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Long-term effect of yolk carotenoid levels on testis size in a precocial bird

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Abstract: Conditions experienced during prenatal development can have long-lasting organizational effects on offspring. Maternal carotenoids deposited in the eggs of birds and other oviparous species play an important role during fast embryonic growth and chick development through their antioxidant properties. However, the long-term consequences of variation in maternal carotenoid transfer for the offspring have seldom been considered. Since plasma carotenoid levels at adulthood are known to influence testis size and yolk carotenoid levels influence the ability to extract carotenoids later in life, we hypothesized that maternally transmitted carotenoids might influence gonad size at adulthood. Here, we showed that male Japanese quail (*Coturnix japonica*) originating from a carotenoid-enriched egg had smaller testes than control individuals at adulthood. This result shows that yolk carotenoids have long-term organizational effects. In addition, given that carotenoid intake at sexual maturity increases sperm quality and that a decreased testis size is associated with a lower sperm production, we propose that carotenoid exposure during embryo development might influence a trade-off between ejaculate size and sperm quality.

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1 **LONG-TERM EFFECT OF YOLK CAROTENOID LEVELS ON TESTIS SIZE IN A**
2 **PRECOCIAL BIRD**

3
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21 **ABSTRACT**

22 Conditions experienced during prenatal development can have long-lasting
23 organizational effects on offspring. Maternal carotenoids deposited in the eggs of birds and
24 other oviparous species play an important role during fast embryonic growth and chick
25 development through their antioxidant properties. However, the long-term consequences of
26 variation in maternal carotenoid transfer for the offspring have been seldom considered. Since
27 plasma carotenoid levels at adulthood are known to influence testis size and yolk carotenoid
28 levels influence the ability to extract carotenoids later in life, we hypothesized that maternally
29 transmitted carotenoids might influence gonad size at adulthood. Here, we showed that male
30 Japanese quail (*Coturnix japonica*) originating from a carotenoid-enriched egg had smaller
31 testes than control individuals at adulthood. This result shows that yolk carotenoids have long-
32 term organizational effects. In addition, given that carotenoid intake at sexual maturity increases
33 sperm quality and that a decreased testis size is associated with a lower sperm production, we
34 propose that carotenoid exposure during embryo development might influence the trade-off
35 between ejaculate size and sperm quality.

36 INTRODUCTION

37 Conditions experienced during embryo development can have major organizational
38 effects that can last until adulthood [1]. However, although numerous studies have documented
39 short-term effects of prenatal conditions on offspring phenotype [2], the long-term
40 consequences for fitness-related traits are still poorly understood.

41 Maternally transmitted antioxidants might influence embryonic developmental trajectory due
42 to their capacity to scavenge the reactive oxygen species produced during development and the
43 challenging period of rapid growth [3]. Several studies have examined the importance of these
44 compounds during development using dietary carotenoid supplementation of laying females to
45 indirectly manipulate yolk antioxidants levels (i.e. carotenoids) and have shown effects of this
46 treatment on nestlings' condition or carotenoid levels just a few weeks after hatching [4,5,6].
47 However, these results must be considered cautiously since these effects may have been
48 mediated by other effects of these dietary manipulations on maternal physiology and/or the
49 differential allocation of other maternally transmitted compounds.

50 So far, only two studies have directly manipulated yolk carotenoid concentrations through *in*
51 *ovo* injections, showing that these maternally transmitted compounds have the potential to
52 influence chick growth, immunocompetence and antioxidation capacity [7,8,9]. The potential
53 long-term effects of prenatal antioxidant exposure have so far only been examined in one study
54 in which male barn swallows (*Hirundo rustica*) hatched from eggs injected with vitamin E
55 arrived earlier at their breeding grounds than controls [10]. We thus need more studies where
56 the long-term consequences of yolk antioxidant manipulations (through *in ovo* injections) are
57 examined to determine the potential organizational effect of these maternally transmitted
58 compounds.

59 Nutritional conditions during development and at adulthood have been shown to influence
60 gonadal development in a variety of taxa (cockroach, [11]), humans [12], mallard [13]), but
61 information on the importance of specific antioxidants (such as carotenoids) on gonadal growth
62 is scarce. Carotenoids are present in both testes and seminal fluid and it has been proposed that
63 these molecules might limit oxidative stress and allow optimal cell growth in testes [14], a tissue
64 where the high rate of cell division might generate high levels of free radicals [15]. Given that
65 variation in yolk carotenoid levels can influence the ability to extract or assimilate these
66 compounds later in life [4] and that dietary carotenoid availability at adulthood influenced testes
67 size [16], we hypothesized that maternally transmitted carotenoid may influence gonad size at
68 adulthood. To test this hypothesis, we experimentally manipulated yolk lutein levels in Japanese
69 quail eggs and measured the consequences of this treatment for the sons' testis size at adulthood.

70

71 **METHODS**

72 Unincubated Japanese quail (*Coturnix japonicus*) eggs were collected from 55 females
73 of our captive breeding population and injected with either 15 µg of carotenoids (FloraGLO
74 Lutein 20%, Kemin Foods, Des Moines, Iowa) dissolved in 15µL of safflower oil or with only
75 safflower oil as a control. Lutein was chosen since it is the most abundant carotenoid found in
76 Japanese quail eggs and the dose injected represents approximately one standard deviation of
77 the yolk carotenoid content in this species [17]. The overall hatching success was 41.1%
78 (Control = 38.5%, Carotenoid = 44.1%) and comparable to previous studies in Japanese quail
79 [18, 19]. See ESM for details on the incubating and rearing conditions. One year post-hatch, 48
80 males were randomly selected and euthanized (29 control and 19 birds from carotenoid-injected
81 eggs, from 40 females and 430 eggs injected ((226 control-injected and 204 carotenoid-

82 injected)). Both testes were collected and weighed to the nearest 1 mg. Tarsus length was
83 measured to the nearest 0.1 mm.

84

85 *Statistics*

86 Testis mass was significantly repeatable between the left and right sides ($F_{1, 45} = 23.45$,
87 $P < 0.001$), we thus used average values per bird for our analyses. In order to avoid pseudo-
88 replication, we used the mean average testis mass of all male offspring per mother in our
89 analyses since six mothers had more than one son. We ran general linear models to test whether
90 the yolk carotenoid manipulation and bird size (tarsus length), as well as their interaction,
91 affected testis size. All statistical analyses were run in R 3.01 (R Core Team 2013).

92

93 **RESULTS**

94 Bigger birds had bigger testes ($F_{1,37} = 7.77$, $P = 0.008$). Furthermore, testis size was
95 significantly affected by the carotenoid treatment ($F_{1,37} = 4.52$, $P = 0.04$) with males originating
96 from carotenoid-injected eggs having smaller testes than males originating from control eggs
97 (figure 1). The interaction effect between carotenoid treatment and tarsus length was non-
98 significant ($F_{1, 36} = 0.030$, $P = 0.86$). Yolk carotenoid treatment did not influence body size at
99 adulthood ($F_{1, 38} = 1.41$, $P = 0.24$).

100

101 **DISCUSSION**

102 Here, we show that prenatal exposure to carotenoids has long-term effects on a primary
103 sexual trait. Male Japanese quail originating from a carotenoid-injected egg had smaller testes
104 than controls one year post-hatching. This result is in line with the idea that yolk carotenoids
105 have organizational effects that last until adulthood, potentially due to their antioxidant

106 properties [20, but see 21] and / or their effect on gene expression, cell proliferation, and cell–
107 cell communication [22, 23].

108 Previous studies have indirectly manipulated yolk carotenoid levels through supplementation
109 of laying females and showed that chick absorption and utilization of carotenoids,
110 immunocompetence and plumage coloration were influenced by this dietary manipulation
111 several weeks after hatching [4,5,24]. Our result confirms these long-lasting effects of yolk
112 carotenoid levels using a direct *in ovo* manipulation of these maternally-transmitted compounds
113 and by measuring a primary sexual trait several months after the end of the developmental
114 phase. Given that only one carotenoid has been manipulated in our study, further work is needed
115 to investigate if also other yolk carotenoids have long-term effects on fitness-related traits.

116

117 We propose three non-mutually exclusive hypotheses that might explain the reduction
118 of testis size in males originating from carotenoid-enriched eggs. First, carotenoids may
119 influence the trade-off between ejaculate size and sperm quality [25]. Recent studies have
120 shown that an increased dietary intake of carotenoid leads to a reduction of testes size in
121 mallards (*Anas platyrhynchos*) [13] and that testis size and testis carotenoid concentrations are
122 negatively correlated in house finches (*Haemorhous mexicanus*) [16]. Given that testis size is
123 positively associated with sperm production in birds [26], this strongly suggests that high
124 carotenoid intake decreases ejaculate size in birds. However, studies in various taxa have also
125 shown that an increased carotenoid intake at sexual maturity increases sperm quality [27, 28,
126 29], potentially through their antioxidant properties or the recycling of vitamin E molecules, a
127 major actor of spermatozoa protection from oxidative damage [29]. Thus, carotenoids seem to
128 stimulate the production of high-quality sperm, but in smaller amounts. Future studies should
129 test this hypothesis by simultaneously measuring ejaculate size and sperm quality in individuals

130 supplemented or not with carotenoid at different life stages (before or after hatching). Second,
131 prenatal exposure to high levels of carotenoid may influence the trade-off between self-
132 maintenance and survival toward a reduced reproductive investment during the first breeding
133 event. However, this hypothesis seems unlikely since carotenoid supplementation has been
134 shown to increase several components of reproductive investment in various species [30].
135 Finally, while we acknowledge the possibility that prenatal carotenoid exposure may have
136 detrimental consequences for testes maturation and thus sperm production in our study, we
137 believe that it is inconsistent with the accumulating evidence that carotenoid supplementation
138 improves sperm quality [27, 29, but see 31]. In addition, the injected carotenoid dose was well
139 within the natural range (Peluc *et al.* 2012) and yolk carotenoid levels after injection were not
140 unnaturally high since females were fed with a low-carotenoid diet during the whole
141 experiment.

142 To conclude, we show for the first time that yolk carotenoid levels have long-term
143 effects on a primary sexual trait, strongly suggesting that maternally-transmitted antioxidants
144 influence offspring fitness.

145

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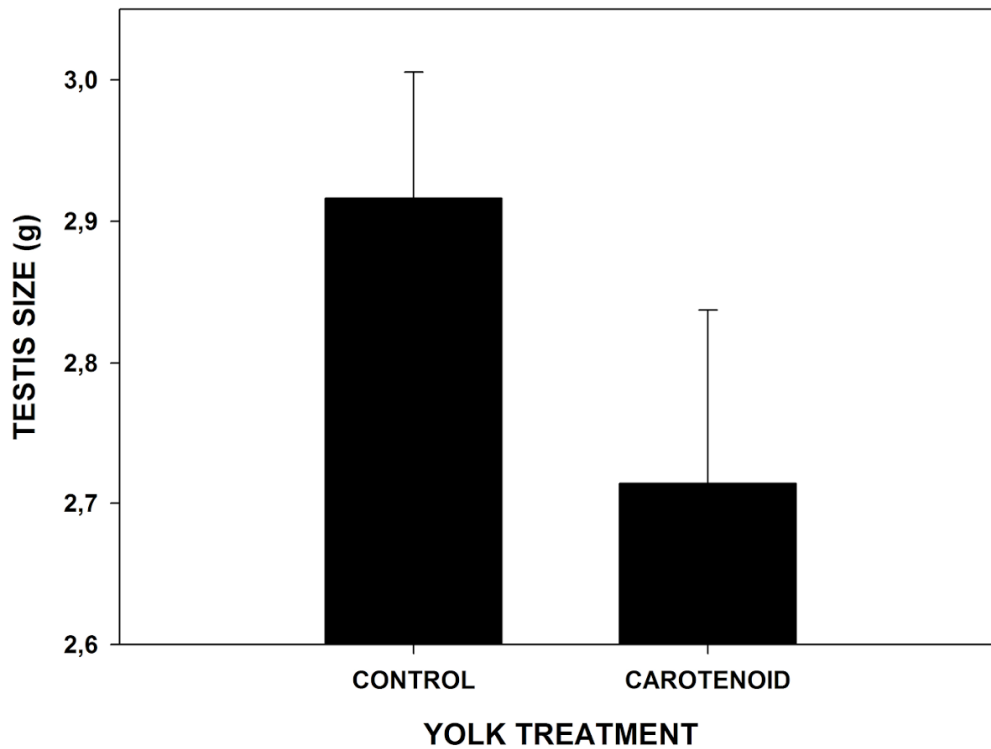
155 **Authors' contributions:** MG and AZ collected the data, MG and BT designed the study, BT
156 analyzed the data. MG and BT wrote the manuscript. All authors agree to be held accountable
157 for the content therein and gave final approval for publication.

158 **Competing interests:** We declare we have no competing interests.

159 Figure 1: Effect of yolk carotenoid injection on testis size one year post-hatching. Means \pm 1 SE
160 are shown.

161

162



163 **REFERENCES**

- 164 1. Mousseau TA, Fox CW. 1998 Maternal Effects as Adaptations. Oxford University
165 Press, New York.
- 166 2. Groothuis TGG, Wendt M, von Engelhardt N, Carere C, Eising C. 2005 Maternal
167 hormones as a tool to adjust offspring phenotype in avian species. *Neurosci. Biobehav.*
168 *Rev.* **29**, 329–352.
- 169 3. Surai PF, Speake BK, Sparks NHC. 2001 Carotenoids in avian nutrition and embryonic
170 development. 2. Antioxidant properties and discrimination in embryonic tissues. *J.*
171 *Poultry Sci.* **38**, 117–145.
- 172 4. Koutsos EA, Clifford AJ, Calvert CC, Klasing KC. 2003 Maternal carotenoid status
173 modifies the incorporation of dietary carotenoids into immune tissues of growing
174 chickens (*Gallus gallus domesticus*). *J. Nutr.* **133**, 1132–1138.
- 175 5. McGraw KJ, Adkins-Regan E, Parker RS. 2005 Maternally derived carotenoid pigments
176 affect offspring survival, sex ratio, and sexual attractiveness in a colorful songbird.
177 *Naturwissenschaften* **92**, 375–380.
- 178 6. Grether GF, Kolluru GR, Lin K, Quiroz MA, Robertson G, Snyder AJ. 2008 Maternal
179 effects of carotenoid consumption in guppies (*Poecilia reticulata*). *Funct. Ecol.* **22**,
180 294–302.
- 181 7. Saino N, Ferrari RP, Romano M, Martinelli R, Møller AP. 2003 Experimental
182 manipulation of egg carotenoids affects immunity of barn swallow nestlings. *Proc R*
183 *Soc Lond B Biol Sci.* **270**, 2485–2489.
- 184 8. Saino N, Romano M, Caprioli M, Rubolini D, Ambrosini R. 2011 Yolk carotenoids
185 have sex-dependent effects on redox status and influence the resolution of growth trade-
186 offs in yellow-legged gull chicks. *Behav. Ecol.* **22**, 411–421.

- 187 9. Romano M, Caprioli M, Ambrosini R, Rubolini D, Fasola M, Saino N. 2008 Maternal
188 allocation strategies and differential effects of yolk carotenoids on the phenotype and
189 viability of yellow-legged gull chicks in relation to sex and laying order. *J. Evol. Biol.*
190 **21**, 1826–1840.
- 191 10. Moller AP, Biard C, Karadas F, Rubolini D, Saino N, Surai PF. 2011 Maternal effects
192 and changing phenology of bird migration. *Climate research* **49**, 201-210.
- 193 11. Barrett ELB, Hunt J, Moore AJ, Moore PJ. 2009 Separate and combined effects of
194 nutrition during juvenile and sexual development on female life-history trajectories: the
195 thrifty phenotype in a cockroach. *Proc. R. Soc. B* **276**, 3257–3264.
- 196 12. Lummaa V. 2003 Early developmental conditions and reproductive success in humans:
197 downstream effects of prenatal famine, birthweight, and timing of birth. *Am. J. Hum.*
198 *Biol.* **15**, 370–379.
- 199 13. Butler MW, Karanfilian B, Homsher M, McGraw KJ. 2013 Carotenoid supplementation
200 during adulthood, but not development, decreases testis size in mallards. *Comparative*
201 *Biochemistry and Physiology, Part A* **166**, 465–469.
- 202 14. Blount JD, Møller AP, Houston DC. 2001 Antioxidants, showy males and sperm
203 quality. *Ecol. Letters* **4**, 393-396.
- 204 15. Taylor CT. 2001 Antioxidants and reactive oxygen species in human fertility. *Environ.*
205 *Tox. Pharm.* **10**, 189–198.
- 206 16. Rowe M, Tourville EA, McGraw KJ. 2012 Carotenoids in bird testes: links to body
207 carotenoid supplies, plumage coloration, bodymass and testes mass in house finches.
208 *Comp. Biochem. Physiol. B* **163**, 285–291.
- 209 17. Peluc SI, Reed WL, McGraw KJ, Gibbs P. 2012 Carotenoid supplementation and GnRH
210 challenges influence female endocrine physiology, immune function, and egg-yolk

- 211 characteristics in Japanese quail (*Coturnix japonica*). *J. Comp. Physiol. B.* **182**, 687–
212 702.
- 213 18. Daisley JN, Bromundt V, Mostl E, Kotrschal K. 2005 Enhanced yolk testosterone
214 influences behavioral phenotype independent of sex in Japanese quail chicks *Coturnix*
215 *japonica*. *Horm. Behav.* **47**, 185–194.
- 216 19. Hegyi G, Schwabl H. 2010 Do different yolk androgens exert similar effects on the
217 morphology or behaviour of Japanese quail hatchlings *Coturnix japonica*? *J. Avian Biol.*
218 **41**, 258–265.
- 219 20. Halliwell B, Gutteridge J. 2007 Free radicals in biology and medicine. Oxford: Oxford
220 University Press.
- 221 21. Costantini D, Møller AP. 2008 Carotenoids are minor antioxidants for birds. *Funct Ecol.*
222 **22**, 367–370.
- 223 22. Edge R, McGarvey DJ, Truscott TG. 1997 The carotenoids as antioxidants- a review. *J*
224 *Photochem Photobiol B.* **41**, 189–200.
- 225 23. Chew BP, Park JS. 2004 Carotenoid action on the immune response. *J Nutr.* **134**, 257S–
226 261S.
- 227 24. Biard C, Surai PF, Møller AP. 2007 An analysis of pre- and post-hatching maternal
228 effects mediated by carotenoids in the blue tit. *J Evol Biol.* **20**, 326–339.
- 229 25. Immler S, Pitnick S, Parker GA, Durrant KL, Lüpold S, Calhim S, Birkhead T. 2011
230 Resolving variation in the reproductive tradeoff between sperm size and number. *PNAS*
231 **108**, 5325–5330.
- 232 26. Møller AP. 1988 Testes size, ejaculate quality and sperm competition in birds. *Biol. J.*
233 *Linn. Soc.* **33**, 273–283.

- 234 27. Helfenstein F, Losdat S, Møller AP, Blount JD, Richner H. 2010 Sperm of colourful
235 males are better protected against oxidative stress. *Ecol. Lett.* **13**, 213–222.
- 236 28. Taş M, Güney Saruhan B, Kurt D, Yokuş B, Denl M. 2010 Protective role of lycopene
237 on aflatoxin B1 induced changes sperm characteristics and testicular damages in rats.
238 Kafkas Univ. *Vet. Fak. Derg.* **16**, 597–604.
- 239 29. Almbro M, Dowling DK, Simmons LW. 2011 Effects of vitamin E and beta-carotene
240 on sperm competitiveness. *Ecol. Lett.* **14**, 891–5.
- 241 30. Blount JD, Houston DC, Surai PF, Møller AP. 2004 Egg-laying capacity is limited by
242 carotenoid pigment availability in wild gulls *Larus fuscus*. *Proc. R. Soc. B* **271**, S79–
243 S81.
- 244 31. Sullivan M, Brown AC, Clotfelter ED. 2014 Dietary carotenoids do not improve
245 motility or antioxidant capacity in cichlid fish sperm. *Fish Physiology and Biochemistry*
246 **40(5)**, 1399-1405.

ESM

Eggs were artificially incubated for 14 days at a temperature of 37.6°C and 55% humidity and then at 37.6°C and 80% humidity for the last 3 days. Chicks were reared in mixed treatment cohorts of 40 chicks for 2 weeks and in cohorts of 20 chicks for three more weeks. At the age of 5 weeks, chicks were released into outdoor aviaries. Adults and chicks received *ad libitum* water and commercial game bird mix low in carotenoid content during the whole experiment.