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1 **Influence of mate preference and laying order on maternal allocation in a monogamous**  
2 **parrot species with extreme hatching asynchrony**

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17 **Abstract**

18

19 It is well established that in many avian species, prenatal maternal resource allocation varies  
20 both between and within clutches and may affect offspring fitness. Differential allocation of  
21 maternal resources, in terms of egg weight and yolk composition, may therefore allow the  
22 female to adjust brood reduction and to fine-tune reproductive investment in accordance with  
23 the expected fitness returns. The adaptive value of such maternal resource allocation is  
24 thought to be context-dependent as well as species-specific. We investigated the effects of  
25 female preference for her mate on the allocation of prenatal maternal resources in the  
26 budgerigar, *Melopsittacus undulatus*, a monogamous species of parrot that shows an extreme  
27 hatching asynchrony. We assessed mate preferences in a two-way preference test and allowed  
28 females two breeding rounds: one with the preferred and one with the non-preferred partner.  
29 We found no effect of preference on either latency to lay or clutch size, but females mated  
30 with the preferred partner laid eggs that contained significantly more yolk. Their eggs also  
31 contained significantly more androstenedione but not testosterone. Our results suggest that in  
32 this species, female preference may influence maternal resource allocation, and that the  
33 functional roles of each androgen in the yolk should be considered separately. In addition, we  
34 found a significant effect of laying order on egg and yolk weight as well as on yolk  
35 testosterone and androstenedione levels. These measures, however, did not change linearly  
36 with the laying order and render it unlikely that female budgerigars compensate for the  
37 extreme hatching asynchrony by adjusting within-clutch allocation of prenatal maternal  
38 resources.

39

40 **Keywords:** Maternal effects, Differential allocation, Male attractiveness, Yolk hormones,  
41 Hatching asynchrony, Androstenedione, Testosterone, Reproductive investment,  
42 *Melopsittacus undulatus*

43

## 44 **Introduction**

45

46 Early maternal allocation of resources provides a non-genetic mechanism for the female to  
47 modify the phenotype of the offspring in accordance with the environmental conditions that  
48 she experiences and that her offspring are likely to face after hatching (Mousseau and Fox,  
49 1998). In oviparous animals, such as birds, the egg provides a sealed system in which the  
50 embryo develops and once the egg is laid, investment in the offspring through maternal  
51 resources is restricted. Birds are therefore excellent subjects for studying the causes and  
52 consequences of differential maternal allocation during the prenatal phase.

53 In birds, maternal resources that determine egg quality include egg weight and various  
54 yolk compounds (e.g. antibodies, antioxidants, androgens; Blount et al., 2002; Groothuis et  
55 al., 2005a; Krist, 2011; Müller et al., 2004; von Engelhardt and Groothuis, 2011). These  
56 maternal resources may vary both within and between clutches. Within clutches, species-  
57 specific deposition patterns of maternal resources over the laying order have been  
58 documented for several species (e.g. androgen deposition and egg weight; Groothuis et al.,  
59 2005a; Muller and Groothuis, 2013; Slagsvold et al., 1984). This has typically been  
60 interpreted in the context of hatching asynchrony, which is caused by the fact that females  
61 may lay more than one egg, but eventually start incubating before the clutch is completed.  
62 The resulting hatching spread and associated size hierarchy in the nest negatively affect the  
63 survival of chicks from later-laid eggs as they experience a competitive disadvantage  
64 compared to their older siblings (Massemin et al., 2002). It has been proposed that differential  
65 allocation patterns of maternal resources within a clutch enable the female to adjust brood  
66 reduction, thereby maximizing her own fitness (Schwabl, 1996; Wagner and Williams, 2007).  
67 An increase in maternal allocation with the laying order may counteract the competitive  
68 disadvantage of the chicks from later-laid eggs, because chicks that hatch from eggs with  
69 heavier yolks or higher levels of the maternal androgens androstenedione (A4) and/or  
70 testosterone (T) may hatch earlier, beg more vigorously, grow faster and have higher survival  
71 rates (e.g. Eising et al., 2001; Groothuis et al., 2005a; Krist, 2011; Schwabl, 1996; von  
72 Engelhardt and Groothuis, 2011; Wagner and Williams, 2007). A decrease in resources on the  
73 contrary may further enhance brood reduction (Groothuis et al., 2005a). However, the  
74 situation may be more complex, as in some species certain maternal resources such as yolk  
75 androgens have mainly negative effects on offspring fitness (Groothuis et al., 2005a, b; von  
76 Engelhardt and Groothuis, 2011). Together with the fact that species vary considerably with  
77 respect to within-clutch allocation patterns, this suggests that the adaptive value of maternal

78 allocation is species-specific and may differ according to the type of resource. To increase our  
79 understanding of the phylogenetic and life-history factors that potentially underlie within-  
80 clutch allocation patterns, studies that measure different parameters of egg quality in a large  
81 variety of species of different taxa are necessary (Gil et al., 2007; Love et al., 2009).

82 Variation in maternal allocation between clutches has been attributed to a range of  
83 environmental determinants and factors intrinsic to the female (e.g. social density, food  
84 availability, condition, age; Christians, 2002, Hargitai et al., 2009; Remes, 2011; Safran et al.,  
85 2008, 2010; Vergauwen et al., 2012; reviewed in: von Engelhardt and Groothuis, 2011).  
86 Reproduction is costly for the female, and there is thus a trade-off between investment in the  
87 current reproductive attempt and future reproduction (Williams, 1966). Hence, females are  
88 thought to adjust their reproductive effort in accordance to the expected fitness return. One  
89 important factor that may influence the reproductive decisions of the female is mate quality  
90 (Burley, 1988). Females may experience higher fitness returns when investing more heavily  
91 in reproduction when mated with a more attractive male (differential allocation hypothesis;  
92 Burley, 1988; Sheldon, 2000), because attractive males may provide more direct benefits such  
93 as parental care or territory quality and indirect benefits in terms of offspring genetic quality  
94 (Andersson, 1994). The effects of male attractiveness on maternal allocation have been  
95 examined in a variety of species, but the resulting data are inconclusive (reviewed in: Cucco  
96 et al., 2011; Horvathova et al., 2012; Kingma et al., 2009). Interestingly, in most studies, male  
97 attractiveness was determined based on previous work and it was not specifically tested  
98 whether females indeed preferred the males that were considered to be more attractive (e.g.  
99 Alonso-Alvarez et al., 2012; Garcia-Fernandez et al., 2013; Gil et al., 1999, 2004; Grana et  
100 al., 2012; Kingma et al., 2009; Safran et al., 2008; but see: Bluhm and Gowaty, 2004; Cucco  
101 et al., 2011; von Engelhardt et al., 2004). In order to estimate male attractiveness as perceived  
102 by the female, it is necessary to allow females to actively choose between potential partners.

103 The budgerigar, *Melopsittacus undulatus*, is a small monogamous species of parrot  
104 (Juniper, 1998). Female budgerigars of self-selected pairs show a shorter latency to lay and  
105 produce larger clutches (Massa et al., 1996), which indicates that mating preferences may be  
106 an important factor influencing maternal allocation. It has not yet been investigated whether  
107 females flexibly adjust maternal allocation of resources, such as yolk weight and androgen  
108 deposition, according to mate preference. Unlike many other bird species, parrots produce  
109 clutches with extreme hatching asynchrony (budgerigar broods hatch over a span of up to 16  
110 days). Despite the apparent lower competitive abilities of younger chicks, brood reduction is  
111 uncommon (Brockway, 1964; Stamps et al., 1985). To the best of our knowledge, it has not

112 been reported in the literature whether or not female budgerigars compensate for the  
113 competitive disadvantage of younger chicks as caused by the extreme hatching asynchrony  
114 via differential prenatal maternal allocation to the eggs, as has been found for many species  
115 with less pronounced asynchrony.

116 In the current study, we assessed female preferences in a two-way choice test (von  
117 Engelhardt et al., 2004). Subsequently, the females were allowed two consecutive breeding  
118 rounds in a cross-over design. In one breeding round, they were paired with their preferred  
119 partner (i.e. the more attractive male as perceived by the female) and in the other with the  
120 non-preferred partner (i.e. the less attractive male as perceived by the female). Our aims were  
121 twofold. First, we tested whether females increase the allocation of resources when they are  
122 paired with the preferred partner by studying the effects of female preference on latency to  
123 lay, clutch size and the following six maternal resources that have been shown to influence  
124 egg quality: egg weight, yolk weight and the concentrations and the total amounts of the  
125 androgenic hormones T and A4 in the yolk. Finally, we asked whether maternal allocation  
126 varies over the laying order by investigating within-clutch variation in these six maternal  
127 resources.

128  
129

## 130 **Material and methods**

131

### 132 *Ethics statement*

133 The budgerigars of this study were domesticated animals, which were used to human  
134 presence. Because budgerigars are social birds, we always allowed the birds at least vocal  
135 interactions with each other. We did not observe abnormal behavior performed by any of the  
136 birds during the experimental procedures or during the daily routine checks. Handling time  
137 was minimized and did not exceed 3 min per individual for any of the procedures. All  
138 experimental procedures were performed in agreement with the Belgian and Flemish laws.

139

### 140 *Study species and housing*

141 We randomly selected 39 unpaired female and 76 unpaired male budgerigars from our captive  
142 stock population. The birds had been obtained from local breeders as juveniles and had been  
143 maintained in our captive stock for and least one and up to two years. All females showed a  
144 green plumage and were adults of approximately one year old with no breeding experience.  
145 The males varied in plumage coloration and were approximately between one and two years

146 old. Before the experiment, the birds had been housed in two single-sex outdoor aviaries (8m  
147 wide x 2.5m deep x 2.3m high). One week before the start of the preference tests (see  
148 'Preference tests'), the birds were moved to indoor single-sex cages (males: 120cm wide x  
149 40cm deep x 50cm high, females: 150cm wide x 40cm deep x 80cm high). The birds were  
150 housed in groups of up to 10 individuals and there was no visual or auditory contact between  
151 members of the opposite sex. Throughout the experiment, the birds were maintained on a light  
152 regime of 15:9 (L:D), unless stated otherwise. Food (commercial budgerigar seed mix, Nifra  
153 Van Camp bvba, Belgium), greens and water were provided ad libitum.

154

#### 155 *Male stimulus sets*

156 The females were allowed to choose between two males of a stimulus set (see 'Preference  
157 tests'). The males of a stimulus set were matched for body weight. We also matched males  
158 with higher and lower UV reflectance of the chest feathers, because female budgerigars may  
159 show mating preferences based on this trait (Griggio et al., 2010a; Zampiga et al., 2004). The  
160 color of the chest was measured with an USB4000 spectrophotometer (Ocean Optics, Duiven,  
161 The Netherlands), using an Ocean Optics DH-2000 BAL deuterium/halogen lamp. Before the  
162 measurement session, we took a dark current measurement on the chest of a randomly  
163 selected live bird and a white standard reference measurement (WS-1, Diffuse Reflectance  
164 Standard, Ocean Optics, Duiven, The Netherlands) for calibration purposes (Cuthill et al.,  
165 1999; Lahaye et al., 2014). Next, the chest of all males was measured three times by the same  
166 person (Lahaye et al., 2014). From the measurements, we calculated UV chroma as the  
167 proportion of total reflectance occurring between 320-400nm ( $R_{320-400}/R_{320-700}$ ) (Montgomerie,  
168 2006). For each male, we first calculated UV chroma separately for the three spectra that were  
169 measured, and the mean of these three values was used in the statistical analyses (Lahaye et  
170 al., 2014). Each stimulus set contained a male with a higher value for UV chroma of the chest  
171 (mean:  $0.302 \pm 0.0031$ ) and a male with a lower value ( $0.255 \pm 0.0027$ ). The males of a  
172 stimulus set showed a mean difference of  $0.048 \pm 0.0017$  ( $15.73 \pm 0.49\%$ ) for UV chroma of  
173 the chest, which was statistically significant (paired t-test:  $t_{26}=-28.9$ ,  $P<0.0001$ ). A difference  
174 of this size is detectable by females of this species (Griggio et al., 2010a; Zampiga et al.,  
175 2004) and is therefore biologically relevant.

176

#### 177 *Preference tests*

178 Female preferences were assessed in a two-way choice test during six consecutive days (May  
179 27 – June 1, 2010). Our preference test protocols have been described previously (Lahaye et

180 al., 2013). Briefly, ten preference tests were conducted simultaneously in one single room  
181 between 0800 and 1300 hours local time. The preference test apparatus consisted of two small  
182 cages (35cm wide x 35cm deep x 50 cm high) which each contained one male. The small  
183 cages were placed in front of a large central cage (120cm wide x 40cm deep x 50cm high; fig.  
184 1) in which a female was housed. Each male had access to one perch. The female had access  
185 to three perches: one perch in the center of the cage (i.e. no-choice area, fig. 1) and one perch  
186 in front of each stimulus male (i.e. choice area, fig. 1). The males were visually separated and  
187 the female could only see the males when she was positioned in the choice area, preventing  
188 her from seeing the males simultaneously and from seeing the males while she was positioned  
189 in the no-choice area (Griggio et al., 2010a, fig. 1). All individual preference test apparatus  
190 were visually separated from each other. The experimental cages were illuminated by  
191 artificial full spectrum light (Philips True Light, 58W/5500) and natural light through  
192 windows. A similar apparatus has been used in previous studies investigating mating  
193 preferences in a variety of species, including budgerigars (e.g. Griggio et al., 2010a; Lahaye et  
194 al., 2013; Moravec et al., 2010; Rutstein et al., 2007).

195 The afternoon before each preference test, the birds were moved to the preference test  
196 apparatus. The birds were visually, but not acoustically, isolated, and they were allowed to  
197 adjust to the new environment overnight. The following morning, the artificial lights were  
198 turned on, the partitions that visually separated the female from both stimulus males were  
199 removed, and the birds were allowed a habituation period of 50 min (von Engelhardt et al.,  
200 2004). After 50 min, the position of the males was switched, and a video recording of 50 min  
201 was made using digital camcorders (DCR-SX30E, Sony, Tokyo, Japan). During preliminary  
202 testing of our apparatus, we noticed that in many cases the females initially showed little  
203 mobility and only interacted with one stimulus male without visiting the second. By switching  
204 the position of the males before the first recording, we could increase the probability that all  
205 females had interacted with both males at the start of the actual test phase. A second 50-min  
206 recording was made, after switching the position of the males again to control for side  
207 preferences of the female.

208 Preliminary testing of our apparatus also revealed that the females often stayed  
209 immobile for up to 30 min after the disturbance caused by switching the position of the males.  
210 Highly social species, such as the budgerigar, may cope less well with disturbance when they  
211 are housed individually, which was the case during our preference tests (Cohen and Wills,  
212 1985). Therefore, we analyzed 15 min of each recording, excluding the first 30 min and last 5  
213 min to avoid potential disturbance caused by manually switching the test cages, by entering



214 and leaving the testing facility, and by starting and stopping the cameras. We calculated a  
215 preference score by scoring the position of the female every 10 seconds (left choice area, right  
216 choice area or central), yielding a total of 90 score points per video recording. We calculated  
217 the total association score for a male by summing the score obtained for the males over the  
218 two video recordings of a preference test. This score was used in further data analysis. We  
219 considered a preference test to be successful if the female was responsive during the  
220 preference test (i.e. the female spent at least 60% of the choice period ( $\geq 108$  score points) in  
221 the left or right choice area; Enstrom et al., 1997; McGlothlin et al., 2004) and if the female  
222 exhibited a preference (i.e. the preference score for both males differed significantly; one-  
223 tailed binomial test,  $P < 0.05$ ). Females that failed to meet these criteria were re-tested with a  
224 different set of stimulus males. The male stimulus sets were used to test the preferences of up  
225 to three different females. Only females that met our criteria were included in the experiment.  
226 A total of 11 females showed a significant preference during their first preference test. We re-  
227 tested the preference of 21 females and in their second preference test, 16 females showed a  
228 significant preference, resulting in a total of 27 females that met our criteria. Females were  
229 tested with unrelated, unfamiliar males. All females present in the testing room at any time  
230 were unfamiliar with all males that were present in the same room.

231

### 232 *Pairing*

233 We assessed female preferences from the video recordings within two hours of the preference  
234 test. Subsequently, females that showed a significant preference were paired with either their  
235 preferred male or their non-preferred male by moving the male into the central cage with the  
236 female. The second male (i.e. the other male, that was part of the male stimulus set which was  
237 presented during the preference test, but that was not moved into the central cage with the  
238 female) remained visible in the small cage for one hour to increase the time the female was  
239 exposed to both the preferred and non-preferred male (von Engelhardt et al., 2004). After one  
240 hour, the paired birds were moved into a breeding cage in the breeding facilities (see  
241 'Breeding'). In our experimental design, we applied a statistically strong cross-over  
242 experimental design. A total of 15 females were first paired with the preferred male and  
243 subsequently with the non-preferred one, and 12 females the other way around. Therefore, the  
244 second male of the female's preference test, was kept apart in the single-sex outdoor aviary, to  
245 be used in the second breeding round.

246

247

248 *Breeding*

249 Following the preference tests, the pairs were moved to the breeding facilities. All pairs were  
250 housed individually in breeding cages (60cm wide x 40cm deep x 50cm high) supplied with a  
251 nestbox (15cm wide x 15cm deep x 25cm high). Because the budgerigar is a highly social  
252 species which breeds colonially, breeding success may be low when individual pairs are  
253 deprived of auditory and visual contact with other breeding pairs (Brockway, 1964;  
254 Wyndham, 1981; our personal observation). Hence, all pairs were housed in separate breeding  
255 cages but in one single room, and we allowed the pairs auditory and visual contact to increase  
256 breeding success. After the introduction of the pairs, the nestboxes were inspected daily for  
257 the presence of eggs. Latency to lay was calculated as the number of days between the pairing  
258 of the birds and the appearance of the first egg. Eggs were collected within 24 hours after  
259 laying and replaced by plastic dummy eggs to avoid interruption of egg laying. All eggs were  
260 individually marked using a non-toxic pen, measured, weighed and frozen at -20°C until  
261 hormone analysis. Upon collection of the first clutch, the male was removed from the  
262 breeding cage. After five days, the second male from the female's preference test (see  
263 'Pairing') was introduced into the breeding cage and the female and the second male were  
264 allowed to produce a clutch (Gil et al., 2004). The males from the pairs that had failed to  
265 produce a clutch after one month were also removed from the breeding cages and, after five  
266 days, they were replaced with the second male from the female's preference test. The  
267 collecting and handling of the eggs of the second clutch were done identically as for the first  
268 clutch. Upon completion of the second clutch, or eight weeks after the introduction of the  
269 second male, all pairs were separated and the birds were put back in our captive stock.

270

271 *Hormone assay*

272 Our protocols to measure the concentrations of T and A4 in the yolk have been described  
273 previously (Goerlich et al., 2009). Briefly, yolks were weighed to the nearest 0.001g, 1:1  
274 diluted with dematerialized water and homogenized thoroughly. We then weighed ca. 200 mg  
275 of each yolk mixture for extraction. Prior to the extraction we added ca. 5000 cpm radioactive  
276 labeled T to each sample in order to account for losses due to the extraction procedure. Each  
277 sample was extracted three times with 2.0 mL diethyl ether/petroleum benzene 70:30  
278 (DEE/PB, vol:vol). After vortexing (60 sec), centrifuging (5 min, 2000 rpm, 4°C) and snap  
279 freezing, the organic phase was decanted and the extract was dried under a stream of nitrogen.  
280 The extraction procedure was repeated twice with an additional 2.0 mL of DEE/PB, vortexed  
281 for 30 sec and 15 sec, respectively. Next, the extracts were dried under nitrogen. Hormone

282 extracts were rinsed in 2.0 mL of 70 % methanol to precipitate any lipids. The samples were  
283 vortexed until the complete dried pellet was dissolved. Samples were frozen at -20°C  
284 overnight and then centrifuged (5 min, 2000 rpm, 4°C), decanted and dried. The pellet was re-  
285 dissolved in 600 µL phosphate buffered saline with gelatine (PBSG). Recoveries of the  
286 initially added labeled T were measured in a subsample of this solution. Average recovery  
287 was  $77.6 \pm 0.3$  % (mean  $\pm$  SE). Concentrations of T and A4 in the yolk were determined  
288 using commercial Radioimmunoassay (RIA) kits (*Spectria*<sup>®</sup> T Coated-Tube RIA kit, Orion  
289 Diagnostica Espoo, Finland; detection limit: 0.03 ng/mL, antibody cross-reactivity: 100% T,  
290 4.5% 5 $\alpha$ -dihydrotestosterone, and 0.007% A4; *Active*<sup>®</sup> A4 Coated-Tube RIA DSL-3800 kit,  
291 Diagnostic Systems Laboratories, Beckman Coulter, The Netherlands; detection limit:  
292 0.03ng/mL, antibody cross-reactivity: 100% A4, 0.33% Androsterone, and 0.08% 5 $\alpha$ -  
293 dihydrotestosterone). Samples were distributed over two RIAs for T as well as A4 such that  
294 all samples from one female were measured within the same assay and with both preference  
295 groups (i.e. females that were first paired with the preferred and subsequently with the non-  
296 preferred partner and vice versa) distributed equally. The assay kits were validated by  
297 ensuring parallelism of serial dilutions of four random yolk samples with the standard curve.  
298 For A4 there was no perfect parallelism, so a correction factor was calculated by dividing the  
299 slope of the standard curve by the average of the slopes of the four serial dilutions. RIA  
300 concentrations were corrected for initial yolk mass and calculated as pg/mg yolk. We included  
301 a selection of pool samples in both assays to estimate intra-assay variability (T-assays: n=24;  
302 A4-assays: n=15). The average intra-assay coefficients of variation (CV,  
303  $(\text{stddev}(\text{avrg})/\text{avrg}) \times 100$ ) were 2.15% for T, and 2.51% for A4. Repeatability for the pool  
304 samples measured in both assays was very high for both T and A4 ( $r > 0.95$ ,  $P < 0.0001$  in both  
305 cases; Lessells and Boag, 1987).

306

### 307 *Data Analysis*

308 We analyzed all data using the statistical package SAS<sup>®</sup> 9.3 (SAS Institute, Cary, NC, 2008).  
309 Because we did not know whether the concentrations or the amounts of androgens in the yolk  
310 are biologically more relevant in our study species, we modeled (i) concentrations, and (ii)  
311 total amounts of both T and A4 in the yolk, obtained by multiplying concentrations of  
312 respectively T and A4 by yolk mass. Six eggs of six different clutches (one first, second, third  
313 and fifth egg and two fourth eggs) were not included in the analyses because they were  
314 broken in the nest or because the egg contained two yolks.

315 We performed Pearson's correlations to investigate the relationships among the six

316 types maternal resources of a clutch that were measured in this study (mean egg and yolk  
317 weight, mean concentrations and total amounts of both T and A4 in the yolk). We used a  
318 mixed modelling approach to examine the effects of mate preference on maternal allocation.  
319 In each of the six models, we included preference (clutch produced with preferred or non-  
320 preferred male), clutch (1-2) and laying order (egg 1-8) as fixed factors and clutch size (3-8)  
321 as a covariate. Laying order was fitted as a categorical variable because the effects of laying  
322 order may not be linear (Kozłowski and Ricklefs, 2010; Salomons et al., 2006), which is of  
323 particular interest within the scope of our research aim. We also included two-way  
324 interactions between the fixed effects (see table 1 and 2). Our dataset contains two levels of  
325 nested random effects, as a series of eggs were collected in two clutches of the same female.  
326 We nested individual egg within clutch as a random coefficient for laying order and clutch  
327 within female as a random coefficient for preference (cf. random slopes model; Schielzeth  
328 and Forstmeier, 2009). We also included female condition (body weight) as a covariate in all  
329 models, but this factor was not significant in any of the models ( $P > 0.3$  in all cases) and thus  
330 we excluded the variable from all models. To analyze the effects of preference on latency to  
331 lay and clutch size we used two similar mixed models with preference, clutch and the  
332 preference\*clutch interaction term as fixed effects. Both models were corrected for the  
333 random coefficient of clutch, nested within individual female. Several covariance structures  
334 (i.e. compound symmetry, serial autocorrelation and unstructured) were tested in all the above  
335 models to select the best fitting regression based on Bayesian information criterion (BIC)  
336 values, which turned out to be the compound symmetry model in all cases. All response  
337 variables were normally distributed (Shapiro-Wilk, all  $W \geq 0.90$ ), except latency to lay which  
338 had to be log-transformed to meet this model assumption ( $W = 0.95$ ). We always started with  
339 the full model and subsequently excluded, step by step, all non-significant terms. At each  
340 step, the term that gave the smallest contribution (largest  $P$ -value) was excluded. Eta-squared  
341 effect sizes ( $\eta^2$ ) were calculated as the ratio of variance explained by a predictor based on the  
342 final model, thus only including significant terms. Similarly, effect size of the covariate  
343 (clutch size) is estimated by the regression coefficient ( $r$ ). Effect sizes for pair-wise  
344 comparisons are given as Cohen's  $d$ , i.e. as the difference between two means divided by the  
345 standard deviation. Values are consistently reported as mean  $\pm$  SE and a significance level of  
346  $P < 0.05$  was used throughout.

347

348

349

350 **Results**

351

352 *Female preference, latency to lay and clutch size*

353 A total of 27 females showed a significant preference for one of the two males ( $P < 0.025$  in all  
354 cases, fig. 2). The male with the higher value for UV chroma of the chest was preferred by 17  
355 females, while 10 females associated more with the male with the lower value (one-tailed  
356 binomial test:  $X^2_1 = 1.82$ ,  $P = 0.18$ , fig. 2). A total of 16 females produced a clutch with both  
357 males. Four females produced only one clutch; two with the preferred and two with the non-  
358 preferred male. Seven females did not lay eggs with either male (fig. 2).

359 The first egg was laid between 9 and 43 days after pair formation ( $18.47 \pm 1.46$  days).  
360 Latency to lay was not significantly affected by preference ( $F_{1, 17} = 0.34$ ,  $P = 0.57$ ; preferred:  
361  $18.83 \pm 1.97$  days; non-preferred:  $18.11 \pm 2.21$  days) or clutch ( $F_{1, 17} = 1.67$ ,  $P = 0.21$ ; first  
362 clutch:  $19.05 \pm 1.26$  days; second clutch:  $17.75 \pm 2.94$  days) and the preference\*clutch  
363 interaction term was also not significant ( $F_{1, 18} = 0.23$ ,  $P = 0.63$ ).

364 Clutch size varied between 3 and 8 eggs ( $6.03 \pm 0.19$  eggs). For females with the  
365 preferred partner, the average clutch size for the first clutch was  $6.17 \pm 0.42$  eggs ( $n = 12$ ; of  
366 which two females produced only one clutch) and for the second clutch  $6.66 \pm 0.21$  eggs ( $n = 6$   
367 females). For females with the non-preferred partner, the average clutch size for the first  
368 clutch was  $5.45 \pm 0.45$  eggs ( $n = 8$  females, of which two females only produced one clutch)  
369 and for the second clutch  $5.90 \pm 0.32$  eggs ( $n = 10$  females). There was no significant effect of  
370 preference on clutch size ( $F_{1, 14} = 0.62$ ,  $P = 0.44$ ). There was also no significant effect of clutch  
371 ( $F_{1, 12} = 0.12$ ,  $P = 0.74$ ) and the preference\*clutch interaction term was also not significant ( $F_{1, 14} = 0.28$ ,  $P = 0.61$ ).

373

374 *Egg weight and yolk weight*

375 Mean egg weight and yolk weight of a clutch were positively correlated ( $r = 0.63$ ,  $P < 0.0001$ ,  
376  $n = 36$  clutches). We found a significant overall effect of preference on yolk weight ( $\eta^2 = 0.05$ ),  
377 but not on egg weight (table 1, fig. 3a and 3b). Yolk weight of the second clutch was  
378 significantly higher (table 1, fig. 3a and 3b;  $\eta^2 = 0.02$ ). Clutch size significantly affected both  
379 egg weight and yolk weight (egg weight:  $r = 0.41$ ; yolk weight:  $r = 0.21$ ). Larger clutches  
380 contained heavier eggs with more yolk (table 1, fig. 4a). None of the interactions were  
381 significant for egg weight and for yolk weight, there was only a significant effect of the  
382 preference\*clutch interaction term (table 1;  $\eta^2 = 0.08$ ). Tukey adjusted post-hoc tests revealed  
383 that females that were paired with the preferred partner for the second clutch produced eggs

384 with the heaviest yolks (compared to the same females with the non-preferred partner for the  
385 first clutch:  $t_{72}=-3.54$ ,  $P_a=0.004$ ,  $d=0.53$ , 95% CI [0.09-0.96]; and compared to females with  
386 the preferred partner for the first clutch:  $t_{72}=-2.92$ ,  $P_a=0.024$ ,  $d=0.87$ , 95% CI [0.46-1.29] and  
387 the non-preferred partner for the second clutch:  $t_{72}=2.66$ ,  $P_a=0.047$ ,  $d=0.71$ , 95% CI [0.28-  
388 1.13]).

389

### 390 *Yolk androgens*

391 With regard to both concentrations and total amounts, we found that the yolk contained on  
392 average approximately five times more A4 than T, irrespective of treatment (fig. 3c-f). Mean  
393 concentrations of T and A4 in the yolks of a clutch were positively correlated ( $r=0.80$ ,  
394  $P<0.0001$ ,  $n=36$  clutches) and this was also the case for mean total amounts of T and A4 in  
395 the yolk ( $r=0.85$ ,  $P<0.0001$ ,  $n=36$  clutches). Mean concentrations of T and A4 in the yolks of  
396 a clutch were not correlated with either mean egg weight (T:  $r=0.27$ ,  $P=0.11$ ; A4:  $r=0.10$ ,  
397  $P=0.57$ ,  $n=36$  clutches) or mean yolk weight of that clutch (T:  $r=-0.044$ ,  $P=0.80$ ; A4:  $r=-0.25$ ,  
398  $P=0.15$ ,  $n=36$  clutches). Both mean total amounts of T and A4 in the yolk were positively  
399 correlated with mean egg weight as well as mean yolk weight of a clutch ( $r>0.45$ ,  $P<0.01$  in  
400 both cases,  $n=36$  clutches).

401 For concentrations and total amounts of T in the yolk, no significant effect of  
402 preference was found (table 2, fig. 3c and 3e). Eggs laid by females paired with the preferred  
403 partner contained significantly higher total amounts and tended to contain higher  
404 concentrations of A4 in the yolk (table 2, fig. 3d and 3f; total amounts:  $\eta^2=0.02$ ;  
405 concentrations:  $\eta^2=0.02$ ). The concentrations of T in the yolk, but not the total amounts, were  
406 significantly higher in the first clutch (table 2, fig. 3c and 3e;  $\eta^2=0.03$ ). No significant effect  
407 of clutch was found for A4 (table 2, fig. 3d and 3f). Clutch size significantly affected maternal  
408 androgen deposition (table 2). The yolks of larger clutches contained significantly higher  
409 concentrations and total amounts of both T and A4 (fig. 4b and 4c; concentrations of T:  
410  $r=0.29$ ; total amounts of T:  $r=0.33$ ; concentrations of A4:  $r=0.36$ ; total amounts of A4:  
411  $r=0.43$ ). For concentrations and total amounts of A4 in the yolk, we found a significant  
412 preference\*clutch size interaction term, which indicates that the effect of preference was  
413 stronger for larger clutches (table 2, fig. 4c). None of the other interaction terms were  
414 significant (table 2).

415

416

417

### 418 *Effects of laying order*

419 There was a significant effect of laying order for all six types of maternal resource that we  
420 measured in this study (tables 1 and 2). The second egg of a clutch contained a significantly  
421 heavier yolk than the fifth, sixth and seventh egg ( $t_{96}>3.20$ ,  $P_a<0.042$  and  $d>0.46$ , in all cases,  
422 fig. 5a) and the third egg contained a heavier yolk than the sixth and seventh egg ( $t_{96}>3.40$ ,  
423  $P_a<0.02$  and  $d>0.49$ , in both cases, fig. 5a). For egg weight, only egg 2 was significantly  
424 heavier than egg 6 ( $t_{96}=3.12$ ,  $P_a=0.047$ ,  $d=0.24$ , 95% CI [-0.26-0.74], fig. 5a). For both T and  
425 A4, the values for concentrations as well as total amounts in the yolk were lower in the first  
426 egg compared to the second egg and then remained more or less stable or gradually declined  
427 from the second egg on (fig. 5b and 5c). The effects of laying order were not affected by  
428 preference, clutch or clutch size (tables 1 and 2).

429

430

### 431 **Discussion**

432

433 In this study, female budgerigars were allowed to choose between two males, and we  
434 subsequently investigated the effects of female preference for her mate on maternal allocation  
435 of resources to the eggs. Additionally, we tested whether females increase allocation of  
436 resources to the eggs with the laying order to compensate for the competitive disadvantage of  
437 younger chicks due to the extreme hatching asynchrony as observed in this species.

438

### 439 *Maternal resources and female preference*

440 We found no significant effects of female preference for her mate on either the latency to lay  
441 or clutch size. A previous study in budgerigars reported significant effects of mate choice on  
442 both traits, but this effect disappeared after the first clutch (Massa et al., 1996). The different  
443 results may be due to the fact that the experimental design was not the same. Massa et al.  
444 (1996) also used inexperienced birds, but they allowed birds to form pairs during a three  
445 month period, and then compared self-selected pairs with randomly paired individuals. The  
446 randomly paired birds may have included individuals which had previously established pair  
447 bonds with other partners and that were thus artificially divorced. While in our experiment,  
448 unpaired females were paired with their preferred and non-preferred partner based on  
449 preference scores obtained from an experimental preference test.

450 Maternal allocation in terms of yolk weight but not egg weight was affected  
451 significantly by mate preference and differed between the first and second clutch. The

452 preference\*clutch interaction term was only significant for yolk weight. Our significant  
453 results for yolk weight seem to be largely due to the fact that females with the preferred  
454 partner produced heavier eggs with heavier yolks only in the second clutch. The differential  
455 allocation hypothesis predicts that females should invest more in clutches that are produced  
456 when mated with attractive or preferred partners because reproduction is costly and return on  
457 investment is higher when mated with attractive males (Burley, 1988; Sheldon, 2000).  
458 Producing large and heavy eggs is thought to be costly to the female (Nager, 2006; Vezina  
459 and Williams, 2002; Williams, 2005) and hatching from heavier eggs with heavier yolks has  
460 been shown to be beneficial for the offspring (Christians, 2002; Krist, 2011; Wagner and  
461 Williams, 2007; Williams, 1994). In accordance with the differential allocation hypothesis,  
462 experimental studies on several species report that females invest more in clutches of  
463 attractive males by increasing egg size and/or mass, although some studies did not find such a  
464 relationship (reviewed in: Cucco et al., 2011; Horvathova et al., 2012).

465 We found that female budgerigars that were paired with their preferred partner laid  
466 eggs with yolks that contained more A4 but not T. Females of a variety of species have been  
467 found to increase maternal androgen allocation when mated with attractive partners while  
468 other studies, mainly in wild populations, did not report such an effect (reviewed in: Kingma  
469 et al., 2009). In contrast to maternal investments in terms of egg production (e.g. egg weight,  
470 yolk weight), which are thought to be costly to the female (Nager, 2006; Vezina and  
471 Williams, 2002; Williams, 2005), there is little evidence to support that females experience  
472 direct costs from maternal androgen deposition (Groothuis et al., 2005a; Groothuis and  
473 Schwabl, 2008). As maternal androgens impose no direct costs to the female, the differential  
474 allocation theory cannot explain why females allocated more androgens to the eggs when they  
475 were mated with their preferred partner. Possibly, the females increased the allocation of  
476 androgens to stimulate the male to feed more (Groothuis et al., 2005a; Moreno-Rueda, 2007;  
477 Müller et al., 2007) as exposure to higher levels of maternal androgens during early  
478 development may increase offspring begging behavior (e.g. Eising and Groothuis, 2003;  
479 Schwabl, 1996; von Engelhardt et al., 2006). In species in which male attractiveness is  
480 negatively correlated with food provisioning, females should thus allocate more androgens to  
481 the eggs of attractive males (Moreno-Rueda, 2007). It seems unlikely that this hypothesis can  
482 be applied here, as male budgerigars usually provide little direct paternal care during the  
483 nestling phase (Stamps et al., 1985), but it could be that begging behavior affects male  
484 provisioning indirectly by increased courtship feeding.

485 Alternatively, even if the deposition of maternal androgens per se is not costly to the



486 female, there may be an indirect cost due to trade-offs at the level of the offspring (Groothuis  
487 et al., 2005a). Chicks may benefit from more maternal androgens because they hatch earlier,  
488 grow faster, are more alert and competitive and have higher survival rates (review in:  
489 Groothuis et al., 2005a). Nevertheless, several studies indicate that higher levels of T in the  
490 yolk may also induce immune suppression, elevated metabolic rate, oxidative stress and  
491 parasite susceptibility (Andersson et al., 2004; Cucco et al., 2008; Groothuis et al., 2005b;  
492 Müller et al., 2005; Navara et al., 2005; Sandell et al., 2009; Tobler et al., 2007; but see:  
493 Navara et al., 2006; Noguera et al., 2011; Rubolini et al., 2006; Tschirren et al., 2005). Such  
494 negative effects on the fitness of the offspring may even continue through adult life (Müller et  
495 al., 2009; Schwabl et al., 2012). Possibly, we found that females allocate more A4 in clutches  
496 produced with preferred males, because the resulting offspring are of higher genetic quality.  
497 As higher quality offspring may be better able to cope with the costs of maternal androgens,  
498 they may benefit more from the associated positive effects (Gil et al., 2004). This hypothesis  
499 may especially apply to species which breed colonially and/or in nest cavities, such as the  
500 budgerigar (Brockway, 1964; Wyndham, 1981). In such species, chicks are more likely to  
501 encounter parasites and pathogens in the nest (Berkunsky et al., 2005; Brown and Brown,  
502 1986; Tomas et al., 2007, Walls et al., 2012), which may render them more susceptible to the  
503 potential immuno-suppressive effects of yolk androgens (Gil et al., 2007; Müller et al., 2004).

504 Interestingly, we found that females allocated more A4 but not T to the yolk when  
505 mated with their preferred partner. Although the yolk contains at least three androgens (T, A4  
506 and 5 $\alpha$ -dihydrotestosterone; Schwabl, 1993), so far most research has focused on T, largely  
507 neglecting the potential functions of the other androgens in the yolk (Gil et al., 2007).  
508 However, the functional roles of different androgens in the yolk may not be the same, as  
509 indicated in a number of recent publications (e.g. Eising et al., 2001; Eising and Groothuis,  
510 2003; Hegyi and Schwabl, 2010; Hegyi et al., 2011; Muriel et al., 2013; Tschirren et al.,  
511 2014). Moreover, it seems that maternal androgens such as T and A4 are differently  
512 influenced by genes and the environment and are under opposing directional selection, which  
513 provides further supports for the notion that these two maternal hormones may play different  
514 functional roles (for a discussion on this topic, see: Tschirren et al., 2009; 2014). In contrast to  
515 T, A4 shows a very low affinity to the androgen receptor, and high levels of A4 are probably  
516 less harmful to the embryo. A4 is a metabolite of the ovarian steroid hormone synthesis  
517 pathway and can be converted to T by 17 $\beta$ -hydroxysteroid dehydrogenase (Groothuis and  
518 Schwabl, 2008; Horton and Tait, 1966). By allocating higher levels of A4 to the yolk, the  
519 female may provide the embryo with a source of T, while avoiding potential toxic effects of

520 high T levels (Bruggeman et al., 2002; Gil et al., 2007; Groothuis and Schwabl, 2002).  
521 Although A4 is thought to impose low immediate costs to the developing embryo, we  
522 hypothesize that the eggs produced with preferred males contain higher levels of A4 because  
523 the offspring of higher quality males may benefit more from increased T levels. This depends  
524 on the conversion of A4 to T by enzymes produced by the embryo in the course of  
525 development, needed for its own endogenous T and estrogen production. Differences in the  
526 functional significance of different androgens may also explain why we found, similarly to  
527 many other avian species, higher levels of A4 than T in budgerigar yolks (Gil et al., 2007).  
528 Our findings suggest that the functional role of each androgen in yolk should be considered  
529 separately, based on their presence and concentrations in a study species. Many questions  
530 regarding the adaptive significance of maternal hormones in the yolk still remain unanswered.  
531 Important new insights may come from studies focusing on the physiological mechanisms  
532 through which these hormones may affect the offspring (Carere and Balthazart 2007;  
533 Pfannkuche et al. 2011; von Engelhardt and Groothuis 2011).

534         A total of 28% of the females showed a significant preference the first time they were  
535 tested and 76% of the females were successful during their second preference test. Similar  
536 rates of success have been reported for previous preference experiments (e.g. von Engelhardt  
537 et al., 2004). Possibly, many preference tests were not successful because the males of a  
538 stimulus set did not differ sufficiently enough to enable the females to discriminate between  
539 them. The males of a stimulus set did not differ in body weight, but they differed significantly  
540 in UV chroma of the chest, a trait that had been shown to reflect male quality and to be  
541 involved in female mate choice (Griggio et al., 2010a, b; Zampiga et al., 2004). This renders it  
542 unlikely that the females could not discriminate between the males. It may also be that some  
543 females were affected by the social situation (i.e. being housed individually with limited  
544 possibilities for social interactions) during the preference tests (Cohen and Wills, 1985).  
545 Budgerigars are a highly social species (Brockway, 1964), and although we allowed the birds  
546 to have a long habituation period prior to the preference tests, the social situation may have  
547 influenced the behavior of some females. If this was the case, females were eventually more  
548 used to the experimental conditions during their second preference test, as more females were  
549 responsive during the second test. Although the preference test apparatus we used allows for  
550 unambiguous and fast measurement of preferences, it is possible that there are limitations as  
551 to how accurately female budgerigar preferences can be inferred from our experimental  
552 apparatus (Rutstein et al., 2007). We are, however, convinced that our method is reliable as  
553 preferences measured in the same species, using similar apparatus, have been shown to be

554 repeatable between studies (Griggio et al., 2010a; Zampiga et al., 2004) and to be reflected in  
555 pairing patterns (Moravec et al., 2006, 2010). This indicates that the preference scores  
556 obtained using this method, are a good measure for sexual preferences.

557 We found that females did not prefer males with higher values for UV chroma of the  
558 chest. This result was unexpected as two independent previous studies have found that UV  
559 chroma of the chest significantly predicts female choice (Griggio et al., 2010a; Zampiga et al.,  
560 2004), but it may be caused by the rather small sample size. Although we did not find a  
561 significant effect of chest coloration on female preference, there was a weak trend that  
562 females preferred to associate with males that expressed higher values for UV chroma.  
563 Furthermore, in contrast to the previous studies, which allowed females to choose between  
564 males treated with UV blocker and untreated males (Griggio et al., 2010a; Zampiga et al.,  
565 2004), we only measured natural variation in UV chroma. This might explain why the females  
566 in our study did not seem to use UV chroma of the chest as a decisive cue during mate  
567 preference in all cases. Other male traits, such as the production of warbling song and contact  
568 call similarity, have also been found to be important cues in female mate choice in the  
569 budgerigar (Moravec et al., 2006, 2010), but these traits could not be considered in this study.

570

#### 571 *Maternal resources and laying order*

572 Our results show that all six maternal resources measured in this study, varied significantly  
573 over the laying order. These measures, however, neither changed linearly with the laying  
574 order, nor in interaction with any of the other factors measured. Hence, females did not  
575 compensate for the consequences of hatching asynchrony, giving that chicks hatching from  
576 larger or heavier eggs with heavier yolks and more yolk androgens would have benefited from  
577 hatching earlier, weighing more, growing faster and having higher survival rates (Christians,  
578 2002; Groothuis et al., 2005a; Krist, 2011; Schwabl, 1996; Wagner and Williams, 2007;  
579 Williams, 1994). In contrast, we found that in budgerigar clutches earlier-laid eggs were  
580 heavier and contained more yolk. We also found that the concentrations of both T and A4 in  
581 the yolk are higher in the second egg compared to the first, while being lower again among  
582 the last eggs. For other species of parrot similar allocation patterns have been described  
583 (Budden and Beissinger, 2005; Kozłowski and Ricklefs, 2010). The functional consequences  
584 have as yet to be studied, but it seems unlikely that the observed allocation of maternal  
585 resources functions to reduce hatching asynchrony and/or its negative effects on the younger  
586 chicks. However, one may also have to consider whether and to what extent these patterns  
587 reflect physiological processes during laying (Christians, 2002; Groothuis et al., 2005a;

588 Groothuis and Schwabl, 2008; Kilpi et al., 1996; Nilsson and Svensson, 1993).

589         Interestingly, brood reduction is - despite the allocation patterns we found -  
590 uncommon in parrots, and usually the majority of chicks survive until fledging (e.g. Krebs,  
591 1999; Masello and Quillfeldt 2002; Spoon et al., 2006; Stamps et al., 1985; Wyndham, 1981).  
592 Similarly, in species of several other avian taxa, maternal allocation does not increase with the  
593 laying order although brood reduction is uncommon (Eising et al., 2009; Ellis et al., 2001; Gil  
594 et al., 1999; Reed and Vleck, 2001). It may be that in these species, alternative mechanisms  
595 are more important in overcoming the competitive disadvantages of later-hatched chicks.  
596 These alternative mechanisms may be especially important in the budgerigar, and in other  
597 species in which the chicks hatch over a long period of time, because differential maternal  
598 allocation is potentially insufficient to compensate for the extreme hatching asynchrony  
599 (Muller and Groothuis, 2013; Winkler, 1993).

600         In the budgerigar, preferential feeding by the female may provide such an alternative  
601 mechanism. Newly hatched budgerigar chicks show little begging activity, yet female  
602 budgerigars are known to feed the smallest chick first, irrespective of the begging behavior of  
603 its siblings (Stamps et al., 1985; our personal observation). This female strategy may diminish  
604 the competitive disadvantage of the younger chicks due to the hatching asynchrony, even  
605 without affecting the asynchrony itself. Actually, one may wonder why females do not control  
606 the degree of hatching asynchrony simply by postponing the onset of incubation if hatching  
607 asynchrony were disadvantageous (Groothuis et al., 2005a). This consideration may  
608 especially be relevant in cavity-nesting species such as the budgerigar, as in these species  
609 early incubation is less likely to be necessary in order to protect the eggs from predation or  
610 precipitation (Magrath, 1990; although other factors such as ambient temperature or microbial  
611 infection of the egg shell may potentially affect the onset of incubation; Cook et al., 2003;  
612 Stoleson and Beissinger, 1999; but see: Walls et al., 2012). In contrast, female budgerigars  
613 usually initiate incubation after laying the first or second egg, thereby stimulating  
614 asynchronous hatching (Brockway, 1964). This suggests that hatching asynchrony may be  
615 adaptive in budgerigars for reasons other than brood reduction. For example, size differences  
616 between the chicks due to the hatching asynchrony, may enable the spread of the brood's peak  
617 food demands or may reduce sibling competition if it results in the formation of a stable size  
618 hierarchy (Hahn, 1981; Kozlowski and Ricklefs, 2010; Magrath, 1990; Stamps et al., 1985;  
619 Wiebe and Bortolotti, 1994). If this is the case, it may not be surprising that the deposition  
620 patterns of maternal resources we found in budgerigar clutches do not appear to be  
621 unambiguously related to the hatching asynchrony. Whether or not maternal resources in the

622 egg serve other adaptive functions in the budgerigar remains to be elucidated.

623

624 To summarize, we found that female budgerigars when mated with their preferred partner did  
625 not initiate egg laying earlier and did not produce larger clutches, but they increased  
626 reproductive investment by producing clutches with eggs that contained more yolk and more  
627 A4 but not T in the yolk. Interestingly, we did not find evidence that the high chick survival –  
628 despite the extreme hatching asynchrony in budgerigars – is due to differential allocation of  
629 prenatal resources by the female.

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637 Table legends

638

639 **Table 1: Mixed models investigating the effects of female preference on egg weight and**  
640 **yolk weight.** Preference, laying order, clutch and their interactions were included as fixed  
641 effects and clutch size as a covariate. The degrees of freedom, F-value and *P*-value are given  
642 for the last step where the variable was included in the model. Significant effects ( $P < 0.05$ ) are  
643 highlighted in bold.

	Egg weight			Yolk weight		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
Preference	1, 72	2.54	0.12	1, 72	5.43	<b>0.023</b>
laying order	7, 96	2.21	<b>0.040</b>	7, 96	5.11	<b>&lt;0.0001</b>
clutch	1, 14	2.99	0.11	1, 14	9.71	<b>0.008</b>
clutch size	1, 72	7.03	<b>0.010</b>	1, 72	5.38	<b>0.023</b>
preference *laying order	6, 55	0.28	0.94	6, 55	0.54	0.77
preference *clutch	1, 60	0.16	0.69	1, 72	4.50	<b>0.037</b>
preference*clutch size	1, 55	0.00	0.98	1, 60	0.33	0.57
clutch*laying order	6, 66	1.96	0.084	6, 60	0.86	0.53
clutch*clutch size	1, 60	0.59	0.44	1, 60	0.23	0.63
laying order*clutch size	7, 60	1.11	0.37	7, 66	1.45	0.20

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652 **Table 2: Mixed models investigating the effects of female preference on the concentrations and the total amounts of both T and A4 in the**  
 653 **yolk.** Preference, laying order, clutch and their interactions were included as fixed effects and clutch size as a covariate. The degrees of freedom,  
 654 F-value and *P*-value are given for the last step where the variable was included in the model. Significant effects (*P*<0.05) are highlighted in bold.

655

656

	Concentrations of yolk T			Concentrations of yolk A4			Total amounts of yolk T			Total amounts of yolk A4		
	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>	df	<i>F</i>	<i>P</i>
preference	1, 72	0.24	0.63	1, 72	3.65	0.060	1, 72	0.90	0.35	1, 72	5.05	<b>0.028</b>
laying order	7, 96	15.89	<b>&lt;0.0001</b>	7, 96	5.03	<b>&lt;0.0001</b>	7, 96	16.38	<b>&lt;0.0001</b>	7, 96	8.25	<b>&lt;0.0001</b>
clutch	1, 14	8.29	<b>0.012</b>	1, 14	0.73	0.41	1, 14	1.33	0.27	1, 14	0.84	0.38
clutch size	1, 72	9.88	<b>0.002</b>	1, 72	13.82	<b>0.0004</b>	1, 72	19.1	<b>&lt;0.0001</b>	1, 72	17.91	<b>&lt;0.0001</b>
preference*laying order	6, 60	1.56	0.18	6, 60	1.09	0.38	6, 60	1.72	0.13	6, 60	1.24	0.30
preference*clutch	1, 55	0.00	0.95	1, 60	0.66	0.42	1, 55	0.97	0.33	1, 55	0.36	0.55
preference*clutch size	1, 55	0.24	0.62	1, 72	4.91	<b>0.030</b>	1, 55	0.13	0.72	1, 72	6.54	<b>0.012</b>
clutch*laying order	6, 55	1.73	0.13	6, 55	0.80	0.57	6, 55	1.40	0.23	6, 55	0.99	0.44
clutch*clutch size	1, 55	0.01	0.94	1, 55	0.02	0.88	1, 55	0.31	0.58	1, 66	1.33	0.25
laying order*clutch size	7, 66	1.67	0.13	7, 66	1.33	0.25	7, 66	1.85	0.10	7, 66	1.50	0.18



657 Figures Legends

658 **Figure 1: Diagram of the preference test apparatus used in the experiment.**

659 The black lines represent the perches. The gray squares represent the food and water  
660 containers. Dotted lines represent grated cage walls. The hatched areas indicate the left and  
661 right choice area; the open area in the large central part of the cage indicates the no-choice  
662 area.

663

664 **Figure 2: Female preference based on association score points.** Association score points  
665 above and below the 45° line represent respectively females that showed a preference  
666 ( $P < 0.05$ ) for the male with the higher and the male with the lower value for UV chroma of the  
667 chest. Points further from the 45° line indicate a stronger preference. Breeding results  
668 according to preference are represented as follows: females that produced a clutch with: both  
669 the preferred and the non-preferred partner (open squares), neither the preferred nor the non-  
670 preferred partner (black circles), only the preferred partner (open triangles), only the non-  
671 preferred partner (gray triangles).

672

673 **Figure 3: Maternal allocation according to preference.**

674 Maternal resource allocation (mean  $\pm$  SE) to the eggs produced by females paired with the  
675 preferred (open bars;  $n_{\text{clutch1}}=12$ ;  $n_{\text{clutch2}}=6$ ) and non-preferred partner (gray bars;  $n_{\text{clutch1}}=8$ ;  
676  $n_{\text{clutch2}}=10$ ): A) egg weight, B) yolk weight, C) concentrations of T in the yolk, D)  
677 concentrations of A4 in the yolk, E) total amounts of T in the yolk, F) total amounts of A4 in  
678 the yolk. The females of each group laid at maximum two clutches and the order of the  
679 preference treatment (mated with preferred or non-preferred partner) was reversed for the two  
680 groups of females. Four females only produced a first clutch (two with the preferred and two  
681 with the non-preferred partner).

682

683 **Figure 4: Maternal allocation and clutch size.**

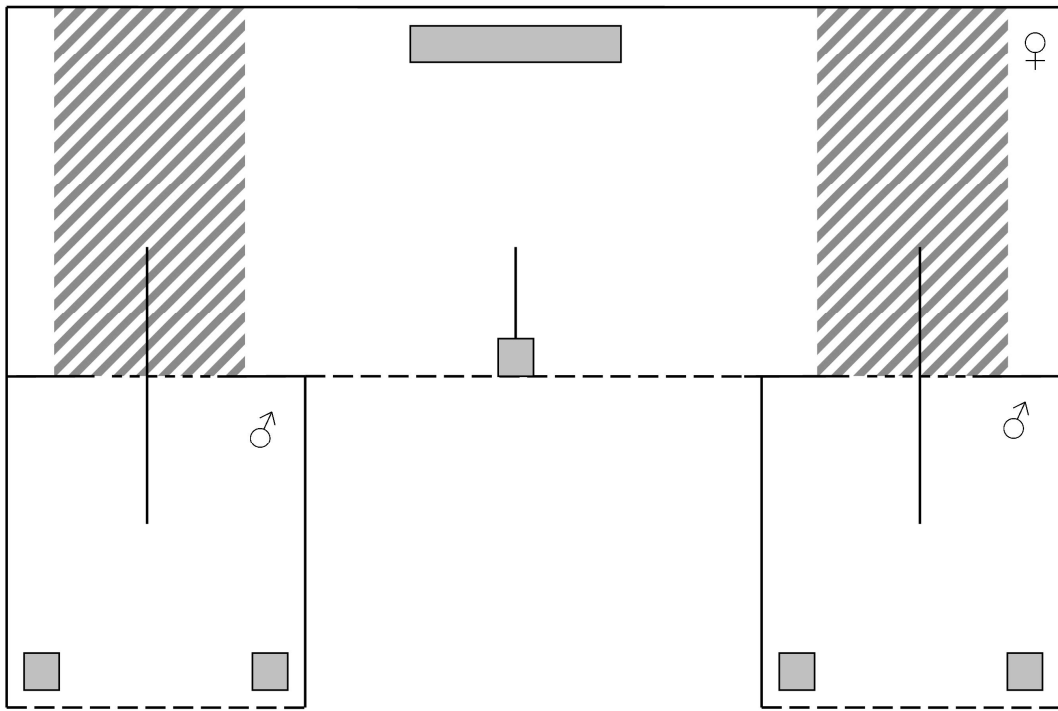
684 Maternal resources (mean  $\pm$  SE) in relation to clutch size for the eggs produced by females  
685 mated with the preferred (open squares) or non-preferred partner (black squares) for A) yolk  
686 weight and egg weight (SE for egg weight are too small to be represented by error bars; range:  
687 0.018 – 0.051), B) concentrations and total amounts of T in the yolk and C) concentrations  
688 and total amounts of A4 in the yolk. Clutch size ranged from 3 to 8 eggs. Sample size for  
689 females with the preferred and non-preferred partner was respectively:  $n_{3\text{eggs}}=1:1$ ,  $n_{4\text{eggs}}=0:1$ ,  
690  $n_{5\text{eggs}}=2:3$ ,  $n_{6\text{eggs}}=9:8$ ,  $n_{7\text{eggs}}=3:5$ ,  $n_{8\text{eggs}}=3:0$ .

691

692 **Figure 5: Effects of laying order.**

693 Variation in maternal resources (mean  $\pm$  SE) with laying order: A) egg weight (open circles)  
694 and yolk weight (black circles), B) concentrations of T in the yolk (black circles) and  
695 concentrations of A4 in the yolk (open circles), C) total amounts of T in the yolk (black  
696 circles) and total amounts of A4 in the yolk (open circles). Levels not connected by the same  
697 letter are significantly different (Tukey adjusted post-hoc tests,  $P_a < 0.05$ ).

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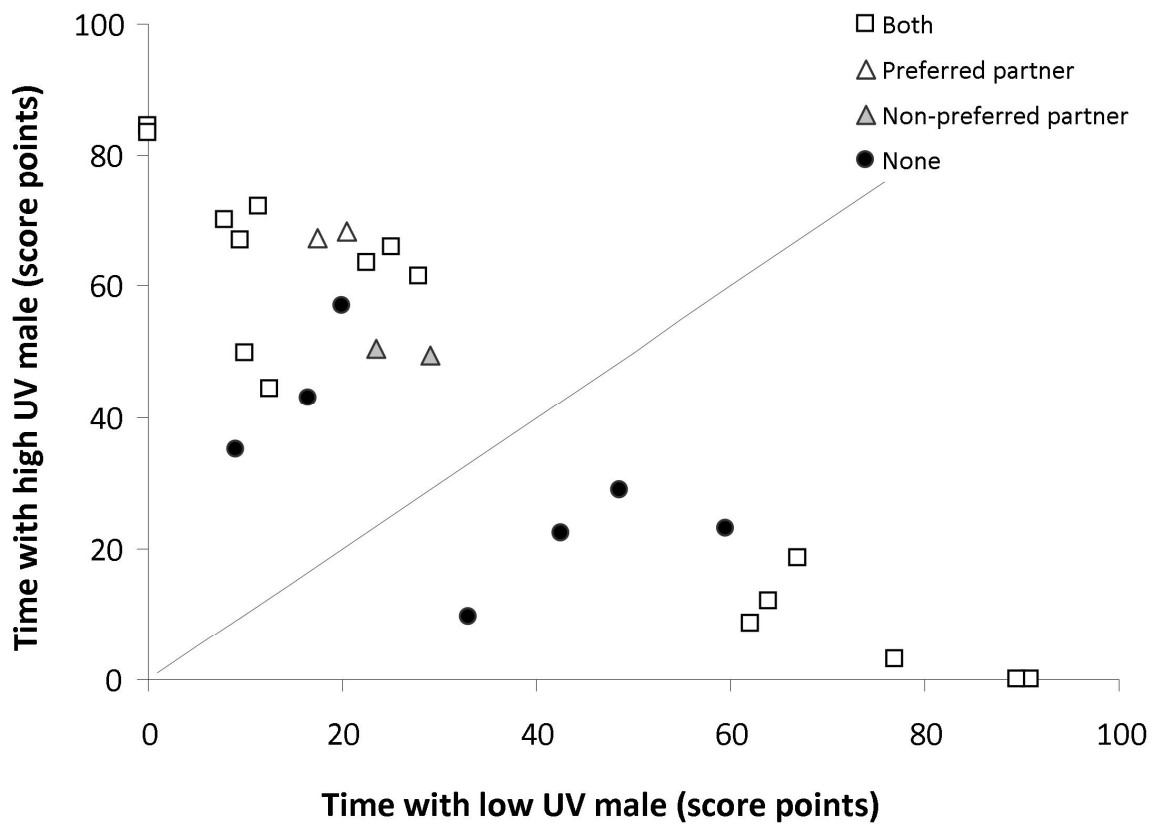


Figure 2

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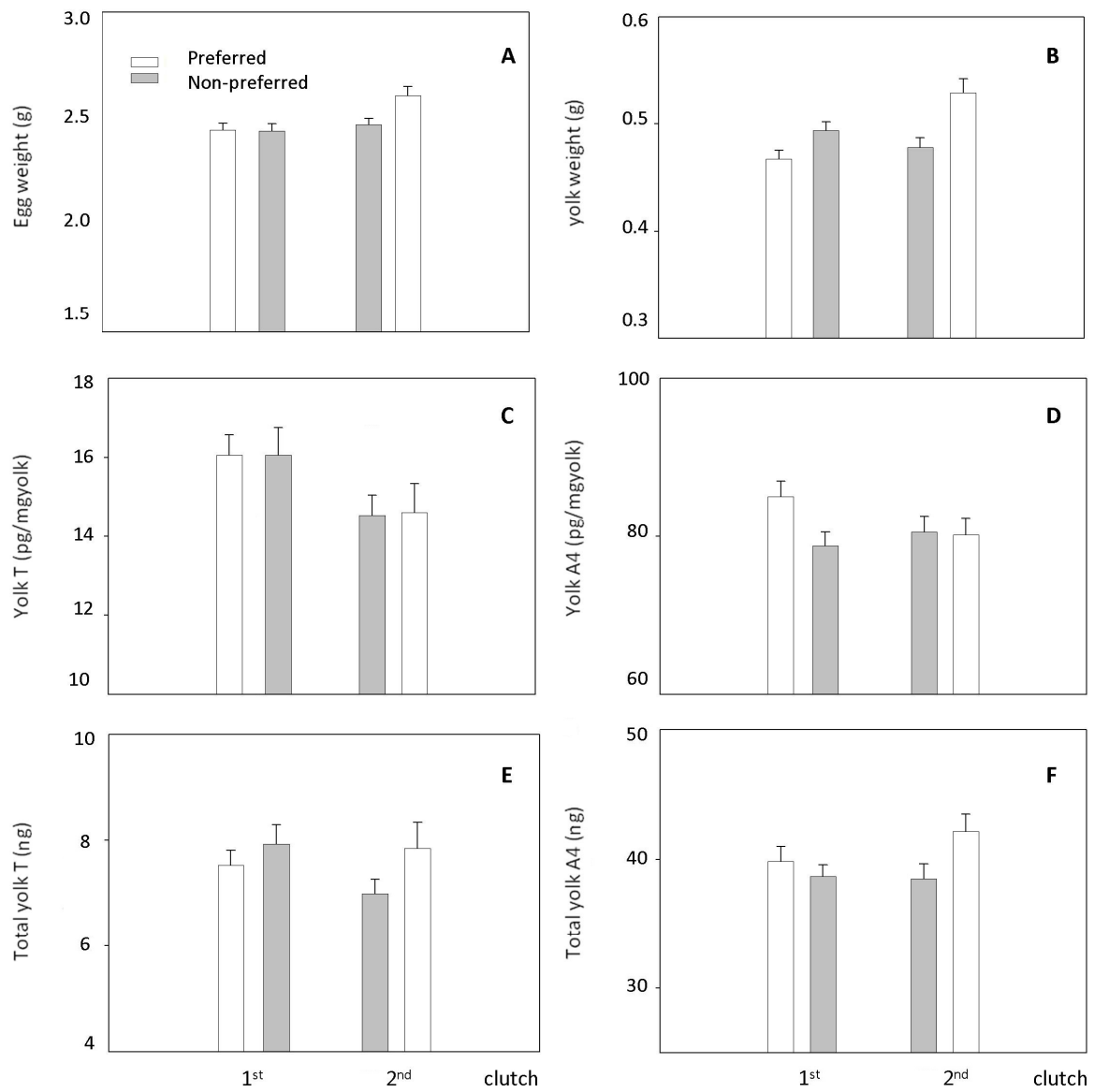
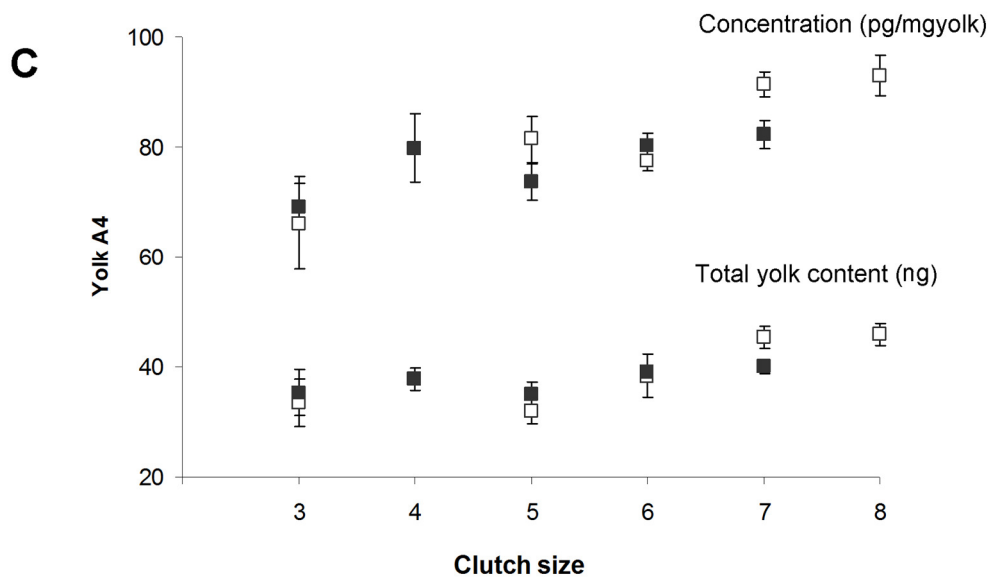
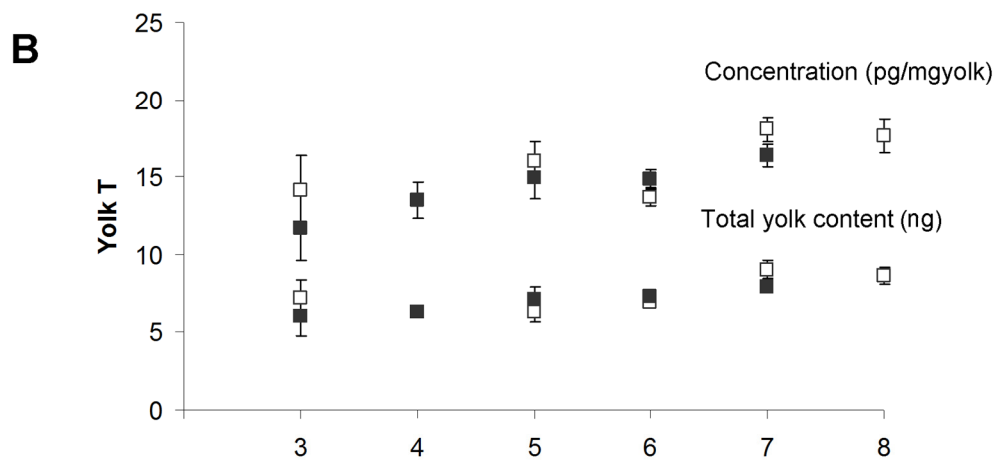
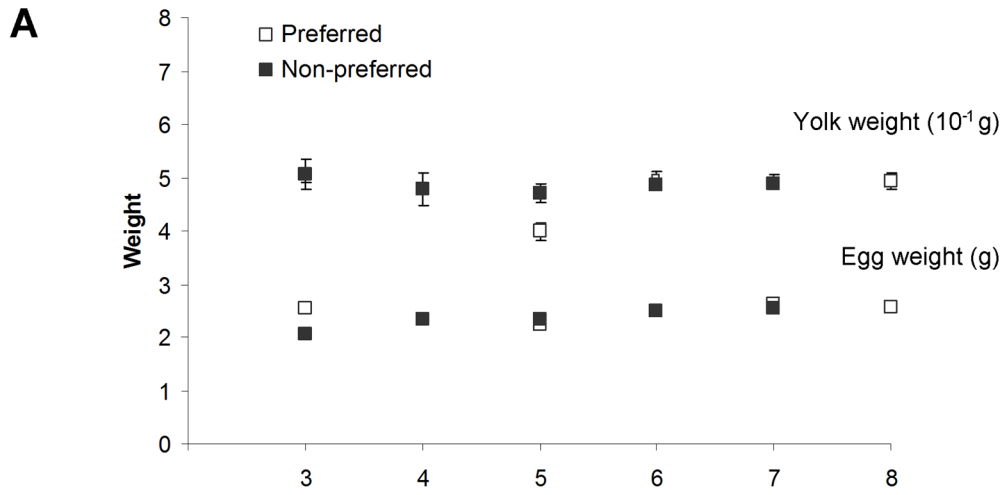


Figure 3

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Figure 4

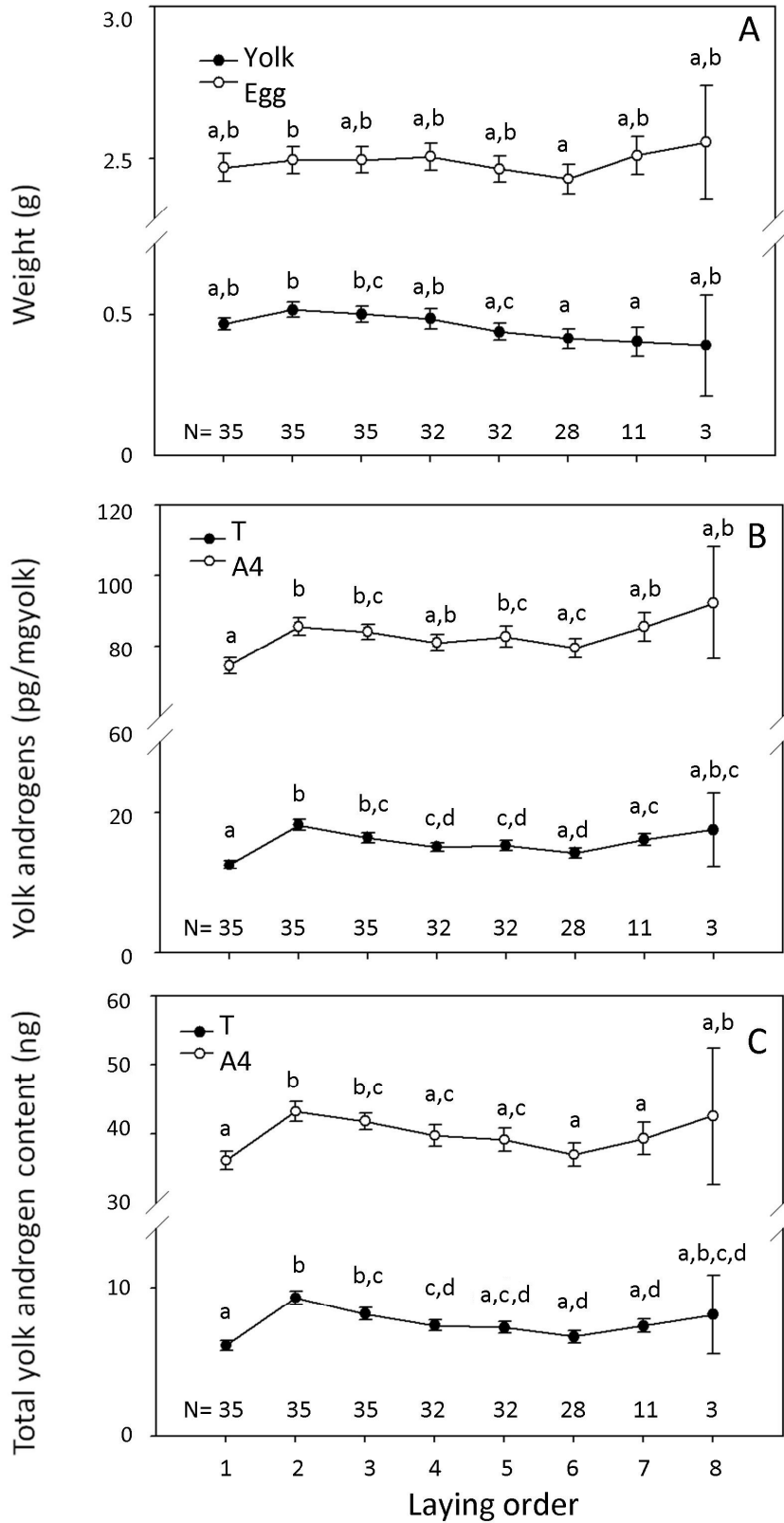


Figure 5

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721 References

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723 Alonso-Alvarez, C., Lorenzo Pérez-Rodríguez, L., Ferrero, M.E., García de-Blas, E., Casas,  
724 F., Mougeot F., 2012. Adjustment of female reproductive investment according to  
725 male carotenoid-based ornamentation in a gallinaceous bird. *Behav. Ecol. Sociobiol.*  
726 66, 731-742.

727 Andersson, M., 1994. *Sexual selection*. Princeton University Press, Princeton, New Jersey.

728 Andersson, S., Uller, T., Lohmus, M., Sundstrom, F., 2004. Effects of egg yolk testosterone  
729 on growth and immunity in a precocial bird. *J. Evol. Biol.* 17, 501-505.

730 Berkunsky, I., Formoso, A., Aramburu, R., 2005. Ectoparasitic load of blue-fronted parrot  
731 (*Amazona aestiva*, Psittacidae) nestlings. *Ornitol. Neotrop.* 16, 573-578.

732 Blount, J.D., Surai, P.F., Nager, R.G., Houston, D.C., Møller, A.P., Trewby, M.L., Kennedy,  
733 M.W., 2002. Carotenoids and egg quality in the lesser black-backed gull *Larus fuscus*:  
734 A supplemental feeding study of maternal effects. *Proc. R. Soc. B* 269, 29-36.

735 Bluhm, C.K., Gowaty, P.A., 2004. Social constraints on female mate preferences in mallards,  
736 *Anas platyrhynchos*, decrease offspring viability and mother productivity. *Anim.*  
737 *Behav.* 68, 977-983.

738 Brockway, B.F., 1964. Ethological studies of the budgerigar (*Melopsittacus undulatus*): Non-  
739 reproductive behavior. *Behavior* 22, 193-222.

740 Brown, C.R., Brown, M.B., 1986. Ectoparasitism as a cost of coloniality in cliff swallows  
741 (*Hirundo pyrrhonota*). *Ecology* 67, 1206-1218.

742 Bruggeman, V., Van As, P., Decuypere, E., 2002. Developmental endocrinology of the  
743 reproductive axis in the chicken embryo. *Comp. Biochem. Physiol. A Mol. Integr.*  
744 *Physiol.* 131, 839-846.

745 Budden, A.E., Beissinger, S.R., 2005. Egg mass in an asynchronously hatching parrot: Does  
746 variation offset constraints imposed by laying order? *Oecologia* 144, 318-326.

747 Burley, N., 1988. The differential allocation hypothesis - an experimental test. *Am. Nat.* 132,  
748 611-628.

749 Carere, C., Balthazart, J., 2007. Sexual versus individual differentiation: The controversial  
750 role of avian maternal hormones. *Trends Endocrinol. Metab.* 18, 73-80.

751 Christians, J.K., 2002. Avian egg size: Variation within species and inflexibility within  
752 individuals. *Biol. Rev.* 77, 1-26.

753 Cohen, S., Wills, T.A., 1985. Stress, social support, and the buffering hypothesis. *Psychol.*  
754 *Bull.* 98, 310-357.

755 Cook, M.I., Beissinger, S.R., Toranzos, G.A., Rodriguez, R.A., Arendt, W.J., 2003. Trans-  
756 shell infection by pathogenic micro-organisms reduces the shelf life of non-incubated  
757 bird's eggs : a constraint on the onset of incubation? *Proc. R. Soc. B* 270, 2233-2240.

758 Cucco, M., Guasco, B., Malacarne, G., Ottonelli, R., Tanvez, A., 2008. Yolk testosterone  
759 levels and dietary carotenoids influence growth and immunity of grey partridge  
760 chicks. *Gen. Comp. Endocrinol.* 156, 418-425.

761 Cucco, M., Grenna, M., Pellegrino, I., Malacarne, G., 2011. Egg-sequence rather than mating  
762 preference influences female egg investment in the red-legged partridge. *Ethol. Ecol.*



- 763 Evol. 23, 343-357.
- 764 Cuthill, I.C., Bennett, A.T.D., Partridge, J.C., Maier, E.J., 1999. Plumage reflectance and the  
765 objective assessment of avian sexual dichromatism. *Am. Nat.* 153, 183-200.
- 766 Eising, C.M., Eikenaar, C., Schwabl, H., Groothuis, T.G.G., 2001. Maternal androgens in  
767 black-headed gull (*Larus ridibundus*) eggs: Consequences for chick development.  
768 *Proc. R. Soc. B* 268, 839-846.
- 769 Eising, C.M., Groothuis, T.G.G., 2003. Yolk androgens and begging behaviour in black-  
770 headed gull chicks: An experimental field study. *Anim. Behav.* 66, 1027-1034.
- 771 Eising, C.M., Robles, R., Lasthuizen, M., Groothuis, T.G.G., 2009. Lesser double-collared  
772 sunbirds *Nectarinia chalybea* do not compensate for hatching asynchrony by adjusting  
773 egg mass or yolk androgens. *J. Avian Biol.* 40, 635-639.
- 774 Ellis, L.A., Borst, D.W., Thompson, C.F., 2001. Hatching asynchrony and maternal  
775 androgens in egg yolks of house wrens. *J. Avian Biol.* 32, 26-30.
- 776 Enstrom, D.A., Ketterson, E.D., Nolan, V., 1997. Testosterone and mate choice in the dark-  
777 eyed junco. *Anim. Behav.* 54, 1135-1146.
- 778 Garcia-Fernandez, V., Draganoiu, T.I., Ung, D., Lacroix, A., Malacarne, G., Leboucher, G.,  
779 2013. Female canaries invest more in response to an exaggerated male trait. *Anim.*  
780 *Behav.* 85, 679-684.
- 781 Gil, D., Graves, J., Hazon, N., Wells, A., 1999. Male attractiveness and differential  
782 testosterone investment in zebra finch eggs. *Science* 286, 126-128.
- 783 Gil, D., Leboucher, G., Lacroix, A., Cue, R., Kreutzer, M., 2004. Female canaries produce  
784 eggs with greater amounts of testosterone when exposed to preferred male song.  
785 *Horm. Behav.* 45, 64-70.
- 786 Gil, D., Biard, C., Lacroix, A., Spottiswoode, C.N., Saino, N., Puerta, M., Møller, A.P., 2007.  
787 Evolution of yolk androgens in birds: Development, coloniality, and sexual  
788 dichromatism. *Am. Nat.* 169, 802-819.
- 789 Goerlich, V.C., Dijkstra, C., Schaafsma, S.M., Groothuis, T.G.G., 2009. Testosterone has a  
790 long-term effect on primary sex ratio of first eggs in pigeons - In search of a  
791 mechanism. *Gen. Comp. Endocrinol.* 163, 184-192.
- 792 Grana, S.C., Sakaluk, S.K., Bowden, R.M., Doellman, M.A., Vogel, L.A., Thompson, C.F.,  
793 2012. Reproductive allocation in female house wrens is not influenced by  
794 experimentally altered male attractiveness. *Behav. Ecol. Sociobiol.* 66, 1247-1258.
- 795 Griggio, M., Hoi, H., Pilastro, A., 2010a. Plumage maintenance affects ultraviolet colour and  
796 female preference in the budgerigar. *Behav. Processes* 84, 739-744.
- 797 Griggio, M., Zanollo, V., Hoi, H., 2010b. UV plumage color is an honest signal of quality in  
798 male budgerigars. *Ecol. Res.* 25, 77-82.
- 799 Groothuis, T.G.G., Schwabl, H., 2002. Determinants of within- and among-clutch variation in  
800 levels of maternal hormones in black-headed gull eggs. *Funct. Ecol.* 16, 281-289.
- 801 Groothuis, T.G.G., Müller, W., von Engelhardt, N., Carere, C., Eising, C., 2005a. Maternal  
802 hormones as a tool to adjust offspring phenotype in avian species. *Neurosci. Biobehav.*  
803 *Rev.* 29, 329-352.
- 804 Groothuis, T.G.G., Eising, C.M., Dijkstra, C., Müller, W., 2005b. Balancing between costs  
805 and benefits of maternal hormone deposition in avian eggs. *Biol. Lett.* 1, 78-81.

- 806 Groothuis, T.G.G., Schwabl, H., 2008. Hormone-mediated maternal effects in birds:  
807 Mechanisms matter but what do we know of them? *Philos. Trans. R. Soc. Lond. B*  
808 *Biol. Sci.* 363, 1647-1661.
- 809 Hahn, D.C., 1981. Asynchronous hatching in the laughing gull - cutting losses and reducing  
810 rivalry. *Anim. Behav.* 29, 421-427.
- 811 Hargitai, R., Arnold, K.E., Herenyi, M., Prechl, J., Torok, J., 2009. Egg composition in  
812 relation to social environment and maternal physiological condition in the collared  
813 flycatcher. *Behav. Ecol. Sociobiol.* 63, 869-882.
- 814 Hegyi, G., Schwabl, H., 2010. Do different yolk androgens exert similar effects on the  
815 morphology or behaviour of Japanese quail hatchlings *Coturnix japonica*? *J. Avian*  
816 *Biol.* 41, 258-265.
- 817 Hegyi, G., Herenyi, M., Szollosi, E., Rosivall, B., Torok, J., Groothuis, T.G.G., 2011. Yolk  
818 androstenedione, but not testosterone, predicts offspring fate and reflects parental  
819 quality. *Behav. Ecol.* 22, 29-38.
- 820 Horton, R., Tait, J.F., 1966. Androstenedione production and interconversion rates measured  
821 in peripheral blood and studies on possible site of its conversion to testosterone. *J.*  
822 *Clin. Invest.* 45, 301-313.
- 823 Horvathova, T., Nakagawa, S., Uller, T., 2012. Strategic female reproductive investment in  
824 response to male attractiveness in birds. *Proc. R. Soc. B* 279, 163-170.
- 825 Juniper, T., Parr, M., 1998. *Parrots: A guide to the parrots of the world.* Pica Press, Sussex.
- 826 Kilpi, M., Hillstrom, L., Lindstrom, K., 1996. Egg-size variation and reproductive success in  
827 the herring gull *Larus argentatus*: Adaptive or constrained size of the last egg? *Ibis*  
828 138, 212-217.
- 829 Kingma, S.A., Komdeur, J., Vedder, O., von Engelhardt, N., Korsten, P., Groothuis, T.G.G.,  
830 2009. Manipulation of male attractiveness induces rapid changes in avian maternal  
831 yolk androgen deposition. *Behav. Ecol.* 20, 172-179.
- 832 Kozlowski, C.P., Ricklefs, R.E., 2010. Egg size and yolk steroids vary across the laying order  
833 in cockatiel clutches: A strategy for reinforcing brood hierarchies? *Gen. Comp.*  
834 *Endocrinol.* 168, 460-465.
- 835 Krebs, E.A., 1999. Last but not least: Nestling growth and survival in asynchronously  
836 hatching crimson rosellas. *J. Anim. Ecol.* 68, 266-281.
- 837 Krist, M., 2011. Egg size and offspring quality: A meta-analysis in birds. *Biol. Rev.* 86, 692-  
838 716.
- 839 Lahaye, S.E.P., Eens, M., Darras, V.M., Pinxten, R., 2013. Hot or not: The effects of  
840 exogenous testosterone on female attractiveness to male conspecifics in the  
841 budgerigar. *Plos One* 8, e74005.
- 842 Lahaye, S.E.P., Eens, M., Darras, V.M., Pinxten, R., 2014. Bare-part color in female  
843 budgerigars changes from brown to structural blue following testosterone treatment  
844 but is not strongly masculinized. *Plos One* 9, e86849.
- 845 Lessells, C.M., Boag, P.T., 1987. Unrepeatable repeatabilities - a common mistake. *Auk* 104,  
846 116-121.
- 847 Love, O.P., Gilchrist, H.G., Bety, J., Wynne-Edwards, K.E., Berzins, L., Williams, T.D.,  
848 2009. Using life-histories to predict and interpret variability in yolk hormones. *Gen.*

- 849           Comp. Endocrinol. 163, 169-174.
- 850 Magrath, R.D., 1990. Hatching asynchrony in altricial birds. *Biol. Rev.* 65, 587-622.
- 851 Masello, J.F., Quillfeldt, P., 2002. Chick growth and breeding success of the burrowing  
852 parrot. *Condor* 104, 574-586.
- 853 Massa, R., Galanti, V., Bottoni, L., 1996. Mate choice and reproductive success in the  
854 domesticated budgerigar, *Melopsittacus undulatus*. *It. J. Zool.* 63, 243-246.
- 855 Massemin, S., Korpimäki, E., Poyri, V., Zorn, T., 2002. Influence of hatching order on growth  
856 rate and resting metabolism of kestrel nestlings. *J. Avian Biol.* 33, 235-244.
- 857 McGlothlin, J.W., Neudorf, D.L.H., Casto, J.M., Nolan, V., Ketterson, E.D., 2004. Elevated  
858 testosterone reduces choosiness in female dark-eyed juncos (*Junco hyemalis*):  
859 Evidence for a hormonal constraint on sexual selection? *Proc. R. Soc. B* 271, 1377-  
860 1384.
- 861 Montgomerie, R., 2006. Analyzing colors. in: Hill, G.E., McGraw, K.J. (Eds.), *Bird*  
862 *coloration*. Harvard University Press, Cambridge, MA, pp. 90-147.
- 863 Moravec, M.L., Striedter, G.F., Burley, N.T., 2006. Assortative pairing based on contact call  
864 similarity in budgerigars, *Melopsittacus undulatus*. *Ethology* 112, 1108-1116.
- 865 Moravec, M.L., Striedter, G.F., Burley, N.T., 2010. 'Virtual Parrots' confirm mating  
866 preferences of female budgerigars. *Ethology* 116, 961-971.
- 867 Moreno-Rueda, G., 2007. Yolk androgen deposition as a female tactic to manipulate paternal  
868 contribution. *Behav. Ecol.* 18, 496-498.
- 869 Mousseau, T.A., Fox, C.W., 1998. The adaptive significance of maternal effects. *Trends Ecol.*  
870 *Evol.* 13, 403-407.
- 871 Müller, W., Groothuis, T.G.G., Dijkstra, C., Siitari, H., Alatalo, R.V., 2004. Maternal  
872 antibody transmission and breeding densities in the black-headed gull *Larus*  
873 *ridibundus*. *Funct. Ecol.* 18, 719-724.
- 874 Müller, W., Groothuis, T.G.G., Kasprzik, A., Dijkstra, C., Alatalo, R.V., Siitari, H., 2005.  
875 Prenatal androgen exposure modulates cellular and humoral immune function of  
876 black-headed gull chicks. *Proc. R. Soc. B* 272, 1971-1977.
- 877 Müller, W., Lessells, C.M., Korsten, P., von Engelhardt, N., 2007. Manipulative signals in  
878 family conflict? On the function of maternal yolk hormones in birds. *Am. Nat.* 169,  
879 84-96.
- 880 Müller, W., Vergauwen, J., Eens, M., 2009. Long-lasting consequences of elevated yolk  
881 testosterone levels on female reproduction. *Behav. Ecol. Sociobiol.* 63, 809-816.
- 882 Muller, M., Groothuis, T.G.G., 2013. Within-clutch variation in yolk testosterone as an  
883 adaptive maternal effect to modulate avian sibling competition: Evidence from a  
884 comparative study. *Am. Nat.* 181, 125-136.
- 885 Muriel J., Pérez-Rodríguez L., Puerta M., Gil D., 2013. Differential effects of yolk  
886 testosterone and androstenedione in embryo development and nestling growth in the  
887 spotless starling (*Sturnus unicolor*). *Gen. Comp. Endocrinol.* 194, 175-182.
- 888 Nager, R.G., 2006. The challenges of making eggs. *Ardea* 94, 323-346.
- 889 Navara, K.J., Hill, G.E., Mendonca, M.T., 2005. Variable effects of yolk androgens on  
890 growth, survival, and immunity in eastern bluebird nestlings. *Physiol. Biochem. Zool.*

- 891 78, 570-578.
- 892 Navara, K.J., Hill, G.E., Mendonca, M.T., 2006. Yolk testosterone stimulates growth and  
893 immunity in house finch chicks. *Physiol. Biochem. Zool.* 79, 550-555.
- 894 Nilsson, J.A., Svensson, E., 1993. Causes and consequences of egg mass variation between  
895 and within blue tit clutches. *J. Zool.* 230, 469-481.
- 896 Noguera, J.C., Alonso-Alvarez, C., Kim, S.Y., Morales, J., Velando, A., 2011. Yolk  
897 testosterone reduces oxidative damages during postnatal development. *Biol. Lett.* 7,  
898 93-95.
- 899 Pfannkuche, K.A., Gahr, M., Weites, I.M., Riedstra, B., Wolf, C., Groothuis, T.G.G., 2011.  
900 Examining a pathway for hormone mediated maternal effects – Yolk testosterone  
901 affects androgen receptor expression and endogenous testosterone production in young  
902 chicks (*Gallus gallus domesticus*). *Gen. Comp. Endocrinol.* 172, 487-493.
- 903 Reed, W.L., Vleck, C.M., 2001. Functional significance of variation in egg-yolk androgens in  
904 the American coot. *Oecologia* 128, 164-171.
- 905 Remes, V., 2011. Yolk androgens in great tit eggs are related to male attractiveness, breeding  
906 density and territory quality. *Behav. Ecol. Sociobiol.* 65, 1257-1266.
- 907 Rubolini, D., Romano, M., Martinelli, R., Saino, N., 2006. Effects of elevated yolk  
908 testosterone levels on survival, growth and immunity of male and female yellow-  
909 legged gull chicks. *Behav. Ecol. Sociobiol.* 59, 344-352.
- 910 Rutstein, A.N., Brazill-Boast, J., Griffith, S.C., 2007. Evaluating mate choice in the zebra  
911 finch. *Anim. Behav.* 74, 1277-1284.
- 912 Safran, R.J., Pilz, K.M., McGraw, K.J., Correa, S.M., Schwabl, H., 2008. Are yolk androgens  
913 and carotenoids in barn swallow eggs related to parental quality? *Behav. Ecol.*  
914 *Sociobiol.* 62, 427-438.
- 915 Safran, R.J., McGraw, K.J., Pilz, K.M., Correa, S.M., 2010. Egg-yolk androgen and  
916 carotenoid deposition as a function of maternal social environment in barn swallows  
917 *Hirundo rustica*. *J. Avian Biol.* 41, 470-478.
- 918 Salomons, H.M., Müller, W., Dijkstra, C., Eising, C.M., Verhulst, S., 2006. No sexual  
919 differences in embryonic period in jackdaws *Corvus monedula* and black-headed gulls  
920 *Larus ridibundus*. *J. Avian Biol.* 37, 19-22.
- 921 Sandell, M.I., Tobler, M., Hasselquist, D., 2009. Yolk androgens and the development of  
922 avian immunity: An experiment in jackdaws (*Corvus monedula*). *J. Exp. Biol.* 212,  
923 815-822.
- 924 Schielzeth, H., Forstmeier, W., 2009. Conclusions beyond support: overconfident estimates in  
925 mixed models. *Behav. Ecol.* 20, 416-420.
- 926 Schwabl, H., 1993. Yolk is a source of maternal testosterone for developing birds. *Proc. Natl.*  
927 *Acad. Sci. U S A* 90, 11446-11450.
- 928 Schwabl, H., 1996. Maternal testosterone in the avian egg enhances postnatal growth. *Comp*  
929 *Biochem. Physiol. A Physiol.* 114, 271-276.
- 930 Schwabl, H.H., Holmes, D., Strasser, R., Scheurelein, A., 2012. Embryonic exposure to  
931 maternal testosterone influences age-specific mortality patterns in a captive passerine  
932 bird. *Age* 4, 87-94.
- 933 Sheldon, B.C., 2000. Differential allocation: Tests, mechanisms and implications. *Trends*

- 934 Ecol. Evol. 15, 397-402.
- 935 Slagsvold, T., Sandvik, J., Rofstad, G., Lorentsen, O., Husby, M., 1984. On the adaptive value  
936 of intraclutch egg-size variation in birds. *Auk* 101, 685-697.
- 937 Spoon, T.R., Millam, J.R., Owings, D.H., 2006. The importance of mate behavioural  
938 compatibility in parenting and reproductive success by cockatiels, *Nymphicus*  
939 *hollandicus*. *Anim. Behav.* 71, 315-326.
- 940 Stamps, J., Clark, A., Arrowood, P., Kus, B., 1985. Parent-offspring conflict in budgerigars.  
941 *Behavior* 94, 1-40.
- 942 Stoleson, S.H., Beissinger, S.R., 1999. Egg viability as a constraint on hatching synchrony at  
943 high ambient temperatures. *J. Anim. Ecol.* 68, 951-962.
- 944 Tobler, M., Nilsson, J., Nilsson, J.F., 2007. Costly steroids: egg testosterone modulates  
945 nestling metabolic rate in the zebra finch. *Biol. Lett.* 3, 408-410.
- 946 Tomas, G., Merino, S., Moreno, J., Morales, J., 2007. Consequences of nest reuse for parasite  
947 burden and female health and condition in blue tits, *Cyanistes caeruleus*. *Anim.*  
948 *Behav.* 73, 805-814.
- 949 Tschirren, B., Saladin, V., Fitze, P.S., Schwabl, H., Richner, H., 2005. Maternal yolk  
950 testosterone does not modulate parasite susceptibility or immune function in great tit  
951 nestlings. *J. Anim. Ecol.* 74, 675-682.
- 952 Tschirren, B., Sendecka, J., Groothuis, T.G.G., Gustafsson, L., Doligez, B., 2009. Heritable  
953 variation in maternal yolk hormone transfer in a wild bird population. *Am. Nat.* 174,  
954 557-564.
- 955 Tschirren, B., Postma, E., Gustafsson, L., Groothuis, T.G.G., Doligez, B., 2014. Natural  
956 selection acts in opposite ways on correlated hormonal mediators of prenatal maternal  
957 effects in a wild bird population. *Ecol. Lett.* 17, 1310-1315.
- 958 Vergauwen, J., Goerlich, V.C., Groothuis, T.G.G., Eens, M., Müller, W., 2012. Food  
959 conditions affect yolk testosterone deposition but not incubation attendance. *Gen.*  
960 *Comp. Endocrinol.* 176, 112-119.
- 961 Vezina, F., Williams, T.D., 2002. Metabolic costs of egg production in the European starling  
962 (*Sturnus vulgaris*). *Physiol. Biochem. Zool.* 75, 377-385.
- 963 von Engelhardt, N., Groothuis, T.G.G., Brintjes, R., C., D., 2004. Effects of male  
964 attractiveness on yolk androgens, offspring sex ratio and survival in zebra finches,  
965 *Taeniopygia guttata*. in: von Engelhardt, N. (Ed.), Proximate control of avian sex  
966 allocation, a study in zebra finches. P.h.D. dissertation thesis, Univerisity of  
967 Groningen, Groningen.
- 968 von Engelhardt, N., Carere, C., Dijkstra, C., Groothuis, T. G. G., 2006. Sex-specific effects of  
969 yolk testosterone on survival, begging and growth of zebra finches. *Proc. R. Soc. B*  
970 273, 65-70.
- 971 von Engelhardt, N., Groothuis, T. G. G., 2011. Maternal hormones in avian eggs. in: Norris,  
972 D.O., Lopez, K.H. (Eds.), Hormones and reproduction of vertebrates. Vol. 4 Birds.  
973 Academic Press, New York, pp. 91-127.
- 974 Wagner, E.C., Williams, T.D., 2007. Experimental (antiestrogen-mediated) reduction in egg  
975 size negatively affects offspring growth and survival. *Physiol. Biochem. Zool.* 80.  
976 293-305.

- 977 Walls, J.G., Hepp, G.R., Eckhardt, L.G., 2012. Effects of nest reuse and onset of incubation  
978 on microbial growth and viability of wood duck eggs. *Condor* 114. 720-725.
- 979 Wiebe, K.L., Bortolotti, G.R., 1994. Energetic efficiency of reproduction - the benefits of  
980 asynchronous hatching for american kestrels. *J. Anim. Ecol.* 63, 551-560.
- 981 Williams, G.C., 1966. Natural selection costs of reproduction and a refinement of Lack's  
982 principle. *Am. Nat.* 100, 687-690.
- 983 Williams, T.D., 1994. Intraspecific variation in egg size and egg composition in birds - effects  
984 on offspring fitness. *Biol. Rev.* 69, 35-59.
- 985 Williams, T.D., 2005. Mechanisms underlying the costs of egg production. *Bioscience* 55, 39-  
986 48.
- 987 Winkler, D.W., 1993. Testosterone in egg yolks - an ornithologists perspective. *Proc. Natl.*  
988 *Acad. Sci. U S A* 90, 11439-11441.
- 989 Wyndham, E., 1981. Breeding and mortality of budgerigars *Melopsittacus undulatus*. *Emu* 81,  
990 240-243.
- 991 Zampiga, E., Hoi, H., Pilastro, A., 2004. Preening, plumage reflectance and female choice in  
992 budgerigars. *Ethol. Ecol. Evol.* 16. 339-349.
- 993