



Libraries and Learning Services

University of Auckland Research Repository, ResearchSpace

Version

This is the Accepted Manuscript version. This version is defined in the NISO recommended practice RP-8-2008 <http://www.niso.org/publications/rp/>

Suggested Reference

Cain, K. E., & Ketterson, E. D. (2013). Individual variation in testosterone and parental care in a female songbird; The dark-eyed junco (*Junco hyemalis*). *Hormones and Behavior*, 64(4), 685-692. doi: [10.1016/j.anbehav.2015.07.034](https://doi.org/10.1016/j.anbehav.2015.07.034)

Copyright

Items in ResearchSpace are protected by copyright, with all rights reserved, unless otherwise indicated. Previously published items are made available in accordance with the copyright policy of the publisher.

This is an open-access article distributed under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivatives](https://creativecommons.org/licenses/by-nc-nd/4.0/) License.

For more information, see [General copyright](#), [Publisher copyright](#), [SHERPA/RoMEO](#).

1 RESEARCH ARTICLE: Individual variation in testosterone and parental care in a female songbird;
2 the dark-eyed junco (*Junco hyemalis*)

3

4

5 Kristal E. Cain* ^{1,2,3} & Ellen D. Ketterson ^{2,3}

6 ¹Research School of Biology, The Australian National University, ACT 0200, Australia

7 ²Department of Biology, Indiana University, Bloomington, IN 47405, USA

8 ³Center for the Integrative Study of Animal Behavior, Bloomington, IN 47405

9

10 * Address correspondence to:

11 Kristal Cain, kristalcain@gmail.com

12 Research School of Biology; Evolution, Ecology and Genetics

13 The Australian National University, ACT 0200, Australia

14

15 Keywords: life history tradeoffs; endogenous steroids; testosterone; gonadotropin releasing hormone
16 (GnRH); maternal care; yolk hormones; dark-eyed junco (*Junco hyemalis*)

17

18 **SUMMARY**

19 When competition for sex-specific resources overlaps in time with offspring production
20 and care, trade-offs can occur. Steroids hormones, particularly testosterone (T), play a
21 crucial role in mediating such trade-offs in males, often increasing competitive behaviors
22 while decreasing paternal behavior. Recent research has shown that females also face such
23 trade-offs; however, we know little about the role of T in mediating female phenotypes in
24 general, and the role of T in mediating trade-offs in females in particular. Here we examine
25 the relationship between individual variation in maternal effort and endogenous T in the
26 dark-eyed junco, a common songbird. Specifically, we measure circulating T before and
27 after a physiological challenge (injection of gonadotropin releasing hormone, GnRH), and
28 determine whether either measure is related to provisioning, brooding, or the amount of T
29 sequestered in egg yolk. We found that females producing more T in response to a
30 challenge spent less time brooding nestlings, but provisioned nestlings more frequently,
31 and deposited more T in their eggs. These findings suggest that, while T is likely important
32 in mediating maternal phenotypes and female life history tradeoffs, the direction of the
33 relationships between T and phenotype may differ from what is generally observed in
34 males, and that high levels of endogenous T are not necessarily as costly as previous work
35 might suggest.

36

37 INTRODUCTION

38

39 Because time and energy can be spent only once, animals are often functionally constrained
40 from maximally investing in all potentially beneficial traits (Lessells, 2008). Such constraints
41 produce tradeoffs, which are key to understanding the evolution of life histories (Roff et al.,
42 2002; Stearns, 1992). One of the most important behavioral tradeoffs occurs when competition
43 for crucial sex-specific resources overlaps in time with the need to care for offspring (Magrath
44 and Komdeur, 2003). This tradeoff between resource acquisition and offspring care is often
45 portrayed as a continuum with males at one end, investing more in competition for mates, and
46 females at the opposite end, investing mainly in offspring production and care (Rosvall, 2013;
47 Shuster and Wade, 2003; Trivers, 1972). However, recent research has shown that females also
48 benefit from increased competitive ability in reproductive contexts (Clutton-Brock, 2009;
49 Langmore, 1998; LeBas, 2006; Rosvall, 2011; Stockley and Bro-Jørgensen, 2011; Tobias et al.,
50 2012). Less is known about whether, and to what extent, females trade-off competitive ability
51 with parental care, but research has shown that increased investment in traits that appear
52 important for competitive ability (i.e. *competitive traits*: ornaments, armaments, aggression, etc.
53 (Cain & Ketterson, 2012; West-Eberhard, 1983)) is often associated with decreased maternal
54 effort (Bell et al., 2011; Dantzer et al., 2011; Fite et al., 2005; Fitzpatrick et al., 1995; Nordeide
55 et al., 2006; Packer et al., 1995; Rosvall, 2011).

56

57 In vertebrates, the sex steroids are often important in mediating this tradeoff. In particular,
58 among males, high levels of testosterone (T) are generally associated with increased
59 investment in traits used to compete for reproductive resources (e.g., mates, territories), at the
60 expense of parental care and self-maintenance (Adkins-Regan, 2005; Ketterson and Nolan,
61 1999; Wingfield et al., 2001); but see (Lynn et al., 2002). Relative to males, we know
62 considerably less about the role of T in mediating female phenotypes in general and life history

63 tradeoffs in particular (Ketterson et al., 2005; Staub and De Beer, 1997). However, the available
64 data suggest that T may affect female trait expression in a manner similar to males. For
65 example, experimentally elevating plasma T levels in females often increases the expression of
66 traits used in same-sex competition, e.g. aggression (Rosvall, 2013; Sandell, 2007; Veiga et al.,
67 2004; Zysling et al., 2006), and often decreases some forms of maternal care (Clotfelter et al.,
68 2004; O'Neal et al., 2008; Rosvall, 2013; Rutkowska et al., 2005; Veiga and Polo, 2008).
69 However, some components of maternal care are unaffected by experimentally elevated T
70 (Clotfelter et al., 2004; DeVries and Jawor, 2013; Ketterson et al., 2005; O'Neal et al., 2008), and
71 endogenous measures of T are often unrelated to female competitive traits (Elekonich, 2000;
72 Hau et al., 2004; Jawor et al., 2006b) and maternal care (DeVries and Jawor, 2013). As a result,
73 the relative importance of T in mediating female phenotypes and life history tradeoffs is
74 currently unclear.

75
76 In addition to shaping a female's parental phenotype, female T production may also have
77 important effects on offspring phenotype via maternal effects. In oviparous vertebrates,
78 individual females vary in the amount of T that they deposit in eggs (Gil, 2003; Groothuis et al.,
79 2005; Groothuis and Schwabl, 2008). Experimental studies have shown that variation in the
80 amounts of steroid hormone in the egg can have large effects on developing offspring both in
81 the short-term and long-term, facilitating rapid growth and begging, and shaping adult
82 morphology and competitive ability, but can also be costly in terms of immune function
83 (Groothuis et al., 2005; Groothuis and Carere, 2005; Navara et al., 2005). Further, T has been
84 implicated as a factor influencing primary sex ratios in birds (Correa et al., 2011; Rutkowska
85 and Cichoń, 2006). Despite these potentially important consequences, less is known about the
86 proximate mechanisms controlling the amount of T sequestered, and it is unclear whether the
87 transfer of hormones is actively controlled by the female, or due to passive transfer (Groothuis
88 and Schwabl, 2008; Müller et al., 2011; Schwabl, 1993). Previous studies have failed to find a

89 relationship with circulating T and yolk T (Navara et al., 2005). However, other work has
90 shown that individual ability to produce T predicts the amount of T a female deposits in her
91 eggs (Jawor et al., 2007; Müller et al., 2011). Understanding the relationship between
92 individual T profiles and yolk T is crucial to developing a greater understanding of the role of
93 steroid-mediated maternal effects in shaping offspring development and phenotypic evolution.
94
95 Currently, the majority of studies examining the role of T in mediating female phenotypes,
96 particularly in birds, have utilized a phenotypic engineering approach, in which T is
97 experimentally altered (Adkins-Regan, 2005; Ketterson et al., 2005; Williams, 2008).
98 Experimental studies of this type are crucial for revealing the causal relationship between T
99 and phenotype. However, there are limits to what we can learn from phenotypic engineering
100 (McGlothlin et al., 2008; 2007; 2010). We currently have very little information regarding the
101 role of individual variation in endogenous testosterone in mediating female behavior or
102 adjusting offspring phenotype via maternal effects (Groothuis et al., 2005; Williams, 2012;
103 2008). To fully understand the evolution of hormonally mediated phenotypes we must also
104 examine the relationship between naturally existing hormonal and behavioral variation (Cain
105 and Ketterson, 2012; DeVries and Jawor, 2013; McGlothlin et al., 2007; Moore et al., 2002).
106
107 Here we examine the relationships between endogenous T and maternal effort (behavior and
108 yolk hormone deposition), in the dark-eyed junco (*Junco hyemalis carolinensis*), a common
109 songbird often used as a model for exploring the relationships between hormones and
110 behavior in free-living animals (e.g. (Deviche et al., 2001; Holberton et al., 2008; Ketterson and
111 Nolan, 1999; Ketterson et al., 1992; McGlothlin et al., 2010; Raouf et al., 1997). Previous work
112 has shown that experimentally elevated T in female juncos leads to increased aggression
113 (Zysling et al., 2006), and reduced maternal care in some, but not all, measures (Clotfelter et al.,
114 2004; O'Neal et al., 2008). More recently, we found that endogenous T production ability is

115 positively related to female-female aggression (Cain and Ketterson, 2012), and that females
116 tradeoff some forms of maternal care (brooding and egg size) with intra-sexual aggression
117 (Cain & Ketterson, in press). To determine whether T may play a role in mediating this tradeoff
118 or maternal phenotype in general, we examined the relationships between two measures of
119 endogenous T, circulating T before and after a physiological challenge in the form of an
120 injection of gonadotropin releasing hormone (or GnRH), and three measures of maternal
121 effort: brooding, provisioning, and the amount of T sequestered in the yolk. Based on previous
122 studies using experimentally elevated T, we predicted that endogenous T measures would be
123 negatively related to maternal behavior, and positively related to yolk T. Alternatively, other
124 work suggests that the relationships between T and maternal care may be more complicated
125 (Dantzer et al., 2011; DeVries and Jawor, 2013; Spinney et al., 2006; Swett and Breuner, 2009;
126 Veiga and Polo, 2008).

127

128 **METHODS**

129 *STUDY SPECIES, SITE, AND GENERAL METHODS*

130 This study took place on and around Mountain Lake Biological Station, in Giles Co., Virginia
131 (37°22'N, 80°32'W), from April 15-August 10 2008, 2009, and 2010. Juncos are a socially
132 monogamous, mildly dimorphic songbird (Nolan et al., 2002). Females build nests, incubate
133 eggs, and brood nestlings; males assist in feeding and nest defense. General field methods are
134 described in detail elsewhere (McGlothlin et al., 2010). Briefly, resident individuals were
135 captured using baited potter traps and mist nets, and marked with numbered metal bands and
136 unique combinations of color bands. Females were aged as young (first breeding season) or old
137 (after first breeding season) using plumage, eye coloration and mark-recapture data from
138 previous years (Nolan et al., 2002). We searched the study site daily for nests of all resident
139 females. When located, nests were marked and then monitored to determine the social pair

140 and the commencement of egg laying. Within 24h of clutch completion, the third laid egg of
141 each female's first nesting attempt was collected (2008, n= 42; 2009, n= 35; 2010, n= 31). If egg
142 order was unknown we selected the largest egg, as the 3rd egg is often largest (Nolan et al.,
143 2002).

144

145 *PARENTAL BEHAVIOR*

146 In 2009 and 2010, we measured maternal behavior for all females that had both been
147 administered GnRH challenges (see below), and had a nest that survived to the nestling stage,
148 which limited the sample size (2009: $n = 12$; 2010: $n = 16$). To quantify maternal behavior we
149 videotaped each nest for 4 consecutive hours (0900-1700) at day 3 post-hatching. A single
150 observer watched all tapes at a later date and determined the number of female feeding trips,
151 the number of brooding bouts, and the length of each brooding bout. Mean brooding bout was
152 calculated by summing the total amount of time spent brooding and dividing by the number of
153 brooding bouts. If the female was still brooding at the end of the tape that brooding event was
154 excluded. Mean brood bouts ranged from 244s – 1588s, mean of 663s. We used a Campbell
155 CR10 data logger located on the study site to record ambient temperature at 1400 (mid-point
156 for most recordings). A female's age category (young or old) had no effect on brooding or
157 provisioning (mean brooding bout: $t_{28} = -0.11$, $P = 0.91$; provisioning rate: $t_{28} = -1.06$, $P = 0.30$),
158 nor did year (provisioning rate; $t_{28} = -1.27$, $P = 0.22$, mean brooding bout; $t_{28} = 1.15$, $P = 0.26$),
159 ambient temperature (mean brooding bout, $R^2 = 0.01$, $P = 0.62$; provisioning rate, $R^2 = 0.002$, $P =$
160 0.87), or date (mean brooding bout, $R^2 = 0.002$, $P = 0.79$; provisioning rate, $R^2 = 0.003$, $P = 0.77$).
161 There was a negative relationship between the two measures of parental behavior ($R^2 = 0.28$,
162 $P = 0.0047$, $n = 28$); i.e. females that brooded most tended to feed less frequently.

163

164 *GNRH CHALLENGES*

165 Testosterone production is regulated by the hypothalamic-pituitary-gonadal (HPG) axis. The
166 hypothalamus responds to a variety of external and internal stimuli by releasing
167 gonadotropin-releasing hormone (GnRH), stimulating the pituitary to release luteinizing
168 hormones (LH), which then travels via the bloodstream to the gonads, which response by
169 releasing sex steroids, including T (Adkins-Regan, 2005; (Jawor et al., 2006a)). To measure
170 individual variation in ability to produce testosterone, we challenged females' HPG axis by
171 administering an injection of GnRH to produce a transient increase in circulating T (2008: n=
172 18; 2009: n= 26; 2010: n= 19). This procedure stimulates individuals to release maximal T
173 levels (Cain and Ketterson, 2012; Jawor et al., 2007; 2006a; McGlothlin et al., 2007; Moore et
174 al., 2002; Wingfield et al., 1991), and is repeatable in both sexes (Jawor et al., 2006a; Rosvall &
175 Bergeon Burns unpublished). In female juncos the most robust response to GnRH is during the
176 7 days prior to oviposition, when females are rapidly yolking eggs (Jawor et al., 2007). We
177 challenged females only during this stage, identifying females that were heavy and had the
178 distinctive "torpedo-like" shape typical of yolking songbirds (Cain and Ketterson, 2012). Mean
179 mass for female juncos early in the breeding season is $21.5\text{g} \pm 0.18$ (mean, s.e.) (Cain and
180 Ketterson, 2012), mean mass for challenged females was 24.7 ± 1.44 . Challenged females were
181 captured during the pre-breeding season and transported to a central processing area. An
182 initial blood sample (initial T) was taken from the wing, followed by an intramuscular injection
183 of 50 μL of a solution containing 1.25 μg of chicken GnRH- I (Sigma L0637; American Peptide
184 54-8-23). After exactly 30 minutes a second blood sample was taken (post-challenge T).
185 Samples were centrifuged and the plasma was drawn off and frozen at -20 C° until assayed.
186 We recorded time at capture (capture time) and the total amount of time elapsed between
187 capture and the initiation of the challenge (handling time, mean = 1696s).

188

189 *PLASMA TESTOSTERONE ASSAYS*

190 Plasma T concentrations for 2009 and 2010 were determined using commercially available
191 EIA kits (Assay Designs, Inc., #901-065) as described elsewhere (Clotfelter et al., 2004; Jawor
192 et al., 2007). We added 2000cpm of tritiated T (H3-T) to calculate recovery efficiencies prior to
193 two rounds of diethyl ether extractions. Samples were then dried down with N2 and re-
194 suspended in 300ul of assay buffer and 50ul of ethanol. All samples were run in duplicate; a
195 four-parameter logistic curve-fitting program (Microplate Manager; Bio-Rad Laboratories, Inc.)
196 was used to determine concentrations. Plasma T concentrations for 2008 were determined
197 using long-column chromatography followed by a radio-immunoassay as part of a separate
198 experiment, described in Cain & Ketterson 2012. These values were used only for examining
199 the relationship between plasma and yolk T. For all samples, values were corrected for
200 incomplete recoveries; average recovery was 90%. Both initial and post-challenge T values
201 were normal after a logit transformation (Shapiro-Wilk test: $P > 0.15$ for both). Three samples
202 were excluded because there was a problem with the challenge (bled at the wrong time) or the
203 hormone assay (unusually high or low recoveries). The time of day the challenge occurred had
204 no detectable relationship with initial or post-challenge T (all $P > 0.35$).

205

206 *YOLK TESTOSTERONE ASSAYS*

207 The concentration of testosterone in yolk was determined using a testosterone enzyme-
208 immunoassay kit (EIA), (Catalog # ADI-901-065) (Enzo Life Sciences Intl., Inc., Plymouth
209 Meeting, PA), following a modified ethanol extraction (Kozlowski et al., 2009). Previously
210 frozen eggs were allowed to semi-thaw to permit separation of the yolk from the albumin. We
211 recorded the total egg mass and yolk mass and transferred yolks to 2ml Eppendorf tubes. Mean
212 yolk mass was $0.504\text{g} \pm 0.006$ (mean, s.e.). After the yolk thawed fully, we added 500ul of
213 distilled water and several glass mixing beads, then vortexed thoroughly until yolks were
214 completely homogenized. For hormone extraction, 50ul of the yolk solution was transferred to
215 a clean 1.5ml Eppendorf, and then further diluted with an additional 100ul of distilled water.

216 Tritiated testosterone (2000 cpm H³-T) was added to the homogenate for calculation of
217 extraction recoveries, and mean sample recovery was 82%. Homogenate was then vortexed
218 and incubated at 37C for 1hr to allow the native and tritiated hormone to equilibrate. After
219 incubation, 300ul of 100% ethanol was added to each sample. Samples were again vortexed
220 and put on a shaker at 500rpm for 5min. Samples then were allowed to sit at room
221 temperature for 10 min to incubate and settle. After incubation, samples were spun in a micro-
222 centrifuge for 10min at 13,000 rpm. The supernatant was decanted into a 13x100 borosilicate
223 culture tube and dried down using a forced air manifold and warm water bath. Dried samples
224 were then rehydrated with 50ul of 100% ethanol and 300ul of assay buffer. Assays were run
225 in accordance with kit directions and as described for plasma samples with two exceptions.
226 First, because mean testosterone levels were above optimal levels for the assay, all samples
227 were run at a 1:10 dilution. Second, kit standards were replaced by standards made from
228 pooled and diluted yolk extract in order that samples and standards be of comparable matrices.
229 Values were normally distributed after natural log transformation (Shapiro-Wilk *W* test, *P*>
230 0.15).

231 *ASSAY VALIDATION*

232 To determine whether substances present in the extract interfered with the accuracy of the
233 assay, or introduced bias (Engelhardt and Groothuis, 2005), we pooled extracts from 5 yolks
234 prepared as above and performed a serial dilution of 1:4, 1:8, 1:16, 1:32, 1:64 and 1:128. Serial
235 dilutions were parallel to the standard curve (ANCOVA, dilution by concentration interaction,
236 *P*= 0.47). We also created five replicates of the diluted sample pool (1:50) and spiked each with
237 a serial dilution of a known standard (i.e. 1000, 630, 320, 125, and 62.5 pg/ml) provided with
238 the assay kit. Spiked recoveries were 91% of expected values.

239

240 This modified technique produced T concentration similar to what has been reported
241 previously in this species. T concentration in yolks using this method ranged from 1.58 to

242 18.79 pg/mg, (mean, s.e.) 7.107 ± 0.454 pg/mg, and recoveries averaged 82.85%. Lipar et al.
243 (1999) used long column RIAs to determine yolk T and report mean T value of 7.63 pg/mg,
244 with recoveries of 71%; Jawor et al. (2007) also used RIAs and reported a mean T value of 1.71
245 pg/mg, with 58% recoveries.

246 *STATISTICAL ANALYSIS*

247 All analyses were done in JMP 10 (SAS Institute Inc.). To examine the relationship between
248 hormone measures and parental behavior, we used the hormone measure as the dependent
249 variable, allowing us to examine the relationship between T and the behavior of interest while
250 holding the other variables constant (e.g. date, mass, year, capture time, handling time) (Jawor
251 et al. 2006a; McGlothlin et al. 2007). However, we make no assumption about whether the
252 relationship is causal, or in which direction any causality may operate. We used BIC (Bayesian
253 Information Criterion) minimum scores to objectively select the predictive variables. The
254 beginning model included the parental behavior of interest, year, and factors that have
255 previously been shown to influence response (date, mass, capture time, handling time).
256 For most of the females that received a GnRH challenge we also collected an egg (2008, n=26;
257 2009, n=11; 2010, n=19). Females were only included in this analysis if they received the GnRH
258 challenge in the same year the egg was collected. The final model was run in a multiple
259 regression analysis. To examine the relationships between yolk T, initial T and post-challenge
260 T we used multiple regression models with yolk T as the dependent variable, with year and
261 initial or post-challenge T as predictors.

262

263 To illustrate relationships between post-challenge T and behavior we calculated leverage plots
264 pairs. Leverage pairs are derived from the residual error without the effect in the model and
265 the actual residuals from the best-fit line, similar to a partial correlation (Sall, 1990). Finally, to
266 visualize potential patterns of covariation between variables of interest we constructed a
267 network model, see Figure 2 (Huffman et al., 2012). Specifically, we used the calculated

268 coefficient of determination (adjusted R^2) from each multiple regression model, i.e. tests
269 examining relationship between hormones measures and maternal behavior/yolk T, to
270 construct a correlation matrix between measures of hormonal phenotype and maternal care.
271 We then used Cytoscape software (version 2.3) to construct a force-weighted network model
272 to visualize the emerging network. Each node represents a variable of interest, colored
273 according to type of trait (hormone measures are blue, maternal effort is orange, egg metrics
274 are yellow). Lines connecting nodes denote the nature of the relationship between the
275 variables (solid lines are positive; dashed lines are negative, faint lines indicate no detectable
276 relationship). The length of the line is inversely related to the strength of the relationship;
277 short lines indicate stronger relationships.

278

279 **RESULTS**

280 *MATERNAL BEHAVIOR AND TESTOSTERONE*

281 Initial T was negatively, but not significantly, related to brooding ($F_{1,16} = 2.74$, $R^2 = 0.16$, $P =$
282 0.12), and unrelated to provisioning ($P > 0.45$). Post-challenge T was negatively related to
283 brooding; females that produce more T brooded for shorter intervals (Fig. 1 & 3, Table 1,
284 Overall model Adj. $R^2 = 0.98$, $F_{6,12} = 33.03$, $P = 0.0007$; mean brooding bout, $b = -0.002$, $P =$
285 0.0011). In the final model, date, year, mass, capture time and handling time, were all
286 significant variables. Post-challenge T was positively related to provisioning behavior; females
287 with higher T provisioned their young more often than females with lower T (Fig 1 & 3, Table 1.
288 Overall model Adj. $R^2 = 0.80$, $F_{4,12} = 12.27$, $P = 0.0028$: provisioning rate, $b = 3588$, $P = 0.0468$). In
289 the final model, year, mass, and handling time, were significant variables.

290 *YOLK T AND MATERNAL T*

291 Controlling for year, there was a positive correlation between yolk T and post-challenge T
292 (Figure 2 & 3, Full model, $F_{2,47} = 3.20$, Adj. $R^2 = 0.13$, $P = 0.0324$; post challenge T, $P = 0.0128$; year,

293 $P=0.13$). There was no detectable relationship between initial T and yolk T ($P>0.25$). Yolk mass
294 and yolk T were not related ($R^2= 0.003$, $P= 0.88$), and neither yolk mass nor yolk T differed
295 between years (all $P> 0.25$). Yolk mass was unrelated to post-challenge T ($P>0.50$). However,
296 there was a positive trend between yolk mass and initial T (Fig. 3, $R^2= 0.05$, $P=0.1244$).

297

298 **DISCUSSION**

299 We examined the relationships between endogenous T and three measures of maternal effort
300 (provisioning, brooding and yolk T deposition) in females to determine whether endogenous T
301 is potentially mediating female parental phenotypes and if so, whether the relationship in
302 females is similar to that in males and previous experimental studies on females. We found
303 that females capable of producing more T in response to a physiological challenge (injection of
304 GnRH) spent less time brooding nestlings, congruent with predictions based on previous
305 experimental studies. However, in contrast to experimental studies in both females and males,
306 we found that high T females provisioned more frequently. Finally, we found that females
307 producing more T in response to GnRH also deposited greater concentrations of T in the yolk.

308 *STRONGER RELATIONSHIPS WITH POST-CHALLENGE T*

309 We found significant relationships between maternal effort and post-challenge T but not
310 between behavior and initial T (Fig. 3). The lack of relationship between initial T and behavior
311 is similar to other studies reporting no association between endogenous T and female behavior
312 (DeVries and Jawor, 2013; Elekonich, 2000; Hau et al., 2004; Jawor et al., 2006a). This lack of
313 association may be because circulating levels can rapidly change in response to uncontrollable
314 sources of variation, thereby obscuring relationships, e.g. social interactions, time of day, etc.
315 Consequently, the stronger relationships we observed with post-challenge T may have arisen
316 because GnRH induced T, is less perturbable by the stimuli that may have influenced initial T,
317 allowing us to detect patterns that would normally be obscured. Alternatively, the costs

318 associated with high levels of circulating T (García-Vigón et al., 2008; Gerlach and Ketterson,
319 2013; Ketterson et al., 2005; O'Neal et al., 2008; Rosvall, 2013; Rutkowska et al., 2005; Veiga
320 and Polo, 2008) may favor females that maintain circulating T at low levels, particularly once
321 breeding has commenced. In support of this, research in juncos has shown that captive females
322 did not elevate T after an aggressive interaction (Jawor et al., 2006a), circulating T measured
323 immediately after a simulated intrusion during incubation was unrelated to aggression
324 (Rosvall et al., 2012), and that incubating and brooding females do not respond to the GnRH
325 challenge (Jawor et al., 2007).

326

327 However, females do increase T in response to GnRH before incubation begins, and individual
328 ability to produce T during this period *is* related to fitness relevant behaviors weeks later (Cain
329 & Ketterson 2012, this study). This suggests that individual GnRH responses during this critical
330 period, when competitive ability may influence mate or territory selection, is likely to be an
331 informative proxy for individual responsiveness to stimuli or sensitivity to steroids more
332 generally, a possibility supported by several lines of research in juncos. Individual response to
333 GnRH is repeatable in both sexes, and the source of individual variation appears to be
334 individual differences in gonad function, suggesting that GnRH response is a property of the
335 individual (Bergeon Burns & Rosvall, unpublished). Further, female aggression, which is
336 positively related to post-challenge T (Cain and Ketterson, 2012), is also related to individual
337 differences in the level of steroid receptor mRNA (Rosvall et al., 2012). Finally, it is important
338 to note that in the final models examining these relationships, other factors were also
339 significantly related to post-challenge T (see Table 1), as has been reported in previous work
340 (Cain et al., 2013; Cain and Ketterson, 2012; Jawor et al., 2007; 2006a; McGlothlin et al., 2010;
341 2007). This suggests than individual responsiveness the GnRH may be affected by
342 downstream factors (Jawor et al., 2007; 2006a), which could also be important in regulating T
343 and T-mediated phenotypes in females.

345 *TESTOSTERONE & MATERNAL CARE*

346 Our finding that females capable of producing more T in response to the GnRH challenge also
347 brood for less time is consistent with the generality that higher T levels are associated with
348 reductions in parental care in both sexes (Ketterson et al., 2005; 1992; Rosvall, 2013; Stoehr
349 and Hill, 2000; Trainor and Marler, 2001; Veiga and Polo, 2008; Wingfield et al., 2001). More
350 specifically, it is congruent with previous work in juncos that found reduced brooding in
351 females with T elevated experimentally (O'Neal et al., 2008). Together, these findings support
352 the possibility that T may mediate tradeoffs between reproductive competition and maternal
353 care, suggesting an important cost for high T in females. A recent examination of the costs of
354 aggression in junco females found that more aggressive females, which tend to produce more T
355 in response to GnRH (Cain and Ketterson, 2012), have hatchlings of lower mass than do less
356 aggressive females (Cain and Ketterson, in press); mass is an important predictor of survival in
357 songbird nestlings (Starck and Ricklefs, 1998). The negative relationship reported here
358 between T and brooding may partially explain this pattern. Reduced brooding or incubation,
359 may lead to slower developmental rates, forcing nestlings to devote more energy to
360 thermoregulation and reducing the amount of energy available for growth (Ardia et al., 2010).
361 Because only females brood in this species, reduced brooding may present an important cost
362 for females with high T, and lead to selection favoring reduced T in females. If male and female
363 T levels are genetically correlated, these costs could constrain male T (Ketterson et al., 2005;
364 2009).

365

366 In contrast to the typical pattern of negative relationships between parental care and
367 testosterone, we found a positive relationship between provisioning and T. However, because
368 the relationship between T and female maternal behavior is so rarely examined, it is difficult to
369 say whether this relationship is unusual. In experimental studies, females with elevated T

370 either show reduced maternal care relative to controls, or there is no effect, depending on the
371 form of care and the species examined. In spotless starlings (*Sturnus unicolor*), T females
372 showed delayed egg laying and reduced provisioning rates (Veiga and Polo, 2008); in tree
373 swallows, T-females showed reduced incubation and hatching success (Rosvall, 2013); in
374 juncos, T-females showed reduced brooding, nest defense, and nest success, but there was no
375 effect on incubation, provisioning, nestling quality or extra-pair offspring production
376 (Clotfelter et al., 2004; Gerlach et al., 2013; O'Neal et al., 2008). In contrast, though white-
377 striped morph white-throated sparrow females (*Zonotrichia albicollis*) are more aggressive
378 and engage less in maternal care relative to tan morphs, the morphs do not differ in circulating
379 T levels (Spinney et al., 2006; Swett and Breuner, 2009). Our understanding of why within a
380 species T affects some traits and not others, and why species differ in trait sensitivity, is
381 currently quite limited. However, there is some indication that species' trait sensitivity to T
382 depends on female life histories and the relative importance of competition versus maternal
383 care for those females (Rosvall, 2013).

384

385 Only one previous study has examined covariation between female T production ability (i.e.
386 post-challenge T) and provisioning rates. That study addressed this question in female
387 northern cardinals (*Cardinalis cardinalis*), and found no relationship between the two
388 measures (DeVries and Jawor, 2013). The observed difference in the relationships between T
389 and provisioning in cardinal and junco females likely stems from differences in female life
390 histories as well as important differences in study methodology. The cardinal is a non-
391 migratory resident that defends territories year-round (Halkin and Linville, 1999), while the
392 junco migrates away from the breeding grounds and winters in flocks (Nolan et al., 2002).
393 Further, in the cardinal study GnRH challenges were administered during the nestling period.
394 However, cardinal females, like juncos females, did not elevate T in response to GnRH during
395 the nestling period (DeVries and Jawor, 2013; Jawor et al., 2007). In contrast, we administered

396 GnRH challenges in the pre-breeding season, during the period that female juncos exhibit the
397 most robust response to a GnRH challenge (Jawor et al., 2007).

398

399 A final alternative explanation for the positive relationship between T and provisioning is that
400 current definitions of maternal care, and of what makes a 'good mother', may be overly
401 simplistic. While T does often lead to reductions in some forms of maternal care, high T levels
402 may be important for other forms of maternal care, particularly when successful reproduction
403 requires competition (Rosvall, 2013). In the junco, females capable of producing more T in the
404 pre-breeding season may be more competitive, and acquire higher quality mates or territories
405 than lower T females (Cain and Ketterson, 2012; Cain et al., 2011). As a result, though they
406 brood less, they may be able to provision more and thus neutralize some negative effects of
407 higher T (Cain & Ketterson, in press). Previous work in this population found that more
408 aggressive females, which produce more T in response to GnRH, have greater nest success in
409 some years (Cain & Ketterson, 2012; Cain & Ketterson, in press), suggesting that these high T
410 females may actually be better mothers, if the metric used is greater offspring survival.

411

412 A similar pattern can be seen in other species. For example, in red squirrel females
413 (*Tamiasciurus hudsonicus*), increased fecal androgens are associated with less time in the nest
414 but more time devoted to territory defense and resource acquisition, which may increase
415 juvenile survival and optimize female reproductive success (Dantzer et al., 2011).

416 Experimentally elevating T in female tree swallows decreases incubation but increases
417 aggression (Rosvall, 2013), which is important for acquiring a nest cavity, a necessary
418 reproductive resource (Rosvall, 2008), and similar findings were reported in spotless starlings
419 (Veiga and Polo, 2008). This pattern suggests that T may facilitate female ability to acquire
420 resources important for indirect offspring care. This may generate tradeoffs with other forms
421 of maternal care, but nevertheless may lead to improved reproductive success. Thus, caution is

422 warranted when interpreting findings; a reduction in one form of maternal care or behavior
423 may not inherently be poor mothering (Rosvall, 2013; Stiver and Alonzo, 2009).

424

425 *YOLK TESTOSTERONE*

426 In addition to maternal behavior, females can also affect offspring phenotype via the amount of
427 steroid sequestered in the yolk (Gil, 2003; Groothuis et al., 2005; Schwabl, 1993). We found a
428 positive relationship between T produced in response to a GnRH challenge and the amount of T
429 deposited in yolk, replicating a finding from a previous study in juncos (Jawor et al., 2007), and
430 in canaries (*Serinus canaria*) (Müller et al., 2011). In most species studied to date, yolk T
431 functions as an anabolic steroid, stimulating growth and begging, although often at the cost of
432 reduced immune function (Gil, 2003; Groothuis et al., 2005; Groothuis and Carere, 2005; Lipar
433 and Ketterson, 2000; Schwabl, 1993); but see (Cox, 2005; Navara et al., 2005; Sockman and
434 Schwabl, 2000). Exposure to T in the egg can also alter offspring ability to produce T later in
435 life (Cain et al., 2013; Müller et al., 2011; Pfannkuche et al., 2011), and influence primary sex
436 ratio (Correa et al., 2011; Rutkowska and Cichoń, 2006), suggesting another potential avenue
437 by which females can influence offspring and grand-offspring fitness (Clutton-Brock et al.,
438 1986; Trivers and Willard, 1973).

439

440 Previous studies on females in this population have found that aggressive females produce
441 more T in response to GnRH (Cain and Ketterson, 2012), and in some years, produce smaller
442 eggs and lighter hatchlings (Cain and Ketterson, in press). However, those nestlings gained
443 mass faster than nestlings of less aggressive females, potentially neutralizing any negative
444 effects that stem from the production of smaller eggs. The positive relationship between post-
445 challenge T and yolk T suggests one way that nestlings of aggressive females may “catch-up”. If
446 more aggressive females deposit more T in their eggs, this may stimulate greater growth. In
447 tree swallows, higher aggressive interaction rates were positively correlated with yolk T

448 (Whittingham and Schwabl, 2002), supporting this possibility. The faster growth rates of
449 aggressive female nestlings may have also been further fueled by greater provisioning rates
450 observed in high T females. Thus, individual variation in yolk T may be another way that
451 females influence offspring quality and survival, outside of traditional measures of maternal
452 care (Gil, 2003; Groothuis et al., 2005; Jawor et al., 2007; Ruuskanen et al., 2012). Another
453 possibility is that high levels of yolk T may result in increased begging, motivating parents to
454 provision more frequently. Thus, the positive relationship female T production and
455 provisioning may be an indirect product of yolk T initiating behavioral differences in chicks,
456 rather than a direct effect of T on female behavior. Differences in yolk T levels may also
457 contribute to the year observed differences in nest success, e.g. females with high T levels may
458 be more successful under competitive conditions, but not in conditions where resources are
459 plentiful (Cain & Ketterson, in press). As the results reported here are correlative, determining
460 which of these possibilities underlies the observed patterns in the junco will require further
461 empirical study.

462

463 CONCLUSION

464 Steroid hormones play a key role in mediating life-history tradeoffs (Adkins-Regan, 2005;
465 Ketterson and Nolan, 1999; Wingfield et al., 2001; Zera and Bottsford, 2001), however we
466 know little about the tradeoff between competition and maternal effort in females. The
467 physiological mechanisms underlying this tradeoff in females have rarely been examined
468 directly, particularly in regards to endogenous steroid levels (DeVries and Jawor, 2013;
469 Rosvall, 2013). The findings reported here support the possibility that T may play a similar role
470 in females as has been reported in males, increasing competitive behavior at the expense of
471 maternal effort. However, we also found unexpected positive relationships between some
472 measures of maternal behavior and T. Together these results suggest that while T is likely
473 important in maternal phenotypes and mediating tradeoffs, high transient levels of T are not

474 necessarily as costly as previous work using experimental elevations of T might suggest (e.g.
475 Gerlach et al., 2013, Veiga and Polo, 2008). These incongruent findings underscore the need for
476 additional data on T in females, particularly on the relationships between endogenous T and
477 behavior. Such data is essential if we are to further our understanding of how selection shapes
478 T-mediated phenotypes in females in specific, and proximate regulation of life histories more
479 generally.

480

481 **ACKNOWLEDGEMENTS**

482 Research was supported by National Science Foundation (NSF) grants to EK (BSC 05-19211
483 and IOS 08-20055) and an NSF Doctoral Dissertation Improvement Grant to KC (0910036). KC
484 was also supported by an NSF Graduate Research Fellowships Program fellowship and
485 National Institute of Health training grant, Common Themes in Reproductive Diversity (NIH no.
486 HD 049336-04). This research adhered to the Association for the Study of Animal
487 Behavior/Animal Behavior Society Guidelines for the Use of Animals in Research, the legal
488 requirements of the United States of America (USFWS special use permit number MB093279-2,
489 USGS banding permit number 20261), the states of Indiana and Virginia, and was conducted in
490 compliance with the University of Virginia and Indiana University Institutional Animal Care
491 and Use Committee (protocol #09-037). The authors thank S. Wanamaker and A. Dapper for
492 assistance with data collection; R. Stewart for guidance with steroid assays; the Ketterson Lab,
493 C. Ziegenfus, and the United Junco Workers for the field assistance; S. Pryke, K.A. Rosvall and
494 D.M. O'Neal for helpful discussions; S. Hoobler for additional support; and Mountain Lake
495 Biological Station (B. Brodie III, Director and E. Nagy, Associate Director) and Mountain Lake
496 Hotel for permission to work on their property.

497

498 **LITERATURE CITED**

- 499 Adkins-Regan, E., 2005. *Hormones And Animal Social Behavior*. Princeton University Press,
500 Princeton.
- 501 Ardia, D.R., Pérez, J.H., Clotfelter, E.D., 2010. Experimental cooling during incubation leads to
502 reduced innate immunity and body condition in nestling tree swallows. *Proc. Biol. Sci.* 277,
503 1881–1888.
- 504 Bell, M.B.V., Nichols, H.J., Gilchrist, J.S., Cant, M.A., Hodge, S.J., 2011. The cost of dominance:
505 suppressing subordinate reproduction affects the reproductive success of dominant female
506 banded mongooses. *Proc. Biol. Sci.* 279, 619–624.
- 507 Cain, K.E., Burns, C.M.B., Ketterson, E.D., 2013. Testosterone production, sexually dimorphic
508 morphology, and digit ratio in the dark-eyed junco. *Behav. Ecol.* 24, 462–469.
- 509 Cain, K.E., Ketterson, E.D., 2012. Competitive females are successful females; phenotype,
510 mechanism and selection in a common songbird. *Behav. Ecol. Sociobiol.* 66, 241–252.
- 511 Cain, K.E., Ketterson, E.D., in press. Costs and benefits of competitive traits in females;
512 aggression, maternal care and reproductive success. *PLOS ONE*
- 513 Cain, K.E., Rich, M.S., Ainsworth, K., Ketterson, E.D., 2011. Two sides of the same coin?
514 Consistency in aggression to conspecifics and predators in a female songbird. *Ethol.* 117,
515 786–795.
- 516 Clotfelter, E.D., O'Neal, D.M., Gaudio, J.M., Casto, J.M., Parker-Renga, I.M., Snajdr, E.A., Duffy,
517 D.L., Nolan, V., Ketterson, E.D., 2004. Consequences of elevating plasma testosterone in
518 females of a socially monogamous songbird: evidence of constraints on male evolution?
519 *Horm. Behav.* 46, 171–178.
- 520 Clutton-Brock, T.H., 2009. Sexual selection in females. *Anim. Behav.* 77, 3–11.
- 521 Clutton-Brock, T.H., Albon, S.D., Guinness, F.E., 1986. Great expectations: dominance, breeding
522 success and offspring sex ratios in red deer. *Anim. Behav.* 34, 460–471.
- 523 Correa, S.M., Horan, C.M., Johnson, P.A., Adkins-Regan, E., 2011. Copulatory behaviors and body

524 condition predict post-mating female hormone concentrations, fertilization success, and
525 primary sex ratios in Japanese quail. *Horm. Behav.* 59, 556–564.

526 Cox, R.M., 2005. Testosterone has opposite effects on male growth in lizards (*Sceloporus* spp.)
527 with opposite patterns of sexual size dimorphism. *J. Exp. Biol.* 208, 4679–4687.

528 Dantzer, B., McAdam, A.G., Palme, R., Humphries, M.M., Boutin, S., Boonstra, R., 2011. Maternal
529 androgens and behaviour in free-ranging North American red squirrels. *Anim. Behav.* 81,
530 469–479.

531 Deviche, P., Breuner, C., Orchinik, M., 2001. Testosterone, corticosterone, and photoperiod
532 interact to regulate plasma levels of binding globulin and free steroid hormone in dark-
533 eyed juncos, *Junco hyemalis*. *Gen. Comp. Endocrinol.* 122, 67–77.

534 DeVries, M.S., Jawor, J.M., 2013. Natural variation in circulating testosterone does not predict
535 nestling provisioning rates in the northern cardinal, *Cardinalis cardinalis*. *Anim. Behav.*

536 Elekonich, M.M., 2000. Female song sparrow, *Melospiza melodia*, response to simulated
537 conspecific and heterospecific intrusion across three seasons. *Anim. Behav.* 59, 551–557.

538 Engelhardt, von, N.K., Groothuis, T.G., 2005. Measuring steroid hormones in avian eggs. *Ann. NY*
539 *Acad. Sci.* 1046, 181–192.

540 Fite, J.E., French, J.A., Patera, K.J., Hopkins, E.C., Rukstalis, M., Ross, C.N., 2005. Elevated urinary
541 testosterone excretion and decreased maternal caregiving effort in marmosets when
542 conception occurs during the period of infant dependence. *Horm. Behav.* 47, 39–48.

543 Fitzpatrick, S., Berglund, A., Rosenqvist, G., 1995. Ornaments or offspring: costs to reproductive
544 success restrict sexual selection processes. *Biol. J. Linnean Soc* 55, 251–260.

545 García-Vigón, E., Cordero, P.J., Veiga, J.P., 2008. Exogenous testosterone in female spotless
546 starlings reduces their rate of extrapair offspring. *Anim. Behav.* 76, 345–353.

547 Gerlach, N.M., Ketterson, E.D., 2013. Experimental elevation of testosterone lowers fitness in
548 female dark-eyed juncos. *Horm. Behav.*

549 Gil, D., 2003. Golden eggs: maternal manipulation of offspring phenotype by egg androgen in

550 birds. *Ardeola* 50, 281–294.

551 Groothuis, T.G.G., Carere, C., 2005. Avian personalities: characterization and epigenesis.
552 *Neurosci. Biobehav. Rev.* 29, 137–150.

553 Groothuis, T.G.G., Müller, W., Engelhardt, von, N.K., Carere, C., Eising, C., 2005. Maternal
554 hormones as a tool to adjust offspring phenotype in avian species. *Neurosci. Biobehav. Rev.*
555 29, 329–352.

556 Groothuis, T.G.G., Schwabl, H., 2008. Hormone-mediated maternal effects in birds: mechanisms
557 matter but what do we know of them? *Phil. Trans. Roy. Soc. B* 363, 1647–1661.

558 Halkin, S.L., Linville, S.U., 1999. Northern Cardinal (*Cardinalis cardinalis*). Cornell Lab of
559 Ornithology, Ithaca.

560 Hau, M., Stoddard, S.T., Soma, K.K., 2004. Territorial aggression and hormones during the non-
561 breeding season in a tropical bird. *Horm. Behav.* 45, 40–49.

562 Holberton, R.L., Boswell, T., Hunter, M.J., 2008. Circulating prolactin and corticosterone
563 concentrations during the development of migratory condition in the Dark-eyed Junco,
564 *Junco hyemalis*. *Gen. Comp. Endocrinol.* 155, 641–649.

565 Huffman, L.S., Mitchell, M.M., O'Connell, L.A., Hofmann, H.A., 2012. Rising StARs: Behavioral,
566 hormonal, and molecular responses to social challenge and opportunity. *Horm. Behav.* 61,
567 631–641.

568 Jawor, J.M., McGlothlin, J.W., Casto, J.M., Greives, T.J., Snajdr, E.A., Bentley, G.E., Ketterson, E.D.,
569 2006a. Seasonal and individual variation in response to GnRH challenge in male dark-eyed
570 juncos (*Junco hyemalis*). *Gen. Comp. Endocrinol.* 149, 182–189.

571 Jawor, J.M., McGlothlin, J.W., Casto, J.M., Greives, T.J., Snajdr, E.A., Bentley, G.E., Ketterson, E.D.,
572 2007. Testosterone response to GnRH in a female songbird varies with stage of
573 reproduction: implications for adult behaviour and maternal effects. *Funct. Ecol.* 21, 767–
574 775.

575 Jawor, J.M., Young, R., Ketterson, E.D., 2006b. Females competing to reproduce: Dominance

576 matters but testosterone may not. *Horm. Behav.* 49, 362–368.

577 Ketterson, E.D., Atwell, J.W., McGlothlin, J.W., 2009. Phenotypic integration and independence:
578 Hormones, performance, and response to environmental change. *Integrative and*
579 *Comparative Biology* 49, 365–379.

580 Ketterson, E.D., Nolan, V., 1999. Adaptation, exaptation, and constraint: a hormonal
581 perspective. *Am. Nat.* 154, S4–S25.

582 Ketterson, E.D., Nolan, V., Sandell, M., 2005. Testosterone in females: mediator of adaptive
583 traits, constraint on sexual dimorphism, or both? *Am. Nat.* 166, S85–S98.

584 Ketterson, E.D., Nolan, V., Wolf, L., Ziegenfus, C., 1992. Testosterone and avian life histories:
585 effects of experimentally elevated testosterone on behavior and correlates of fitness in the
586 dark-eyed junco (*Junco hyemalis*). *Am. Nat.* 980–999.

587 Kozlowski, C.P., Bauman, J.E., Caldwell Hahn, D., 2009. A simplified method for extracting
588 androgens from avian egg yolks. *Zoo Biol.* 28, 137–143.

589 Langmore, N.E., 1998. Functions of duet and solo songs of female birds. *Trends Ecol. Evol.* 13,
590 136–140.

591 LeBas, N.R., 2006. Female finery is not for males. *Trends Ecol. Evol.* 21, 170–173.

592 Lessells, C.M., 2008. Neuroendocrine control of life histories: what do we need to know to
593 understand the evolution of phenotypic plasticity? *Phil. Trans. Roy. Soc. B* 363, 1589–1598.

594 Lipar, J.L., Ketterson, E.D., 2000. Maternally derived yolk testosterone enhances the
595 development of the hatching muscle in the red-winged blackbird *Agelaius phoeniceus*. *Proc.*
596 *Biol. Sci.* 267, 2005–2010.

597 Lynn, S.E., Hayward, L.S., Benowitz-Fredericks, Z.M., Wingfield, J.C., 2002. Behavioural
598 insensitivity to supplementary testosterone during the parental phase in the chestnut-
599 collared longspur, *Calcarius ornatus*. *Anim. Behav.* 63, 795–803.

600 Magrath, M.J.L., Komdeur, J., 2003. Is male care compromised by additional mating
601 opportunity? *Trends Ecol. Evol.* 18, 424–430.

602 McGlothlin, J.W., Jawor, J.M., Greives, T.J., Casto, J.M., Phillips, J.L., Ketterson, E.D., 2008.
603 Hormones and honest signals: males with larger ornaments elevate testosterone more
604 when challenged. *J. Evol. Biol.* 21, 39–48.

605 McGlothlin, J.W., Jawor, J.M., Ketterson, E.D., 2007. Natural variation in a testosterone-mediated
606 trade-off between mating effort and parental effort. *Am. Nat.* 170, 864–875.

607 McGlothlin, J.W., Whittaker, D.J., Schrock, S.E., Gerlach, N.M., Jawor, J.M., Snajdr, E.A., Ketterson,
608 E.D., 2010. Natural selection on testosterone production in a wild songbird population. *Am.*
609 *Nat.* 175, 687–701.

610 Moore, I.T., Perfito, N., Wada, H., Sperry, T.S., Wingfield, J.C., 2002. Latitudinal variation in
611 plasma testosterone levels in birds of the genus *Zonotrichia*. *Gen. Comp. Endocrinol.* 129,
612 13–19.

613 Müller, W., Groothuis, T.G.G., Goerlich, V.C., Eens, M., 2011. GnRH - A missing link between
614 testosterone concentrations in yolk and plasma and its intergenerational effects. *PLoS ONE*
615 6, e22675.

616 Navara, K.J., Hill, G.E., Mendonca, M.T., 2005. Variable effects of yolk androgens on growth,
617 survival, and immunity in eastern bluebird nestlings. *Physiol. Biochem. Zool.* 78, 570–578.

618 Nolan, V., Ketterson, E.D., Cristol, D.A., Rogers, C.M., Clotfelter, E.D., Schoech, S.J., RC, T., Snajdr,
619 E.A., 2002. Dark-eyed Junco. Cornell Lab of Ornithology, Philadelphia.

620 Nordeide, J.T., Rudolfsen, G., Egeland, E.S., 2006. Ornaments or offspring? Female sticklebacks
621 (*Gasterosteus aculeatus L.*) trade off carotenoids between spines and eggs. *J. Evol. Biol.* 19,
622 431–439.

623 O'Neal, D.M., Reichard, D.G., Pavilis, K., Ketterson, E.D., 2008. Experimentally-elevated
624 testosterone, female parental care, and reproductive success in a songbird, the Dark-eyed
625 Junco (*Junco hyemalis*). *Horm. Behav.* 54, 571–578.

626 Packer, C., Collins, D.A., Sindimwo, A., Goodall, J., 1995. Reproductive constraints on aggressive
627 competition in female baboons. *Nature* 373, 60–63.

628 Pfannkuche, K.A., Gahr, M., Weites, I.M., Riedstra, B., Wolf, C., Groothuis, T.G.G., 2011. Examining
629 a pathway for hormone mediated maternal effects--yolk testosterone affects androgen
630 receptor expression and endogenous testosterone production in young chicks (*Gallus*
631 *gallus domesticus*). Gen. Comp. Endocrinol. 172, 487–493.

632 Raouf, S.A., Parker, P.G., Ketterson, E.D., Nolan, V., Ziegenfus, C., 1997. Testosterone affects
633 reproductive success by influencing extra-pair fertilizations in male dark-eyed juncos
634 (*Aves: Junco hyemalis*). Proc. Biol. Sci. 264, 1599–1603.

635 Roff, D.A., Mostow, S., Fairbairn, D.J., 2002. The evolution of trade-offs: testing predictions on
636 response to selection and environmental variation. Evol. 56, 84–95.

637 Rosvall, K.A., 2008. Sexual selection on aggressiveness in females: evidence from an
638 experimental test with tree swallows. Anim. Behav. 75, 1603–1610.

639 Rosvall, K.A., 2011. Intrasexual competition in females: evidence for sexual selection? Behav.
640 Ecol. 22, 1131–1140.

641 Rosvall, K.A., 2013. Life history trade-offs and behavioral sensitivity to testosterone: An
642 experimental test when female aggression and maternal care co-occur. PLoS ONE 8,
643 e54120.

644 Rosvall, K.A., Burns, C.M.B., Barske, J., Goodson, J.L., Schlinger, B.A., Sengelaub, D.R., Ketterson,
645 E.D., 2012. Neural sensitivity to sex steroids predicts individual differences in aggression:
646 implications for behavioural evolution. Proc. Biol. Sci. 279, 3547–3555.

647 Rutkowska, J., Cichoń, M., 2006. Maternal testosterone affects the primary sex ratio and
648 offspring survival in zebra finches. Anim. Behav. 71, 1283–1288.

649 Rutkowska, J., Cichoń, M., Puerta, M., Gil, D., 2005. Negative effects of elevated testosterone on
650 female fecundity in zebra finches. Horm. Behav. 47, 585–591.

651 Ruuskanen, S., Doligez, B., Gustafsson, L., Laaksonen, T., 2012. Long-term effects of yolk
652 androgens on phenotype and parental feeding behavior in a wild passerine. Behav. Ecol.
653 Sociobiol. 66, 1201–1211.

654 Sall, J., 1990. Leverage plots for general linear hypotheses. *Am Stat* 44, 308–315.

655 Sandell, M.I., 2007. Exogenous testosterone increases female aggression in the European
656 starling (*Sturnus vulgaris*). *Behav. Ecol. Sociobiol.* 62, 255–262.

657 Schwabl, H., 1993. Yolk is a source of maternal testosterone for developing birds. *Proc Natl*
658 *Acad Sci USA* 90, 11446–11450.

659 Shuster, S.M., Wade, M.J., 2003. *Mating Systems and Strategies: (Monographs in Behavior and*
660 *Ecology)*. Princeton University Press, Princeton.

661 Sockman, K.W., Schwabl, H., 2000. Yolk androgens reduce offspring survival. *Proc. Biol. Sci.*
662 267, 1451–1456.

663 Spinney, L.H., Bentley, G.E., Hau, M., 2006. Endocrine correlates of alternative phenotypes in
664 the white-throated sparrow (*Zonotrichia albicollis*). *Horm. Behav.* 50, 762–771.

665 Starck, J.M., Ricklefs, R.E., 1998. *Avian Growth and Development*. Oxford University Press on
666 Demand.

667 Staub, N.L., De Beer, M., 1997. The role of androgens in female vertebrates. *Gen. Comp.*
668 *Endocrinol.* 108, 1–24.

669 Stearns, S.C., 1992. *The Evolution of Life Histories*. Oxford University Press on Demand.

670 Stiver, K.A., Alonzo, S.H., 2009. Parental and mating effort: Is there necessarily a trade-off?
671 *Ethol.* 115, 1101–1126.

672 Stockley, P., Bro-Jørgensen, J., 2011. Female competition and its evolutionary consequences in
673 mammals. *Biol. Rev.* 86, 341–366.

674 Stoehr, A.M., Hill, G.E., 2000. Testosterone and the allocation of reproductive effort in male
675 house finches (*Carpodacus mexicanus*). *Behav. Ecol. Sociobiol.* 48, 407–411.

676 Swett, M.B., Breuner, C.W., 2009. Plasma testosterone correlates with morph type across
677 breeding substages in male white-throated Sparrows. *Physiol. Biochem. Zool.* 82, 572–579.

678 Tobias, J.A., Montgomerie, R.D., Lyon, B.E., 2012. The evolution of female ornaments and
679 weaponry: social selection, sexual selection and ecological competition. *Phil. Trans. Roy.*

680 Soc. B 367, 2274–2293.

681 Trainor, B.C., Marler, C.A., 2001. Testosterone, paternal behavior, and aggression in the
682 monogamous California mouse (*Peromyscus californicus*). *Horm. Behav.* 40, 32–42.

683 Trivers, R., 1972. Parental Investment and Sexual Selection, in: Campbell, B. (Ed.), *Sexual*
684 *Selection and the Descent of Man 1871 – 1971*. Aldine, Chicago, pp. 136–179.

685 Trivers, R.L., Willard, D.E., 1973. Natural selection of parental ability to vary the sex ratio of
686 offspring. *Science* 179, 90–92.

687 Veiga, J.P., Polo, V., 2008. Fitness consequences of increased testosterone levels in female
688 spotless starlings. *Am. Nat.* 172, 42–53.

689 Veiga, J.P., Viñuela, J., Cordero, P.J., Aparicio, J.M., Polo, V., 2004. Experimentally increased
690 testosterone affects social rank and primary sex ratio in the spotless starling. *Horm. Behav.*
691 46, 47–53.

692 Whittingham, L.A., Schwabl, H., 2002. Maternal testosterone in tree swallow eggs varies with
693 female aggression. *Anim. Behav.* 63, 63–67.

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

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

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

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

703 Zera, A.J.A., Bottsford, J.J., 2001. The endocrine-genetic basis of life-history variation: the
704 relationship between the ecdysteroid titer and morph-specific reproduction in the wing-
705 polymorphic cricket *Gryllus firmus*. *Evolution* 55, 538–549.

706 Zysling, D.A., Greives, T.J., Breuner, C.W., Casto, J.M., Demas, G.E., Ketterson, E.D., 2006.
707 Behavioral and physiological responses to experimentally elevated testosterone in female
708 dark-eyed juncos (*Junco hyemalis carolinensis*). *Horm. Behav.* 50, 200–207.
709
710

711 **Figure and table captions**

712 **Figure 1:** Scatter plots relating parental behavior (left: brooding behavior; right: provisioning
713 behavior) to ability to produce T in response to GnRH challenge. Points in the scatter plots
714 for T are leverage pairs, akin to partial correlation (see statistical methods), *P* values are
715 from single effects in multiple regression.

716
717 **Figure 2:** Scatterplot illustrating the correlation between female ability to produce T in
718 response to a GnRH challenge (post challenge T) and the concentration of yolk T (ng/g)
719 deposited in eggs. Lines and symbols differ according to year; 2008, cross and dashed line;
720 2009, x and dotted line, 2010 square and solid line.

721
722 **Figure 3:** Network model illustrating the relationships between measures of hormonal
723 phenotype and maternal behaviors. Each node represents a variable of interest, colored
724 according to type of trait (hormone measures are blue, maternal effort is orange, egg
725 metrics are yellow). Lines connecting nodes denote the nature of the relationship between
726 the variables (solid lines are positive; dashed line is negative, faint lines indicate a trend
727 that is not statistically significant). The length of the line is inversely related to the strength
728 of the relationship; short lines are stronger. Notice tight clustering around post-challenge T
729 as compared to initial T.

730

731

732 **Table 1:** Final multiple regression models of the relationship between ability to produce
733 testosterone in response to a GnRH challenge and measures of parental effort.