

Biological Sciences

2 Physiology/Ecology

## 4 **Testosterone drives status-specific patterns of cooperation and transmission of behavior in a social network**

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**Short title:** Testosterone modulates cooperation

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### ETHICS

30 All methods were approved by the Smithsonian ACUC (protocol #17-11) and the Ecuadorean  
Ministry of the Environment (MAE-DNB-CM-2015-0008).

32

### DATA AVAILABILITY

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

36 <https://figshare.com/s/d36c1a5c822e00abc581>

### 38 AUTHOR CONTRIBUTIONS

TBR, BMH, and ITM designed research

40 TBR, RD, BMH, BJV, and ITM performed research

RD, TBR, and BSE analyzed the data

42 TBR and RD wrote the manuscript

All authors edited the manuscript

44

### CONFLICT OF INTEREST

46 We have no conflict of interest.

## Abstract

48 Stable social partnerships require physiological mechanisms that maintain a balance between  
50 competition and cooperation. Although the endocrine system is known to facilitate competitive  
52 behavior, we know very little about how steroid hormones affect cooperation and the stability of  
54 partnerships. Here, we examine how testosterone (T) modulates social behavior in wire-tailed  
56 manakins (*Pipra filicauda*), a species in which territorial and non-territorial males form  
58 cooperative coalitions within a larger social network. Our approach links repeated hormone  
60 sampling and a hormone-manipulation experiment with an automated telemetry system that  
62 captured >30,000 male-male social interactions. Using 528 repeated T samples from 209 males,  
64 we find that circulating T is repeatable with individual differences accounting for 19% of the  
66 total variation, and that among-individual differences in baseline T can explain 17% of the  
68 variation in cooperative behavior. Patterns of hormone-mediated behavior were status-specific  
whereby T significantly increased cooperative behaviors in floaters whereas it tended to decrease  
the same behaviors in territorial males. Experimental elevation of T caused significant decreases  
in both the number of partners and frequency of cooperative interactions received by territory-  
holders, with the strongest effect observed in lower-T individuals. This status-specificity  
suggests that hormone-mediated behavior is under stabilizing selection and that ontogenetic  
plasticity in hormone-regulatory networks facilitated the evolution of a stable cooperative  
society. Our analyses also reveal that one male's T can indirectly affect the behavior of other  
individuals with whom he interacts (explaining 1-3% of variation), indicating that hormones are  
a mechanism underlying the transmission of behavior in social networks.

## 70 Significance Statement (max 120 words)

72 The hormone testosterone is often linked to aggressive behaviors that aid in competition. In  
74 cooperative societies, hormone-mediated behavior must be modified to allow stable partnerships.  
76 We studied testosterone in the wire-tailed manakin, a gregarious bird whose cooperative  
78 partnerships are crucial for male mating success. Using observational and experimental data, we  
show that testosterone has opposing effects on male manakins from two different status classes.  
Older, territory-holding males form fewer cooperative partnerships after their testosterone is  
experimentally increased, whereas testosterone in young males is positively correlated with  
social behavior. We also show that circulating hormones may underlie how behaviors are  
socially transmitted across social networks. Our results highlight that developmental changes in  
the androgen system can facilitate a stable cooperative society.

82 What are the proximate and ultimate drivers of cooperation among non-kin (Clutton-Brock,  
2009)? The stability of these partnerships is predicated on biological mechanisms that maintain a  
84 delicate balance between self-interest and the interests of social partners. In systems where  
cooperation is used as a reproductive strategy, group members often differ in status, behavior,  
86 and physiology (Stacey and Koenig, 1990). These repeatable differences among individuals can  
facilitate stable cooperation (Bergmuller et al., 2010) as well as the dominance hierarchies that  
88 ultimately determining an individual's status and reproductive success (Ryder et al., 2008; Ryder  
et al., 2009). However, within-individual plasticity is also essential to competition and  
90 cooperation, as it allows individuals to adjust their behavior to the current social environment  
(Taborsky and Oliveira, 2012). Thus, a key question is how selection has shaped hormone-  
92 mediated behavior to balance cooperation and competition when both are important for fitness.

Steroid hormones are well known for their pleiotropic effects on reproductive traits  
94 (Ketterson et al., 1996; Wingfield et al., 2001; Adkins-Regan, 2005; Hau, 2007), but can also act  
as potent facilitators of social behavior (Oliveira, 2004). Testosterone, in particular, has been  
96 well studied in male vertebrates, where it can either facilitate or constraint the adaptive plasticity  
of diverse physiological, reproductive, and social behaviors (Adkins-Regan, 2005; Hau, 2007).  
98 Although heritable variation in endocrine systems has been hypothesized to be a key mechanism  
underlying individual differences in behavior, the supporting evidence is limited (Adkins-Regan,  
100 2005; Ball and Balthazart, 2008). To date, the vast majority of the work on hormone-behavior  
relationships has focused on archetypal behaviors (e.g., aggression) that occur in simple social  
102 systems. As a result, the role of steroids in shaping complex behaviors, like cooperation, remain  
virtually unknown (Soares et al., 2010).

104 Our understanding of how hormones modulate social behavior is further complicated by  
the fact that both steroid hormones and behavior are intrinsically labile and responsive to the  
106 social environment. For example, steroids not only affect behavior, but they can also  
dynamically respond to it, and this social modulation is thought to optimize behaviors for the  
108 current social environment (Wingfield et al., 1990; Goymann, 2009; Oliveira, 2009).  
Furthermore, the behaviors expressed by one individual can affect the behaviors of others (Wolf  
110 et al., 1998), and hormones are one mechanism through which social influence and transmission  
of behavior could occur. Thus, understanding complex hormone-mediated traits requires a  
112 repeated sampling design and analytical approaches that enable partitioning of within- and  
among-individual phenotypic variation, while accounting for the dynamic social environment  
114 (Dingemanse and Araya-Ajoy, 2015).

Here, we designed such a study to evaluate how testosterone modulates cooperative  
116 behavior and social influence in the wire-tailed manakin (*Pipra filicauda*; Fig. 1A). Wire-tailed  
manakins have a complex social system in which territorial and non-territorial (floater) males  
118 differ in their circulating testosterone and behavior (Fig. 1A-B; Ryder et al., 2008; Ryder et al.,  
2011b). Manakins in both status classes also interact to perform cooperative displays and overt  
120 aggression among males has only been rarely observed over 16 years of study. Previous work  
has demonstrated that floater males with many cooperative partners have an increased  
122 probability of territory inheritance, a prerequisite for mating (Ryder et al., 2008), and territorial  
males with more partners have increased reproductive success (Ryder et al., 2009). These  
124 partnerships are not based on kinship and although they can last for many years, there is also  
substantial day-to-day variation in the social network of male manakins (Ryder et al., 2011a;  
126 Dakin and Ryder, preprint in review).

128 Our goal in this study was to evaluate the relative contribution of three hormonal  
129 mechanisms of social behavior: (i) within- and (ii) among -individual standing variation in  
130 circulating testosterone (loop arrows in Fig. 1A); as well as (iii) the hormonal phenotype of a  
131 male's social partner(s) as a mechanism of social influence (straight arrows in Fig. 1A). Towards  
132 this end, we collected repeated measures of circulating testosterone from a large population of  
133 209 male manakins and verified that hormonal concentrations varied both within and among  
134 individuals (Fig. 1C-D). To match these hormone samples to repeated behaviors, we developed  
135 an autonomous data-logging system to monitor social behavior and coalition partnerships that  
136 occur on the territories where male manakins perform their cooperative displays (Ryder et al.,  
2012; Dakin and Ryder, preprint in review).

We used these data to quantify a suite of six behavioral phenotypes that capture a male's  
138 daily sociality and the behaviors that underlie stable cooperative partnerships (Fig. 2A). These  
139 included a measure of attendance on the display territories (effort); the number of territories  
140 visited (dispersal); the total frequency of cooperative interactions with any partner (total  
141 strength); the average frequency of cooperation per partner (avg. strength); the total number of  
142 unique cooperative partners (degree); and the exclusivity of his partnerships (importance). We  
143 consider effort and dispersal to be measures of a male's status-seeking behavior and strength,  
144 degree, and importance to be measures of his cooperative tendencies. Because our focus was on  
145 hormonal effects on behavior, we took behavioral recordings at a given subset of territories after  
146 capturing the birds for blood samples. We also paired this observational approach with a  
147 hormone manipulation of territorial males to verify the causal relationship between circulating  
148 testosterone and cooperation.

We hypothesized that circulating T would explain variation in cooperation and tested  
150 three mutually-exclusive predictions. Specifically, we predicted that if the action of T is  
151 conserved (i.e., T promotes competitive ability and aggression; Wingfield et al., 1987), high T  
152 would be antagonistic to cooperation (negative slope, Fig. 2B). In contrast, if there has been a  
153 reversal in hormone action in this species, T could actually facilitate cooperation (positive slope,  
154 Fig. 2B). Finally, given that hormonal mechanisms of behavior are known to show among-  
155 individual variation (Rosvall et al., 2012), we predict that T could facilitate cooperation in one  
156 status class and antagonize it in the other (status- or dose-specific slopes, Fig. 2B). This final  
157 prediction assumes some ontogenetic plasticity in the way in which hormones shape behavior.

## 158 **Results**

159 Although most variation in circulating T occurred within individuals, a significant proportion  
160 (19% of the total variation in T was due to differences among males (95% CI = 11-30%; Fig. 1  
161 and Table S1). How do these among- and within-individual variation in androgen levels  
162 influence male social behavior? To partition these sources of variation, we compared the fit of  
163 models that included either baseline T (repeatable, among-individual variation), residual T  
164 (within-individual variation), or no T variable at all (the "null T" model, Table S3). The results  
165 revealed that T has highly status-specific effects that are primarily driven by among-individual  
166 differences in standing T variation (Fig. 3, Table S4). Among floater males, baseline T positively  
167 covaried with lek attendance (effort), the number of territories visited (dispersal), and the  
168 frequency of cooperative interactions (total/average strength; see also Fig. S3). These results  
169 indicate that T is a key mechanism driving differences among floater individuals, whereby high  
170 baseline T promotes status-seeking and cooperation. Previous research has shown that these  
171 behaviors increase the probability that floater males will ascend in status (Ryder et al., 2008). In  
172

174 contrast, the relationships between the same behaviors and standing variation in baseline T  
among territorial males were more negative, as evidenced by the significant slope differences  
between status classes (Fig. 3 and Table S4).

176 Residual T had a more limited role, as it did not explain plastic within-individual  
variation in most social behaviors. This is not surprising, given that T is highly labile, and our  
178 proximity system captured behaviors that occurred with a median time lag of 12 days after our T  
samples. Despite this constraint, residual T did predict at least one phenotype, a male's social  
180 importance. This behavioral phenotype captures the exclusivity of male's partnerships. When  
floaters had high T relative to their baseline (i.e., more positive residual T), their social  
182 partnerships became more exclusive, whereas the opposite pattern was observed in territory-  
holding males (Fig. 3, Table S4). This suggests that residual T has status- or dose-dependent  
184 effects on the stability of a male's social relationships. Viewed cumulatively, these results  
support the prediction that a high T may antagonize social behavior once males ascend in status  
186 and maintain higher circulating hormone concentrations.

We next used a hormone manipulation experiment to directly test if T negatively affects  
188 cooperative tendencies. We chose territorial males for this procedure, prior to any analysis,  
because their high site fidelity makes them readily available for post-manipulation behavioral  
190 data collection. Fourteen males were randomly assigned to receive either a control (Blank, n = 8)  
or testosterone (T, n = 9) implant; three of those males received opposite treatments in  
192 subsequent years. Each male's social behavior was tracked for an average of 8 days before and 4  
days after treatment. The analysis tested whether the T implant significantly altered social  
194 behavior, and whether this change was greater than that observed in the blank control treatment,  
after correcting for multiple comparisons (Table S5).

196 Elevating a territorial male's T significantly decreased his cooperative behavior, both in  
terms of the number of partners and the frequency of social interactions. This occurred as a result  
198 of a major decline in cooperation within the T-implanted male's own territory (i.e., in-degree and  
in-strength; Fig. 4A-D), demonstrating that elevated T directly decreased a male's cooperation  
200 with, and/or attractiveness to, other, visiting males. Further analysis revealed that these implant  
effects also depended on a male's initial baseline T (Fig. 4E-F, Table S6). Low-T males  
202 exhibited a much stronger decrease, such that they received fewer cooperative partners and had a  
lower frequency cooperative interactions in their territories. The T implants also decreased other  
204 behaviors, including effort, average strength, and importance, but those analyses did not reach  
statistical significance (Fig. S6). Overall, this experiment confirms that elevating a male's T can  
206 have a direct negative effect on his position in the cooperative social network. These results,  
combined with the observational results reported above, highlight that a number of physiological  
208 and extrinsic factors can influence how T modulates social behavior.

In addition to intrinsic hormone levels, social behavior is also influenced by dynamic  
210 network partnerships (Dakin and Ryder, preprint in review). Hence, our final step was to  
evaluate whether males show a plastic response to their partners' circulating T (Fig. 5A). We  
212 focused this analysis on the baseline T of the partners, because the repeatable component of T is  
the best predictor of a bird's average behavioral phenotype (Table S4). Building on the best-fit  
214 models of manakin social behavior from our previous analysis, we added the baseline T of recent  
social partners as another covariate. Because wire-tailed manakins have multiple coalition  
216 partners (Dakin and Ryder, preprint in review), this analysis tests whether partner T induces  
behavioral plasticity.



218 Our results show that the hormone phenotype of recent partners can explain a small but  
219 significant proportion of the variance in all six behavioral phenotypes, ranging from 1-3%  
220 (compare to up to 17% for a bird's own T; Fig. 5B). Notably, the variance explained by partner T  
221 was similar to that explained by a bird's own T for three of the six behaviors: effort, dispersal,  
222 and importance. These results are summarized in Figs. 5C and S7. Interacting with high-T  
223 floaters tended to increase the subsequent lek attendance (effort) and territorial visitation  
224 (dispersal) of other floaters. In contrast, interacting with high-T territory-holders tended to  
225 decrease a male's effort and dispersal. Another phenotype that was associated with partner T was  
226 importance, which captures social exclusivity as a measure of social standing. Interacting with  
227 high-T floaters tended to decrease the importance of territorial males and interacting with high-T  
228 territorial males decreased the importance of floaters. This last result implies that one  
229 individual's T phenotype can indirectly affect the stability cooperative partnerships across the  
230 broader social network.

## 232 Discussion

233 How do endocrine systems promote and constrain the evolution of adaptive behavioral traits  
234 (Hau, 2007; Ketterson and Nolan, 1999)? Like many cooperative systems, manakin sociality  
235 evolved from territorial ancestors (Prum, 1994). In territorial social systems, androgens are well  
236 known to facilitate aggression, competitive abilities, and dominance during the breeding season  
237 (e.g., Ketterson et al., 1996; Wingfield et al., 2001). The evolution of stable cooperation  
238 therefore requires heritable changes in endocrine systems that optimize the expression of  
239 behavior for the appropriate social context (Adkins-Regan, 2005; Ryder et al., 2011b).

240 Here, we show that individual wire-tailed manakins differ moderately, but significantly,  
241 in their circulating T levels (Fig. 1C, D). This finding, of among-individual differences, is  
242 consistent with growing evidence that hormone-phenotypes are repeatable and heritable  
243 (Williams, 2008; Mills et al., 2009; While et al., 2010; van Oers et al., 2011; Iserbyt et al., 2015),  
244 although estimates from wild populations, like those presented here, are still exceedingly rare  
245 (Cox et al., 2016). Our results also show that among-individual variation in T explains as much  
246 as 15-17% of the total variation in total/avg. strength, direct measures of the rate of cooperative  
247 behavior (Fig. 5B). Notably, these effects sizes are, if anything, underestimates, because of the  
248 measurement error associated with a limited number of repeat hormone samples (Fig. 1C; range  
249 =1-8) for free-living birds. Thus, the fact that hormonal differences can account for any of the  
250 variation in behavior is an important result (Ball and Balthazart, 2008). Given that repeatable  
251 variation often indicates heritable variation (Dohm, 2002; Williams, 2008), our results also  
252 support the hypothesis that endocrine systems can facilitate and/or constrain adaptive behavioral  
253 traits (Ketterson and Nolan, 1999; Adkins-Regan, 2005; Hau, 2007).

254 Our results also reveal how these hormone-behavior relationships depend on an  
255 individual's status (Figs. 3, 5). Floater males with high T, relative to other floater males, were  
256 observed to attend the display territories more often and engage in more cooperative interactions  
257 (Fig. 3). In contrast, T had very different effects on older territory-holding males, i.e., it tended to  
258 decrease their social behavior. It is possible that our correlational results arise from the reciprocal  
259 feedback between hormones and behavior, whereby an individual's previous behavior can also  
260 influence his own circulating hormones (Wingfield et al., 1990; Goymann, 2009; Oliveira,  
261 2009). Although decoupling cause and effect is difficult with observational data, our hormone  
262 manipulation experiment (Fig. 4) confirms that elevated T in territory-holders decreases

264 cooperation, a trait that is strongly linked to fitness in this social system (Ryder et al., 2008; Ryder et al., 2009).

266 These results suggest that wire-tailed manakin social behavior and its heritable hormonal  
268 basis are the result of stabilizing selection, such that intermediate hormone concentrations  
270 balance competitive behaviors (for status and dominance) with behaviors that promote stable  
272 cooperation. Higher T in floaters facilitates a suite of status-seeking behaviors (Eisenegger et al.,  
274 2011), such as territory attendance and visitation (Fig. 3). At the same time, standing variation in  
276 floater T, which is on average lower than that of territorial males (Fig. 1), does not antagonize  
278 cooperation, and indeed promotes floaters to interact with coalition partners at a higher  
280 frequency (Fig. 3). Given that territoriality is a prerequisite for reproductive success and  
282 coalition partnerships predict social ascension (Ryder et al., 2008), higher T may maximize  
284 floater fitness by increasing the probability that an individual ultimately acquires a territory.  
Once a male acquires a territory, his fitness depends both on the number of cooperative partners  
and the stability of those partnerships (Ryder et al., 2009; Ryder et al., 2011a). Our experiments  
confirm that when circulating T is too high, it will compromise a male's ability to attract and  
maintain stable partnerships (Fig. 4) and hence, his reproductive success. These status-specific  
patterns of hormone-mediated behavior suggest that ontogenetic plasticity of the androgen  
system (also see Maruska and Fernald, 2010) would maximize individual fitness, by allowing a  
manakin to express higher levels of T as a floater, but lower levels as a territory-holder.  
Moreover, coalition partnerships are most common among males in different status classes  
(Ryder et al. 2011), such that plasticity in hormone-mediated behavior prevents social instability  
and promotes stable cooperation.

286 The plasticity in the androgen system may result from a number of potential  
288 physiological mechanisms that are not mutually exclusive. One possibility is that T is only  
290 antagonistic to cooperative behavior above some threshold that most floater males have not yet  
292 reached. The idea of dose-dependent hormonal modulation of cooperative behavior is supported  
294 by the fact that manipulated territory-holders with lower T had larger antagonistic effects on  
296 cooperation (Fig. 3). A second possibility is direct transcriptional effects, whereby status-specific  
298 expression of steroidogenic enzymes and/or receptors can cause among-individual and/or status-  
specific differences in the neural sensitivity to T (Rosvall et al., 2012). Finally, steroid hormones  
like T can have indirect effects on other key neurohormones that also influence behavior (e.g.,  
arginine vasotocin, and mesotocin; Kimura et al., 1999; De Vries and Panzica, 2006; Kabelik et  
al., 2010), and these pathways may differ among age and/or status classes. Each of these  
hypotheses represents a promising option for identifying how variation in the hormone-  
regulatory network facilitate cooperation in the manakin lineage. Future work examining status-  
specific plasticity in the expression of key genes, steroidogenic enzymes, and neuropeptides  
across the brain is a pivotal next step.

300 Cooperation is an emergent property of repeated interactions in a social network, and  
302 these interactions are integral to determining the costs and benefits of cooperative behavior  
304 (Ohtsuki et al., 2006). Moreover, each social partner represents a potential extrinsic proximate  
306 influence on a focal individual's behavior (Fehl et al., 2011; Rand et al., 2011). Previous work  
308 has established that individual manakins differ in their effect on others (Dakin and Ryder,  
preprint in review). Here, our analyses provide evidence that partner T is one key mechanism  
driving the social influence and transmission of behaviors among coalition partners within the  
social network. Moreover, we find that in some cases partner T can explain as much of a focal  
individual's behavior as the focal's own T (Fig. 5B). High-T in both status classes also

310 apparently suppresses social importance in opposite-status individuals (Fig. 5C). This suggests  
311 that one bird's elevated T can have complex ripple effects in the broader social network that  
312 would erode the stability of cooperative partnerships. These results raise key questions about  
313 how individual physiology and the interacting phenotypes of coalition partners scale up to shape  
314 the emergent properties of social networks like topology, clustering, modularity and temporal  
dynamics.

315 Understanding how selection shapes complex phenotypes is a long-standing challenge  
316 that requires integrative approaches (Garland et al., 2016). The discovery here that repeatable  
317 variation in T at first facilitates, and then antagonizes, cooperative behavior as a male ascends  
318 the social hierarchy is an exciting result that highlights how selection can optimize hormone-  
319 mediated behavior for social context. These results specifically demonstrate that endocrine  
320 systems can both facilitate and constrain behavioral evolution. Although ontogenetic behavioral  
321 and physiological plasticity are known for other complex social systems (e.g., cichlid fish;  
322 Maruska and Fernald, 2010; Maruska and Fernald, 2011), here we show that variation in  
323 hormone-regulatory networks can promote stable cooperative partnerships within social  
324 networks. Ultimately, we believe this work begins linking two biological networks () to  
325 understand how the mechanisms that regulate individual behavior scale-up to influence  
326 population-level processes.

## 328 **Materials and Methods**

### 330 **Study System**

331 We studied wire-tailed manakins (*Pipra filicauda*) at the Tiputini Biodiversity Station, Orellana  
332 Province, Ecuador (0° 38' S, 76° 08' W, approx. 200 m elevation), a population that has been  
333 observed and color-banded since 2002. The present study was conducted over three breeding  
334 seasons, from December to March in 2015-16, 2016-17, and 2017-18. Sample sizes were not  
335 predetermined, but instead we aimed to sample all males in the population and to recapture as  
336 many as possible for up to three repeated hormone samples per season.

### 338 **Testosterone Assay**

339 We caught birds with mist-nets and recorded the time of day and duration of restraint (Vernasco  
340 et al., 2018). Blood (~75uL) was collected from the brachial vein and stored in a cooler before  
341 being centrifuged for 5 min to separate plasma. The volume of plasma was measured to the  
342 nearest 0.25ul and stored in 100% ethanol prior to double extraction with dichloromethane  
343 (Goymann et al., 2007). We used direct radioimmunoassay to determine the total plasma T  
344 concentration (ng/mL), adjusted by extraction efficiency (Eikenaar et al., 2011). Any sample that  
345 fell below the detection limit was assigned that value as its T concentration. Extraction efficiency  
346 ranged from 62-73% and the intra-assay coefficients of variation were 6.6%, 11.6% and 9.2% for  
347 2015-16, 2016-17, 2017-18, respectively. The inter-assay coefficient of variation was 19.5%.

348

### **Social Behavior**

350 Territorial status was determined by direct observation (Ryder et al., 2008). We used an  
351 automated proximity system (Ryder et al., 2012) to quantify a range of social behaviors as shown  
352 in Fig. 2. Males were outfitted with coded nano-tags (NTQB-2, Lotek Wireless; 0.35 g) that  
353 transmitted unique signals at an average rate of one per 20 s. Proximity data-loggers (hereafter  
354 DL; SRX-DL800, Lotek Wireless) were placed in each cooperative display territory to record the



356 tag signals (pings) from 0600 to 1600 after the territory-holder had been captured. DLs remained  
357 at a territory for recording sessions lasting ~6 days ( $\pm 1$  SD) before being moved to a new  
358 location (for further details of the tracking method, see Dakin and Ryder, preprint in review). In  
359 total, we recorded 29,760 hours (2015-16: 49 territories, mean = 16 recording days per territory;  
360 2016-17: 54 territories, mean = 21 days; 2017-18: 48 territories, mean = 22 days, SD =  $\pm 4$  days  
361 in all years). The number of males color-banded and tagged in each study year was  $n_{15-16} = 100$ ,  
362  $n_{16-17} = 114$ , and  $n_{17-18} = 82$ , for a total  $n_{\text{tagged}}$  of 179.

363 Effort and dispersal were quantified using a male's pings (Fig. 2A). To define strength,  
364 degree, and importance, the proximity data were first filtered to identify times when two males  
365 co-occurred at a display territory as an indication of cooperative interactions (Ryder et al., 2008;  
366 Ryder et al., 2012). A detailed description of the filtering algorithm is provided in (Dakin and  
367 Ryder, preprint in review); a ground-truthing experiment confirmed that this method identified  
368 birds in close proximity. In total, we identified approx. 37,000 unique social interactions. To  
369 calculate a male's importance, we first found the proportion of each of his partner's interactions  
370 that were with the focal male, and then took the weighted average, based on the focal male's  
371 interaction frequencies. Thus, a male whose partners often interacted with other individuals  
372 would obtain an importance near 0, whereas a male whose partners interacted with him  
373 exclusively would obtain an importance of 1. All behavioral phenotypes were significantly  
374 repeatable, ranging from 25% for degree to 45% for effort (Dakin and Ryder, preprint in review).

### 375 **Hormone Experiment**

376 A within-subjects hormone manipulation was performed on 14 randomly-selected territorial  
377 males in 2016-17 and 2017-18. Subjects received a silastic implant containing either T or blank  
378 (the control). Surgeries were scheduled in between DL recording sessions, such that each  
379 implanted male was monitored first for several days within the 4-week period prior to  
380 implantation, and then again within 10 days after implantation.

### 381 **Baseline and Residual T**

382 All analyses were performed in R (R Core Team, 2018). Standing variation in T can include both  
383 among-individual (baseline) and within-individual (residual) variation. To partition these two  
384 sources of hormonal phenotype, we fit a mixed-effects model of log-transformed T in the lme4  
385 package (Bates et al., 2018). The model included a bird's ID as a random effect, as well as fixed  
386 effects of status and the following variables summarizing the conditions of a male's capture:  
387 year, Julian date, time of day, and duration of restraint (net time; see Vernasco et al., 2018)  
388 (Table S1). For 54 captures that were missing net time data, we assumed the yearly average net  
389 time (range 16-19 min). Baseline T was defined using each bird's random intercept from the  
390 fitted model (i.e., one value per male, representing the individually-repeatable component of  
391 variation in T). Residual T was defined as the variation in T not explained by the model. Hence,  
392 these metrics account for capture conditions that would influence T samples but are expected to  
393 be independent of a male's behavior in the ensuing days. To estimate the repeatability of T  
394 across males in both classes, we dropped status from the model above and calculated the  
395 proportion of total variance due to individual differences.

### 397 **Analyses of Behavior**

398 We used a model selection analysis in the MuMIn package (Bartoń, 2018) to determine whether  
399 behavioral phenotypes were best explained by models with baseline T, residual T, the interaction  
400

402 between each of these T variables and status, or a model without T (Tables S3-S4). All candidate  
403 models included ID (random effect) as well as fixed effects of status, year, date, temperature, and  
404 days since capture when the behavior was recorded. Residual T was determined using the most  
405 recent prior hormone sample within four weeks. Thus, the analysis was limited to 2,544 daily  
406 recordings of 162 control males with a matching T sample within this time-frame. Statistical  
407 significance was determined using the lmerTest and jtools packages (Kuznetsova et al., 2018;  
408 Long, 2018).

409 To analyze the experiment, we fit models that accounted for ID (random effect) as well as  
410 fixed effects of year, temperature, and implant (either pre-implant, blank, or T; Table S5). We  
411 used post-hoc Tukey's tests in the multcomp package (Hothorn et al., 2017) to compare implant  
412 treatments, and corrected the p-values using the step-up false discovery rate procedure in the  
413 multtest package (Pollard et al., 2018). To determine if baseline T modulated the influence of the  
414 T implants, we compared the fit of models with, and without, the baseline T x implant interaction  
(Table S6).

415 To investigate the influence of partner T, we refit models from Table S4 with additional  
416 fixed effects that included the weighted average baseline T and status of a male's partners on the  
417 previous day (Table S7). Hence, this analysis is limited to 1,082 daily recordings of 121 control  
418 individuals whose partners were known on the previous day. The first model included  
419 interactions between the focal male's own status and these partner effects, and we subsequently  
420 removed interactions with  $p > 0.10$ .  $R^2$  estimates for these mixed-effects models were obtained  
421 using the r2glmm package (Jaeger, 2017).

422 All data and materials necessary to reproduce these results are provided at:  
423 <https://figshare.com/s/d36c1a5c822e00abc581>

424

### Acknowledgments

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428 Interdisciplinary Graduate Program in Global Change at Virginia Tech, Millersville University,  
429 and the Tiputini Biodiversity Station of the Universidad San Francisco de Quito.

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560

## FIGURE LEGENDS

562

**Fig. 1. How does testosterone modulate social behavior in a cooperative network?** (A) Wire-tailed manakins have a social system in which males of two status classes, territorial (black) and non-territorial (red), form cooperative display coalitions. Both intrinsic (loop arrows) and extrinsic social factors (straight arrows) are hypothesized to drive variation in male social behavior. (B) Steroid hormones are a key mechanism controlling sociality and males show variation with and among status classes and individuals. (C) To assess the relationship between T and behavior, we collected repeated samples of a steroid hormone, testosterone (T), while simultaneously monitoring the behavior of a large population of males ( $n = 528$  T samples from 209 individuals). (D) Examples of repeated T samples from five manakins. Note that each bird's T levels are arranged in descending order along the x-axis, rather than temporally, to highlight within- and among-individual differences. One individual in (D) transitioned from floater to territory-holder during the sampling period. Once a male attains territorial status, he retains it for life. See Fig. S1 and Table S1 for details.

576

**Fig. 2. Social phenotypes and the relationship between T and cooperation.** (A) We used an automated proximity system to obtain repeated daily measures of six key social behaviors important for reproductive coalitions: effort is a measure of lek attendance; dispersal is the number of territories visited; total strength is the frequency of cooperative interactions; average strength is the frequency of interactions on a per-partner basis; degree is the number of unique cooperative partners; and importance is the exclusivity of a male's social partners. Violin plots show the distributions of individual means ( $n = 170$  males); the vertical lines are the medians. (B) These data were used to test the hypotheses that T may inhibit, facilitate, or have status-specific effects on cooperative behavior. See Fig. S2 and Table S2 for additional data.

586

**Fig. 3. Circulating testosterone explains variation in social behavior in a status-specific manner.** Floaters (top row) with high baseline T have greater lek attendance (effort), visit more territories (dispersal), and interact with other males at higher frequencies (strength) as compared to floater with low baseline T. Also, if a floater male's residual T is elevated relative to his baseline, he subsequently attains more exclusive social partners (importance). Despite these positive effects of T on the cooperative behavior of floaters, T is either independent of, or negatively related to, these same behaviors in territory-holders (bottom row). Each scatterplot shows the residual behavioral variation after accounting for other predictors in the analysis. To visualize the differences among individuals, one average value is shown per male. Results for behaviors predicted by baseline T are shown  $\pm$  SD in the y-axis, whereas results for residual T have a separate regression line for each bird to illustrate the within-individual variance. Heavy black lines show status-specific estimates that are also indicated by the effect sizes in the middle row ( $\pm$  95% confidence intervals). Note that the pattern observed here for total strength is the same as that observed for average strength in Fig. S3. Further details of this analysis are provided in Tables S3-S4.

602

**Fig. 4. Experimental elevation of testosterone decreases cooperation in territory-holders.** Fourteen territorial males were given control (Blank,  $n = 8$ ) or testosterone (T,  $n = 9$ ) implants. (A-B) Average pre- and post-manipulation measures of two cooperative behaviors, the frequency of interactions (in-strength) and the number of partners (in-degree) interacting with the focal bird

606

608 in his own territory. Lines connect data from the same individual. The repeatability of behavior,  
R, is given below each panel. (C-D) The average change in individual behavior as estimated  
610 from Tukey contrasts (blank implant, “ $B_{\text{post}} - B_{\text{pre}}$ ”; T implant, “ $T_{\text{post}} - T_{\text{pre}}$ ”; and a comparison  
612 of the T and blank effects, “ $T - B$ ”). We conclude that T influenced behavior if both “ $T_{\text{post}} -$   
614  $T_{\text{pre}}$ ” and “ $T - B$ ” were statistically significant ( $p < 0.05$ ); all results are corrected for multiple  
616 comparisons (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). The sample size for this analysis was  $n =$   
211 observations of 14 individuals. All effect sizes are based on standardized data to be  
comparable across the figure. (E-F) Further analysis demonstrated that the magnitude of these  
effects depended on the subject male’s baseline T. Specifically, stronger negative effects were  
observed for territory-holding males with lower pre-implant baseline T levels. See also Figs. S5-  
S6 and Tables S5-S6.

618  
**Fig. 5. An individual’s behavior is associated with the hormone phenotype of his recent social partners.** (A) This analysis evaluates the influence of previous social partners’ baseline T on the focal bird, after accounting for the focal bird’s own T and other factors. (B) The percent of behavioral variance explained by own vs. partner T. (C) Heatmap showing standardized effect sizes for partner T in the analysis (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). High-T floaters stimulated lek attendance (effort), territory visitation (dispersal), as well as the number and frequency of cooperative partnerships (degree and strength, respectively). In contrast, high-T territory-holders inhibited effort and dispersal. Social importance is inhibited by high-T floaters and territory-holders. Floaters were generally more strongly influenced by their partners’ T than territory-holders. The sample size was 1,165 measures of 126 control individuals. See Table S7 and Fig. S7 for further details.

## 630 SUPPLEMENTAL FIGURES

632  
**Fig. S1. Circulating testosterone varies within and among individuals.** (A) Repeated T samples (y-axis) were taken from 209 individuals (x-axis) at multiple time points. Filled circles in (A) show individual T measurements and vertical lines show the SE for males with more than one sample. The individuals are ordered along the x-axis according to their (B) baseline T phenotype, which was determined using the random intercepts from a multilevel model that accounted for study year, date, time of day, net time (restraint), and status. Note that the residuals from this analysis were also used to define our residual T variable (i.e., within-individual variation in circulating T).

642  
**Fig. S2. Variation in a suite of behaviors is positively correlated.** The heatmaps show Pearson’s correlations,  $r$ , estimated from the posteriors of a multivariate model after accounting for study year and status. The “among-individual” level (A) represents repeatable individual differences. A positive correlation at this level indicates that males who consistently score highly on one phenotype also score highly on the other. In contrast, the “within-individual” level (B) represents the plasticity of expression within individuals. A positive correlation here indicates that at times when a male expresses a high level of one phenotype (relative to his baseline), he tends to express a high level of the other. Most of the behaviors we analyzed are positively correlated at both levels, with the exception that dispersal and importance vary independently ( $n = 4,481$  measurements of 170 control individuals).

652

654 **Fig. S3. Average strength in relation to circulating testosterone.** Baseline T was positively  
associated with the frequency of interactions (average strength) among floaters, but negatively  
656 associated with this same behavior among territory-holders. Each scatterplot shows the residual  
variation in average strength (y-axis) on a log scale, after accounting for other factors in the best-  
fit model (Table S4). All other features follow Fig. 3 of the main text.

658  
660 **Fig. S4. The percent of behavioral variance explained by circulating testosterone.** Overall  
bar height gives an estimate of the percent of total variance in behavior,  $R^2$ , that can be explained  
662 by all of the fixed effects in Table S4. Each bar can be further divided into variance explained  
by: (i) study year, Julian date, and temperature; (ii) behavioral differences among territorial and  
664 floater males; and (iii) predictors related to a male's circulating testosterone (baseline or residual  
T). Circulating T explained significant variation in several behaviors. In particular, baseline T  
666 can explain average strength, total strength, effort, and dispersal. In contrast, a bird's importance  
was best explained by residual T. Akaike weight is the likelihood of the best-fit model relative to  
the candidate set, on a scale from 0 to 1. See also Tables S3-S4.

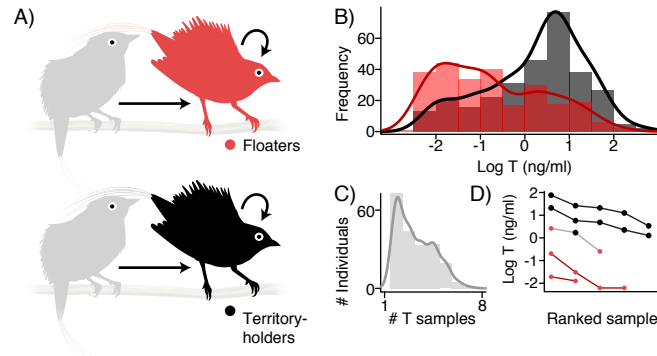
668  
670 **Fig. S5. Effect of testosterone implants on the total frequency and number of cooperative  
partnerships received by territorial males.** All features follow Fig. 4 of the main text. See also  
Tables S5-S6.

672  
674 **Fig. S6. Additional results of the testosterone implant experiment.** (A-C) The effort, average  
strength, and importance of territorial males tended to decrease following T implantation, but  
676 these changes were not statistically significant. The effect of T manipulation on dispersal (D)  
was dependent on a male's baseline T phenotype, such that lower T males increased their  
dispersal behavior following T implantation, whereas higher T males decreased it. All features  
678 follow Fig. 4 of the main text. See also Tables S5-S6.

680 **Fig. S7. Analysis of social influence.** The top rows (A-B) show residual behavioral variation (y-  
axis) in relation to partner T, after accounting for other predictors (Table S7). The bottom row  
682 (C) shows the influence of partner status from the same analyses. For clarity, only the  
statistically significant results from Fig. 5 are shown. Because the social environment varies  
684 within focal individuals, a separate linear regression line is plotted for each focal individual; the  
heavy black line shows the estimated population effect. Note that all phenotypes except  
686 importance are plotted and analyzed on a log scale.

# PREPRINT VERSION

Fig. 1

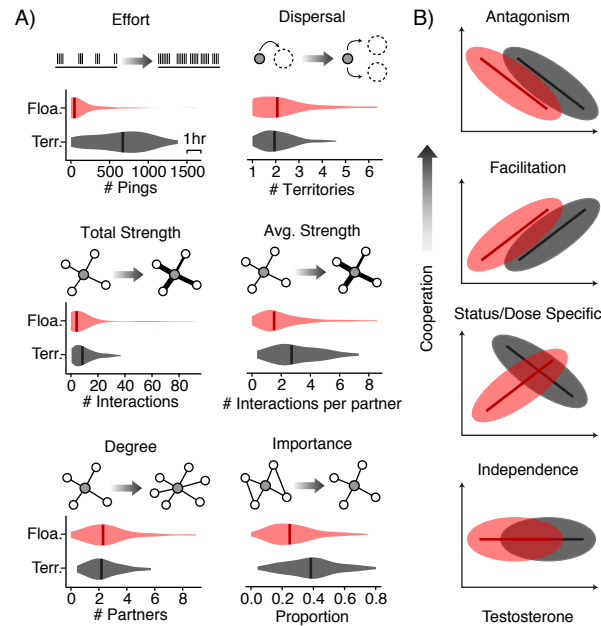


**Fig. 1. How does testosterone modulate social behavior in a cooperative network?** (A) Wire-tailed manakins have a social system in which males of two status classes, territorial (black) and non-territorial (red), form cooperative display coalitions. Both intrinsic (loop arrows) and extrinsic social factors (straight arrows) are hypothesized to drive variation in male social behavior. (B) Steroid hormones are a key mechanism controlling sociality and males show variation with and among status classes and individuals. (C) To assess the relationship between T and behavior, we collected repeated samples of a steroid hormone, testosterone (T), while simultaneously monitoring the behavior of a large population of males ( $n = 528$  T samples from 209 individuals). (D) Examples of repeated T samples from five manakins. Note that each bird's T levels are arranged in descending order along the x-axis, rather than temporally, to highlight within- and among-individual differences. One individual in (D) transitioned from floater to territory-holder during the sampling period. Once a male attains territorial status, he retains it for life. See Fig. S1 and Table S1 for details.



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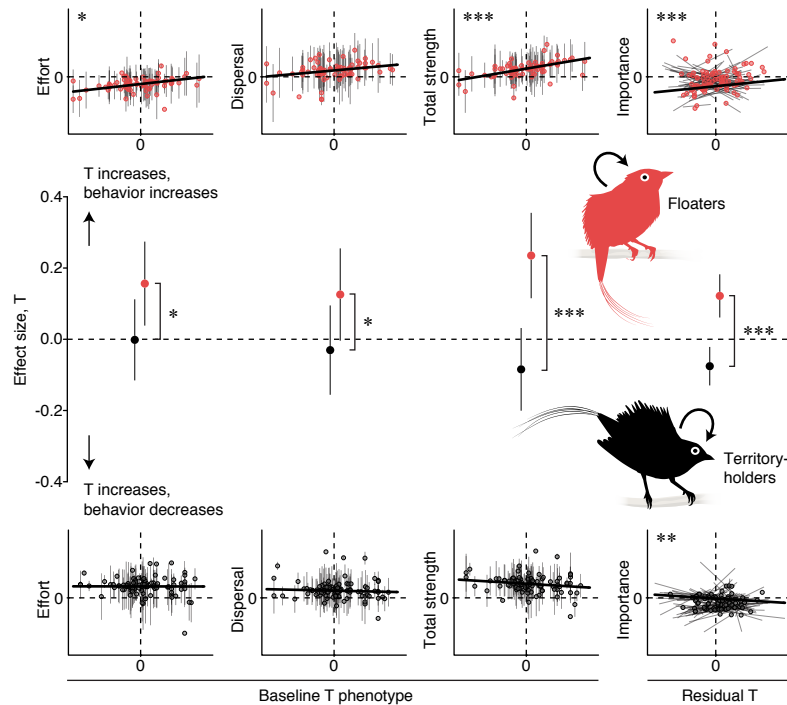
Fig. 2



**Fig. 2. Social phenotypes and the relationship between T and cooperation.** (A) We used an automated proximity system to obtain repeated daily measures of six key social behaviors important for reproductive coalitions: effort is a measure of lek attendance; dispersal is the number of territories visited; total strength is the frequency of cooperative interactions; average strength is the frequency of interactions on a per-partner basis; degree is the number of unique cooperative partners; and importance is the exclusivity of a male's social partners. Violin plots show the distributions of individual means ( $n = 170$  males); the vertical lines are the medians. (B) These data were used to test the hypotheses that T may inhibit, facilitate, or have status-specific effects on cooperative behavior. See Fig. S2 and Table S2 for additional data.

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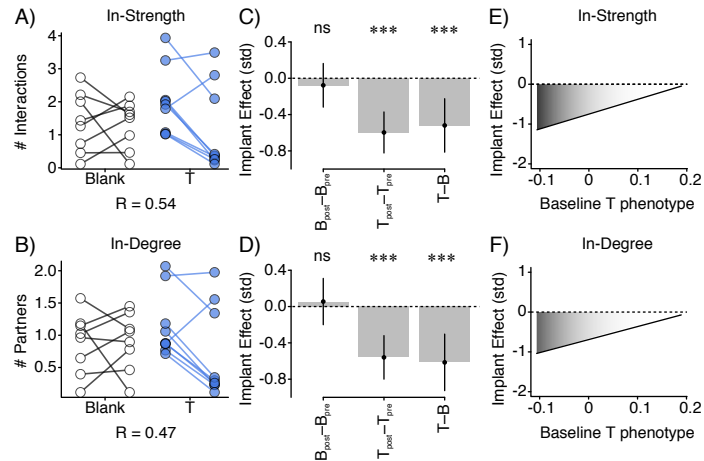
Fig. 3



**Fig. 3. Circulating testosterone explains variation in social behavior in a status-specific manner.** Floaters (top row) with high baseline T have greater lek attendance (effort), visit more territories (dispersal), and interact with other males at higher frequencies (strength) as compared to floater with low baseline T. Also, if a floater male's residual T is elevated relative to his baseline, he subsequently attains more exclusive social partners (importance). Despite these positive effects of T on the cooperative behavior of floaters, T is either independent of, or negatively related to, these same behaviors in territory-holders (bottom row). Each scatterplot shows the residual behavioral variation after accounting for other predictors in the analysis. To visualize the differences among individuals, one average value is shown per male. Results for behaviors predicted by baseline T are shown  $\pm$ SD in the y-axis, whereas results for residual T have a separate regression line for each bird to illustrate the within-individual variance. Heavy black lines show status-specific estimates that are also indicated by the effect sizes in the middle row ( $\pm$ 95% confidence intervals). Note that the pattern observed here for total strength is the same as that observed for average strength in Fig. S3. Further details of this analysis are provided in Tables S3-S4.

PREPRINT VERSION

Fig. 4

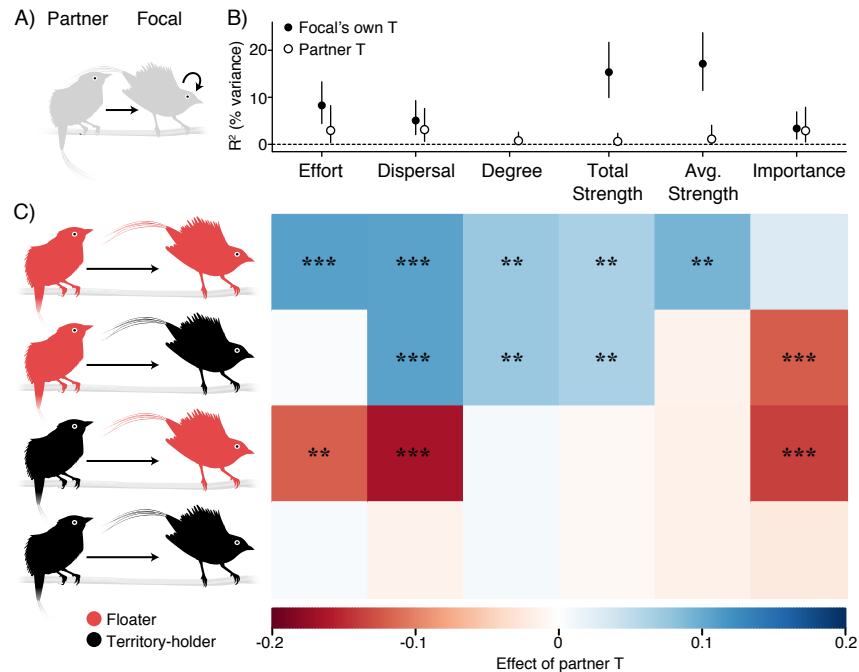


**Fig. 4. Experimental elevation of testosterone decreases cooperation in territory-holders.**

Fourteen territorial males were given control (Blank,  $n = 8$ ) or testosterone (T,  $n = 9$ ) implants. (A-B) Average pre- and post-manipulation measures of two cooperative behaviors, the frequency of interactions (in-strength) and the number of partners (in-degree) interacting with the focal bird in his own territory. Lines connect data from the same individual. The repeatability of behavior,  $R$ , is given below each panel. (C-D) The average change in individual behavior as estimated from Tukey contrasts (blank implant, “ $B_{post} - B_{pre}$ ”; T implant, “ $T_{post} - T_{pre}$ ”; and a comparison of the T and blank effects, “ $T - B$ ”). We conclude that T influenced behavior if both “ $T_{post} - T_{pre}$ ” and “ $T - B$ ” were statistically significant ( $p < 0.05$ ); all results are corrected for multiple comparisons (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). The sample size for this analysis was  $n = 211$  observations of 14 individuals. All effect sizes are based on standardized data to be comparable across the figure. (E-F) Further analysis demonstrated that the magnitude of these effects depended on the subject male’s baseline T. Specifically, stronger negative effects were observed for territory-holding males with lower pre-implant baseline T levels. See also Figs. S5-S6 and Tables S5-S6.

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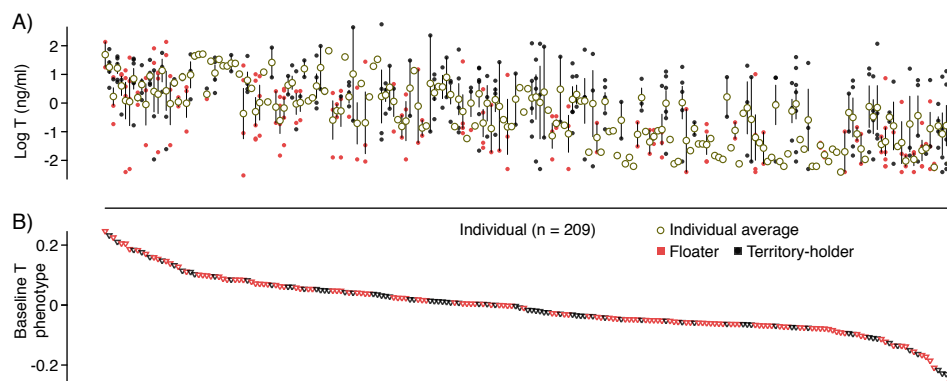
Fig. 5



**Fig. 5. An individual's behavior is associated with the hormone phenotype of his recent social partners.** (A) This analysis evaluates the influence of previous social partners' baseline T on the focal bird, after accounting for the focal bird's own T and other factors. (B) The percent of behavioral variance explained by own vs. partner T. (C) Heatmap showing standardized effect sizes for partner T in the analysis (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). High-T floaters stimulated lek attendance (effort), territory visitation (dispersal), as well as the number and frequency of cooperative partnerships (degree and strength, respectively). In contrast, high-T territory-holders inhibited effort and dispersal. Social importance is inhibited by high-T floaters and territory-holders. Floaters were generally more strongly influenced by their partners' T than territory-holders. The sample size was 1,165 measures of 126 control individuals. See Table S7 and Fig. S7 for further details.

# PREPRINT VERSION

Fig. S1

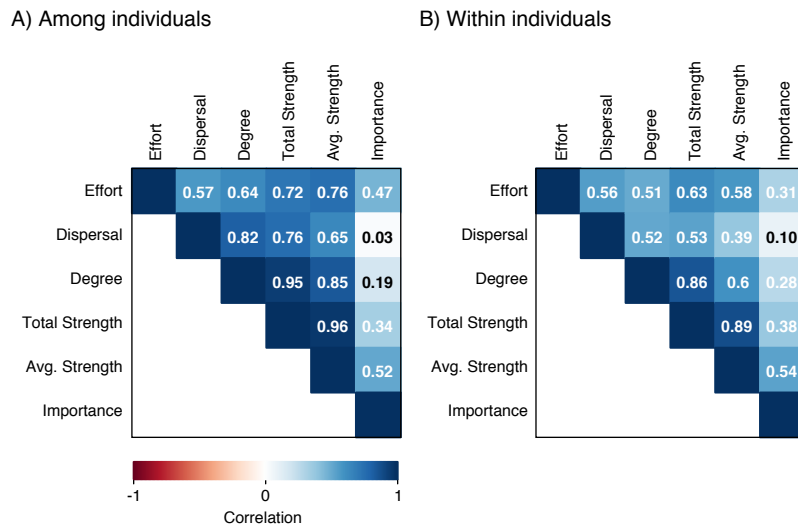


**Fig. S1. Circulating testosterone varies within and among individuals.** (A) Repeated T samples (y-axis) were taken from 209 individuals (x-axis) at multiple time points. Filled circles in (A) show individual T measurements and vertical lines show the SE for males with more than one sample. The individuals are ordered along the x-axis according to their (B) baseline T phenotype, which was determined using the random intercepts from a multilevel model that accounted for study year, date, time of day, net time (restraint), and status. Note that the residuals from this analysis were also used to define our residual T variable (i.e., within-individual variation in circulating T).



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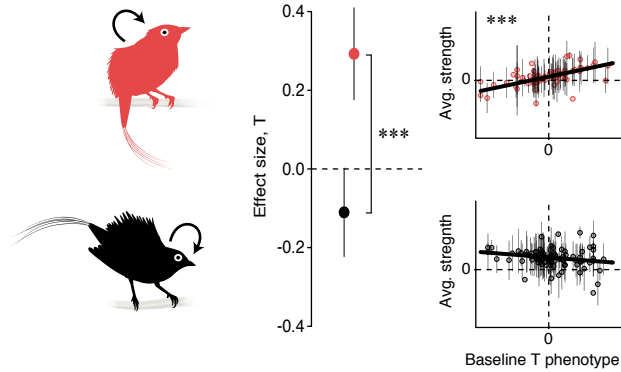
Fig. S2



**Fig. S2. Variation in a suite of behaviors is positively correlated.** The heatmaps show Pearson's correlations,  $r$ , estimated from the posteriors of a multivariate model after accounting for study year and status. The "among-individual" level (A) represents repeatable individual differences. A positive correlation at this level indicates that males who consistently score highly on one phenotype also score highly on the other. In contrast, the "within-individual" level (B) represents the plasticity of expression within individuals. A positive correlation here indicates that at times when a male expresses a high level of one phenotype (relative to his baseline), he tends to express a high level of the other. Most of the behaviors we analyzed are positively correlated at both levels, with the exception that dispersal and importance vary independently ( $n = 4,481$  measurements of 170 control individuals).

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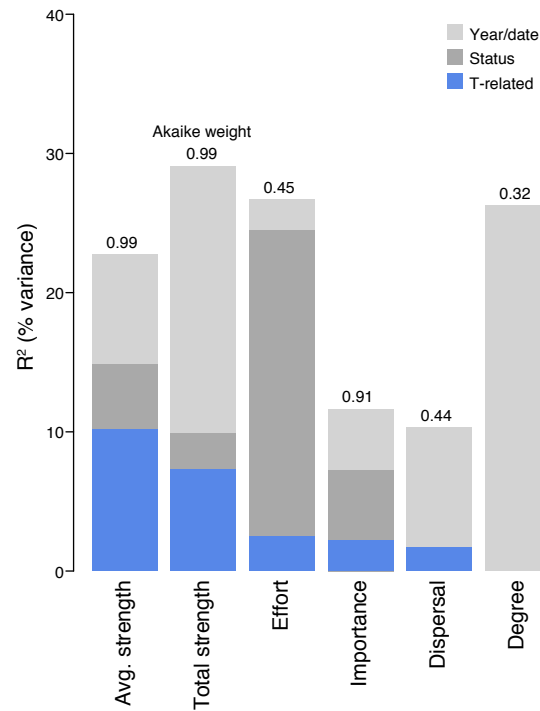
Fig. S3



**Fig. S3. Average strength in relation to circulating testosterone.** Baseline T was positively associated with the frequency of interactions (average strength) among floaters, but negatively associated with this same behavior among territory-holders. Each scatterplot shows the residual variation in average strength (y-axis) on a log scale, after accounting for other factors in the best-fit model (Table S4). All other features follow Fig. 3 of the main text.

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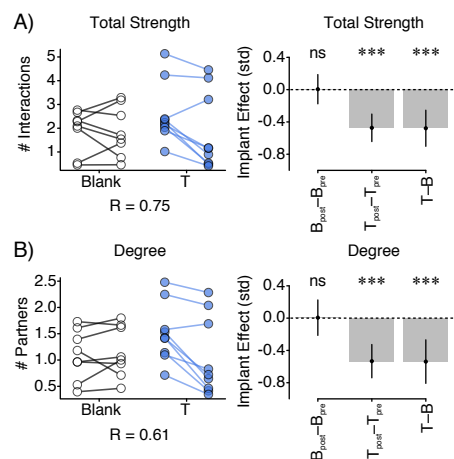
Fig. S4



**Fig. S4. The percent of behavioral variance explained by circulating testosterone.** Overall bar height gives an estimate of the percent of total variance in behavior,  $R^2$ , that can be explained by all of the fixed effects in Table S4. Each bar can be further divided into variance explained by: (i) study year, Julian date, and temperature; (ii) behavioral differences among territorial and floater males; and (iii) predictors related to a male's circulating testosterone (baseline or residual T). Circulating T explained significant variation in several behaviors. In particular, baseline T can explain average strength, total strength, effort, and dispersal. In contrast, a bird's importance was best explained by residual T. Akaike weight is the likelihood of the best-fit model relative to the candidate set, on a scale from 0 to 1. See also Tables S3-S4.

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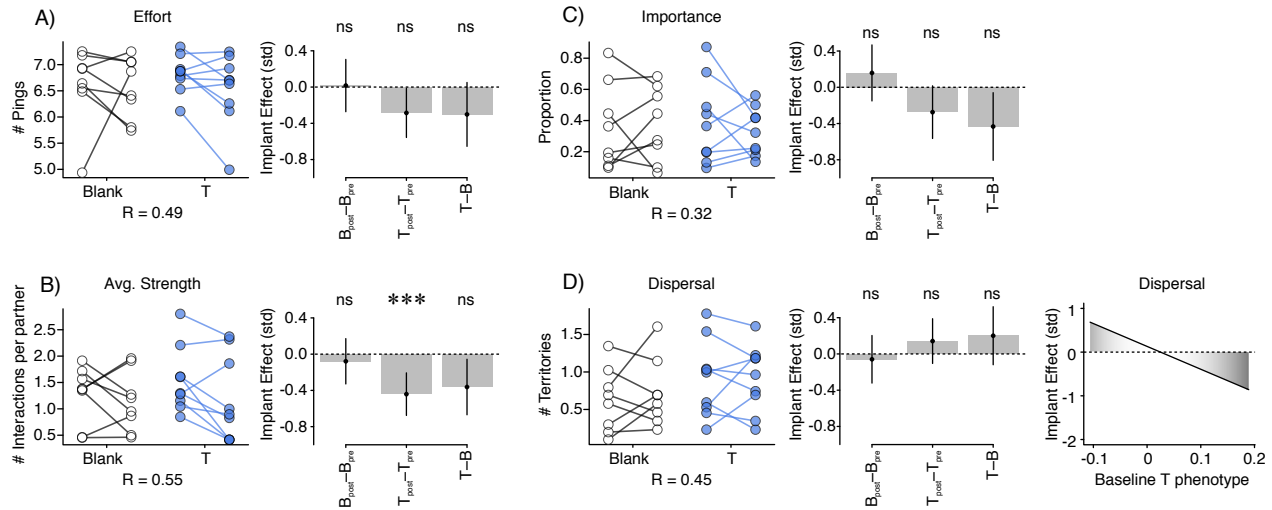
Fig. S5



**Fig. S5. Effect of testosterone implants on the total frequency and number of cooperative partnerships received by territorial males.** All features follow Fig. 4 of the main text. See also Tables S5-S6.

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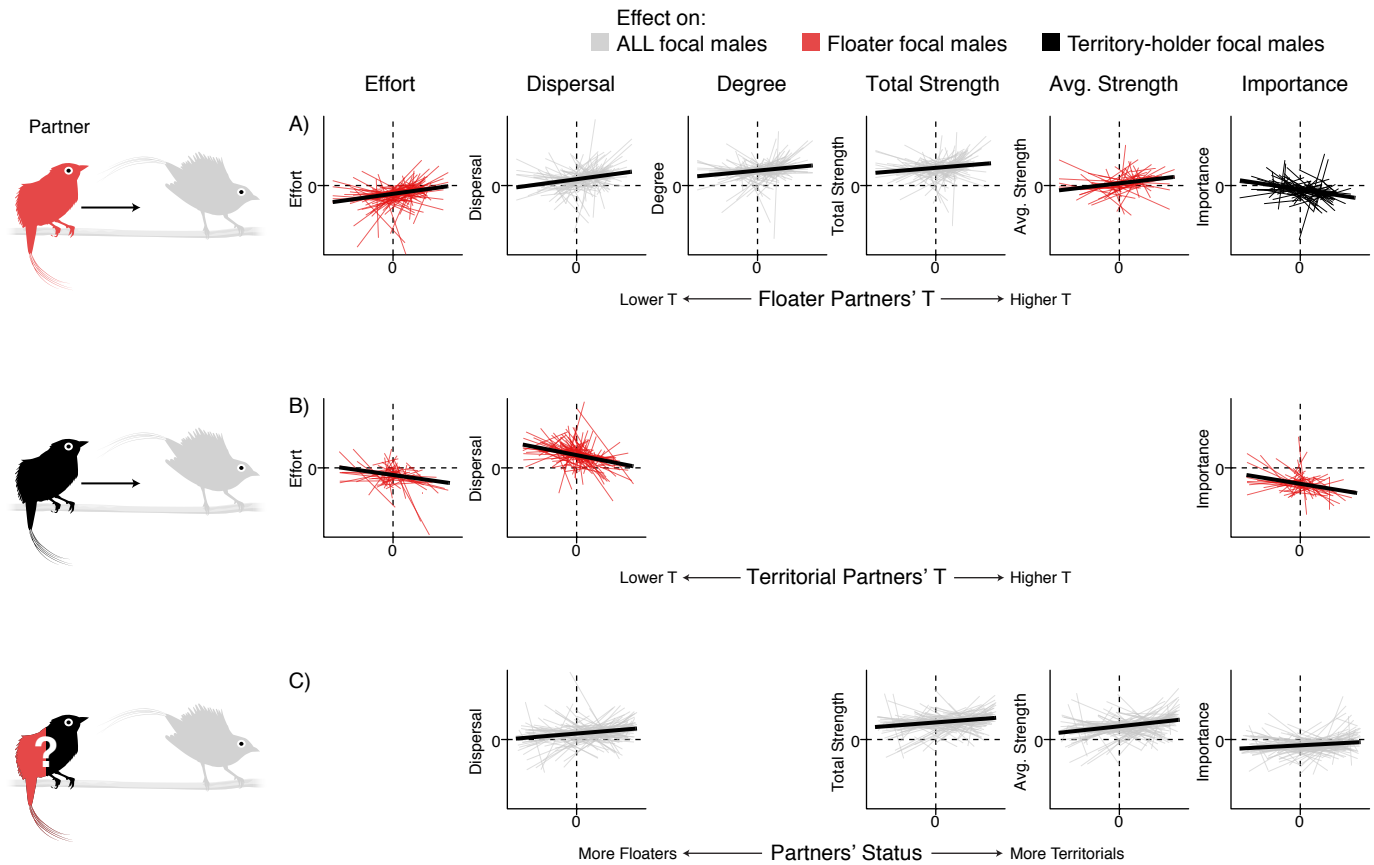
Fig. S6



**Fig. S6. Additional results of the testosterone implant experiment.** (A-C) The effort, average strength, and importance of territorial males tended to decrease following T implantation, but these changes were not statistically significant. The effect of T manipulation on dispersal (D) was dependent on a male's baseline T phenotype, such that lower T males increased their dispersal behavior following T implantation, whereas higher T males decreased it. All features follow Fig. 4 of the main text. See also Tables S5-S6.

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Fig. S7



**Fig. S7. Analysis of social influence.** The top rows (A-B) show residual behavioral variation (y-axis) in relation to partner T, after accounting for other predictors (Table S7). The bottom row (C) shows the influence of partner status from the same analyses. For clarity, only the statistically significant results from Fig. 5 are shown. Because the social environment varies within focal individuals, a separate linear regression line is plotted for each focal individual; the heavy black line shows the estimated population effect. Note that all phenotypes except importance are plotted and analyzed on a log scale.