-laying period, both males and females aggressively defend the territory against intruders. To investigate the effects of maternal social environment on yolk steroid and carotenoid deposition, we manipulated the number of intra-specific interactions experienced by females during egg formation by changing the nest site characteristics of experimental pairs. After territory establishment, we placed an elevated platform in the centre of their territory. The platform was large enough for one bird to stand on and be clearly visible from the surrounding territories. As visibility enhances intra-specific competition in fish (e.g. Sundbaum and Näslund 1998) and gulls use objects near the nest as landmarks (Tinbergen 1953), we expected that the introduction of the platform in a dense breeding colony of gulls would increase nest defence behaviour of both the focal pair and its neighbours. If social environment influences yolk testosterone deposition, we hypothesised that more testosterone would be present in eggs laid by females that experienced more intra-specific interactions. We also investigated if social environment had any effect on the carotenoid content of the eggs, in case testosterone and carotenoid concentrations in eggs are related. In addition, because the relative fitness costs and benefits of depositing maternal resources in male and female eggs may differ (Anderson et al. 1997; Cordero et al. 2000, 2001; Petrie et al. 2001; Müller et al. 2002; Blanco et al. 2003; Margrath et al. 2003; Saino et al. 2003a), we investigated whether egg composition differed in relation to offspring sex.

Methods

Experimental protocol

Data were collected in a large colony of lesser black-backed gulls on Walney Island, Cumbria, UK. Control ($n=11$) and experimental pairs ($n=11$) were randomly selected from the centre of the breeding colony. These pairs were observed for 45 min, at least once every 3 days, starting on 7 April 2002. On 19 April ($n=7$), 23 April ($n=1$) and 29 April ($n=3$), elevated platforms (height 15 cm, diameter 20 cm) were placed in the experimental territories. To minimise the possibility of territory holders losing the platform to other birds, platforms were always introduced after territory establishment and pair formation, i.e. when a pair was seen defending the same area during at least two consecutive 45-min observation periods. Nests, which were usually built within 2 m distance of the platform, were inspected daily. As soon as eggs were laid, they were collected, weighed and replaced with dummy eggs. Behavioural observations continued until the last egg was laid. All but one pair laid 3 eggs and the total number of collected eggs was 65 (one experimental female laid only 2 eggs). Eggs were kept in an incubator (Curfew 747 Professional) at 38°C for 4 days to allow embryonic growth and facilitate sex determination using molecular methods (Griffiths et al. 1998), before being stored at -20°C. In five eggs, embryo development was insufficient for reliable sex determination, so embryo sex was only available for 60 eggs. Androgen production by the embryo does not begin until 6–8 days after the start of incubation in domestic fowl (Ottinger 1989; Elf and Fivizzani 2002; Eising et al. 2003), which show a similar incubation period to lesser black-backed gulls. However, for an unknown reason, androgen levels may sharply decrease from day one of incubation (Elf and Fivizzani 2002). Therefore, androgens measured as early as 4 days may not be the same as what was maternally allocated. Although the results should be interpreted with caution, we assume that eggs laid by control and experimental females are comparable, because all eggs were given exactly the same incubation period.

Behavioural observations

During 45-min observation periods, we recorded the amount of time each pair member was present in the territory. The amount of time the male and the female were aggressive, resting or involved in courtship behaviour during each observation period was also recorded and categorised according to Tinbergen (1953). Aggressive behaviour included threat posture, grass pulling, long calls, walking or flying towards, or away from an opponent and actual fights. The total number of intra-specific interactions (i.e. aggressive encounters with conspecifics other than nest mates), as well as the number of intra-specific interactions won (resulting in the intruder moving back), were also counted. Courtship behaviour comprised nest building, choking, head tossing, begging, courtship feeding and copulation. When a bird was preening or sleeping it was classified as resting. The sex of the birds studied was determined by the presence of colour rings (in which case individuals had been part of a previous study and sexed using molecular techniques) or, if the bird was not ringed, by its size relative to its partner (males are larger than females) and sex-specific behaviour (begging, courtship feeding, copulation). For the purpose of this study on egg composition we focus on female behaviour only.

Yolk testosterone analysis

Eggs were dissected while frozen, and yolk, albumen and shell weighed individually. Prior to hormone assay, whole yolks were thoroughly mixed and a weighed sample (50–100 mg) diluted with an equal amount of water (1:1 w/w). Before extraction, 6,000 cpm tritiated testosterone was added to allow calculation of extraction recovery. After adding 2 ml methanol, the samples were vortexed for 40 min and then centrifuged at -8°C for 10 min. 300 μ l of the

supernatant was transferred to a new vial and diluted with 2,700 μ l water. The samples were purified using pre-conditioned Isolute C18 columns (International Sorbent Technology, UK). After running the sample through the column, the column was washed with 3 ml water. Testosterone was eluted with 3 ml 70% methanol. Extraction efficiency (estimated from recovery of tritiated testosterone) for the yolk samples averaged 74.2 \pm 0.98%. Three 200- μ l aliquots of each extract were dried under a stream of air and resuspended in 100 μ l assay buffer (0.05 M phosphate buffered saline with 0.25% bovine serum albumin). Testosterone concentrations were measured in triplicate with double antibody radioimmunoassay using a modification of an established protocol (Sheffield and O'Shaughnessy 1989). Each yolk sample was randomly allocated to one of three different assays. The inter-assay CV values at 50% and 21% displacement points averaged 5.6% and 2.5%, respectively, and the mean sensitivity (two standard deviations of buffer controls) averaged 2.0 pg/mg yolk. The mean intra-assay CV value was 21.1%.

Yolk carotenoid analysis

Yolk carotenoid content was estimated visually using the Roche Yolk Colour Fan (scale 1–15; e.g. Nys 2000). Homogenised yolks were scored twice by the same observer who was unaware of the experimental treatment. The repeatability of the two colour scores was high ($r=0.96$, $n=65$, $P<0.001$). In addition, we measured the carotenoid concentration in 21 third laid eggs using HPLC analysis. Yolk samples (~200 mg) were extracted twice with 2 ml hexane after protein precipitation with ethanol. Extracts were dried down under nitrogen gas and dissolved in 0.3 ml dichloromethane and 0.3 ml methanol. Samples (10 μ l) were injected into a Spherisorb type S5NH₂ reverse-phase column 25 \times 4.6 mm (Phase separation, Clwyd, UK) with a mobile phase of methanol-distilled water (97:3), at a flow rate of 1.5 ml min⁻¹ as described by H \ddot{o} rak et al. (2002). Total carotenoid concentration was measured at an absorbance of 445 nm, and results were calculated in relation to lutein as a standard (Sigma, Poole, UK).

Statistical analyses

In lesser black-backed gulls, the process of yolk formation takes approximately 10 days (Carey 1996). Because we were interested in the effect of social environment on egg composition, we examined female behaviour during the egg formation period and analysed all behavioural data collected from 10 days before the appearance of the first egg until the last egg of the clutch was laid. At the time pairs were assigned to different treatments, the laying date of the first egg of the clutch was not yet known. To control for seasonal variation in female behaviour, each experimental female was matched with a control female that had laid her first egg on the same day (± 1 day). For the purpose of the analysis, control females were assigned the same treatment start date as the experimental pair they were matched with, although no territorial changes were instigated on that date.

As eggs laid by, and behavioural observations from, each female are not independent, the data were analysed using generalised linear mixed models (GLMM) with female identity as a random effect (SAS version 8.2). The significance of the variance component was tested using a Z-test. This indicated whether or not variability existed among females. The position of an egg in the laying order (first, second or third), treatment (control or platform), and embryo sex (male or female) were modelled as fixed effects. The significance of these factors was tested by means of stepwise backward elimination, starting with a full model and testing two-way interactions first. Main effects were kept in the model whenever interactions were significant. The correct degrees of freedom for every estimate and test of interest was calculated using the Satterthwaite approximation (Littell et al. 2002). To ensure that within- and between-nest effects in yolk testosterone concentrations were not due to confounding inter-assay effects, we added a factor indicating assay number to the model. This factor was not signif-

icant in any of the analyses (all $P>0.36$) and was therefore removed from the models. For proportional data such as the percentage of interactions won and the proportion of male offspring we used GLMM with logit link function and binomial error structure (GLMMIX macro, SAS version 8.2). This was done to account for unequal variances and differences in sample size (i.e. total number of interactions, respectively brood size).

Results

Female behaviour

At the start of the experiment, before the platforms were placed in the territory, the average number of intra-specific interactions per hour did not differ between control and experimental females (mixed model with female identity as a random effect, $F_{1,20}=0.34$, $P=0.57$). Because controls were allocated based on laying date, control and experimental females did not differ in the date at which they laid their first egg (control: 8.1 May \pm 2.0 days ($n=11$), experiment: 8.4 May \pm 2.2 days ($n=11$), $F_{1,20}=0.03$, $P=0.93$). On average, the first egg was laid 15.8 \pm 1.6 days after a platform was placed in the territory. During the 10-day yolk formation period, control and experimental females did not differ in the amount of time they were present on the territory and, while present, females in the two groups did not differ in the amount of time spent in aggressive, resting and courtship behaviour (Table 1). The proportion of intra-specific interactions won did not differ between the two groups. However, the total number of intra-specific interactions in which females were involved was significantly higher in the experimental group than in the control group (Table 1). This indicates that the introduction of a platform altered maternal social environment during the egg formation period and that experimental females were challenged by conspecifics more often.

Egg mass

Fresh egg mass differed among females ($Z=2.66$, $n=22$, $P=0.004$) and declined with the position of an egg in the

laying sequence (mixed model with female identity as a random effect, $F_{2,38.9}=13.65$, $P<0.001$). Interestingly, third eggs of experimental females were heavier than third eggs of control females (mixed model with female identity as a random effect, interaction laying order \times treatment: $F_{2,38.9}=4.58$, $P=0.002$; Fig. 1a). As expected, the mass of most egg components declined with the position of an egg in the laying sequence (mixed model with female identity as a random effect, yolk: $F_{2,39.1}=7.49$, $P=0.002$; albumen: $F_{2,38.8}=6.70$, $P=0.003$; shell: $F_{2,38.3}=2.89$, $P=0.068$). There was no significant difference in yolk mass or shell mass between eggs laid by control or experimental females (mixed model with female identity as a random effect, controlled for laying order, yolk mass: $F_{1,19.8}=1.07$, $P=0.31$; shell mass: $F_{1,18.9}=0.34$, $P=0.57$). The higher total egg mass of last eggs in the clutches of experimental females was due to a disproportional increase in albumen mass across the laying sequence (mixed model with female identity as a random effect, interaction laying order \times treatment, $F_{2,38.8}=4.29$, $P=0.021$). Egg mass did not differ between eggs containing a male or a female embryo (mixed model with female identity as a random effect, controlled for laying order and treatment, $F_{1,38.2}=0.60$, $P=0.44$).

Yolk testosterone

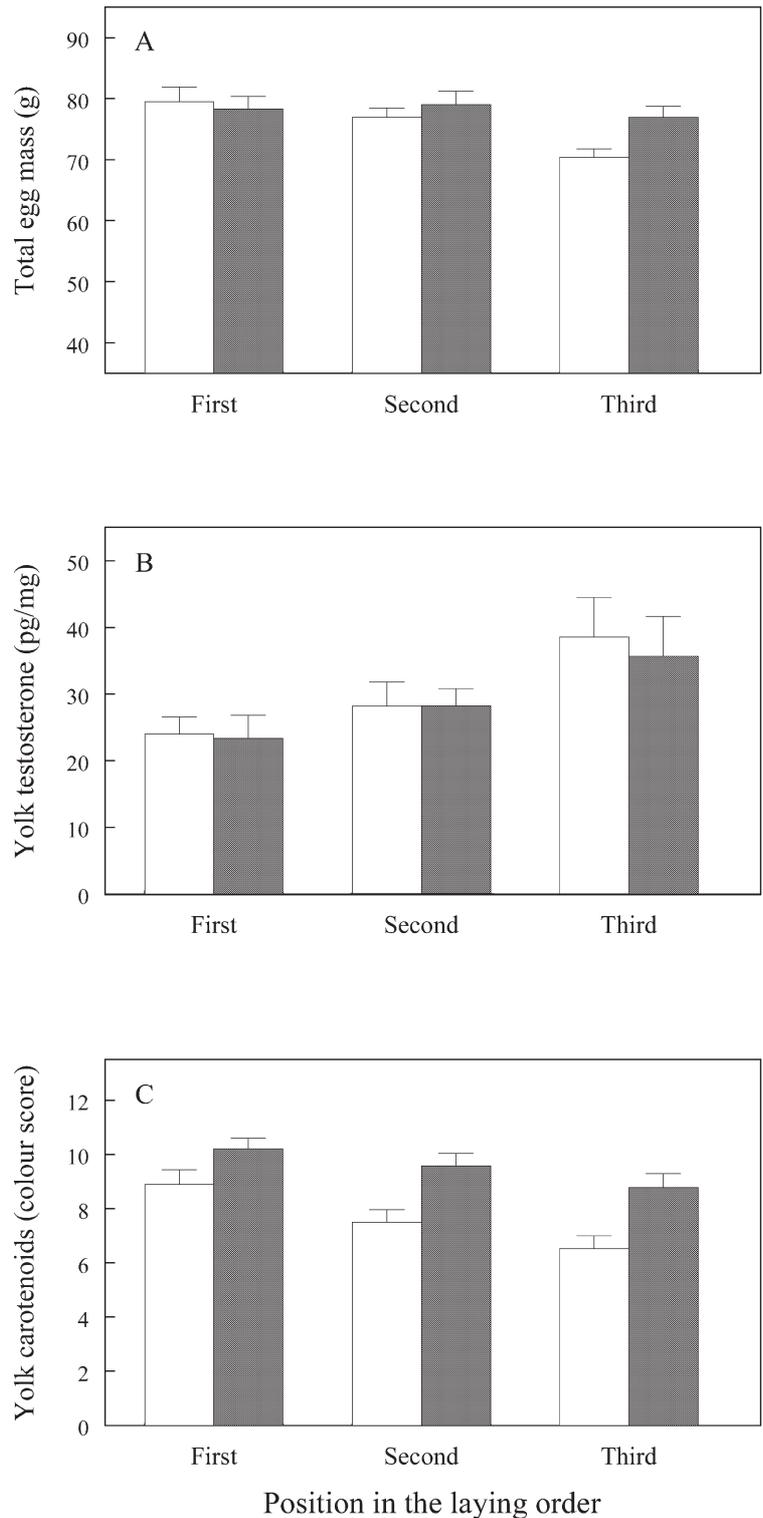
The yolk testosterone concentration did not differ among eggs laid by different females ($Z=0.83$, $n=22$, $P=0.20$). The yolk testosterone concentration increased with the position of an egg in the laying sequence (mixed model with female identity as a random effect, $F_{2,41.8}=6.07$, $P=0.005$). There was no difference in yolk testosterone concentration between eggs in the clutch of control and experimental females (mixed model with female identity as a random effect, controlled for laying order, $F_{1,20.3}=0.09$, $P=0.77$; Fig. 1b), demonstrating that an experimental increase in intra-specific interactions during the egg formation period did not result in higher yolk testosterone concentrations. Taking into account differences in yolk mass and analysing the total amount of testosterone per egg did not change the results (mixed

Table 1 The amount of time in min/h that control and experimental female lesser black-backed gulls (*Larus fuscus*) were absent during the egg formation period (i.e. from 10 days before the first egg until the third egg was laid) and the behaviour while present on the territory. Female behaviour is summarised in three categories (aggressive, courtship and resting behaviour; Tinbergen 1953). The

frequency of intra-specific interactions (i.e. aggressive encounters with conspecifics other than nest mates), as well as the proportion of intra-specific interactions won (i.e. resulting in the intruder moving back) are also given. The average number of observations per female was 4.5 \pm 0.22 (range 3–7)

| | Control Mean \pm SE min/h (11 females) | Experimental Mean \pm SE min/h (11 females) | GLMM analysis | P-value |
|--------------------------------|---|--|-------------------|---------|
| Absent | 3.66 \pm 1.33 | 4.63 \pm 1.49 | $F_{1,106}=0.24$ | 0.63 |
| Aggressive | 0.50 \pm 0.20 | 1.24 \pm 0.88 | $F_{1,106}=0.67$ | 0.41 |
| Courtship | 2.26 \pm 0.51 | 1.89 \pm 0.47 | $F_{1,106}=0.28$ | 0.60 |
| Resting | 57.3 \pm 0.57 | 57.65 \pm 0.57 | $F_{1,106}=0.23$ | 0.63 |
| No. interactions per h | 0.61 \pm 0.20 | 1.93 \pm 0.52 | $F_{1,106}=5.63$ | 0.02 |
| Proportion of interactions won | 0.43 | 0.45 | $F_{1,15.4}=1.50$ | 0.24 |

Fig. 1 a Mean total egg mass \pm SE, **b** mean yolk testosterone concentration \pm SE and **c** yolk carotenoids (mean colour score \pm SE, measured using the Roche Yolk Colour Score Fan) in first, second and third laid eggs of control female lesser black-backed gulls (*Larus fuscus*) (*white*, $n=11$) and females with experimentally increased number of intra-specific interactions (*black*, $n=11$)



model with female identity as a random effect, laying sequence: $F_{2,41.8}=4.63$, $P=0.015$; treatment: $F_{1,20.3}=0.13$, $P=0.72$). Yolk testosterone concentration did not differ between male and female eggs (mixed model with female identity as a random effect, controlled for laying order, $F_{1,54.3}=0.34$, $P=0.56$).

Yolk carotenoids

Yolk colour scores on the Roche scale were positively correlated with the total carotenoid concentration as measured with HPLC in a sub-sample of third laid eggs ($r=0.77$, $P<0.001$, $n=21$; Fig. 2), indicating that colour

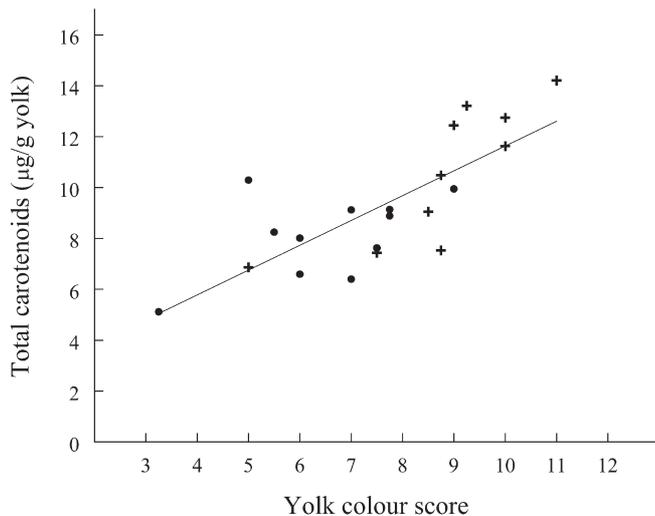


Fig. 2 Total yolk carotenoid concentration measured with HPLC plotted against the yolk colour score measured using the Roche Yolk Colour Fan for third laid eggs of 11 control (●) and 10 experimental clutches (+). Third laid eggs of experimental females had significantly higher total carotenoid concentrations than third laid eggs of control females ($F_{1,19}=6.55$, $P=0.019$). Controlling for differences in egg mass or yolk mass did not change these results (treatment effect controlled for egg mass: $F_{1,18}=4.28$, $P=0.05$; controlled for yolk mass: $F_{1,18}=6.71$, $P=0.018$)

scores could be used to estimate total yolk carotenoid concentration. Yolk carotenoid content based on colour scores differed between eggs laid by different females ($Z=4.50$, $n=22$, $P<0.001$) and decreased with the position of an egg in the laying order (mixed model with female identity as random effect, $F_{2,40,7}=14.21$, $P=0.002$). Compared to control females, experimental females that were exposed to more aggressive interactions produced eggs with higher carotenoid content throughout their clutch ($F_{1,19,5}=12.12$, $P=0.002$; Fig. 1c). The interaction treatment \times laying order was not significant ($F_{2,38,6}=1.41$, $P=0.26$), indicating that the treatment difference was the same for eggs at different positions in the laying order. Within the control group, eggs with a female embryo contained less carotenoids than eggs with a male embryo (mixed model with female identity as a random effect, controlled for laying order, $F_{1,21,1}=6.19$, $P=0.021$). This was not the case within the experimental group (mixed model with female identity as a random effect, controlled for laying order, $F_{1,22,8}=1.31$, $P=0.26$). The introduction of a platform in the territory thus enhanced the carotenoid content of female, but not of male, eggs (mixed model with female identity as a random factor, controlled for laying order, interaction treatment \times embryo sex: $F_{1,46,4}=4.96$, $P=0.031$; Fig. 3). In this model, there was no significant interaction between laying order and embryo sex ($F_{1,42,5}=0.65$, $P=0.53$), indicating that the sex differences found in the control group were the same across the laying sequence. Differences in carotenoid levels in relation to embryo sex were not due to variation in the proportion of male embryos in eggs laid by control or experimental females (mixed model with female identity

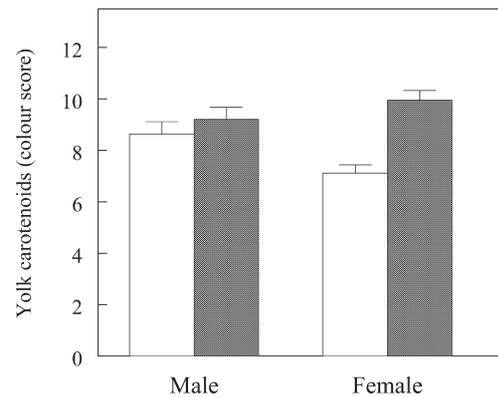


Fig. 3 Yolk carotenoids (mean colour score \pm SE, measured using the Roche Yolk Colour Fan) in male and female eggs laid by control (white) and experimental females (black)

as a random effect, treatment: $F_{1,20,7}=0.97$, $P=0.34$), or in the proportion of males in eggs laid at different positions in the laying sequence (mixed model with female identity as a random effect, laying order: $F_{1,39,5}=1.03$, $P=0.37$). The observed sex differences thus suggest sex-specific allocation of carotenoids in relation to maternal social environment.

Discussion

The placement of an elevated platform in the territory of lesser black-backed gulls altered the social environment of females during egg laying. Compared to control females, the females provided with a platform were involved in a higher number of intra-specific interactions per hour during the 10-day egg formation period. Egg composition differed between eggs laid by control and experimental females. The last egg in clutches of experimental females was heavier and contained more albumen than the last egg in clutches of control females. Moreover, female eggs laid by experimental birds contained more carotenoids throughout the clutch than did female eggs laid by control birds. However, the yolk testosterone concentration did not differ between eggs of control and experimental females. Our results show that maternal social environment can influence conditions for offspring development, through differences in egg composition.

A previous study conducted in tree swallows (*Tachycineta bicolor*) has documented that yolk testosterone concentration was positively correlated with the frequency of territorial intrusions (Whittingham and Schwabl 2002). However, effects of female quality and nest site characteristics, such as breeding density, may confound the interpretation of this result. In an attempt to control for such confounding effects, we experimentally manipulated maternal social environment by increasing the frequency of intra-specific interactions during the egg formation. While changing the characteristics of the territory increased the frequency of intra-specific interactions, our results on egg composition did not show any

effect of the treatment on yolk testosterone concentrations. This result contrasts with that of Mazuc et al. (2003) who found that the introduction of a caged intruder increased yolk testosterone concentrations in house sparrow (*Passer domesticus*) eggs. However, their change in yolk testosterone concentrations was associated with an increase in the duration of female aggressive behaviour. Although our manipulation increased the frequency of intra-specific interactions, it did not significantly increase the amount of time experimental females were aggressive towards neighbours, which may explain why yolk testosterone concentrations in this study were not elevated.

Alternatively, the lack of an effect of our territorial manipulation on yolk testosterone could be due to an ability of female lesser black-backed gulls to control the amount of testosterone they deposit into their eggs, independently of changes in social environment and maternal hormonal state. Although a positive correlation between female plasma testosterone and yolk testosterone has been found (Schwabl 1997b), and experimental elevation of hormones in maternal circulation has been shown to result in elevated levels of these hormones in the egg (Arcos 1972; Adkins-Regan et al. 1995; Wilson and McNabb 1997; Hayward and Wingfield 2004), it is likely that testosterone could also enter the yolk directly following local synthesis in the cells surrounding the developing follicle (Hackl et al. 2003). This local deposition of testosterone into the egg yolk could occur independently of the amount of testosterone present in maternal circulation. Indeed, negative relationships between maternal plasma and yolk testosterone concentrations have been observed (Mazuc et al. 2003; Verboven et al. 2003). If females are able to regulate the transfer of maternal hormones to offspring, yolk testosterone concentrations may remain unaffected, despite changes in maternal hormonal state as a consequence of increased territorial behaviour.

While testosterone concentrations were not affected by the experimental manipulation, other egg characteristics, that could have implications for offspring fitness, were altered in association with the observed increase in intra-specific interactions, namely egg mass and carotenoid concentration. An increase in egg mass was due to higher albumen content in eggs laid by experimental females. Other studies on egg composition in gulls also found that larger eggs contained relatively more albumen (Parsons 1976; Meathrel and Ryder 1987; Nager et al. 2000). It is interesting to note that the effects on egg albumen content were only observed in the last laid eggs in the clutch, which had potentially been exposed to increased intra-specific interactions for longest. The mechanism by which social interactions could affect the amount of albumen present within in the egg deserves further study, because albumen, as the primary source of water, protein and lysozymes in the egg (Carey 1996), is an important determinant of hatchling size (e.g. Finkler et al. 1998) and offspring performance.

Yolk colour scores on the Roche Yolk Colour Fan proved a reliable measure of the total carotenoid con-

centration in the egg yolk. While yolk testosterone concentration increased with the position of an egg in the laying sequence, the carotenoid content declined towards the last egg of the clutch (see also Blount et al. 2002a). These opposite within-clutch patterns confirm the results of a previous study (Royle et al. 2001) and suggest that the two egg components may indeed be linked. Our results, however, also show that an increase in yolk carotenoids is possible without changes in yolk testosterone concentrations, because females that were involved in more intra-specific interactions produced eggs with more carotenoids throughout their clutch, despite there being no change in testosterone concentrations.

Carotenoids are taken up from the yolk in tissues of the growing embryo, where they improve offspring immune function (Saino et al. 2003b) and provide protection against oxidative stress during a period of rapid growth (Haq et al. 1996; Surai and Speake 1998). This may be of particular importance in a dense breeding colony where offspring are likely to experience a high level of intra-specific interactions, placing high demands on the chick's immune system due to increased contact rate with parasites and diseases (Hario and Rudbäck 1999). In this situation, females could enhance the fitness of their offspring by depositing a relatively large amount of antioxidants in the yolk of their eggs. However, animals cannot synthesise carotenoids *de novo* (Goodwin 1984) and, although a certain range of metabolic transformations are possible (Møller et al. 2000; Blount et al. 2002b), animals must obtain carotenoids in their diet. It has therefore been hypothesised that antioxidants are a scarce, limiting resource (Olson and Owens 1998; Møller et al. 2000) and that parent birds provisioning antioxidants to their offspring may incur significant costs by compromising their own antioxidant defence. Females that are subject to high levels of aggression and elevated levels of plasma testosterone may differ in the way they utilise their antioxidant defence mechanism, which may lead to different amounts and/or different composition of carotenoids in egg yolk.

The effect of maternal social environment on yolk carotenoid content was not uniform across the study and, interestingly, an increase in carotenoid concentration was only present in female eggs of birds exposed to increased intraspecific interactions. In the control group, female eggs contained significantly less carotenoids than male eggs. Only a few studies have investigated yolk carotenoid concentration in male and female eggs. Sex differences were not found in the eggs of barn swallows (*Hirundo rustica*; Saino et al. 2003a). In contrast to lesser black-backed gulls, male and female barn swallows do not differ in size. Differential allocation of maternal resources to male and female eggs may be favoured in sexually size-dimorphic species, where male and female offspring exhibit different growth rates. In lesser black-backed gulls, male hatchlings are larger than female hatchlings (Griffiths 1992) and, as nestlings, males are more vulnerable to adverse effects of poor egg quality (Nager et al. 1999). Because males grow faster, they may

be exposed to more oxidative stress in the egg and as hatchlings. Therefore, females may benefit from depositing higher amounts of carotenoids in male eggs. Molecular mechanisms of differential allocation of carotenoids to egg yolk depending on the sex of the embryo need further investigation. Interestingly, sex differences in yolk carotenoid content disappeared when females were more frequently involved in aggressive interactions.

Our results show that female involvement in intra-specific interactions at the time of egg laying can have a profound effect on egg characteristics. Maternal social environment may thus influence the conditions under which embryos develop. Differences in embryonic conditions may have adaptive value for offspring developing under different environmental circumstances. Alternatively, as physiological processes are often inter-related, influencing a wide range of different tissues, phenotypic variation in egg composition may be the result of underlying physiological mechanisms acting as constraints on the evolution of life-history traits. To understand individual differences in resource allocation patterns of egg laying females, it is necessary to combine behavioural studies with an investigation of the physiological mechanism of egg formation as well as consequences of egg composition for offspring fitness.

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