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Testosterone modulates status-specific patterns of cooperation in a social network

Short title: Testosterone modulates cooperation

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20   **Summary**

Stable cooperation requires plasticity whereby individuals are able to express competitive or

22   cooperative behaviors depending on social context. To date, however, the physiological

mechanisms that underlie behavioral variation in cooperative systems are poorly understood. We

24   studied hormone-mediated behavior in the wire-tailed manakin (*Pipra filicauda*), a gregarious

songbird whose cooperative partnerships are crucial for fitness. We used automated telemetry to

26   monitor > 36,000 cooperative interactions among male manakins over three field seasons, and

we examined how circulating testosterone affects cooperation using > 500 hormone samples.

28   Observational data show that in non-territorial floater males, high testosterone is associated with

increased cooperative behaviors and subsequent ascension to territorial status. In territory-

30   holding males, however, both observational and experimental evidence demonstrate that high

testosterone antagonizes cooperation. Moreover, circulating testosterone explains significant

32   variation (2-8%) in social behavior within each status class. Collectively, our findings show that

the hormonal control of cooperation depends on a male's social status. We propose that the

34   status-dependent reorganization of hormone-regulatory pathways can facilitate stable cooperative

partnerships, and thus provide direct fitness benefits for males.

36

Keywords: cooperation, social networks, behavioral endocrinology, androgens, phenotypic

38   plasticity

Introduction

40   Behavioral plasticity is hypothesized to play a central role in the evolution of stable cooperation

(Bergmuller et al. 2010; Díaz-Muñoz et al. 2014). For example, individuals must balance

42   competition for mating opportunities with the benefits of engaging in cooperation (Clutton-

Brock 2009; Cockburn 1998). These negotiations within cooperative social systems are mediated

44   by physiological mechanisms that govern behavioral plasticity (Soares et al. 2010) over the

course of different temporal scales. Over the long-term, the plasticity that drives among-

46   individual differences in behavior and social status can facilitate stable cooperative partnerships

(Bergmuller et al. 2010) as well as the competitive behaviors essential for establishing

48   dominance and acquiring status within social groups (Ryder et al. 2008, 2009). Over shorter

timeframes, within-individual plasticity is thought to facilitate the expression of cooperative or

50   competitive behaviors that are appropriate for the current social context (Taborsky and Oliveira

2012). To date, however, the proximate underpinnings of the behaviors that promote stable

52   cooperation are poorly understood (Soares et al. 2010). Thus, a key question is how

physiological mechanisms modulate behavior in social systems where fitness requires a balance

54   of cooperative and competitive behaviors.

Steroid hormones are well known for their pleiotropic and often antagonistic effects on

56   numerous aspects of physiology and behavior (Ketterson et al. 1996; Wingfield et al. 2001;

Adkins-Regan 2005; Hau 2007). Androgens, in particular, have been well studied in male

58   vertebrates, where they can either facilitate or constrain behavioral plasticity in reproductive and

social contexts (Adkins-Regan 2005; Hau 2007). Yet, the vast majority of work on androgen-

60   mediated traits has focused on archetypal behaviors (e.g., aggression), with very few studies

attempting to link hormones to individual variation in cooperation (Khan et al. 2001; Soares et

62   al. 2010; Díaz-Muñoz et al. 2014). Although studies of cooperative social systems have

documented status-specific differences in circulating testosterone levels (Wingfield et al. 1990;

64   Schoech et al. 1991; Peters et al. 2001; Ryder et al. 2011*b*), including the suppression of

androgens in subordinates (Brouwer et al. 2009), no study has examined how androgens mediate

66   the dynamics of cooperation and status within a social network.

Circulating hormones have long been recognized as a key mechanism that may underlie

68   individual differences in behavior. Mounting evidence suggests that hormonal phenotypes (i.e.,

the expression of consistently low or high hormone levels) are often repeatable and may thus act

70   as a source of heritable variation driving individual differences in behavior (Williams 2008;

Mills et al. 2009; While et al. 2010; van Oers et al. 2011; Iserbyt et al. 2015). However,

72   empirical studies linking among-individual differences in circulating hormones to behavioral

variation are challenging (Niemelä and Dingemanse 2018), in part, because hormone-mediated

74   behaviors are regulated by complex signaling pathways (Adkins-Regan 2005; Ball and

Balthazart 2008; Rosvall et al. 2012; Fuxjager et al. 2018). Indeed, the complexity of steroid-

76   signaling pathways can produce nonlinear dose-dependent responses to circulating hormones,

and these non-monotonic relationships may be key to understanding how hormones shape within

78   and among-individual differences in behavior (Lema 2014).

In addition to the above challenges, both steroid hormones and behavior are intrinsically

80   labile and responsive to the social environment. Steroids not only affect behavior, but they can

also dynamically respond to it, and this social modulation of hormone-behavior relationships is

82   thought to optimize behaviors to the current social landscape (Wingfield et al. 1990; Goymann

2009; Oliveira 2009; Goymann et al. 2019). Engaging in social interactions can rapidly alter

84   circulating testosterone levels, androgen sensitivity in neural pathways, and the patterns of neural

gene expression that influence motivation and shape status-specific behavioral phenotypes

86 (Burmeister et al. 2007; Maruska and Fernald 2010; Fuxjager et al. 2010). Transitions in social

status can likewise alter circulating hormones and their impact on behavioral neural circuits

88 (Romeo et al. 2002; Maruska and Fernald 2010).

In an effort to expand our understanding of how hormones regulate social behavior, we

90 asked how circulating testosterone modulates cooperative behavior in a lek-breeding bird, the

wire-tailed manakin (*Pipra filicauda*; Fig. 1A). Wire-tailed manakins have a social system with

92 two male status classes: territory-holders and floaters who do not yet hold a territory. Males from

both classes form coalitions and perform a cooperative courtship display on territories at long-

94 established lek sites (Heindl 2002). These coalitions are important for reproductive success, as

territorial males with more display partners sire more offspring, and floaters with more partners

96 have a higher probability of eventually inheriting a territory, which is a prerequisite for fitness

(Ryder et al. 2008, 2009). Wire-tailed manakins do not exhibit overt aggression during display

98 bouts, but instead competition maintains dominance hierarchies among coalition partners (Heindl

2002; Ryder et al. 2008). As such, cooperative coalitions have clear fitness benefits, but they also

100 involve male-male competition and play a role in the ascension to territorial status. Many

coalition partnerships are stable from year to year, but males also show substantial day-to-day

102 variation in their social interactions, creating a dynamic social network (Ryder et al. 2011*a*;

Dakin and Ryder 2018). Previous work on this system has established that the status classes

104 differ in their circulating testosterone levels, with territory-holders having higher levels than

floaters (Ryder et al. 2011*b*), and that cooperation is socially contagious (Dakin and Ryder

106 2018).

Our goal in this study was to evaluate the role of a male's circulating testosterone in

108   shaping his behavioral phenotype (Fig. 1A). To quantify cooperative behaviors and identify

coalition partners, we utilized an autonomous behavior-logging system to monitor the social

110   activity and interactions of 180 males (Ryder et al. 2012; Dakin and Ryder 2018). We focused on

three behavioral phenotypes measured for each male on a daily basis (Fig. 1B): "effort",

112   represents the duration of time a male spent attending the leks; "strength", his frequency of

cooperative interactions; and "degree", his number of unique social partners. We consider effort

114   to be a measure of status seeking and status maintenance for floater and territorial males,

respectively, whereas strength and degree are two measures of a male's cooperative tendencies.

116   In addition to repeated measures of behavior, we also collected repeated hormone samples from

210 individuals in our study population to characterize circulating testosterone levels over time;

118   each male was hormone sampled up to three times per field season (Fig. 1C). We used these

hormone data to partition the standing variation in testosterone within and among-individuals,

120   while controlling for capture conditions (Williams 2008; Vernasco et al. 2019), and examined

the relationships between testosterone and behavior.

122   Our analysis evaluates three predictions for the hypothesis that testosterone shapes male

behavioral phenotype (Fig. 1D). First, given that testosterone plays a well-known role promoting

124   competitive ability and aggression in reproductive contexts (Goymann 2009), we consider that

testosterone may be negatively related to cooperative behavior (i.e., "inhibition" in Fig. 1D). In

126   contrast, evidence in humans suggests that testosterone may promote reciprocity and fair

bargaining (Eisenegger et al. 2010; Boksem et al. 2013). As such, our second prediction was that

128   testosterone may be positively related to cooperation (i.e., "facilitation" in Fig. 1D). Third,

substantial evidence suggests that above some threshold, testosterone will no longer be

130   correlated with behavior due to various mechanisms (Grunt and Young 1952; Kohn and Melnick

2002; Adkins-Regan 2005), or it may have detrimental effects on prosocial behaviors (e.g.,

132   parental care; Wingfield et al. 2001). As such, our third prediction was that testosterone may

modulate cooperation in a nonlinear fashion (i.e., inverted U-function in Fig. 1D), whereby

134   individuals with intermediate circulating levels are the most cooperative. Given that wire-tailed

manakins have two status classes, and that social status is known to modulate hormone-behavior

136   relationships in other species (e.g., Maruska and Fernald 2010), our analysis also tested for

status-dependence in the relationship between testosterone and behavior. We also examined the

138   null hypothesis that social behavior may be decoupled from testosterone (i.e., "independence" in

Fig. 1D; Moore et al., 2004; Lynn et al., 2005; Hau, 2007). Finally, to establish a causal

140   relationship between circulating testosterone and cooperation, we paired our observational

analysis with a hormone manipulation experiment involving a subset of territorial males.

142

Methods

144

Study System

146   We studied a population of wire-tailed manakins (*Pipra filicauda*) that has been investigated and

color-banded annually since 2002 at the Tiputini Biodiversity Station in Orellana Province,

148   Ecuador (0° 38' S, 76° 08' W, approx. 200 m elevation). The breeding season lasts up to six

months from November to April. Social status for each individual (i.e., territorial or floater) was

150   determined each year by direct observation (Ryder et al. 2008). Briefly, if an individual was seen

displaying on a territory repeatedly and consistently over several days or weeks, he was assigned

152   as the territory owner. All blood samples and behavioral data were collected during peak

breeding activity (December to March) over three field seasons (2015-16, 2016-17, and 2017-

154   18). Sample sizes were not predetermined, but rather we tried to capture and sample all males at

the studied leks each field season.

156

Testosterone Sampling and Assay

158   Each male manakin was captured using mist-nets up to three times per field season. To maximize

capture rates and minimize disturbance on the display territories (Vernasco et al. 2019), we

160   deployed up to 16 mist-nets simultaneously on a given lek. Nets were checked every 30 minutes.

A previous study demonstrated a subtle but significant effect net time (i.e., time from capture to

162   blood sampling) on circulating testosterone in manakins (Vernasco et al. 2019). Therefore, we

used video monitoring of the mist nets to account for net time in our subsequent statistical

164   analyses (described below). The average span of time between repeated captures was 39 days

(range 8-88 days). Blood samples (75-100uL) were collected from the brachial vein and stored

166   on ice until return from the field when they were centrifuged for 5 min at 10,000 rpm. After

centrifugation, plasma volume was measured to the nearest 0.25ul and stored in 0.75ml of 100%

168   ethanol (Goymann et al. 2007); plasma volumes averaged 38.6uL (SD ± 10.3uL). In the lab,

testosterone was double extracted from plasma with dichloromethane (Ryder et al. 2011*b*). We

170   used direct radioimmunoassay to determine the total plasma testosterone concentration (ng/mL),

adjusted by extraction efficiency and by the plasma volume of the sample (Eikenaar et al. 2011).

172   Any sample that fell below the detection limit was assigned the assay-specific limit of detection

as its testosterone concentration (0.12, 0.08, and 0.09 ng/mL for 2015-16, 2016-17 and 2017-18,

174   respectively). Extraction efficiency ranged from 62-73% and the intra-assay coefficients of

variation were 6.6%, 11.6% and 9.2% for 2015-16, 2016-17 and 2017-18, respectively. The

176   inter-assay coefficient of variation was 19.5%.

178   **Partitioning Variation in Testosterone**

A total of 524 testosterone samples were obtained from 210 individuals (1-8 samples per male;

180   Fig. 1C). To evaluate whether testosterone differed among individuals (Williams 2008; Hau and

Goymann 2015), we fit a mixed-effects model of log-transformed circulating testosterone in the

182   lme4 package (Bates et al. 2018) in R (R Core Team 2018). The model included a bird's ID as a

random effect and controlled for capture conditions that can influence hormone levels by using

184   fixed effects of field season (2015-16, 2016-17, or 2017-18), Julian date within the field season,

time of day, and net time (i.e., see Vernasco et al., 2019). For 51 captures that were missing net

186   time data, we assumed the seasonal average net time (16-19 min) to ensure that the missing

values would not influence the analysis. We then computed the repeatability as the proportion of

188   total variance in circulating testosterone explained by among-individual differences, after

correcting for the fixed effects above. The 95% confidence intervals for repeatability were

190   determined by parametric bootstrapping. To characterize the change in testosterone during social

ascent, we added a fixed effect of four ascent categories to the same analysis (i.e., floaters who

192   did not subsequently ascend, floaters who subsequently acquired a territory, new territory-

holders, and established territory-holders). We then used post-hoc Tukey tests and the

194   Benjamini-Hochberg procedure to compare status and ascent categories.

To examine hormone-behavior relationships, we used "corrected T", which we define as

196   the residuals from the linear model of log-transformed testosterone, after accounting for capture

conditions (i.e., field season, Julian date, time of day, and net time). Next, we used within-

198   subject centering to partition the variation in corrected T within- and among-individuals. The

calculations followed the within-subject centering procedure in (van de Pol and Wright 2009);

200   see Fig. A1 for a detailed illustration of this procedure. Briefly, we first computed each male's

"mean T" as the average of his corrected T values. Next, we computed "relative T" by

202   subtracting each male's mean T from his corrected T values. Hence, mean T is a variable that

captures among-individual variation in testosterone (with one value of mean T per male). In

204   contrast, relative T represents a measure of a male's testosterone in a given blood sample,

relative to his overall average (one value per blood sample).

206

Social Behavior

208   Social behaviors (Fig. 1B) were quantified on the display territories using an automated

proximity data-logging system (described in Ryder et al. 2012; Dakin and Ryder 2018). Males

210   were outfitted with coded nano-tags (NTQB-2, Lotek Wireless; 0.35 g), each of which

transmitted a unique VHF signal ping once per 20 s (range: 19.5-20.5 s). Approximately 10 (±SD

212   7) days after capture, a proximity data-logger (SRX-DL800, Lotek Wireless) was placed within

each territory at that lek to record social behaviors within a detection radius of approximately 30

214   m (i.e., the average diameter of a territory; Ryder et al. 2012; Dakin and Ryder 2018). The data-

loggers recorded all tag pings emitted by males within each territory from 0600 to 1600 local

216   time for ~6 consecutive days (SD ± 1 day). We attempted to recapture males at each lek for

additional blood samples one to two more times per field season, after which social behaviors

218   were again recorded using the procedure described above. Thus, behaviors were monitored after

hormone sampling, and hence we examine the relationship between testosterone levels and

220   subsequent behavior. We recorded a total of 29,760 hours of social activity (2015-16: 49

territories, mean = 16 recording days per territory; 2016-17: 54 territories, mean = 21 days per

222 territory; 2017-18: 48 territories, mean = 22 days per territory, SD = ±4 days in all field seasons).

The number of males tagged for behavioral monitoring was $n_{2015-16} = 100$, $n_{2016-17} = 114$, and

224 $n_{2017-18} = 82$, for a total of $n_{tagged} = 180$ unique individuals. Note that the number tagged

individuals is fewer than the number of blood-sampled males, because some males were captured

226 at unmonitored leks and not tagged. These additional individuals were included in the hormone

repeatability and variance partitioning analysis to obtain more accurate estimates of each effect,

228 but they were not part of the behavioral study.

We defined three behavioral phenotypes. A male's effort, or his rate of lek attendance,

230 was quantified as his number of pings per day at monitored display territories. To define strength

(frequency of cooperative interactions) and degree (number of unique social partners), the

232 proximity data were first filtered to identify times when two males co-occurred on a display

territory as an indication of unique cooperative interactions (Ryder et al. 2008, 2012; Dakin and

234 Ryder 2018). A detailed description of the filtering algorithm is provided in (Dakin and Ryder

2018); a ground-truthing experiment in that study also confirmed that joint detections represent

236 individuals that were on average < 5 m apart. This spatial range corresponds to the visual and

acoustic contact required for a typical display interaction (Heindl 2002). Extensive behavioral

238 observations of our study population have demonstrated that overt aggression (i.e., physical

contact and chasing) is exceedingly rare on the display territories. As such, this automated

240 approach provides a reliable measure of cooperative interactions and increases both the quantity

and quality of social network data relative to other methods (Ryder et al. 2012). An additional

242 validation study also confirmed that the social interactions defined by this automated system

corresponded to cooperative display coalitions that were observed directly (Ryder et al. 2012). In

244   total, we identified 36,885 unique social interactions over the present three-year study. Note that

for floaters, our measures of effort, strength and degree included activity on any territory,

246   whereas for territory-holders, the same measures were limited to a male's activity on his own

territory. Behavioral measures were transformed using $\log(x + 1)$ to meet the assumptions of

248   subsequent statistical analyses, and all measures were significantly repeatable (Dakin and Ryder

2018).

250

Hormone Manipulation Experiment

252   To test whether testosterone has direct effects on male behavior, we conducted a within-subjects

hormone manipulation experiment on a subset of the territory-holding males. We chose the

254   territorial status class for this experiment because their high site fidelity makes them readily

available for post-manipulation behavioral monitoring. Implant surgeries were performed on 14

256   randomly-selected territorial males during the peak of the breeding activity in two field seasons,

2016-17 and 2017-18. Subjects received a subcutaneous 7mm length x 1.47mm internal diameter

258   silastic implant sealed at both ends with ~1mm of silicone. The implants were either filled with

crystalline testosterone (T treatment, n = 9) or left empty (the control, n = 8). Three of the males

260   who were manipulated in 2016-17 also received the opposite treatment in the subsequent field

season (n = 17 implants in total). Implant surgeries were scheduled between data-logger

262   recording sessions, such that each treatment male had a 6-day (SD ± 1 day) pre-implant

behavioral monitoring period within four weeks prior to implantation, and another 6-day (SD ± 1

264   day) post-implant behavioral monitoring period within 10 days after implantation. We verified

that the timing of pre- and post-implant behavioral monitoring did not differ between the two

266   treatment groups (pre-monitoring, p = 0.98; post-monitoring, p = 0.60, Tukey's post-hoc tests on

        linear mixed-effects models).

268   Blood samples were also acquired in the weeks after implantation (mean 13 days, IQR 9-

        15 days) for 17 implant surgeries to verify that the T treatment significantly increased circulating

270   testosterone relative to pre-implant levels (p = 0.04; pre-implant least-squares mean from a

        mixed-effects model = 1.67 ng/mL [95% CI = 0.92, 3.02]; post-implant = 2.93 ng/mL [1.67,

272   5.15]). In contrast, the control implant did not significantly alter circulating testosterone (p =

        0.15; pre-implant = 1.21 ng/mL [95% CI = 0.55, 2.66]; post-implant = 0.57 ng/mL [0.26, 1.25]).

274   Note that although the least-squares mean was lower in the post-implant control group, it was

        still within the 95% CI of the pre-implant control group, indicating no significant change. We

276   also verified that all post-manipulation measures were within the natural (i.e., biologically

        relevant) range (see Fig. 1C and (Ryder et al. 2011*b*)). The average post-implant testosterone

278   level in the T treatment group corresponded to the 78th percentile among unmanipulated

        territory-holding males.

280

        Statistical Analyses

282   Prior to analyzing hormone-behavior relationships in the observational data, we removed data

        from individuals with a single blood sample, because quantifying relative T requires repeated

284   sampling (Appendix Fig. A1). The remaining measures of daily behavior were each assigned a

        relative T value using the bird's most recent prior hormone sample within four weeks (median 12

286   days, IQR 7-18 days). This step filtered the data to 2,363 daily recordings of 125 unmanipulated

        males. Next, we averaged the daily behavioral measures assigned to each blood sample (i.e., one

288   row per unique measure of relative T). The final sample size was 346 hormone-behavior

measures from 125 individuals, including 57 floaters and 76 territory-holders. Note that 56 +

290   76 > 125, because some males were studied at both the floater and territory-holder stage.

To evaluate the hormone-behavior relationships in these data, we used a model selection

292   procedure in the MuMIn package (Bartoń 2018) in R (R Core Team 2018). Specifically, we

evaluated support for competing models representing the hypotheses shown in Fig. 1D and

294   defined in Table A4. All candidate mixed-effects models included a male's identity as a random

effect, as well as fixed effects of field season, Julian date within the field season, temperature

296   (daily averages acquired at the Tiputini Biodiversity Station), the number of days since capture,

social status, and its interaction with testosterone (except in the null model, which did not

298   include testosterone). After the model selection procedure, statistical significance was

determined using the lmerTest package (Kuznetsova et al. 2018) in R. In each case, we also

300   tested for status-dependence of the hormone-behavior relationships by determining whether the

interaction between status and a given testosterone metric was statistically significant. This

302   interaction tested whether the slope of the relationship differed between floater and territorial

males. Given that we found significant status-dependence for all three behaviors, we next

304   repeated the model selection procedure for each status class separately. We present p-values

from the best-fit model for each status class in our main results (see Appendix for complete

306   details on both sets of analyses). To determine the percent of behavioral variance explained by

the testosterone metrics within each status class, we obtained R^2 estimates using the r2glmm

308   package (Jaeger 2017) in R (R Core Team 2018).

To analyze the effect of the hormone manipulation experiment on behavior, we fit mixed-

310   effects models that accounted for a male's identity as a random effect as well as fixed effects of

field season, temperature, and implant (either pre-implant, control, or T treatment; n = 211 daily

312   measures of 14 implanted individuals). We used post-hoc Tukey's tests in the multcomp package

(Hothorn et al. 2017) in R (R Core Team 2018) to compare the treatments, and corrected p-

314   values using the step-up false discovery rate procedure in the multtest package (Pollard et al.

2018). We conducted a further analysis to determine if the effect of the T implant depended on a

316   bird's pre-implant hormone level. To do this, we compared the fit of models with, and without,

the statistical interaction between a male's corrected T and the effect of the implant. We used

318   corrected T for this interaction because the small sample size in the experiment was not

sufficient for variance-partitioning. The corrected T value came from the blood sample taken at

320   the time of surgery.

322   **Results**

Both the floaters and territory-holders expressed a broad range of testosterone levels (Fig. 2A,

324   Table 1). The floaters who successfully acquired a territory had significantly higher levels of

circulating testosterone, as compared to other floaters who did not ascend (p = 0.04, Fig. 2B).

326   Importantly, testosterone levels were significantly repeatable in the population, with 19% of the

standing variation in hormone levels attributed to differences among-individuals. Thus,

328   repeatable differences in hormone levels have the potential to explain at least some of the

among-individual differences in behavior.

330

Table 1. Descriptive statistics for metrics of circulating testosterone. Quartiles are provided

332   because the raw testosterone data have a log-normal distribution[1]. The sample size is n = 524

blood samples from 210 males (247 floater samples, and 277 territory-holder samples).

Testosterone metric	Status class	25th percentile	50th percentile	75th percentile

Testosterone (ng/ml)	Floater	0.17	0.37	1.21
	Territory-holder	0.63	1.62	2.56
Corrected T (residual)	Floater	0.23	0.48	1.61
	Territory-holder	0.73	2.01	3.47
Mean T	Floater	−1.34	−0.65	0.17
	Territory-holder	0.03	0.37	0.89
Relative T	Floater	−0.59	−0.03	0.54
	Territory-holder	−0.52	0.04	0.59

334   [1]See the figshare repository for all data values: https://figshare.com/s/d36c1a5c822e00abc581

336   We found that testosterone could explain significant variation in all three behaviors, but these hormone-behavior relationships were highly status-specific, as evidenced by the significant

338   slope differences between the two status classes (see Fig. 3). For floater males, the individuals with the highest levels of mean T attended the leks more often (effort) and engaged in

340   cooperative interactions more frequently (strength; Fig. 3A-B). Floaters with higher mean T also tended to have more partners, although this relationship was not statistically significant (degree,

342    $p = 0.12$; Fig. 3C). For territory-holding males, the males with higher mean T had significantly lower frequency of cooperative interactions (Fig. 3B), and tended to have fewer partners ($p =$

344   0.08; Fig. 3C). Lek attendance was highest in territory-holders with intermediate levels of mean T (Fig. 3A). In total, we found that the among-individual variation in testosterone (mean T)

346   could explain between 2-8% of the variation (R^2) in behavioral phenotypes within each status class (Fig. 3D). In contrast, the within-individual hormone variation (relative T) had a more

348   limited role, and was primarily related to one behavior, lek attendance (effort), but only in territory-holders (Fig. A2 in the Appendix). The highest level of lek attendance was observed

350   when a territory-holding male's circulating testosterone was closest to his average. Together with the result in Fig. 3A, this indicates that territory-holders reduce their lek attendance above or

352   below some threshold of mean T and relative T.

The territorial males with experimentally-elevated testosterone significantly decreased

354   their cooperative behavior, both in terms of the number of display partners (degree), and the

frequency of cooperative interactions (strength) they received, although the manipulation did not

356   significantly affect their territory attendance (effort; Figure 4A-B). These experiment results are

consistent with the correlative analysis (see Fig. 3), and confirm that testosterone levels above

358   some threshold have a direct negative impact on a territorial male's ability to maintain

cooperative partnerships. Further analysis revealed that the effects of the T treatment also

360   depended on a male's pre-implant hormone level, as males with low testosterone prior to the

implant lost more cooperative interactions and partners (strength and degree) than did males with

362   initially high pre-implant testosterone (Fig. 4C).

364   **Discussion**

The fitness of social animals is often contingent on the ability to express contextually relevant

366   behaviors in response to a dynamic social environment. In the social system of wire-tailed

manakins, floater males must cooperate with territory-holders and compete with other floaters

368   for limited opportunities to acquire a territory (Ryder et al. 2008). However, once an individual

ascends to territorial status, he must behaviorally shift his focus to attracting partners and

370   building stable coalition partnerships on his own territory, because females prefer more

cooperative individuals (Ryder et al. 2009). As such, selection is expected to favor endocrine

372   mechanisms that promote behavioral plasticity to respond appropriately to conspecifics and

ascension opportunities (Kelly and Vitousek 2017). Although we know that hormones can

374   coordinate both within- and among-individual plasticity in behavior and social competence

(Dufty et al. 2002; Hau and Goymann 2015), the vast majority work on androgens has focused

376 on how they facilitate courtship, aggression, competitive abilities, and dominance (Ketterson et

al. 1996; Wingfield et al. 2001; Fusani 2008).

378   Here, we show that testosterone, an androgen long-known for its effect on aggression,

can also influence social behavior in a cooperative system. Higher average testosterone (mean T)

380 was associated with an increased frequency of cooperation in floaters (Fig. 3). Therefore, it does

not appear to antagonize cooperation, but instead may facilitate the social behaviors necessary

382 for territory acquisition (Fig. 1D). Indeed, circulating testosterone was also predictive of which

floaters ascended to territorial status, as the floaters that gained territories had higher testosterone

384 than those who did not obtain territories, both pre- and post-ascension (Fig. 2). Given that

territory ownership and coalition partnerships are both necessary for a male to sire offspring in

386 this species (Ryder et al. 2008), these results suggest that high testosterone during the floater

stage can yield later fitness benefits. Additional experiments are required to decouple the effects

388 of age and testosterone on these correlative results, because the floaters that successfully ascend

the age-graded status queue also tend to be older. Experiments that manipulate hormones or

390 present social stimuli are also needed to determine whether higher testosterone causes status-

seeking and cooperation in floaters, and/or whether testosterone rises in response to these

392 behaviors.

      Once a male acquires a territory, his fitness depends on both the number of cooperative

394 partners and the stability of those partnerships (Ryder et al. 2009, 2011a). The results of our

hormone manipulation experiment on territorial males confirmed that when testosterone in a

396 territorial male is too high, it compromises his ability to attract and maintain stable display

partnerships (Fig. 4). Hence, testosterone levels above some threshold at this stage could

398 decrease reproductive fitness. Our correlative data also support this hypothesis, as territorial

males with lower mean T exhibited the optimal combination of social behaviors (i.e., high effort,

400   strength, and degree in Fig. 3). Therefore, testosterone levels appear to be ultimately constrained

in territory-holders despite their potential benefits at the floater stage.

402   A key finding of our study is that the nature of hormone-behavior relationships depends

on a male's social status (Fig. 3), with all predictions in Fig. 1D represented, depending on the

404   behavior in question and status of the individuals. This finding suggests that a reorganization of

the hormone-regulatory pathways involving testosterone may be required to optimize status-

406   specific social behaviors as males transition from floater to territorial. These changes may result

from a number of physiological mechanisms that are not mutually exclusive. One possibility is

408   that testosterone is only antagonistic to cooperative behavior above some concentration threshold

that is not achieved by most floater males, and thus differential regulation of testosterone

410   secretion according to social status is essential. The threshold at which testosterone inhibits

cooperation may also depend, in part, on an individual's hormonal phenotype or current

412   endocrine status. This possibility is supported by the fact that the negative effect of T-implants

on cooperative behavior was greater for territory-holders with lower pre-implant testosterone

414   (Fig. 4C). Another possibility is that status-specific expression of steroidogenic enzymes and/or

steroid receptors can cause among-individual and/or status-specific differences in the neural

416   sensitivity to testosterone (Romeo et al. 2002; Burmeister et al. 2007; Rosvall et al. 2012; Horton

et al. 2014). Finally, testosterone can also have downstream effects on the release or actions of

418   other neurohormones known to influence social behavior, including steroid-sensitive

neuropeptide systems (e.g., arginine vasotocin, and mesotocin; Kimura et al. 1999; De Vries and

420   Panzica 2006; Kabelik et al. 2010), and these pathways may vary between status classes. Each of

these hypotheses represents a promising direction for identifying how variation in the hormone

422 signaling pathways facilitates cooperation in the manakin lineage. Future work examining status-specific neuroendocrine gene expression and its relationship with behavior in this species is a

424 pivotal next step.

Our repeated hormone sampling efforts also revealed that individual males differ

426 significantly in their circulating testosterone (corrected T; Fig. 1C). This finding of significant repeatability is consistent with growing evidence that hormonal phenotypes are often repeatable

428 and heritable in other systems (Williams 2008; Mills et al. 2009; While et al. 2010; van Oers et al. 2011; Iserbyt et al. 2015), yet robust estimates from wild populations, like those presented

430 here, are still exceedingly rare (Cox et al. 2016). Although the proportion of behavioral variation explained by among-individual variation in testosterone (mean T) was small at 2-8% (Fig. 3D),

432 these R^2 values are likely underestimates because of the measurement error associated with a limited number of repeat hormone samples (mean 2.5 blood samples per male, IQR 1-4; Fig.

434 1C). Moreover, a recent meta-analysis estimated that hormone levels generally account for only 2% of the variation in behavior, on average (Niemelä and Dingemanse 2018). Thus, the fact that

436 among-individual differences in circulating hormones can account for as much as 8% of the variation in the behavior of a free-living animal is an important result (Ball and Balthazart,

438 2008).

The social transmission of behaviors is hypothesized to play an important role in the

440 evolution of cooperation (Trivers 1971; Rankin and Taborsky 2009). Our previous work has established that manakin cooperative behaviors are indeed socially contagious, whereby the

442 cooperative behaviors of one individual stimulate subsequent cooperation in social partners (Dakin and Ryder 2018). Here, we provide evidence that testosterone can influence the

444 expression of socially-contagious behavior, in particular the frequency at which territory-holding

males engage in cooperative interactions (Figs. 3-4). This raises the hypothesis for future study

446 that variation in testosterone levels may influence the collective structure and assortment of the

social network. Because most coalition partnerships involve a territory-holder and a floater

448 (Ryder et al. 2011*a*), status-specific differences in how testosterone mediates behavior may

promote a stable social network, by facilitating cooperation between males of different status

450 classes.

A recently proposed framework suggests that aggression and sociality are inextricably

452 linked and modulated by the same physiological and neural systems (Kelly and Vitousek 2017).

In humans, the effects of testosterone depend on social status; testosterone can drive aggression

454 when individuals are socially challenged, but drive prosocial behaviors in the absence of those

challenges (Boksem et al. 2013). Moreover in other vertebrate taxa, changes in social status may

456 often involve restructuring of the neural circuits and thus the way in which hormones mediate

social behaviors (Romeo et al. 2002; Burmeister et al. 2007; Maruska and Fernald 2011;

458 Maruska 2015; Fernald 2015). In parallel with these discoveries, we show here that both the

optimal level of testosterone and the way it modulates behavior may depend on a male's social

460 status. Overall, our findings support the hypothesis that status-dependent effects of androgens on

social behavior may be a general feature of cooperative social systems where fitness depends on

462 behavioral plasticity.

References

464 Adkins-Regan, E. 2005. Hormones and animal social behavior. Princeton University Press.

Ball, G. F., and J. Balthazart. 2008. Individual variation and the endocrine regulation of
466 behaviour and physiology in birds: a cellular/molecular perspective. Philosophical
Transactions of the Royal Society B: Biological Sciences 363:1699–1710.

468 Bartoń, K. 2018. MuMIn: multi-model inference.

Bates, D., M. Maechler, B. Bolker, S. Walker, R. H. B. Christensen, H. Singmann, B. Dai, et al.
470 2018. lme4: linear mixed-effects models using "Eigen" and S4.

Bergmuller, R., R. Schurch, and I. M. Hamilton. 2010. Evolutionary causes and consequences of
472 consistent individual variation in cooperative behaviour. Philosophical Transactions of
the Royal Society B 365:2751–2764.

474 Boksem, M. A. S., P. H. Mehta, B. Van den Bergh, V. van Son, S. T. Trautmann, K. Roelofs, A.
Smidts, et al. 2013. Testosterone Inhibits trust but promotes reciprocity. Psychological
476 Science 24:2306–2314.

Brouwer, L., T. G. G. Groothuis, O. Vedder, C. Eikenaar, D. S. Richardson, and J. Komdeur.
478 2009. Do primary males physiologically suppress subordinate males? An experiment in a
cooperatively breeding passerine. Ethology 115:576–587.

480 Burmeister, S. S., V. Kailasanath, and R. D. Fernald. 2007. Social dominance regulates androgen
and estrogen receptor gene expression. Hormones and Behavior 51:164–170.

482 Clutton-Brock, T. 2009. Cooperation between non-kin in animal societies. Nature 462:51–57.

Cockburn, A. 1998. Evolution of helping behavior in cooperatively breeding birds. Annual
484 Review of Ecology and Systematics 29:141–177.

Cox, R. M., J. W. McGlothlin, and F. Bonier. 2016. Hormones as mediators of phenotypic and
486 genetic integration: an evolutionary genetics approach. Integrative and Comparative
Biology 56:126–137.

488 Dakin, R., and T. B. Ryder. 2018. Dynamic network partnerships and social contagion drive
cooperation. Proceedings of the Royal Society B: Biological Sciences 285:20181973.

490 De Vries, G. J., and G. C. Panzica. 2006. Sexual differentiation of central vasopressin and
vasotocin systems in vertebrates: different mechanisms, similar endpoints. Neuroscience,
492 Neuroactive Steroids: Old Players in a New Game 138:947–955.

Díaz-Muñoz, S. L., E. H. DuVal, A. H. Krakauer, and E. A. Lacey. 2014. Cooperating to
494 compete: altruism, sexual selection and causes of male reproductive cooperation. Animal
Behaviour 88:67–78.

496 Dufty, A. M., J. Clobert, and A. P. Møller. 2002. Hormones, developmental plasticity and
adaptation. Trends in Ecology & Evolution 17:190–196.

498 Eikenaar, C., M. Whitham, J. Komdeur, M. van der Velde, and I. T. Moore. 2011. Endogenous
testosterone is not associated with the trade-off between paternal and mating effort.
500 Behavioral Ecology 22:601–608.

Eisenegger, C., M. Naef, R. Snozzi, M. Heinrichs, and E. Fehr. 2010. Prejudice and truth about
502 the effect of testosterone on human bargaining behaviour. Nature 463:356–359.

Fernald, R. D. 2015. Social behaviour: Can it change the brain? Animal Behaviour 103:259–265.

504 Fusani, L. 2008. Testosterone control of male courtship in birds. Hormones and Behavior
54:227–233.

506 Fuxjager, M. J., R. M. Forbes-Lorman, D. J. Coss, C. J. Auger, A. P. Auger, and C. A. Marler.
2010. Winning territorial disputes selectively enhances androgen sensitivity in neural

508   pathways related to motivation and social aggression. Proceedings of the National Academy of Sciences 107:12393–12398.

510   Fuxjager, M. J., M. C. Miles, and B. A. Schlinger. 2018. Evolution of the androgen-induced male phenotype. Journal of Comparative Physiology A 204:81–92.

512   Goymann, W. 2009. Social modulation of androgens in male birds. General and Comparative Endocrinology, 9th International Symposium on Avian Endocrinology 163:149–157.

514   Goymann, W., I. T. Moore, and R. F. Oliveira. 2019. Challenge hypothesis 2.0: a fresh look at an established idea. BioScience 69:432–442.

516   Goymann, W., I. Schwabl, M. Trappschuh, and M. Hau. 2007. Use of ethanol for preserving steroid and indoleamine hormones in bird plasma. General and Comparative
518   Endocrinology 150:191–195.

Grunt, J. A., and W. C. Young. 1952. Differential reactivity of individuals and the response of
520   the male guinea pig to testosterone propionate. Endocrinology 51:237–248.

Hau, M. 2007. Regulation of male traits by testosterone: implications for the evolution of
522   vertebrate life histories. BioEssays 29:133–144.

Hau, M., and W. Goymann. 2015. Endocrine mechanisms, behavioral phenotypes and plasticity:
524   known relationships and open questions. Frontiers in Zoology 12:S7.

Heindl, M. 2002. Social organization on leks of the wire-tailed manakin in southern venezuela.
526   The Condor 104:772–779.

Horton, B. M., W. H. Hudson, E. A. Ortlund, S. Shirk, J. W. Thomas, E. R. Young, W. M.
528   Zinzow-Kramer, et al. 2014. Estrogen receptor α polymorphism in a species with alternative behavioral phenotypes. Proceedings of the National Academy of Sciences
530   111:1443–1448.

Hothorn, T., F. Bretz, P. Westfall, R. M. Heiberger, A. Schuetzenmeister, and S. Scheibe. 2017.
532   multcomp: simultaneous inference in general parametric models.

Iserbyt, A., M. Eens, and W. Müller. 2015. Sexually antagonistic selection during parental care is
534   not generated by a testosterone-related intralocus sexual conflict–insights from full-sib comparisons. Scientific Reports 5:17715.

536   Jaeger, B. 2017. r2glmm: computes R squared for mixed (multilevel) models.

Kabelik, D., J. A. Morrison, and J. L. Goodson. 2010. Cryptic regulation of vasotocin neuronal
538   activity but Not anatomy by sex steroids and social stimuli in opportunistic desert finches. Brain, Behavior and Evolution 75:71–84.

540   Kelly, A. M., and M. N. Vitousek. 2017. Dynamic modulation of sociality and aggression: an examination of plasticity within endocrine and neuroendocrine systems. Philosophical
542   Transactions of the Royal Society B: Biological Sciences 372:20160243.

Ketterson, E. D., V. Nolan, M. J. Cawthorn, P. G. Parker, and C. Ziegenfus. 1996. Phenotypic
544   engineering: using hormones to explore the mechanistic and functional bases of phenotypic variation in nature. Ibis 138:70–86.

546   Khan, M. Z., F. M. A. McNabb, J. R. Walters, and P. J. Sharp. 2001. Patterns of testosterone and prolactin concentrations and reproductive behavior of helpers and breeders in the
548   cooperatively breeding red-cockaded woodpecker (*Picoides borealis*). Hormones and Behavior 40:1–13.

550   Kimura, T., K. Okanoya, and M. Wada. 1999. Effect of testosterone on the distribution of vasotocin immunoreactivity in the brain of the zebra finch, *Taeniopygia guttata*
552   *castanotis*. Life Sciences 65:1663–1670.

554 Kohn, M. C., and R. L. Melnick. 2002. Biochemical origins of the non-monotonic receptor-mediated dose-response. Journal of Molecular Endocrinology 29:113–123.

556 Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2018. lmerTest: tests in linear mixed effects models.

558 Lema, S. C. 2014. Hormones and phenotypic plasticity in an ecological context: linking physiological mechanisms to evolutionary processes. Integrative and Comparative Biology 54:850–863.

560 Lynn, S. E., B. G. Walker, and J. C. Wingfield. 2005. A phylogenetically controlled test of
562 hypotheses for behavioral insensitivity to testosterone in birds. Hormones and Behavior 47:170–177.

564 Maruska, K. P. 2015. Social transitions cause rapid behavioral and neuroendocrine changes. Integrative and Comparative Biology 55:294–306.

566 Maruska, K. P., and R. D. Fernald. 2010. Behavioral and physiological plasticity: rapid changes during social ascent in an African cichlid fish. Hormones and Behavior 58:230–240.

568 ———. 2011. Social regulation of gene expression in the hypothalamic-pituitary-gonadal axis. Physiology 26:412–423.

570 Mills, S. C., A. Grapputo, I. Jokinen, E. Koskela, T. Mappes, T. A. Oksanen, and T. Poikonen. 2009. Testosterone-mediated effects on fitness-related phenotypic traits and fitness. The American Naturalist 173:475–487.

572 Moore, I. T., H. Wada, N. Perfito, D. S. Busch, T. P. Hahn, and J. C. Wingfield. 2004.
574 Territoriality and testosterone in an equatorial population of rufous-collared sparrows, Zonotrichia capensis. Animal Behaviour 67:411–420.

576 Niemelä, P. T., and N. J. Dingemanse. 2018. Meta-analysis reveals weak associations between intrinsic state and personality. Proceedings of the Royal Society B: Biological Sciences 285:20172823.

578 Oliveira, R. F. 2009. Social behavior in context: hormonal modulation of behavioral plasticity and social competence. Integrative and Comparative Biology 49:423–440.

580 Peters, A., L. B. Astheimer, and A. Cockburn. 2001. The annual testosterone profile in
582 cooperatively breeding superb fairy-wrens, *Malurus cyaneus*, reflects their extreme infidelity. Behavioral Ecology and Sociobiology 50:519–527.

584 Pollard, K. S., H. N. Gilbert, Y. Ge, S. Taylor, and S. Dudoit. 2018. multtest: resampling-based multiple hypothesis testing.

586 R Core Team. 2018. R 3.5.1: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

588 Rankin, D. J., and M. Taborsky. 2009. Assortment and the evolution of generalized reciprocity. Evolution 63:1913–1922.

590 Romeo, R. D., H. N. Richardson, and C. L. Sisk. 2002. Puberty and the maturation of the male brain and sexual behavior: recasting a behavioral potential. Neuroscience & Biobehavioral Reviews 26:381–391.

592 Rosvall, K. A., C. M. B. Burns, J. Barske, J. L. Goodson, B. A. Schlinger, D. R. Sengelaub, and
594 E. D. Ketterson. 2012. Neural sensitivity to sex steroids predicts individual differences in aggression: implications for behavioural evolution. Proceedings of the Royal Society of London B: Biological Sciences rspb20120442.

596 Ryder, T. B., J. G. Blake, P. G. Parker, and B. A. Loiselle. 2011*a*. The composition, stability, and kinship of reproductive coalitions in a lekking bird. Behavioral Ecology 22:282–290.

598   Ryder, T. B., B. M. Horton, and I. T. Moore. 2011*b*. Understanding testosterone variation in a tropical lek-breeding bird. Biology Letters 7:506–509.

600   Ryder, T. B., B. M. Horton, M. van den Tillaart, J. D. D. Morales, and I. T. Moore. 2012. Proximity data-loggers increase the quantity and quality of social network data. Biology

602   Letters 8:917–920.

Ryder, T. B., D. B. McDonald, J. G. Blake, P. G. Parker, and B. A. Loiselle. 2008. Social

604   networks in the lek-mating wire-tailed manakin (*Pipra filicauda*). Proceedings of the Royal Society B 275:1367–1374.

606   Ryder, T. B., P. G. Parker, J. G. Blake, and B. A. Loiselle. 2009. It takes two to tango: reproductive skew and social correlates of male mating success in a lek-breeding bird.

608   Proceedings of the Royal Society B 276:2377–2384.

Schoech, S. J., R. L. Mumme, and M. C. Moore. 1991. Reproductive endocrinology and

610   mechanisms of breeding inhibition in cooperatively breeding Florida scrub jays (*Aphelocoma c. coerulescens*). The Condor 93:354–364.

612   Soares, M. C., R. Bshary, L. Fusani, W. Goymann, M. Hau, K. Hirschenhauser, and R. F. Oliveira. 2010. Hormonal mechanisms of cooperative behaviour. Philosophical

614   Transactions of the Royal Society B 365:2737–2750.

Taborsky, B., and R. F. Oliveira. 2012. Social competence: an evolutionary approach. Trends in

616   Ecology & Evolution 27:679–688.

Trivers, R. L. 1971. The evolution of reciprocal altruism. The Quarterly Review of Biology

618   46:35–57.

van de Pol, M., and J. Wright. 2009. A simple method for distinguishing within- versus between-

620   subject effects using mixed models. Animal Behaviour 77:753–758.

van Oers, K., K. L. Buchanan, T. E. Thomas, and P. J. Drent. 2011. Correlated response to

622   selection of testosterone levels and immunocompetence in lines selected for avian personality. Animal Behaviour 81:1055–1061.

624   Vernasco, B. J., B. M. Horton, T. B. Ryder, and I. T. Moore. 2019. Sampling baseline androgens in free-living passerines: Methodological considerations and solutions. General and

626   Comparative Endocrinology, Endocrinology of Neotropical Vertebrates 273:202–208.

While, G. M., C. Isaksson, J. McEvoy, D. L. Sinn, J. Komdeur, E. Wapstra, and T. G. G.

628   Groothuis. 2010. Repeatable intra-individual variation in plasma testosterone concentration and its sex-specific link to aggression in a social lizard. Hormones and

630   Behavior 58:208–213.

Williams, T. D. 2008. Individual variation in endocrine systems: moving beyond the 'tyranny of

632   the Golden Mean.' Philosophical Transactions of the Royal Society B: Biological Sciences 363:1687–1698.

634   Wingfield, J. C., R. E. Hegner, Dufty Alfred M., and G. F. Ball. 1990. The "challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating

636   systems, and breeding strategies. The American Naturalist 136:829–846.

Wingfield, J. C., S. E. Lynn, and K. K. Soma. 2001. Avoiding the 'costs' of testosterone:

638   ecological bases of hormone-behavior interactions. Brain, Behavior and Evolution 57:239–251.

640

26

Author contributions

T.B.R., B.M.H., and I.T.M. designed the research

T.B.R., R.D., B.M.H., B.J.V., and I.T.M. performed the research

R.D., T.B.R., and B.S.E. analyzed the data

T.B.R. and R.D. wrote the manuscript

All authors edited the manuscript

Competing interests

The authors declare no competing interests.

Additional information

See the Appendix PDF for Figures A1-A2 and Tables A1-A9.

Data Availability

All materials necessary to reproduce the results including data and R code are available for reviewers at the following link and will be made public upon acceptance:

https://figshare.com/s/d36c1a5c822e00abc581

Ethics

All methods were approved by the Smithsonian ACUC (protocols #12-23, 14-25, and 17-11) and the Ecuadorean Ministry of the Environment (MAE-DNB-CM-2015-0008).

FIGURE LEGENDS

672

Fig. 1. We studied how testosterone influences cooperative behavior in a social network. (A) Wire-tailed manakins have a social system in which males of two status classes, territory-holders (black) and floaters (red), form cooperative display coalitions. Once a male attains territorial status, he retains it for life. (B) We used an automated proximity system to quantify three social behaviors important for male fitness: effort is a measure of lek attendance; strength is the frequency of cooperative interactions; and degree is the number of unique cooperative partners; each was measured on a daily basis. The violin plots show the distributions of individual means (n = 180 individuals); the vertical lines are the medians. See Tables A1 and A2 in the Appendix for additional data. (C) We also collected repeated testosterone samples from 210 individuals (inset; n = 524 blood samples). Analysis of these repeated measures revealed that circulating testosterone (T) varies both among- and within-individuals, allowing us to partition these two sources of variation (Fig. A1 and Table A3 in the Appendix). The plot in (C) shows log-transformed testosterone for males with >1 hormone sample; each bar represents a single male, ordered along the x-axis by his average testosterone after correcting for capture conditions. Each individual's bar spans the 25th and 75th quantiles and is colored according to his status; the dark line connects the medians. (D) We used these data to evaluates the predictions that testosterone may inhibit, facilitate, or have nonlinear (dose-dependent) effects on cooperation; the null hypothesis is that behavior is independent of circulating testosterone. We also considered that these hormone-behavior relationships may differ between the two status classes in (A).

692

Fig. 2. Elevated testosterone is associated with ascension to territorial status. (A)

694   Histograms and probability density curves for log-transformed circulating testosterone in the two

status classes (n = 524 samples). (B) Change in corrected T during ascension to territorial status.

696   Floater males who acquired a territory the following field season had significantly higher

testosterone than floaters who did not acquire a territory. The data points show partial residuals

698   from an analysis that controls for field season and capture conditions (n = 524 measures from

210 individuals, including 15 who acquired territories during the study). Different letters indicate

700   statistically significant Tukey contrasts, after correcting for multiple comparisons.

702   **Fig. 3. Social behavior is related to among-individual variation in circulating testosterone.**

(A-C) Mean T is a measure of among-individual variation in circulating testosterone. Floaters

704   with the highest mean T had greater lek attendance (effort), cooperated with other males at

higher frequencies (strength), and had more unique cooperative partners (degree), although the

706   relationship with degree was not statistically significant (n = 57 floaters). In contrast, territory-

holding males with the highest mean T had lower lek attendance, a lower frequency of

708   cooperation, and fewer partners, although again the relationship with degree was not quite

significant (n = 76 territory-holders). Each scatterplot shows the residuals (y-axis) from the best-

710   fit model after accounting for other predictors; note that all phenotypes were analyzed on a log-

scale. Red and grey asterisks indicate statistical significance within floaters and territory-holders,

712   respectively; the black asterisks compare hormone-behavior relationships between the two status

classes (n.s. non-significant; * p < 0.05; ** p < 0.01). (D) The percent of behavioral variance

714   (R^2) in each phenotype attributed to mean T. Further details are provided in Tables A4-A7 in the

Appendix.

716

Fig. 4. Experimental elevation of testosterone decreased cooperation in territory-holders.

718   Territorial males were given control (n = 8) or testosterone (n = 9) implants. (A) Average pre-

and post-manipulation measures of lek attendance (effort), the frequency of cooperative

720   interactions (strength), and the number of partners (degree). Lines connect data from the same

individual. The repeatability of each behavior, R, is given above each panel. (B) Standardized

722   effect sizes from the statistical analysis (Control treatment, C post vs. pre; T treatment, T post vs.

pre; and a comparison of the T treatment and the Control, T vs. C). Error bars show 95%

724   confidence intervals. Experimentally-elevated testosterone decreased the frequency of

cooperative interactions (strength) and the number of unique partners (degree), because T post

726   vs. pre and T vs. C were both statistically significant after correcting for multiple comparisons

(n.s. non-significant; * p < 0.05; ** p < 0.01; *** p < 0.001). The sample size for this analysis

728   was n = 211 daily measures of 14 individuals. (C) Further analysis indicated that the effect of

elevated testosterone on cooperative behavior (strength and degree) depended on the male's pre-

730   treatment corrected testosterone level. Specifically, stronger negative effects were observed for

territory-holding males with lower pre-implant testosterone. See Tables A8-A9 in the Appendix

732   for additional data. Standardized effects sizes are shown to be comparable across the figure.
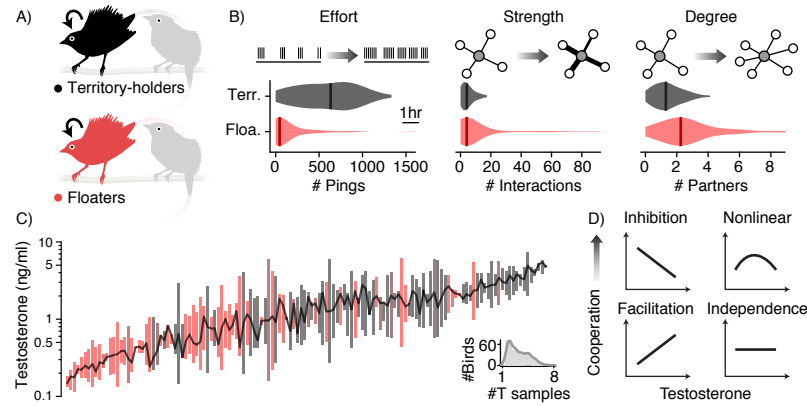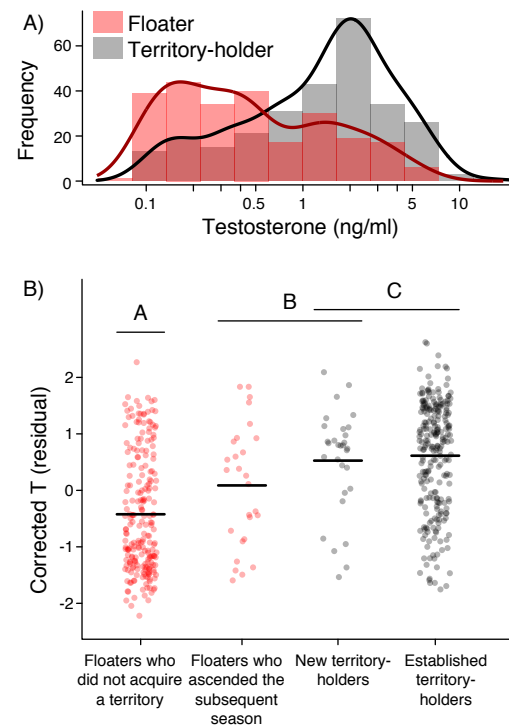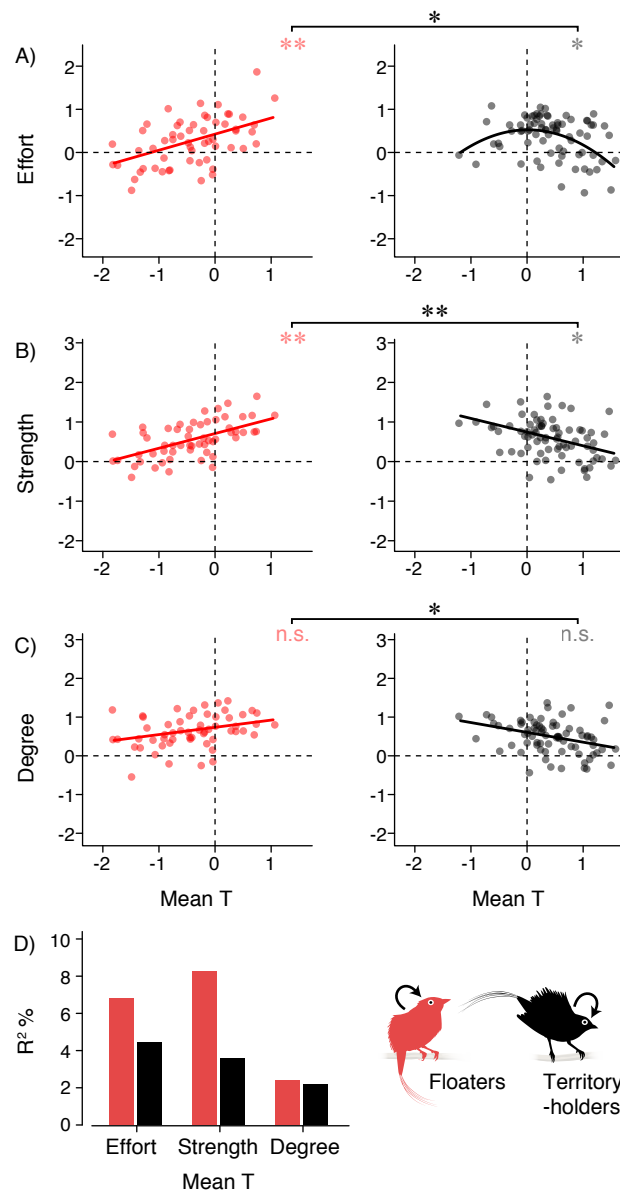
Fig. 1

Fig. 2

Fig. 3

Fig. 4