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1 Short communication

2

3 TITLE: Testosterone production ability predicts breeding success and tracks

4 breeding stage in captive male songbirds

5

6 Running title: Post-challenge T and success

7

8 Key Words: birds, competition, fitness, gonadotropin-releasing hormone (GnRH

9 challenge), testosterone, trade-offs

10

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12

13 Summary statement: We show that individual differences in ability to produce

14 testosterone predicts who will win a reproductive competition, and that circulating

15 testosterone changes with date, but testosterone production ability changes with

16 breeding stage.

17

18

19

20 **Testosterone production ability predicts breeding success and tracks**  
21 **breeding stage in male finches**

22  
23 ABSTRACT

24  
25 Testosterone (T) is an important mediator of reproductive behaviours and potential  
26 target for selection. However, there are few data relating natural variation in T to  
27 fitness estimates. Here, we use the GnRH challenge (an injection of gonadotropin-  
28 releasing hormone which stimulates maximal T release), to examine how individual  
29 differences in T relate to reproductive success and how T changes across date and  
30 breeding stage. We measured pre and post-challenge T, in captive male Gouldian  
31 finch (*Erythrura gouldiae*), before and after introducing females, and across  
32 breeding stage. Post-challenge T before introducing females positively predicted  
33 breeding success. Post-challenge T levels were unrelated to date, but strongly  
34 related to stage; incubating male's ability to produce T was strongly attenuated. Pre-  
35 challenge T levels related only to date. Our results suggest that T production ability  
36 is an important target for selection and that when males invest heavily in parental  
37 care they reduce their sensitivity to GnRH.

38  
39  
40  
41 **Introduction**

42 Testosterone is a well-established mediator of reproductive behaviours, and  
43 appears to be especially important in competitive contexts during the breeding  
44 season (Adkins-Regan, 2005). Because testosterone (T) is a steroid and can regulate  
45 gene expression throughout the body, it has the potential to regulate entire suites of  
46 reproductive traits (McGlothlin & Ketterson, 2008; Ketterson *et al.*, 2009; Atwell *et*  
47 *al.*, 2014). Experimentally elevating T often increases competitive trait expression  
48 (e.g. aggression, dominance badges) and breeding success (Wingfield *et al.*, 1987;  
49 Ketterson *et al.*, 1991; Raouf *et al.*, 1997). However, high levels of T are also  
50 associated with a number of costs, e.g. reduced survival and impaired immune

51 function (Ketterson *et al.*, 1991; Wingfield *et al.*, 2001). Among the most well studied  
52 costs in birds is the negative relationship between T levels and parental care  
53 (Cawthorn *et al.*, 1998; Peters, 2002; Duckworth, 2006; Lynn *et al.*, 2009; Pryke &  
54 Griffith, 2009b; Cain & Ketterson, 2013a). Consequently, animals are expected to  
55 elevate T when competition is essential, but reduce T when possible (Wingfield *et al.*  
56 *et al.*, 1990; 2001) . In support of this possibility, in many species, T levels decrease  
57 across season (Wingfield *et al.*, 2001; Goymann & Hofer, 2010), timed with an  
58 increase in parental care (Ketterson *et al.*, 1992; Ketterson & Nolan, 1994).

59

60 The majority of data on the relationship between T and reproductive behaviour in  
61 male birds stem from two types of studies: 1) correlative studies that relate  
62 circulating T levels to phenotype or fitness, and 2) phenotypic engineering studies  
63 that manipulate T levels and examine the consequences of this manipulation.  
64 Because of the two-way relationship between T and behaviour, correlative studies  
65 can be difficult to interpret (Hau *et al.*, 2008). For example, when aggressive males  
66 have higher T, it is unclear whether T increases aggression or whether aggression  
67 increases T. Phenotypic engineering using exogenous testosterone avoids this issue,  
68 and is a powerful tool for establishing causality (Ketterson *et al.*, 1996; Adkins-  
69 Regan, 2005), but can create artificial phenotypes. For example, testosterone shows  
70 marked daily and seasonal variation (Wingfield *et al.*, 1990) but implants keep  
71 hormones at a static level, and though implants can reveal the proximate basis for  
72 behavioural trade-offs, the optimal resolution for these trade-offs vary according to  
73 individual quality (Roff *et al.*, 2002; McGlothlin *et al.*, 2010). Thus there are  
74 limitations when trying to understand how selection acts on hormonal systems and  
75 hormonally mediated traits (McGlothlin *et al.*, 2007; 2010). To truly understand the  
76 role of T in mediating phenotypes and how selection acts on T mediated traits, we  
77 must pair such studies with examinations of natural variation in T profiles and  
78 production (McGlothlin *et al.*, 2007; Kempnaers *et al.*, 2008; Williams, 2008;  
79 McGlothlin *et al.*, 2010; Cain & Ketterson, 2013b). However, though a strong  
80 relationship between hormones and estimates of fitness is expected, we have  
81 surprisingly little data that detail how individual differences in hormone profiles

82 relate to fitness (McGlothlin *et al.*, 2010; Ouyang *et al.*, 2011; Williams, 2012; Ouyang  
83 *et al.*, 2013; Cain & Ketterson, 2013a).

84

85 Recent research has begun addressing this gap by examining the relationships  
86 between testosterone, phenotype and fitness using a technique that measures  
87 circulating T levels as well as T production ability. Testosterone secretion is  
88 regulated by the hypothalamic-pituitary-gonadal axis (HPG axis). A stimulus  
89 provokes the hypothalamus to release gonadotropin-releasing hormone (GnRH), a  
90 neuropeptide, which then stimulates the receptors on the anterior pituitary to  
91 release gonadotropins, luteinizing hormones (LH) and follicle stimulating hormone  
92 (FSH). The gonadotropins act on receptors in the gonad, provoking the release of  
93 testosterone, and potentially other steroids (Oliveira, 2004; Adkins-Regan, 2005;  
94 Goymann, 2009). Individual, stage or seasonal differences in T levels might arise  
95 from changes in any of these components, as well as other parts of the axis; e.g.  
96 receptors and binding globulins (Adkins-Regan, 2005; Hau, 2007; Rosvall *et al.*,  
97 2012; Bergeon Burns *et al.*, 2014; Rosvall *et al.*, 2016).

98

99 Here, we use an injection of exogenous GnRH (GnRH challenge) to estimate  
100 individual differences in the ability to produce T and determine whether T  
101 production or circulating T predict the outcome of reproductive competition for  
102 resources (mates and nesting cavities) in captive male songbirds. We then follow  
103 these males and examine how T changes with date and breeding stage. By using  
104 GnRH challenges, we can determine whether the overall changes in circulating T is a  
105 product of reduced gonad responsiveness to GnRH, or a reduction of signals  
106 upstream from the gonads (e.g. GnRH or LH). To explore these questions, we use the  
107 Gouldian finch (*Erythrura gouldiae*), an Australian songbird that resides in the  
108 monsoonal tropical region of Northern Australia. Gouldians have three genetically  
109 determined colour morphs that differ in physiology and behaviour, with assortative  
110 pairing (Pryke & Griffith, 2006; Pryke *et al.*, 2007; Pryke & Griffith, 2009a). Mating is  
111 socially monogamous, and both parents incubate and provision young. Previous  
112 work has shown that in species where males invest heavily, male T is low when

113 engaged in paternal care (Ketterson & Nolan, 1994; Van Roo et al., 2003), but it is  
114 unclear whether this is due to a change in gonad responsiveness to GnRH, or a  
115 reduction in the release of GnRH. Here, we use a captive population to examine  
116 whether T levels (pre and post-challenge) prior to female introduction are  
117 predictive of breeding success, and whether T levels (pre and post-challenge)  
118 change across date or breeding stage.

119

## 120 **Methods**

### 121 *Experimental set-up*

122 Males were randomly assigned to 4 indoor/outdoor aviaries (indoor: 2.4 m long, 1.2  
123 m wide, 3 m high; outdoor: 6 m 2.6 m 3 m), each aviary contained 4 males, 2 red  
124 morphs and 2 black morphs. Each aviary also contained 2 nesting cavities boxes,  
125 and all males were in vocal and visual contact with females. Two days after  
126 relocating males to treatment aviaries the first hormone-sampling period occurred.  
127 Two weeks after movement (16 Jan), one female of each morph was added to all  
128 aviaries (2 females per aviary, total of 6 birds per group). Four days after adding  
129 females, the second hormone-sampling period occurred. Birds were challenged  
130 twice more, with a 2-week interval between challenges. Birds were observed on a  
131 daily basis by an observer blind to hormone levels, the observer notes nesting cavity  
132 ownership and pair status.

133

### 134 *Hormone sampling and measurement*

135 We measure pre-challenge circulating T levels, and T in response to an injection of  
136 gonadotropin-releasing hormone (a GnRH challenge). This injection produces a  
137 transient increase in circulating T (Wingfield *et al.*, 1991; Moore *et al.*, 2002; Jawor  
138 *et al.*, 2006; 2007; McGlothlin *et al.*, 2007; Cain *et al.*, 2012; 2016). In dark-eyed  
139 juncos (*Junco hyemalis*), a North American sparrow, males show an increase in T at  
140 30minutes, followed by a decline by hour post injection, by 2 hours post-injection T  
141 levels are back to normal (Jawor et al. 2006a). After capture, we took an initial  
142 blood sample (pre-challenge measure), then administered an injection of 20  $\mu$ L of a  
143 solution containing 2.0  $\mu$ g of chicken GnRH- I (Sigma L0637; American Peptide 54-8-

144 23) (Cain & Pryke, 2016). After exactly 30 minutes, a second sample was taken  
145 (post-challenge measure). Prior to beginning the experiment a subset of males (n=8)  
146 were sampled for initial circulating T levels, these males had never received a GnRH  
147 challenges. Plasma was then stored at -20C until assayed using an established  
148 enzyme immunoassay procedure. Further details on the GnRH challenge protocol  
149 and assay procedures are detailed in Cain and Pryke 2016.

150

### 151 *Statistical Analysis*

152 To determine whether pre or post-challenge T (i.e. T production ability) related to  
153 breeding success (i.e. winning a nesting cavity and attracting a female) we used a  
154 generalized linear model with a binomial error distribution and logit link. Breeding  
155 success was the dependent variable, and morph, pre-challenge T, and post-challenge  
156 T (before introducing females) as predictors. To determine how T levels changes  
157 across date and breeding stage (after female introduction), we built two separate  
158 linear mixed models, with pre and post challenge T measures as the dependent  
159 variables. Predictors for both models included breeding stage, sampling period  
160 (first, second, or third T measurement) and morph, because each male was in both  
161 models three times, we also included male identity as a random factor. To determine  
162 whether repeated GnRH challenges alter circulating male T levels we used a t-test to  
163 compare pre-challenge T levels of males that had received 3 previous challenges to  
164 males that had never received a challenge.

165

### 166 **RESULTS**

167 Five (3 black and 2 red males) of 16 males won nesting cavities; the remaining 3  
168 cavities were not won; no nests were built, no eggs laid. Post-challenge T levels were  
169 positively related to breeding success; i.e. males with greater T production ability  
170 were more likely to win a nesting cavity and mate (Fig 1, Full model  $X^2= 13.12$ ,  
171  $p=0.0044$ ; post-challenge T;  $X^2= 5.38$ ,  $p=0.020$ ). Morph (red or black) and pre-  
172 challenge levels were unrelated to success ( $P=0.33$  and  $P=0.22$  respectively).

173

174 Pre-challenge T levels increased between the first and second sampling period after  
175 introducing females, but did not change significantly in the third period. Pre-  
176 challenge levels were unrelated to male breeding stage (Fig 2, Table 1). In contrast,  
177 post-challenge T levels were unrelated to date (sampling period) but strongly  
178 related to stage; T levels were lowest in males incubating eggs, but there was no  
179 difference in post-challenge T levels of males that has acquired a cavity but not yet  
180 begun incubating and males that did not acquire a cavity (Fig 2, Table 1). Morph was  
181 unrelated to pre or post-challenge T levels ( $p>0.20$ ).

182 There was no detectable difference between pre-challenge T levels in males that had  
183 never received a GnRH challenge ( $n=8$ ) and the experimental males that had  
184 received 3 previous challenges in the course of the experiment ( $n=23$ ) ( $t= -0.32$ ,  $p$   
185  $=0.75$ ).

186

## 187 **Discussion**

188

### 189 *Testosterone predicts breeding success*

190 Because T regulates a number of fitness relevant traits, especially those associated  
191 with reproductive competition, it is expected to be strongly related to fitness  
192 (Ketterson *et al.*, 1992; Wingfield *et al.*, 2001; Adkins-Regan, 2005; McGlothlin &  
193 Ketterson, 2008). However, there are few studies that report such relationships  
194 (McGlothlin *et al.*, 2010), though relationships with components or proxies of fitness  
195 are more common. We examined one aspect of reproductive success that is of  
196 critical importance to Gouldian finches, the ability to acquire and defend a nesting  
197 cavity and attract a female. Individual differences in the ability to produce T in  
198 response to GnRH were positively related to the probability that the male would  
199 later win a cavity and a mate. In contrast, circulating T levels were unrelated to  
200 success. This finding suggests that individual differences in the ability to produce  
201 and secrete T may be related to competitive ability in this species, and a target for  
202 selection.

203



204 The limited available empirical data in wild birds supports this general pattern. In  
205 other bird species, testosterone has been positively related to an important  
206 component of fitness, breeding success. Individual variation in circulating T levels is  
207 positively correlated with mating success in black grouse (*Tetrao tetrix*) (Alatalo et  
208 al., 1996), and satin bowerbirds (*Ptilonorhynchus violaceus*) (Borgia & Wingfield,  
209 1991). Further, in white-striped sparrows (*Zonotrichia albicollis*), the white-stripe  
210 morph, which invests more in pursuit of mates, has slightly higher circulating T  
211 levels. Experimentally elevated T lead to increased mating success in dark-eyed  
212 junco (Reed et al., 2006), and individual differences in the ability to respond to  
213 GnRH with an increase in T are positively related to male mating success  
214 (McGlothlin et al., 2010). A study in European starlings (*Sturnus vulgaris*) found no  
215 relationship between T before competition and success/failure; however, they did  
216 find that winners increased T more in response to competition than did losers  
217 (Gwinner et al., 2002). Importantly, because our study measured T levels prior to  
218 the introduction of females, our findings suggest that T levels are facilitating  
219 competitive ability, rather than increasing in response to success. Taken together,  
220 these results suggest that T production ability is an important potential target for  
221 selection, particularly when competition is strong. However, though these combined  
222 results provide strong support for the possibility that T is facilitating competitive  
223 behaviour, we suggest caution. GnRH may lead to the release of other hormones  
224 such as oestrogen and progesterone, and testosterone may act after aromatization  
225 (Adkins-Regan, 2005).

226

### 227 *Testosterone across time and stage*

228 Testosterone levels often show patterns associated with date and breeding stage.  
229 Disentangling the two is important, but can be difficult because they are generally  
230 strongly correlated (Hegner & Wingfield, 1986). Here we examine both, and found  
231 that pre-challenge T levels were related to date (sampling period), but post-  
232 challenge T levels were related to only to breeding stage. This suggests that  
233 circulating T levels do not track gonad responsiveness to GnRH (i.e. ability to  
234 produce T when stimulated by GnRH). Circulating T levels appear to be a function of

235 competition, increasing as birds acquire and defend nesting cavities or court  
236 females, and then levelling off as the social situation stabilized. This pattern reflects  
237 the general observation that male T often peaks just before the start of the breeding  
238 season (Wingfield *et al.*, 2001; Ketterson *et al.*, 2001).

239

240 In contrast, male gonad responsiveness (T production in response to GnRH ) was  
241 related to breeding stage. Males that had acquired a mate and were engaged in  
242 parental care (incubating) had dramatically reduced post-challenge T levels. This  
243 suggests that when males begin caring for young their ability to respond to  
244 upstream signals (GnRH or LH) was severely attenuated. A number of previous  
245 studies have found that male T is low when engaged in paternal care, and very low  
246 in species that invest heavily, e.g. have equal incubation like the Gouldian finch  
247 (Ketterson & Nolan, 1994; Van Roo *et al.*, 2003). Further, in some species with  
248 essential male care, even experimentally elevating T with implants fails to alter  
249 paternal behaviour, suggesting that these males become insensitive to T (Lynn *et al.*,  
250 2002; Lynn, 2015). However, in species without male incubation, the ability to  
251 increase T in response to GnRH is often retained (Jawor *et al.*, 2006; McGlothlin *et*  
252 *al.*, 2007; Apfelbeck & Goymann, 2011; DeVries & Jawor, 2013; Barron *et al.*, 2015).  
253 Gouldian males invest heavily in parental care, assisting in all stages of offspring  
254 care, including incubation, suggesting that low levels of T are reflective of their life  
255 history strategy. Previous research in Gouldians found that red males increase T  
256 levels when in competitive environments, often at the expense of parental care  
257 (Pryke *et al.*, 2007; Pryke & Griffith, 2009b; Cain & Pryke, 2016). Our results suggest  
258 that though circulating T levels were unrelated to stage, males do reduce their  
259 response to GnRH, which might mitigate this negative effect.

260

261 Conclusion

262 Testosterone is an important mediator of animal phenotypes, but to truly  
263 understand its role in shaping trait expression, and facilitating or constraining  
264 animal responses to the environment, we need to data on how T levels relate to

265 fitness. Our findings add to growing evidence that individual differences in HPG  
266 function is an important target for selection.

267

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271

272 **Literature cited**

- 273 Adkins-Regan, E. 2005. *Hormones and Animal Social Behavior*. Princeton University  
274 Press, Princeton.
- 275 Alatalo, R.V., Hoglund, J., Lundberg, A., Rintamaki, P.T. & Silverin, B. 1996.  
276 Testosterone and male mating success on the black grouse leks. *Proc R Soc Lond*  
277 *B* **263**: 1697–1702.
- 278 Apfelbeck, B. & Goymann, W. 2011. Ignoring the challenge? Male black redstarts  
279 (*Phoenicurus ochruros*) do not increase testosterone levels during territorial  
280 conflicts but they do so in response to gonadotropin-releasing hormone. *Proc R*  
281 *Soc Lond B* **278**: 3233–3242.
- 282 Atwell, J.W., Cardoso, G.C., Whittaker, D.J., Price, T.D. & Ketterson, E.D. 2014.  
283 Hormonal, behavioral, and life-history traits exhibit correlated shifts in relation  
284 to population establishment in a novel environment. *Am. Nat.* **184**: E147–60.
- 285 Barron, D.G., Webster, M.S. & Schwabl, H. 2015. Do androgens link morphology and  
286 behaviour to produce phenotype-specific behavioural strategies? *Anim. Behav.*  
287 **100**: 116–124.
- 288 Bergeon Burns, C.M., Rosvall, K.A., Hahn, T.P., Demas, G.E. & Ketterson, E.D. 2014.  
289 Hormones and Behavior. *Horm. Behav.* **65**: 179–187.
- 290 Borgia, G. & Wingfield, J.C. 1991. Hormonal correlates of bower decoration and  
291 sexual display in the satin bowerbird (*Ptilonorhynchus violaceus*). *Condor* **93**:  
292 935–942.
- 293 Cain, K.E. & Ketterson, E.D. 2013a. Costs and benefits of competitive traits in  
294 females: aggression, maternal care and reproductive success. *PLoS ONE* **8**:  
295 e77816.
- 296 Cain, K.E. & Ketterson, E.D. 2013b. Individual variation in testosterone and parental  
297 care in a female songbird; the dark-eyed junco (*Junco hyemalis*). *Horm. Behav.*  
298 **64**: 685–692.
- 299 Cain, K.E. & Pryke, S.R. 2016. Testosterone production in response to exogenous  
300 gonadotropin releasing hormone (GnRH challenge) depends on social  
301 environment and color polymorphism. *Gen. Comp. Endocrinol.*, doi:  
302 10.1016/j.ygcen.2015.12.029.
- 303 Cain, K.E., Bergeon Burns, C.M. & Ketterson, E.D. 2012. Testosterone production,  
304 sexually dimorphic morphology, and digit ratio in the dark-eyed junco. *Behav.*  
305 *Ecol.* **24**: 462–469.

- 306 Cain, K.E., Jawor, J.M. & McGlothlin, J.W. 2016. Individual variation & selection on  
307 hormone-mediated phenotypes in male and female dark-eyed juncos. In:  
308 *Snowbird* (E. D. Ketterson & J. W. Atwell, eds). University of Chicago Press,  
309 Chicago.
- 310 Cawthorn, M.J., Morris, D., Ketterson, E.D. & Nolan, V. 1998. Influence of  
311 experimentally elevated testosterone on nest defence in dark-eyed juncos. *Anim.*  
312 *Behav.* **56**: 617–621.
- 313 DeVries, M.S. & Jawor, J.M. 2013. Natural variation in circulating testosterone does  
314 not predict nestling provisioning rates in the northern cardinal, *Cardinalis*  
315 *cardinalis*. *Anim. Behav.* **85**: 957–965.
- 316 Duckworth, R.A. 2006. Behavioral correlations across breeding contexts provide a  
317 mechanism for a cost of aggression. *Behav. Ecol.* **17**: 1011–1019.
- 318 Goymann, W. 2009. Social modulation of androgens in male birds. *Gen. Comp.*  
319 *Endocrinol.* **163**: 149–157.
- 320 Goymann, W. & Hofer, H. 2010. Mating systems, social behaviour and hormones. In:  
321 *Animal Behavior: Evolution and Mechanisms* (P. M. Kappeler, ed), pp. 465–501.
- 322 Gwinner, H., Van't Hof, T. & Zeman, M. 2002. Hormonal and behavioral responses of  
323 starlings during a confrontation with males or females at nest boxes during the  
324 reproductive season. *Horm. Behav.* **42**: 21–31.
- 325 Hau, M. 2007. Regulation of male traits by testosterone: implications for the  
326 evolution of vertebrate life histories. *Bioessays* **29**: 133–144.
- 327 Hau, M., Gill, S.A. & Goymann, W. 2008. Tropical field endocrinology: Ecology and  
328 evolution of testosterone concentrations in male birds. *Gen. Comp. Endocrinol.*  
329 **157**: 241–248.
- 330 Hegner, R.E. & Wingfield, J.C. 1986. Behavioral and endocrine correlates of multiple  
331 brooding in the semicolonial house sparrow *Passer domesticus* I. Males. *Horm.*  
332 *Behav.*
- 333 Jawor, J.M., McGlothlin, J.W., Casto, J.M., Greives, T.J., Snajdr, E.A., Bentley, G.E., *et al.*  
334 2006. Seasonal and individual variation in response to GnRH challenge in male  
335 dark-eyed juncos (*Junco hyemalis*). *Gen. Comp. Endocrinol.* **149**: 182–189.
- 336 Jawor, J.M., McGlothlin, J.W., Casto, J.M., Greives, T.J., Snajdr, E.A., Bentley, G.E., *et al.*  
337 2007. Testosterone response to GnRH in a female songbird varies with stage of  
338 reproduction: implications for adult behaviour and maternal effects. *Funct. Ecol.*  
339 **21**: 767–775.
- 340 Kempenaers, B., Peters, A. & Foerster, K. 2008. Sources of individual variation in

- 341 plasma testosterone levels. *Phil. Trans. Roy. Soc. B* **363**: 1711–1723.
- 342 Ketterson, E.D. & Nolan, V. 1994. Male parental behavior in birds. *Annual Review of*  
343 *Ecology and Systematics* 601–628.
- 344 Ketterson, E.D., Atwell, J.W. & McGlothlin, J.W. 2009. Phenotypic integration and  
345 independence: Hormones, performance, and response to environmental change.  
346 *Integrative and Comparative Biology* **49**: 365–379.
- 347 Ketterson, E.D., Nolan, V., Casto, J.M., Buerkle, C.A. & Snajdr, E.A. 2001. Testosterone,  
348 phenotype and fitness: a research program in evolutionary behavioral.
- 349 Ketterson, E.D., Nolan, V., Cawthorn, M.J., Parker, P.G. & Ziegenfus, C. 1996.  
350 Phenotypic engineering: using hormones to explore the mechanistic and  
351 functional bases of phenotypic variation in nature. *Ibis* **138**: 70–86.
- 352 Ketterson, E.D., Nolan, V., Wolf, L. & Ziegenfus, C. 1992. Testosterone and avian life  
353 histories: effects of experimentally elevated testosterone on behavior and  
354 correlates of fitness in the dark-eyed junco (*Junco hyemalis*). *Am. Nat.* 980–999.  
355 JSTOR.
- 356 Ketterson, E.D., Nolan, V., Wolf, L., Ziegenfus, C., Dufty, A.M., Ball, G.F., *et al.* 1991.  
357 Testosterone and avian life histories: the effect of experimentally elevated  
358 testosterone on corticosterone and body mass in dark-eyed juncos. *Horm. Behav.*  
359 **25**: 489–503.
- 360 Lynn, S.E. 2015. Hormones and Behavior. *Horm. Behav.* 1–12. Elsevier Inc.
- 361 Lynn, S.E., Hayward, L.S., Benowitz-Fredericks, Z.M. & Wingfield, J.C. 2002.  
362 Behavioural insensitivity to supplementary testosterone during the parental  
363 phase in the chestnut-collared longspur, *Calcarius ornatus*. *Anim. Behav.* **63**:  
364 795–803.
- 365 Lynn, S.E., Prince, L.E., Schook, D.M. & Moore, I.T. 2009. Supplementary testosterone  
366 inhibits paternal care in a tropically breeding sparrow, *Zonotrichia capensis*.  
367 *Physiol. Biochem. Zool.* **82**: 699–708.
- 368 McGlothlin, J.W. & Ketterson, E.D. 2008. Hormone-mediated suites as adaptations  
369 and evolutionary constraints. *Phil. Trans. Roy. Soc. B* **363**: 1611–1620.
- 370 McGlothlin, J.W., Jawor, J.M. & Ketterson, E.D. 2007. Natural variation in a  
371 testosterone-mediated trade-off between mating effort and parental effort. *Am.*  
372 *Nat.* **170**: 864–875.
- 373 McGlothlin, J.W., Whittaker, D.J., Schrock, S.E., Gerlach, N.M., Jawor, J.M., Snajdr, E.A.,  
374 *et al.* 2010. Natural selection on testosterone production in a wild songbird  
375 population. *Am. Nat.* **175**: 687–701.

- 376 Moore, I.T., Perfito, N., Wada, H., Sperry, T.S. & Wingfield, J.C. 2002. Latitudinal  
377 variation in plasma testosterone levels in birds of the genus *Zonotrichia*. *Gen.*  
378 *Comp. Endocrinol.* **129**: 13–19.
- 379 Oliveira, R.F. 2004. Social modulation of androgens in vertebrates: mechanisms and  
380 function. *Advances in the Study of Behavior* **34**: 165–239. Elsevier.
- 381 Ouyang, J.Q., Sharp, P., Quetting, M. & Hau, M. 2013. Endocrine phenotype,  
382 reproductive success and survival in the great tit, *Parus major*. *J. Evol. Biol.* **26**:  
383 1988–1998.
- 384 Ouyang, J.Q., Sharp, P.J., Dawson, A., Quetting, M. & Hau, M. 2011. Hormone levels  
385 predict individual differences in reproductive success in a passerine bird. *Proc R*  
386 *Soc Lond B* **278**: 2537–2545.
- 387 Peters, A. 2002. Testosterone and the trade-off between mating and paternal effort  
388 in extrapair-mating superb fairy-wrens. *Anim. Behav.* **64**: 103–112.
- 389 Pryke, S.R. & Griffith, S.C. 2009a. Postzygotic genetic incompatibility between  
390 sympatric color morphs. *Evolution* **63**: 793–798.
- 391 Pryke, S.R. & Griffith, S.C. 2006. Red dominates black: agonistic signalling among  
392 head morphs in the colour polymorphic Gouldian finch. *Proc R Soc Lond B* **273**:  
393 949–957.
- 394 Pryke, S.R. & Griffith, S.C. 2009b. Socially mediated trade-offs between aggression  
395 and parental effort in competing color morphs. *Am. Nat.* **174**: 455–464.
- 396 Pryke, S.R., Astheimer, L.B., Buttemer, W.A. & Griffith, S.C. 2007. Frequency-  
397 dependent physiological trade-offs between competing colour morphs. *Biology*  
398 *Letters* **3**: 494–497.
- 399 Raouf, S.A., Parker, P.G., Ketterson, E.D., Nolan, V. & Ziegenfus, C. 1997. Testosterone  
400 affects reproductive success by influencing extra-pair fertilizations in male  
401 dark-eyed juncos (Aves: *Junco hyemalis*). *Proc R Soc Lond B* **264**: 1599–1603.
- 402 Reed, W.L., Clark, M.E., Parker, P.G., Raouf, S.A., Arguedas, N., Monk, D.S., *et al.* 2006.  
403 Physiological effects on demography: a long-term experimental study of  
404 testosterone's effects on fitness. *Am. Nat.* **167**: 667–683.
- 405 Roff, D.A., Mostowy, S. & Fairbairn, D.J. 2002. The evolution of trade-offs: testing  
406 predictions on response to selection and environmental variation. *Evolution* **56**:  
407 84–95.
- 408 Rosvall, K.A., Bergeon Burns, C.M., Barske, J., Goodson, J.L., Schlinger, B.A., Sengelaub,  
409 D.R., *et al.* 2012. Neural sensitivity to sex steroids predicts individual differences  
410 in aggression: implications for behavioural evolution. *Proc R Soc Lond B* **279**:

411 3547–3555.

412 Rosvall, K.A., Bergeon Burns, C.M., Jayaratna, S.P., Dossey, E.K. & Ketterson, E.D.  
413 2016. Gonads and the evolution of hormonal phenotypes. *Integrative and*  
414 *Comparative Biology* **56**: 225-234.

415 Van Roo, B.L., Ketterson, E.D. & Sharp, P.J. 2003. Testosterone and prolactin in two  
416 songbirds that differ in paternal care: the Blue-headed Vireo and the Red-eyed  
417 Vireo. *Horm. Behav.* **44**: 435–441.

418 Williams, T.D. 2012. Hormones, life-history, and phenotypic variation: Opportunities  
419 in evolutionary avian endocrinology. *Gen. Comp. Endocrinol.* **176**: 286–295.

420 Williams, T.D. 2008. Individual variation in endocrine systems: moving beyond the  
421 “tyranny of the Golden Mean.” *Phil. Trans. Roy. Soc. B* **363**: 1687–1698.

422 Wingfield, J.C., Ball, G.F., Dufty, A.M., Hegner, R.E. & Ramenofsky, M. 1987.  
423 Testosterone and aggression in birds. *American Scientist* **75**: 602–608.

424 Wingfield, J.C., Hegner, R.E. & Lewis, D.M. 1991. Circulating levels of luteinizing  
425 hormone and steroid hormones in relation to social status in the cooperatively  
426 breeding white-browed sparrow weaver, *Plocepasser mahali*. *J Zool* **225**: 43–58.

427 Wingfield, J.C., Hegner, R.E., Dufty, A.M. & Ball, G.F. 1990. The challenge hypothesis -  
428 theoretical implications for patterns of testosterone secretion, mating systems,  
429 and breeding strategies. *Am. Nat.* **136**: 829–846.

430 Wingfield, J.C., Lynn, S. & Soma, K.K. 2001. Avoiding the “costs” of testosterone:  
431 ecological bases of hormone-behavior interactions. *Brain Behav. Evolut.* **57**:  
432 239–251.

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435 **Table 1:** Full model results for linear mixed models examining the relationships  
 436 between pre and post-challenge T levels, sampling period, and breeding stage.

<b>OVERALL model</b>	<b>Fixed factor</b>	<b>Estimate</b>	<b>SE</b>	<b>F</b>	<b>p value</b>
	Intercept	5.99	0.18		
	Morph <sup>a</sup>	-0.004	0.14	0.0009	0.98
<b>Pre-challenge T</b>	<b>Sampling period<sup>b</sup></b>				
N= 47	<b>Second</b>	<b>0.38</b>	<b>0.20</b>	<b>3.43</b>	<b>0.046</b>
-2 Loglikelihood =	<b>Third</b>	<b>0.17</b>	<b>0.20</b>		
132.8	<b>Breeding Stage<sup>c</sup></b>				
	Nest defence	0.56	0.30	1.73	0.20
	Incubation	-0.39	0.31		
	Intercept	6.49	0.16		
	Morph <sup>a</sup>	0.056	0.13	0.20	0.66
<b>Post-challenge T</b>	<b>Sampling period<sup>b</sup></b>				
N=47	Second	-0.28	0.15	1.85	0.18
-2 Loglikelihood =	Third	0.18			
113.5	<b>Breeding Stage<sup>c</sup></b>				
	<b>Nest defence</b>	<b>0.65</b>	<b>0.24</b>	<b>4.49</b>	<b>0.021</b>
	<b>Incubation</b>	<b>-0.72</b>	<b>0.25</b>		

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439 <sup>a</sup> Relative to black morph; <sup>b</sup> Relative to first sampling period; <sup>c</sup> Relative to pre-

440 breeding stage

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445 **FIGURE CAPTIONS**

446 **Fig 1:** Relationship between post-challenge T prior to the introduction of females  
447 and eventual success or failure as a breeder. Testosterone levels were plotted  
448 according to whether or not the male was successful at acquiring a cavity and mate.  
449 Line is a logistic regression estimating the probability that a male with a given post-  
450 challenge T level would be successful.

451

452 **Fig 2:** Effect of date and breeding stage on pre (white) and post GnRH-challenge  
453 (grey) T levels. Left panel: T levels according to breeding stage of the individual  
454 male; Right panel: T levels across sampling period, after females were introduced.  
455 Values are natural log transformed. Boxes illustrate median (thick line) and  
456 quartiles (box), whiskers are 90% and 10% quantiles.

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