Maternal effects in the highly communal sociable weaver may exacerbate brood reduction and prepare offspring for a competitive social environment

René E. van Dijk · Corine M. Eising · Richard M. Merrill · Filiz Karadas · Ben Hatchwell · Claire N. Spottiswoode

Received: 11 August 2011 / Accepted: 14 August 2012 © Springer-Verlag 2012

Abstract Maternal effects can influence offspring phenotype with short- and long-term consequences. Yet, how the social environment may influence egg composition is not well understood. Here, we investigate how laying order and social environment predict maternal effects in the sociable weaver, *Philetairus socius*, a species that lives in massive communal nests which may be occupied by only a few to 100+ individuals in a single nest. This range of social environments is associated with variation in a number of phenotypic and life-history traits. We investigate whether maternal effects are adjusted accordingly.

We found no evidence for the prediction that females might benefit from modifying brood hierarchies through an increased deposition of androgens with laying order. Instead, females appear to exacerbate brood reduction by decreasing the costly production of yolk mass and antioxidants with laying order. Additionally, we found that this effect did not depend on colony size. Finally, in accordance with an expected increased intensity of environmental stress with increasing colony size, we found that yolk androgen concentration increased with colony size. This result suggests that females may enhance the competitive ability of offspring raised in larger colonies, possibly preparing the offspring for a competitive social environment.

Keywords Breeding density · Competition · Egg composition · Hatching asynchrony · Maternal investment

Introduction

The social environment in which animals live has important implications for their life-histories (Stearns 2000; Hau et al. 2010; Székely et al. 2010). Breeding density, for instance, is likely to influence the level of competition for resources (Kokko et al. 2004; Alonzo and Sheldon 2010), and parents may be able to improve their fitness by adjusting their offspring’s phenotype to the environment in which they live. One mechanism for parents to achieve this is through maternal effects (Mousseau and Fox 1998), which may be manifested in egg size, egg composition, laying date and parental care (Price 1998).

Deposition of hormones (such as androgens) and carotenoids of maternal origin in avian egg yolk has been shown to depend on the position of the egg in the laying sequence (Schwabl 1993; Kozłowski and Ricklefs 2010),...
and may influence sibling competition arising from hatching asynchrony. Negative effects of elevated yolk androgens may limit the potential benefits of increased androgen levels to both mother and offspring. Such negative effects include increased metabolic activity which can lead to an increase in reactive oxygen species (ROS), potentially detrimental by-products of the respiratory chain (Alonso-Alvarez et al. 2007). The generation of oxidative stress owing to an imbalance between the production of ROS and antioxidant defences (Folstad and Karter 1992; Kettersson and Nolan 1999; Finkel and Holbrook 2000; Owen-Ashley et al. 2004) may cause damage to tissues, cells and DNA (von Schantz et al. 1999; Finkel and Holbrook 2000; Larbi et al. 2007). Further negative effects of elevated levels of yolk androgens may include a reduced immune responsiveness of the offspring (Navara et al. 2005; Sandell et al. 2009), reduced fitness of the offspring (Sockman and Schwabl 2000; Rubolini et al. 2006), or a disruption of a potentially adaptive sex-specific effect of testosterone on offspring begging behaviour and growth (von Engelhardt et al. 2006). Despite these negative effects, mitigation of hatching asynchrony through elevated concentrations of yolk androgens may be beneficial by causing offspring that hatch later in the laying order to be better able to compete with their older siblings (Lipar et al. 1999; Eising et al. 2001). Conversely, exacerbated hatching asynchrony may be beneficial when environmental conditions are adverse as it potentially enhances brood reduction ensuring the survival of at least some offspring (Schwabl et al. 1997).

Adjustment of maternal effects may also depend on the social environment in which the parents live and the offspring are raised (Schwabl 1997; Taborsky et al. 2007; Safran et al. 2010), with increased levels of androgens having been detected with increasing group size, predicted levels of competition, and environmental stress (Gil et al. 2007; Eising et al. 2008; Safran et al. 2010). Offspring experiencing increased levels of maternal androgens may be better competitors, especially when living in potentially stressful and competitive environments (Schwabl 1997; Mazuc et al. 2003; Safran et al. 2010). However, the enhanced competitive abilities of offspring may be traded off against various significant physiological and fitness costs (Hall et al. 2010).

The relationship between the social environment and yolk antioxidants, however, is much less clear (Verbomen et al. 2005; Hargitai et al. 2009; Safran et al. 2010), despite bird eggs being rich in carotenoids that play an important role throughout an individual’s life (Blount et al. 2000; McGraw et al. 2005; Biard et al. 2009). The fitness effects of carotenoids are thus much less controversial than those of androgens, yet there is a clear lack of information regarding the relationship between carotenoids and social environment. Carotenoids are of wide relevance in evolutionary studies including those investigating how antioxidants modulate immunity (von Schantz et al. 1999; Saino et al. 2011b). Carotenoids can be acquired only through diet, so competition for food, which is likely to increase with density, may influence the quantity of carotenoids a female deposits in her eggs. Under environmental stress, a female’s level of circulating carotenoid antioxidants may be lower owing to faster depletion of carotenoids, while an increased production of antioxidants may itself also lead to an increased concentration of ROS (Buttemer et al. 2010). Alternatively, living at higher densities may also increase the incidence of disease (Alexander 1974; Brown and Brown 2004), influencing levels of circulating carotenoid antioxidants in laying females. As such, the social environment of the mother may influence the phenotype of her offspring through adjusted levels of yolk antioxidants, with potentially lifelong effects. Antioxidant protection in adult birds has been shown to predict long-term survival in barn swallows, Hirundo rustica, for example (Saino et al. 2011a).

Here, we use sociable weavers, Philetairus socius, as a model species to investigate whether the concentration of maternal androgens and antioxidants in yolks is predicted by (1) the laying order (2) the social environment, defined as the number of adult individuals in the colony, and (3) an interaction between laying order and social environment. Sociable weavers are a particularly suitable study species to address these objectives: first, they are highly social and live and breed in colonies of variable sizes, ranging from only a few to over 100 individuals living in one, massive communal nest. Second, incubation in this species commences with the first or second egg [mean (±SD) clutch size = 3.2 ± 0.4 eggs], leading to pronounced brood hierarchies (Maclean 1973b). Brood reduction through starvation of later-hatched nestlings is common, especially in larger colonies (Spottiswoode 2007). These differences between colonies are reflected in probably adaptive adjustment of the adult phenotype to the selective conditions in colonies of different sizes, such as rates of parasitism, predation and starvation (Spottiswoode 2007, 2009), and are expected to be associated with different levels of environmental stress. Finally, opportunities for breeding are erratic and food availability during breeding is unpredictable, requiring flexible adjustment of maternal investment in egg size and brood size as well as of the offspring’s phenotype. Variable colony size will influence competition for resources, and will also affect social interaction between females, which may be reflected by circulating steroid or carotenoid levels in the maternal plasma and subsequently in the yolks of the offspring (Adkins-Regan et al. 1995; Marshall and Uller 2007). Colony size may also be associated with prevalence of
parasitism and disease (Spottiswoode 2007), which may impose selection for adaptive maternal investment of antioxidants to enhance the immune defence of their offspring. The predation risk experienced by offspring also increases with colony size (Spottiswoode 2007). Sociable weavers lay replacement clutches after such predation events so that up to nine clutches may be produced by a single female in one season (Maclean 1973b). The costs of re-laying (Stearns 1992; Nager et al. 2001; Nilsson and Råberg 2001) may be traded off against maternal investment, again resulting in an association between colony size and maternal effects.

In this study, we investigated several components of prenatal maternal investment, namely egg mass, yolk mass, yolk androgen content (androstenedione, hereafter A4, and testosterone, hereafter T) and yolk antioxidants (the vitamin E-components α- and γ-tocopherol, the vitamin A-component retinol, and the total carotenoid concentration). First, we tested whether maternal investment was predicted by laying order. The relative importance of different yolk androgens, including A4 and T, is unclear, with previous studies focusing on T rather than on other yolk androgens that are part of the metabolic pathway leading to the production of T (Elf and Fivizzani 2002; Hegyi et al. 2011; Paitz et al. 2011). Although elevated levels of androgens may enhance the survival of later-hatched offspring (Lipar et al. 1999), yolk androgens are also costly for both the mother and offspring owing to the generation of oxidative stress (Ketterson and Nolan 1999; Finkel and Holbrook 2000; Owen-Ashley et al. 2004) and their likely consequent fitness costs (Sockman and Schwabl 2000; Rubolini et al. 2006; von Engelhardt et al. 2006). Such negative effects of yolk androgens may be counteracted by an increase in yolk antioxidants, but high androgen concentrations in the female may reduce the antioxidants available for eggs (Royle et al. 2001; Verboven et al. 2005), while the production of antioxidants itself may increase the concentration of ROS. Hence, antioxidants deposited in the yolk may compromise the female’s own current condition and ability to invest in subsequent offspring.

Second, we tested whether maternal investment is predicted by the size of the colony. For adults, high-density environments are likely to be more stressful (Eising et al. 2008; Hargitai et al. 2009; Alonzo and Sheldon 2010; Saffran et al. 2010), and for their offspring, the future social environment is relatively predictable because individuals sort phenotypically according to colony size (Spottiswoode 2007), and the relatively little natal and adult dispersal that does occur tends to be between similarly-sized colonies (Brown et al. 2003). Therefore, in larger colonies, we predicted positive effects of increased levels of yolk androgens on the offsprings’ competitiveness, thus preparing the offspring for a competitive environment. We also predicted lower concentrations of yolk antioxidants since high androgen concentrations in the female may compromise its availability for deposition in the eggs.

Finally, we investigated whether the effect of laying order depends on colony size. Given that environmental stress and the level of brood reduction are both likely to increase with increasing colony size (Spottiswoode 2007), we expected that maternal effects in response to laying order are likely to vary with the size of the colony, since females probably face different life-history trade-offs across colonies of various sizes.

### Materials and methods

#### Study species and study site

The sociable weaver is a 26- to 32-g passerine, endemic to the semiarid savannahs of southern Africa (Maclean 1973a). It is a colonial, communal and cooperatively breeding species that constructs massive, permanent nests out of stiff grasses. Breeding takes place in chambers on the underside of the nest mass, and pairs may be assisted by up to seven helpers (Maclean 1973b). The mean rate of nest predation is 75 % (Covas 2002), principally by snakes (mostly Cape cobra, *Naja nivea* and boomslang, *Dispolidus typus*). Breeding is strongly rainfall-dependent and may continue for 9 months in favourable conditions (Maclean 1973b). Clutches consist of two to five eggs, typically three to four (Maclean 1973b), and clutch size is unrelated to colony size (Spottiswoode 2007).

The study was conducted at Benfontein Game Farm in South Africa’s Northern Cape Province near Kimberley (28°53′S, 24°50′E) during the breeding season of summer 2004–2005. This summer was not affected by the Southern Oscillation (http://www.cpc.ncep.noaa.gov). About 30 colonies, of which 8 are included in this study, are distributed across an approximately 2,000-ha tract of Kalahari sandveld savannah. Colony size was taken as the number of adults caught at the colony during the year of study (see Covas et al. 2004 for detailed field methods).

#### Sample collection

The size of the eight colonies included in this study varied from 8 to 128 individuals. Clutches consisted of either three (n = 27 clutches) or four eggs (n = 8). Nests were visited daily and eggs were marked to record their position in the laying sequence. If the clutch was discovered only after the second egg was laid, only the order of the subsequent eggs could be recorded. The complete laying order was known at 15 out of a total of 36 clutches, whereas part
of the laying order was known for 14 clutches, and at 7 clutches the laying order was unknown. In total, the position in the laying sequence was known for 65 eggs, drawn from 29 clutches in seven colonies. For androgen and carotenoid assays, eggs were collected under licence from the Northern Cape Department of Tourism, Environment and Conservation (permit number 0965/04). Sampled clutches were initiated within a 2-week period (23 December 2004–2007, January 2005), in order to minimise any seasonal variation in environmental conditions. These clutches were all ‘first clutches’ after at least 1 month of no breeding activity (clutches early in the season, in September or October, are often unsuccessful (R.E.v.D. and C.N.S., unpublished data; Maclean 1973b). Replacement clutches after predation were excluded. Eggs were collected on the fourth day after the clutch was initiated (five-egg clutches are extremely rare; Maclean 1973a). Incubation commences with the appearance of the first egg. This may have influenced the laying order effects we observed. Yet, although egg composition and its consequences for the developing embryo can vary between species and the study of yolk-to-embryo transfer of carotenoids and hormones needs further investigation, the concentration of yolk carotenoids is unlikely to change to the extent which we observed across the laying sequence during these early stages of embryogenesis (Hill 1993; Surai et al. 1996, 2001a, b; but see Mora et al. 2004). The androgen concentration may change during early stages of embryo development, but they appear to stay stable for at least the first 3 days of incubation (Eising et al. 2003; Groothuis and Schwabl 2008; Paitz et al. 2011). The majority of eggs we collected were incubated for a maximum of 3 (three-egg clutches) or 4 (four-egg clutches) days. We therefore expect the potential bias emerging from differential incubation with laying order to be minimal.

At the time of collection, we measured egg dimensions (using a digital calliper accurate to 0.01 mm) and egg mass (using an Acculab PP2060D balance, accurate to 0.001 g). Egg volume was estimated according to the following formula: volume = 0.51 \times \text{length} \times \text{breadth}^2 (Hoyt 1979). Yolks were weighed to the nearest 0.001 g after pouring out the albumen and removing any excess liquid by dabbing the yolk with tissue paper. If the edges of the yolks were at all blurred with the albumen, yolks were not weighed and we excluded these eggs from all analyses concerning androgen and carotenoid concentrations to avoid a downwards bias of these concentrations. In the field, the yolks were divided in two and each half transferred into a separate Eppendorf tube for subsequent analyses of androgens and antioxidants. Egg samples were then stored at \(-18 \, ^\circ\text{C}\) in the field and subsequently after the fieldwork at \(-80 \, ^\circ\text{C}\) until the analyses of androgens began in April 2005 and the carotenoid analyses in September 2006.

Androgen and antioxidant assays

Eggs were analysed for \(A4\) and \(T\) concentrations using radioimmunoassay according to Schwabl (1993). Approximately 200 mg of diluted yolk was used for analysis. Inter-assay coefficients of variation for \(A4\) and \(T\) were 5.15 and 4.93 %, respectively, and overall recovery rates (based on known quantities of radioactively labelled hormone added to the samples prior to assay) were 62.5 and 50.3 %, respectively. Concentrations are given in pg mg\(^{-1}\).

Eggs were analysed for total carotenoid concentration, \(\alpha\)- and \(\gamma\)-tocopherol, and retinol concentration, following established laboratory protocols using high performance liquid chromatography (HPLC; Surai and Speake 1998). Concentrations are given in \(\mu\)g g\(^{-1}\).

Statistical analyses

We used Linear Mixed Models (LMM) implemented using the package nlme in \(R\) (R Development Core Team, 2011) to account for the statistical non-independence of eggs within clutches and clutches within colonies. We entered clutch (where appropriate) and colony as random factors (with clutch nested within colony). Position in the laying order and colony size were included as covariates. The analyses concerning laying order were corrected for clutch size by classifying laying order as ‘first’, ‘middle’, and ‘last’ egg in the laying sequence, averaging the values of the second and third eggs of four-egg clutches and classifying those as ‘middle’. The date of clutch initiation did not contribute significantly to any of the models (\(P > 0.124\)) and was thus excluded from the final models we present here. Normal error distributions were achieved by log- or square-root-transformation of androgen and antioxidant concentrations and of proportional yolk mass prior to analyses. Interactions between laying order and colony size were not significant (see “Results”) and were therefore excluded from the final models regarding laying order and colony size.

To investigate a potential trade-off between maternal androgens and antioxidants, we calculated the mean values of the androgens and antioxidants for each clutch. Analyses used Pearson’s product-moment correlation or Spearman rank correlation if normality was not achieved. In addition, we analysed how maternal antioxidants were associated with maternal androgens by including the antioxidants as a covariate in the LMMs testing whether, at the level of eggs, laying order and colony size predict the concentration of yolk androgens. Colony was included as a random factor with clutch nested within colony. Egg and yolk mass were positively related (\(R = 0.550, t_{89} = 6.215, P < 0.001\)), so we also ran the analyses of androgen and antioxidant concentrations with yolk mass as a proportion of egg mass.

\(\odot\) Springer
Table 1 The means, SD and ranges of weights of eggs and yolks, and of concentrations of yolk androgens and yolk antioxidants in sociable weavers, *Philetairus socius*.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Min</th>
<th>Max</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eggs</td>
<td>Clutches</td>
<td>Colonies</td>
<td></td>
</tr>
<tr>
<td>Egg mass (g)</td>
<td>2.50 ± 0.22</td>
<td>1.92</td>
<td>3.34</td>
<td>110</td>
</tr>
<tr>
<td>Yolk mass (g)</td>
<td>0.56 ± 0.06</td>
<td>0.44</td>
<td>0.70</td>
<td>93</td>
</tr>
<tr>
<td>A4 (pg mg⁻¹)</td>
<td>26.88 ± 48.13</td>
<td>0.50</td>
<td>359.79</td>
<td>85</td>
</tr>
<tr>
<td>T (pg mg⁻¹)</td>
<td>13.41 ± 11.54</td>
<td>0.85</td>
<td>74.1</td>
<td>85</td>
</tr>
<tr>
<td>Total carotenoids (µg g⁻¹)</td>
<td>28.06 ± 13.22</td>
<td>0.06</td>
<td>76.48</td>
<td>103</td>
</tr>
<tr>
<td>Retinol (µg g⁻¹)</td>
<td>2.13 ± 0.62</td>
<td>0.23</td>
<td>3.65</td>
<td>103</td>
</tr>
<tr>
<td>α-Tocopherol (µg g⁻¹)</td>
<td>27.62 ± 10.17</td>
<td>2.95</td>
<td>93.30</td>
<td>104</td>
</tr>
<tr>
<td>γ-Tocopherol (µg g⁻¹)</td>
<td>8.73 ± 5.03</td>
<td>0.35</td>
<td>33.26</td>
<td>104</td>
</tr>
</tbody>
</table>

We provide effect sizes and their 95 % confidence intervals (Lipsey and Wilson 2001).

Owing to the differences in yolk size across the laying sequence (see “Results”), we repeated the analyses for androgens and antioxidants using the absolute quantities per yolk, calculated by multiplying the concentrations with the yolk mass. This did not qualitatively change most of the results, but when it did (for A4 in relation to laying order), we provide those results as well.

**Results**

**Androgen and antioxidant concentrations**

The mean concentrations of A4 and of T were not correlated with each other, whether analysed at the level of clutches (*R* = −0.039, *t*₂₈ = −0.21, *P* = 0.838) or eggs [LMM; model effect estimate ± SE: 0.020 ± 0.128, *t*₅₀ = 0.16, *P* = 0.876, *R* (CI) = 0.018 (−0.205 to 0.240)]. Eggs contained significantly higher concentrations of A4 than of *T* [*R*₂₁ = 3.28, *P* = 0.001, *R* = 0.254 (0.102–0.395); Table 1]. We found no significant correlation between either of the androgens (A4 or T) and any of the antioxidants (α-, γ-tocopherol, retinol, and total carotenoid concentration; all *P* > 0.195, *R* < 0.243, except for A4 versus total carotenoid concentration, where *R* = −0.323 (−0.612 to 0.042), *t*₂₈ = −1.81, *P* = 0.082. However, when the concentration of antioxidants was included in the LMMs analysing whether yolk androgen concentration is predicted by laying order and colony size, we found that the concentrations of α- and γ-tocopherol and of total carotenoids were negatively associated with the concentration of A4 [α-tocopherol: −0.051 ± 0.017, *t*₂₁ = −3.06, *P* = 0.006, *R* = −0.419 (−0.632 to −0.147); γ-tocopherol: −0.085 ± 0.038, *t*₂₁ = −2.22, *P* = 0.037, *R* = −0.318 (−0.557 to −0.030); total carotenoids: −0.034 ± 0.012, *t*₂₂ = −2.70, *P* = 0.013, *R* = −0.377 (−0.602 to −0.097)]. This analysis revealed an increase in concentration of A4 with colony size (see below), but with a corresponding decrease in the concentration of α- and γ-tocopherol and of total carotenoids. The concentration of retinol was not associated with A4 [−0.351 ± 0.237, *t*₂₁ = −1.48, *P* = 0.153, *R* = −0.221 (−0.483 to 0.078)]. None of the antioxidants was associated with the concentration of *T* in these models (*P* > 0.562).

**Maternal effects as a function of laying order**

Clutch size was not associated with egg mass, egg volume, (proportional) yolk mass, or androgen or carotenoid content (*all P* > 0.104), and was therefore not included in the initial models testing for an effect of laying order and colony size (see below). All results concerning the effect of laying order were qualitatively unchanged when we restricted the dataset to complete clutches only while accounting for the effect of clutch size. Egg mass varied markedly with laying order, with later-laid eggs being heavier than earlier-laid eggs (Table 2a; Fig. 1). This effect could merely be a consequence of greater mass loss from earlier-laid eggs that had already experienced some incubation at the time of collection and weighing. However, sociable weaver eggs decrease in mass by on average only 0.025 ± 0.017 g per day (based on 114 eggs weighed on successive days early in the incubation period), which is insufficient to account for the difference between first- and fourth-laid eggs. Furthermore, if mass loss explained this result, then egg volume should not be related to laying order. However, we also found that egg volume increased with laying order (Table 2a), confirming that there is a genuine increase in egg size in successive eggs within a clutch.

Yolk mass may be more relevant to maternal effects than overall egg mass, since androgens and carotenoids are deposited in the yolk. Absolute yolk mass (g) varied significantly with laying order, with later-laid eggs (third- and
fourth-laid eggs) having lighter yolks than earlier-laid (first and second) eggs (Table 2a). When expressed as a proportion of egg mass, yolk mass again decreased significantly with laying order (Table 2a; Fig. 2).

The concentration of $A_4$ and the absolute quantity of $A_4$ per yolk did not depend on laying order (Table 2a). Similarly, neither $T$ concentration, nor the absolute quantity of $T$ per yolk varied with laying order (Table 2a). However, total carotenoid concentration per egg decreased significantly with laying order within clutches (Table 2a; Fig. 3) and the vitamin E components $\alpha$- and $\gamma$-tocopherol also decreased in concentration with laying order (Table 2a). In contrast, the vitamin A component retinol was not associated with laying order (Table 2a).

To summarise this section, laying order effects were found in several components of maternal investment: later-laid eggs were larger in overall mass compared to earlier-laid eggs, but had lighter yolks. However, no effect of

| Table 2 Maternal effects as a function of (a) laying order and (b) colony size in sociable weavers |
|-------------------------------------------------|-------------------------------|-----------------|-----------------|-----------------|
| Model effect estimate ± SE | df | $t$ | $P$ | R (95 % CI) |
| (a) Laying order |
| Egg mass | 0.083 ± 0.022 | 31 | 3.68 | <0.001 | 0.435 (0.203 to 0.620) |
| Egg volume | 5.53 ± 17.552 | 31 | 2.88 | 0.007 | 0.354 (0.110 to 0.558) |
| Absolute yolk mass | -0.022 ± 0.007 | 22 | 3.33 | 0.003 | -0.430 (-0.631 to -0.175) |
| Proportional yolk mass | -0.079 ± 0.013 | 22 | 6.20 | <0.001 | -0.667 (-0.797 to -0.477) |
| $A_4$ concentration | 0.201 ± 0.154 | 28 | 1.31 | 0.202 | 0.182 (-0.096 to 0.433) |
| Absolute quantity of $A_4$ per yolk | 0.263 ± 0.227 | 14 | 1.16 | 0.265 | 0.194 (-0.140 to 0.486) |
| $T$ concentration | 0.114 ± 0.139 | 21 | 0.82 | 0.421 | 0.117 (-0.167 to 0.383) |
| Absolute quantity of $T$ per yolk | 0.172 ± 0.133 | 21 | 1.29 | 0.210 | 0.194 (-0.106 to 0.461) |
| Total carotenoid concentration | -0.244 ± 0.048 | 26 | 5.08 | <0.001 | -0.572 (-0.727 to -0.362) |
| $\alpha$-Tocopherol concentration | -0.139 ± 0.043 | 25 | 3.25 | 0.003 | -0.408 (-0.607 to -0.160) |
| $\gamma$-Tocopherol concentration | -0.276 ± 0.084 | 25 | 3.28 | 0.003 | -0.411 (-0.610 to -0.163) |
| Retinol concentration | -0.031 ± 0.047 | 25 | 0.67 | 0.512 | 0.092 (-0.351 to 0.180) |
| (b) Colony size |
| Egg mass | -0.001 ± 0.001 | 5 | -2.38 | 0.063 | -0.299 (-0.514 to -0.048) |
| Egg volume | -1.093 ± 0.591 | 5 | -1.85 | 0.123 | -0.236 (-0.462 to 0.019) |
| Absolute yolk mass | -0.000 ± 0.000 | 5 | -0.98 | 0.374 | -0.138 (-0.399 to 0.143) |
| Proportional yolk mass | 0.000 ± 0.000 | 5 | 1.23 | 0.274 | 0.175 (-0.109 to 0.432) |
| Mean $A_4$ concentration per clutch | 0.009 ± 0.004 | 28 | 0.41 | 0.678 | 0.092 (0.081 to 0.643) |
| Mean $T$ concentration per clutch | 0.000 ± 0.002 | 25 | 0.00 | 0.949 | 0.001 (-0.319 to 0.338) |
| Model effect estimate ± SE | df | $F$ | $P$ | R (95 % CI) |
| $A_4$ concentration per egg | 0.012 ± 0.003 | 21 | 3.41 | 0.003 | 0.435 (0.184 to 0.633) |
| $T$ concentration per egg | -0.000 ± 0.003 | 4 | 0.13 | 0.902 | 0.019 (-0.261 to 0.296) |
| Total carotenoid concentration | -0.004 ± 0.002 | 5 | -2.62 | 0.047 | -0.339 (-0.554 to -0.081) |
| $\alpha$-Tocopherol concentration | -0.001 ± 0.002 | 5 | -0.60 | 0.575 | -0.082 (-0.340 to 0.187) |
| $\gamma$-Tocopherol concentration | 0.005 ± 0.003 | 5 | 2.12 | 0.088 | 0.279 (0.015 to 0.507) |
| Retinol concentration | -0.001 ± 0.002 | 5 | -0.71 | 0.511 | -0.098 (-0.356 to 0.175) |

Fig. 1 Egg mass (g) in relation to position in the laying order in sociable weaver, *Philetairus socius* clutches. $n$ is the number of clutches. Box plots indicate the median, the interquartile range, the maximum and minimum values excluding outliers, and outliers.
laying order was found on androgens. Total carotenoid and vitamin E concentrations decreased with laying order, whereas vitamin A was unaffected.

Maternal effects as a function of colony size

Neither egg mass, egg volume, nor absolute or proportional yolk mass showed a trend in relation to colony size (Table 2b). We note that a negative effect of colony size on egg mass has, however, been detected in a larger sample (Spottiswoode 2007), while we found only a non-significant trend with a medium effect size (Table 2b). Colony identity did not contribute significantly to the models concerning the effect of colony size on androgen concentration ($P = 0.592$), and was therefore excluded from the final models presented here. The concentration of $A_4$ increased significantly with increasing colony size (Table 2b). When this analysis was repeated using the $A_4$ concentrations per egg rather than means per clutch, we found a qualitatively similar result, i.e. a significant, positive effect of colony size on $A_4$ concentration (Table 2b; Fig. 4). The interaction between colony size and laying order was not significant ($-0.002 \pm 0.003$, $t_{27} = -0.56$, $P = 0.578$, $R = -0.080$ ($-0.345$ to $0.198$)], suggesting that the effect of colony size on concentration of $A_4$ is independent of the position in laying order; this interaction was therefore excluded from the final model. We found no effect of colony size on $T$ concentration either at the level of the clutch or at the level of eggs (Table 2b). Again, the interaction between colony size and laying order was not associated with the concentration of $T$ ($0.001 \pm 0.003$, $t_{26} = -0.29$, $P = 0.783$, $R = -0.040$ ($-0.345$ to $0.241$]), and was therefore excluded from the final model.

The total carotenoid concentration per egg decreased with increasing colony size (Table 2b). Neither $\alpha$-tocopherol, $\gamma$-tocopherol nor retinol concentration was significantly associated with colony size (Table 2b). The interaction between laying order and colony size had no effect on any of the antioxidants we examined ($P > 0.263$). $\gamma$-Tocopherol was excluded from this analysis, because a normal error distribution could not be achieved by log- or square-root-transformation.

Discussion

We found that both laying sequence and social environment, as reflected by colony size, are associated with maternal effects. Later-laid eggs were heavier, but with
lower yolk mass and lower concentrations of total carotenoids and vitamin E components. Colony size was positively associated with the concentration of A4. Total carotenoid concentration showed a marginally significant trend to decrease with colony size, whereas the concentration of retinol and both of the tocopherols did not depend on colony size.

The relationship between laying sequence and maternal effects was not influenced by colony size, as there was no significant interaction between laying order and colony size for any of factors investigated. Thus, contrary to our prediction of an interaction between laying order and colony size, we found no evidence for modification of maternal effects in relation to laying order according to colony size-specific variation in life history. This finding may be explained by the limited variation among colonies in predation and food limitation. Predation is more common in larger colonies, but it is still frequent in smaller colonies (Spottiswoode 2007). Similarly, although competition for food may be fiercer in larger colonies and the greatest impact of food supplementation was on large colonies, food supplementation has been shown to lead to advanced laying date and increased nestling survival in both large and small colonies (Spottiswoode 2009), suggesting that smaller colonies are also food-limited. If so, then brood reduction by an exacerbated effect of hatching asynchrony through maternal effects may be adaptive in all colonies irrespective of colony size.

We also found that concentrations of yolk A4 were higher than those of yolk T, as is the case for most species (Gil et al. 2007). Hegyi et al. (2011) suggested that in such species, yolk A4 and not T may be the yolk androgen that is adaptively adjusted by females, and/or has long-term fitness consequences through the offspring’s phenotype. Our result that A4, but not T, significantly increased with the size of the colony is consistent with their view.

Maternal effects as a function of laying order

Our result that egg size increased with laying order has been commonly found in passerines (reviewed by Slagsvold et al. 1984). However, the decline in yolk mass we observed in our study (and especially in proportional yolk mass) with laying order appears much less common in other species. This decline suggests that eggs later in the laying sequence contain more albumen and may therefore be better provisioned with water and proteins (Sotherland and Rahn 1987; Williams 1994). In a recent study of rockhopper penguins, Eudyptes chrysocome, Poisbleau et al. (2011) reported that yolk mass, egg mass and yolk androgen concentrations all decreased with laying order, but this species exhibits reversed hatching asynchrony. Therefore, in this species, first-laid, later-hatching eggs potentially benefit from increased yolk mass and increased deposition of maternal androgens by mitigating the effects of hatching asynchrony. Another recent study on cockatoos, Nymphicus hollandicus, also found a decline in yolk mass with laying order which was suggested to reinforce the potentially adaptive brood hierarchies increasing parental feeding efficiency (Kozlowski and Ricklefs 2010). In our study, in addition to yolk mass, the concentration of antioxidants also decreased with laying order, suggesting that female sociable weavers may bias their investment in costly components towards the eggs with the highest probability of survival, which in our study population are the first-laid eggs (Spottiswoode 2007). Brood reduction in this species is common, and the enhancement of brood reductions through differential maternal investment may be adaptive when food resources are limited in the unpredictable environment in which they live.

Despite these maternal effects that might enhance the likelihood of brood reduction, increased egg size may, to some extent, still mitigate brood hierarchies in sociable weavers when environmental conditions are favourable. However, an increased metabolic activity by the production of costly eggs may result in elevated levels of ROS towards the later-laid eggs. Again, possibly depending on the favourability of environmental conditions, this could cause an increased stress response by the mother, resulting in depletion of antioxidants towards the end of the laying cycle. The eggs in our study were collected within a 2-week period within which fluctuations in food availability were likely to have been minimal, but across an entire breeding season food abundance may vary substantially and influence maternal investment. The costs of increased egg size may be relatively low for females compared to the costs of yolk and antioxidants (Hipfner et al. 2003; Blount et al. 2004), and may be flexibly adjusted depending on, for example, food availability. How the unpredictable non-social environment of sociable weavers may influence maternal effects deserves further investigation.

The absence of a statistical association between androgens and antioxidants when pooling all eggs irrespective of position in the laying order or colony membership suggests that yolk androgens and antioxidants may be independently deposited in the eggs (Safran et al. 2008; but see Royle et al. 2001). Therefore, an increased level of A4 is not necessarily followed by an increased level of antioxidants to compensate for an increase in ROS causing oxidative stress. This is consistent with our suggestion that females may preferentially invest in first-laid eggs, which have the highest chances of survival. However, given the potential negative consequences that may arise from elevated levels of androgens, the deposition in eggs of androgens and antioxidants are unlikely to be entirely independent effects.
Rather than a preferential investment in first-laid eggs, a decrease in the concentration of carotenoids with laying order may also arise from the generation of ROS associated with egg production. To avoid physiological damage, females may need antioxidants themselves to prevent oxidative stress, resulting in a faster depletion of circulating carotenoids. In accordance with this argument, when taking laying order and colony size into account, we found that an increase in concentration of A4 in response to colony size was associated with a decrease in the concentration of \( \alpha \)- and \( \gamma \)-tocopherol and total carotenoids. This is expected under elevated levels of circulating androgens at higher social densities with potentially increased levels of environmental stress (Alexander 1974; Brown and Brown 2004; Spottiswoode 2007). However, experimental tests are needed to test causation in this relationship.

Maternal effects as a function of colony size

An increase in A4 concentration with colony size suggests that offspring in larger colonies may be better prepared to face aggressive social interactions and competition with offspring of other females than are offspring in smaller colonies. The significant increase in A4 with colony size, and thus potentially higher levels of oxidative stress, is consistent with the generally lower immunocompetence of nestlings in larger colonies, as well as with their high levels of ectoparasite infestation (Spottiswoode 2007). There could also be long-term negative side effects of an increased A4 concentration for the offspring, resulting from a potentially increased concentration of ROS. Hitherto, fitness consequences of elevated levels of yolk androgens remain underexplored and research has provided mixed results, ranging from negative effects to positive ones (Gil 2003; Müller et al. 2009; Tobler et al. 2010). A recent study on house sparrows, *Passer domesticus*, a relative of the sociable weaver, showed that testosterone treatment in the egg increased body mass at the age of 3–4 months and increased survivorship (Schwabl et al. 2012), suggesting potential fitness benefits of yolk androgens. However, experiments are required to test whether A4 is indeed causally related to elevated environmental stress in sociable weavers, and to test the fitness consequences of elevated levels of A4 in this species.

Except for an increase in A4 concentration and the marginally significant trend in the concentration of total carotenoids, neither \( T \), nor egg or yolk size, nor any of the measured antioxidants was related to colony size. Although a decrease in concentration of carotenoids with colony size may appear counterintuitive given the lack of a significant effect for any of the carotenoid components, we note that the effect size was not strong and there was a large CI of the trend for total carotenoid concentration to decrease with colony size. Moreover, other carotenoid components that were not investigated may contribute to the pattern observed for total carotenoids. Additionally, we note that, although \( \gamma \)-tocopherol also shows a medium effect size in the opposite direction of the effect for the total carotenoid concentration, the average concentration of \( \gamma \)-tocopherol is relatively low compared to \( \alpha \)-tocopherol. Nonetheless, if the concentration of total carotenoids decreases with colony size, while the concentration of \( \gamma \)-tocopherol increases, this implies that the carotenoid composition may change depending on the size of the colony.

The predicted increased environmental stress in larger colonies does not appear to be associated with elevated levels of yolk antioxidants. An increased intensity of parasitism may cause the females to require the antioxidants themselves, while an increased production of antioxidants, competition for food, and more frequent replacement due to higher predation levels may all elevate production of ROS. This largely null result is consistent with the findings of the few other studies that have investigated yolk carotenoids in response to social environments (Verboven et al. 2005; Hargitai et al. 2009; Safran et al. 2010). These results are at odds with theoretical predictions that yolk antioxidants are associated with the predicted social interactions the offspring is likely to encounter during its lifetime (Royle et al. 2001; Hargitai et al. 2009).

In conclusion, we have shown that maternal effects in the sociable weaver are associated with both laying sequence and the social environment. Our results suggest that female sociable weavers bias reproductive investment of costly antioxidants to the offspring that are most likely to survive, thereby exacerbating the potentially adaptive brood reduction observed in this species to ensure the survival of at least some of the offspring. When environmental conditions are favourable, they might be better off mitigating the brood hierarchies, but we have not been able to test this possibility in this cross-sectional study. Female sociable weavers additionally appear to prepare their offspring for the social environment they are likely to encounter during their lifetime, by adjusting the A4 concentration depending on the size of the colony.

Acknowledgments  We are grateful to De Beers Consolidated Mines Ltd and Morné du Plessis for the opportunity to work at Benfontein Game Farm, to the Northern Cape Province’s Department of Tourism, and Environment and Conservation (especially Mark Anderson) for research permits, and to Eric Herrmann for his assistance in the field. The androgen assays were carried out at the University of Groningen by kind permission of Ton Groothuis. Nick Davies, Rebecca Kilner, Jane Reid, Indrikis Krams and two anonymous referees provided helpful comments on earlier drafts of the manuscript. R.E.v.D. was supported by a grant of the Natural Environment Research Council (NERC) to B.J.H., and C.N.S. by St John’s College, Cambridge (UK) and the University of Cape Town (South Africa). Our research complied with the current laws of the countries in which it was performed.
Conflict of interest The authors declare that they have no conflict of interest.

References


