Testosterone mediates status-specific patterns of cooperation and transmission of behavior in a social network

Short title: Testosterone mediates cooperation

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Abstract

Stable cooperation requires plasticity whereby individuals are able to express competitive or cooperative behaviors depending on social context. To date, however, the physiological mechanisms that underlie behavioral plasticity in cooperative systems are poorly understood. We 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 monitor cooperative interactions among male manakins over three breeding seasons, and we examined how circulating testosterone affects cooperation. Our results indicate that in non-territorial floater males, high testosterone promotes cooperative behaviors and is associated with subsequent ascension to territorial status. In territory-holding males, however, both observational and experimental evidence demonstrate that high testosterone antagonizes cooperation. Collectively, our findings show that hormonal control of cooperative behavior depends on a male's social status. We propose that status-dependent changes in testosterone signalling facilitate stable cooperative partnerships and thus provide direct fitness benefits for males.

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 competition for mating opportunities with the benefits of engaging in cooperation (Clutton-Brock, 2009; Cockburn, 1998). These behavioral negotiations within cooperative social systems are mediated by physiological mechanisms that govern phenotypic plasticity (Soares et al., 2010) over the course of different temporal scales. Over the long-term, the plasticity that drives among-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 dominance and acquiring status within social groups (Ryder et al., 2008; Ryder et al., 2009). Over shorter timeframes, within-individual plasticity is thought to facilitate the expression of cooperative or competitive behaviors that are appropriate for the current social context (Taborsky and Oliveira, 2012). To date, however, the proximate underpinnings of the behavioral plasticity that promotes stable cooperation are poorly understood (Soares et al., 2010). Thus, a key question is how physiology modulates behavior in social systems where fitness requires a balance of cooperative and competitive behaviors.

Steroid hormones are well known for their pleiotropic and often antagonistic effects on numerous aspects of physiology and behavior (Ketterson et al., 1996; Wingfield et al., 2001; Adkins-Regan, 2005; Hau, 2007). Testosterone, in particular, has been well studied in male vertebrates, where it can either facilitate or constrain behavioral plasticity in both the reproductive and social contexts (Adkins-Regan, 2005; Hau, 2007). Yet, the vast majority of work on androgen-mediated traits has focused on archetypal behaviors (e.g., aggression), with very few studies attempting to link hormones to individual variation in cooperative behaviors (Khan et al., 2001; Soares et 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; Schoech et al., 1991; Peters et al., 2001; Ryder et al., 2011b), including the suppression of testosterone in subordinates (Brouwer et al., 2009), no study has examined how androgens mediate the dynamics of cooperation and competition within a social network.

Circulating hormones have long been recognized as a key mechanism underlying individual differences in behavior. Mounting evidence suggests that hormonal phenotypes (i.e., the expression of relatively low or high hormone levels) are often repeatable and may thus act 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, empirical studies linking among-individual differences in circulating hormones to behavioral variation are challenging, in part, because hormone-mediated behaviors are regulated by complex signaling pathways (Adkins-Regan, 2005; Ball and Balthazart, 2008). The effect of testosterone on male behavior depends not only on circulating hormone levels, but also on the neural sensitivity of tissues, which is determined by the expression of receptors and steroidogenic enzymes (i.e., aromatase, 5α-reductase; (Ball and Balthazart, 2008; Rosvall et al., 2012)), and on the downstream regulation of other neuroendocrine genes (e.g., neuropeptides; reviewed in (Fuxjager et al., 2018)). Indeed, the complexity of steroid-signaling pathways can produce nonlinear dose-dependent responses, and recent work suggests that these non-monotonic relationships may be key to understanding how hormones shape within and among-individual differences in behavior (Lema, 2014).

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

thought to optimize behaviors to the current social landscape (Wingfield et al., 1990; Goymann, 2009; Oliveira, 2009). Engaging in social interactions can rapidly alter circulating testosterone levels, androgen sensitivity in neural pathways, and the patterns of neural gene expression that influence motivation and shape status-specific behavioral phenotypes (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 (Romeo et al., 2002; Maruska and Fernald, 2010). Moreover, the hormone-mediated behavior of one individual can influence that of social partners, providing a potentially indirect pathway for hormones to shape behavioral phenotypes. It is thus possible that testosterone is a key mechanism underlying the vertical and horizontal social transmission of behaviors among individuals (Dufty et al., 2002), especially in cooperative systems. The hypothesis that hormone-mediated behavior can indirectly shape the socially contagious behaviors that facilitate cooperation has yet to be formally tested.

In an effort to understand how hormones regulate social behavior, we asked if circulating testosterone is a mechanism underlying variation in cooperative behavior in a lek-breeding bird, the wire-tailed manakin (*Pipra filicauda*; Fig. 1A). Wire-tailed manakins have a social system with 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-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 have a higher probability of eventually inheriting a territory, which is a prerequisite for breeding (Ryder et al., 2008; Ryder et al., 2009). As such, display coalitions have clear fitness benefits, but they also involve male-male competition and play a role in ascension to territorial status. Many coalition partnerships are stable from year to year, but males also show substantial day-to-day variation in their social interactions, creating a dynamic social network (Ryder et al., 2011a; Dakin and Ryder, 2018). Previous work on this system has established that the status classes differ in their circulating testosterone levels (Ryder et al., 2011b), and that cooperative behavior is socially contagious (Dakin and Ryder, 2018). Thus, wire-tailed manakins provide a unique opportunity to jointly measure how hormones and social interactions shape emergent cooperative behavior. Here, our first goal was to evaluate the role of a male's circulating testosterone in shaping his behavioral phenotype (which we define as the "direct effect" of testosterone; loop arrows in Fig. 1A). Second, we evaluated how a male's testosterone-mediated behavior may affect the subsequent behavior of his social partner(s) (which we define as the socially transmitted "indirect effect" of testosterone; straight arrows in Fig. 1A).

To quantify cooperative behaviors and identify coalition partners, we utilized an autonomous behavior-logging system to monitor the social 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", 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 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. 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; each male was sampled up to three times per season (Fig. 1C). We used these hormone data to partition the standing variation in testosterone within and among-individuals, while controlling for capture conditions (Williams, 2008; Vernasco et al., 2018), and examined the relationships between testosterone and behavior.

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 competitive ability and aggression (Goymann, 2009), we consider that testosterone may be negatively related to cooperative behavior (i.e., "inhibition" in Fig. 1D). In 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 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 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., 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 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 relationships in other species (Maruska and Fernald, 2010), our analysis also tested for status-dependence in the relationship between testosterone and behavior. In all cases, we also examine the null hypothesis that social behavior may be dissociated from testosterone (i.e., "independence" in Fig. 1D; Moore et al., 2004; Lynn et al., 2005; Hau, 2007). Finally, to establish a causal relationship between circulating testosterone and cooperation, we also paired our observational analysis with a hormone manipulation experiment involving a subset of territorial males.

Results

Circulating testosterone levels were significantly repeatable in the wire-tailed manakin population, with 19% of the total standing variation in hormone levels attributed to differences among individuals. Thus, repeatable differences in hormone levels have the potential to explain at least some of the among-individual differences in behavior. To formally test this hypothesis, we partitioned testosterone levels into two sources of variation for our subsequent hormone-behavior analyses: "T-among", defined as an individual's average testosterone level relative to other individuals; and "T-within", defined as the fluctuations in testosterone within an individual male, relative to his overall average.

Our analysis revealed nonlinear hormone-behavior relationships that explained 1-4% of the variance within each status class (Fig. 2). Moreover, these relationships were highly status-specific. For floater males, individuals with the highest levels of T-among expressed greater lek attendance (effort), frequency of cooperative interactions (strength), and number of cooperative partnerships (degree; Fig. 2A-C). For territory-holding males, however, individuals with relatively moderate levels of T-among showed the greatest lek attendance (Fig. 2A), and T-among was not significantly related to either measure of cooperation (Fig. 2B-C). Examining within-individual variation in testosterone, in floater males, the highest levels of T-within were associated with slight increases in the frequency of engaging in cooperative coalitions, but not attendance or number of partners (Fig. 2D-F). However, in territory-holding males, intermediate levels of T-within were associated with the highest levels of all three behaviors (Fig. 2D-F). Thus, above some threshold of T-within, territory-holding males began to reduce their expression of social behavior.

Collectively, these results indicate that circulating testosterone may be a key mechanism driving individual differences in social behavior among floater males, whereby relatively high hormone levels promote status-seeking and cooperation. Previous research has shown that

cooperative interactions with territory-holding males increase the probability that floater males will ascend in status (Ryder et al., 2008). Hence, our results suggest that floater males who express relatively high testosterone levels may be favored. In support of this hypothesis, we also found that the floaters who successfully acquired a territory had significantly higher levels of circulating testosterone as compared to other floaters ($p = 0.04$, Fig. 3).

Our results also suggest that once a male is well-established as a territory-holder, further increases in testosterone may antagonize his cooperative behavior, and hence, reduce his mating success (Ryder et al., 2009). To test whether testosterone has causal effects on male behavior, we conducted a hormone manipulation experiment on a subset of the territory-holding males. We chose the territorial status class for this experiment, prior to any analysis, because their high site fidelity makes them readily available for post-manipulation behavioral monitoring. Fourteen males were randomly assigned to receive either an empty (Control treatment, $n = 8$) or testosterone-filled implant (T treatment, $n = 9$); three of these males received opposite treatments in subsequent years. Each male's social behavior was tracked for an average of 6 days before and 6 days after treatment (SD ± 1 day). The analysis tested whether the testosterone implants altered behavior, and whether behavioral changes in the T treatment group were greater than any change observed in the control group, after correcting for multiple comparisons.

The territorial males with experimentally elevated testosterone significantly decreased 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 significantly affect their territory attendance (effort; Figure 4A-B). Further analysis revealed that the effects of the T treatment on degree depended on a male's pre-implant hormone level, as males with relatively low testosterone prior to implant lost more partners than did males with initially high pre-manipulation testosterone (Fig. 4C). Overall, these results are consistent with the correlative analysis in Fig. 2 and confirm that the highest testosterone levels have a direct negative impact on a territorial male's ability to maintain cooperative partnerships.

To evaluate how a male's testosterone-mediated behavior may affect the subsequent behavior of his social partner(s), we used a path analysis of our observational data. We found that the indirect effect of floater testosterone (T-among) mediated the social transmission of cooperation frequency (strength), and to a lesser extent, lek attendance (effort; Fig. 5A). This suggests that testosterone-mediated cooperation and effort can be socially contagious from floater males to their social partners. We did not detect any significant indirect effects arising through unmanipulated territorial males in our observational data (Fig. 5B). However, the testosterone implants were associated with indirect effects that decreased the frequency of cooperation (strength) and the number of partners obtained (degree) by social partners (Fig. 5C). Moreover, the magnitude of the socially-transmitted decrease in strength was significantly greater than that observed in the control group. Together, these results suggest that elevated testosterone in floaters can promote the spread of cooperative activity to other individuals in the social network, whereas elevated testosterone in territory-holders can constrain it.

Discussion

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

224 coalition partnerships on his territory, because females prefer more cooperative individuals (Ryder et al., 2009). Moreover, male manakins are observed to match the behavior of
226 conspecifics (Dakin and Ryder, 2018), which may help them to maintain their territory, strengthen social partnerships, and attract females. As such, selection is expected to favor
228 endocrine mechanisms that promote behavioral plasticity to respond appropriately to conspecifics and ascension opportunities (Kelly and Vitousek, 2017). Although we know that
230 hormones can 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
232 has focused on how they facilitate courtship, aggression, competitive abilities, and dominance (Ketterson et al., 1996; Wingfield et al., 2001). Here, we show that testosterone, an androgen
234 long-known for its effect on aggression, can also mediate adaptive behavioral plasticity in a cooperative social system.

236 The results of our repeated hormone sampling efforts revealed that individual males differ significantly in their circulating testosterone (Fig. 1C). This finding of significant
238 repeatability is consistent with growing evidence that hormonal phenotypes are often repeatable and heritable in other systems (Williams, 2008; Mills et al., 2009; While et al., 2010; van Oers et
240 al., 2011; Iserbyt et al., 2015), yet robust estimates from wild populations, like those presented here, are still exceedingly rare (Cox et al., 2016). Although the proportion of behavioral variation
242 directly explained by testosterone was small at 1-4% (Table S6), these R^2 values are likely underestimates because of the measurement error associated with a limited number of repeat
244 hormone samples (mean 2.5 blood samples per male, IQR 1-4; Fig. 1C). Thus, the fact that among-individual differences in circulating hormones can account for any of the variation in the
246 behavior of a free-living animal is an important result (Ball and Balthazart, 2008).

Higher testosterone in floaters appears to facilitate status-seeking behaviors (Fig 2; see
248 also Eisenegger et al., 2011), as lek attendance increases the opportunities for establishing coalitions partnerships. At the same time, testosterone levels in floaters (which were, on average,
250 lower than that of those of territorial males; Figs. 1, 2) do not appear to antagonize cooperation, but instead seem to facilitate cooperative interactions (i.e., "facilitation" in Fig. 1D).
252 Territoriality is a prerequisite for reproductive success, and the number of coalition partnerships predicts social ascension (Ryder et al., 2008). Here, we also show that circulating testosterone
254 may be predictive of which males ascend to territorial status. Floaters that gained territories had higher testosterone pre- and post-ascension than floaters who did not obtain territories (Fig. 3).
256 These results raise the possibility that elevated testosterone in floaters may have key fitness benefits. Given the nature of age-graded queues in this system, the floaters that successfully
258 ascend are, on average, older. Therefore, hormone manipulation experiments are required to decouple the effects of age and testosterone on social ascension.

260 Once a male acquires a territory, his fitness depends on both the number of cooperative partners and the stability of those partnerships (Ryder et al., 2009; Ryder et al., 2011a). The
262 results of our hormone manipulation experiment confirm that when testosterone in territorial males is too high, it compromises their ability to attract and maintain stable display partnerships
264 (Fig. 4). Hence, testosterone levels above some threshold would decrease reproductive success. Our correlative data support this hypothesis, as territorial males with moderate testosterone
266 levels exhibited the optimal combination of social behaviors (i.e., "non-linear" hormonal modulation in Fig 1D). Because most coalition partnerships involve a territory holder and floater
268 (Ryder et al., 2011a), status-specific differences in how testosterone mediates behavior may promote social stability by facilitating cooperation between males of different status classes.

The status-specific hormone-behavior relationships reported here support the hypothesis that plasticity in androgen signalling maximizes individual fitness in manakins, whereby the expression of relatively high testosterone levels is favored in floaters, but constrained in territory holders. Plasticity in androgen signalling may result from a number of physiological mechanisms that are not mutually exclusive. One possibility is 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 secretion according to social status is essential. Although our findings for territorial males support a non-linear hormonal modulation of cooperative behavior (i.e., the inverted U function in Fig 1D), the threshold at which testosterone inhibits cooperation may depend, in part, on the hormonal phenotype or current endocrine status of the individual. This possibility is supported by the fact that the negative effect of T-implants on the number of display partners (degree) was greater for territory holders with lower pre-implant T-levels (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 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 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 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 signaling pathways facilitates cooperation in the manakin lineage. Future work examining status-specific expression of key steroid receptors, steroidogenic enzymes, and neuropeptides in the brain is a pivotal next step.

The social transmission of behaviors is hypothesized to play an important role in the evolution of cooperation (Trivers, 1971; Rankin and Taborsky, 2009). Our previous work has established that manakin cooperative behaviors are indeed socially contagious, whereby the cooperative behaviors of one individual stimulate subsequent cooperation in social partners (Dakin and Ryder, 2018). Here, we provide novel evidence that testosterone-mediated behavior may influence the status-dependent transmission of social behaviors among coalition partners, in particular the frequency at which manakins to engage in cooperative interactions (Fig. 5A, C). These findings support the hypothesis that testosterone can indirectly drive the social transmission of behaviors, and ultimately, influence the dynamics of the broader cooperative network.

A recently proposed framework suggests that aggression and sociality are inextricably 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 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 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; 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 male social status. Overall, our findings support the hypothesis that status-dependent and non-linear effects of androgens on social behavior may be a general feature of cooperative social systems where fitness depends on behavioral plasticity.

Methods

Study System

We studied a population of wire-tailed manakins (*Pipra filicauda*) that has been investigated and color-banded since 2002 at the Tiputini Biodiversity Station in Orellana Province, Ecuador (0° 38' S, 76° 08' W, approx. 200 m elevation). Social status (i.e., territorial or floater) was determined each year by direct observation (Ryder et al., 2008). Briefly, if an individual was noted displaying on a territory repeatedly and consistently over several days or weeks, he was assigned as the territory owner. The present study was conducted over three breeding seasons, from December to March in 2015-16, 2016-17, and 2017-18. Sample sizes were not predetermined, but rather we tried to capture and sample all males at the studied leks each season. Further details of sample sizes in each analysis are provided below.

Testosterone Sampling and Assay

We caught manakins with mist-nets up to three times per breeding season, and recorded the time of day and duration of restraint in the net (Vernasco et al., 2018). 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 on ice in the field 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% ethanol (Goymann et al., 2007); plasma volumes averaged 38.6uL (SD ± 10.3uL). For the testosterone assay, steroids were double extracted from plasma with dichloromethane (Ryder et al., 2011b). We used direct radioimmunoassay to determine the total plasma testosterone concentration (ng/mL), adjusted by extraction efficiency (Eikenaar et al., 2011). 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, 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 inter-assay coefficient of variation was 19.5%.

Partitioning Variation in Testosterone

A total of 524 testosterone samples were obtained from 210 individuals (1-8 samples per male; 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 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 fixed effects of breeding season, Julian date, time of day, and duration of restraint (i.e., time in the net; see Vernasco et al., 2018; also see Table S3). For 51 captures that were missing net 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 total variance in circulating testosterone explained by among-individual differences. The 95% confidence intervals for repeatability were determined by parametric bootstrapping. To characterize the change in testosterone during social ascent, we added a fixed effect of four ascent categories (Fig. 3) to the analysis. We then used post-hoc Tukey tests and the Benjamini-Hochberg procedure to compare status and ascent categories.

Given that testosterone was significantly repeatable, we next used a within-individual centering approach to partition the two sources of hormonal variation (T-among and T-within)

(van de Pol and Wright, 2009). We fit a model of log-transformed circulating testosterone using the same fixed effects above; the residuals of this model represent hormonal concentrations after accounting for capture conditions. For each male, we then computed the average of his residuals, which represents the individually-repeatable component of circulating testosterone, or T-among (one value per male). Next, we computed T-within (one value per blood sample) by subtracting each male's T-among from his testosterone residuals (van de Pol and Wright, 2009). Hence, T-within represents a male's circulating testosterone relative to his average level.

Social Behavior

Social behaviors (Fig. 1B; Tables S1-S2) were quantified using an automated proximity data-logging system (described in Ryder et al., 2012; Dakin and Ryder, 2018). Briefly, males were outfitted with coded nano-tags (NTQB-2, Lotek Wireless; 0.35 g), each of which transmitted a unique VHF signal ping at a rate of one ping per 20 s (range: 19.5-20.5 s). Approximately 10 (±SD 7) days after the captures were completed at a given lek, a proximity data-logger (SRX-DL800, Lotek Wireless) was placed within in each territory at that lek to record social behaviors. The data-loggers recorded all tag pings emitted by males within each territory from 0600 to 1600 local 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 season, after which social behaviors 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 subsequent behavior. We recorded a total of 29,760 hours of social activity over three field seasons (2015-16: 49 territories, mean = 16 recording days per territory; 2016-17: 54 territories, mean = 21 days per territory; 2017-18: 48 territories, mean = 22 days per territory, SD = ±4 days in all years). The number of males tagged for behavioral monitoring in each season was $n_{2015\text{-}16}$ = 100, $n_{2016\text{-}17}$ = 114, and $n_{2017\text{-}18}$ = 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 at unmonitored leks. These additional individuals are included in the repeatability and variance partitioning analysis of testosterone to obtain the most accurate measures, but they are not part of the behavioral study.

We defined three behavioral phenotypes using the data-logging system. A male's effort, or his rate of lek attendance, was quantified as his number of pings per day at monitored display territories. To define strength and degree, the 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; Ryder et al., 2012; Dakin and 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 this method identified birds that were indeed in close spatial proximity, a pre-requisite for social interactions. In total, we identified 36,885 unique social interactions over the three-year study. Note that for floaters, our measures of effort, strength and degree included activity on any territory, whereas for territory-holders, the same measures were limited to a male's effort and cooperative activity on his own territory. Behavioral measures were transformed using log(x + 1) to meet the assumptions of subsequent statistical analyses and were all significantly repeatable in a previous study (Dakin and Ryder, 2018).

Hormone Manipulation Experiment

A within-subjects testosterone manipulation was performed on 14 randomly-selected territorial males during the peak of the breeding season in 2016-17 and 2017-18. Subjects received a subcutaneous 7mm length x 1.47mm internal diameter silastic implant in which both ends were sealed with ~1mm of silicone. The implants were either filled with crystalline testosterone (T treatment) or left empty (the control). Three of these males were implanted in both breeding seasons, but with a different implant treatment each year. Implant surgeries were scheduled between data-logger recording sessions, such that each implanted male was monitored first for several days within a 4-week period prior to implantation, and then again for several days within a 10-day period after implantation. Blood samples were also acquired from 15 individuals in the weeks after implantation (mean 13 days, IQR 9-15 days) to verify that the T treatment significantly increased circulating testosterone relative to pre-implant levels ($p = 0.04$; pre-implant least-squares mean = 1.67 ng/mL [95% CI = 0.92, 3.02]; post-implant = 2.93 ng/mL [1.67, 5.15]). In contrast, the control implant did not significantly alter circulating testosterone ($p = 0.15$; pre-implant = 1.21 ng/mL [0.55, 2.66]; post-implant = 0.57 ng/mL [0.26, 1.25]). We also verified that all post-manipulation measures were within the natural (i.e., biologically relevant) range (see Fig. 1C and (Ryder et al., 2011b)). The average post-implant testosterone level in the T treatment group corresponded to the 78th percentile among unmanipulated territory-holding males.

Statistical Analyses

To evaluate the direct relationship between testosterone and behavior from our observational data, we used a model selection analysis in the MuMIn package (Bartoń, 2018) in R (R Core Team, 2018). Specifically, we evaluated support for the competing models representing the hypotheses shown in Fig. 1D and defined in Tables S4-S5. All candidate mixed-effects models included ID (random effect) as well as fixed effects of breeding season, Julian date, temperature (daily averages acquired at the Tiputini Biodiversity Station), days since capture, social status, and its interaction with testosterone (except in the null model which did not include testosterone). Because we were interested in the effects of testosterone on behavior, we recorded behavior after birds were blood sampled, and behavioral observations were assigned values of T-within using the bird's most recent prior hormone sample within four weeks (median 12 days, IQR 7-18 days). Thus, this analysis was limited to 2,565 daily recordings of 165 males for which a testosterone sample was acquired within this timeframe. After the model selection procedure, statistical significance was determined using the lmerTest package (Kuznetsova et al., 2018) in R. In each case, we also tested for status-dependence by determining whether the interactions between status and testosterone were statistically significant. R^2 estimates for the best-fit models were obtained using the r2glmm package (Jaeger, 2017) in R (R Core Team, 2018). Complete details of all candidate models are provided in Tables S4-S5.

To analyze the effect of the hormone manipulation experiment on behavior, we fit mixed-effects models that accounted for ID (random effect) as well as fixed effects of breeding season, temperature, and implant (either pre-implant, control, or T treatment; Table S7; n = 211 daily 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-values using the step-up false discovery rate procedure in the multtest package (Pollard et al., 2018). To determine if the effect of the T implant depended on a bird's pre-implant hormone level, we compared the fit of models with, and without, the implant x T-within interaction (Table S8).

To evaluate the indirect effects of testosterone, we used a path analysis to determine whether one bird's hormone-mediated behavior might explain variation in the same behavior subsequently expressed by their coalition partners. The data were organized by first considering each focal bird-observation, then determining the identities of the focal male's social partners (if any) on that observation date, and then determining the behavior of those social partners on the subsequent day (averaged by status class if the focal had both types of partner). The sample size for this analysis was n = 1,278 daily measures of 84 floater focal individuals, and 1,678 daily measures of 80 territory-holders. Path analyses were fit to the resulting data using the lavaan package (Roseel et al., 2018) in R (R Core Team, 2018) while accounting for repeated measures of the focal individuals using the cluster argument. The paths included the effect of the focal bird's testosterone on his own behavior, the social transmission of the focal bird's behavior on the subsequent behavior of his social partners, and additional covariates to account for season, temperature, and partner status (see Table S9 for complete details; all CFI > 0.96 indicating good model fits). For inference, we examined the mediation coefficient representing the indirect association between the hormone-driven behavior of the focal bird and the downstream behavior of his partners. We also verified that the direct effect of the focal bird's testosterone on subsequent partner behavior was non-significant in all cases (all $p > 0.08$). Finally, we analyzed the indirect effects of the hormone manipulation experiment by analyzing changes in the average behavior of the implanted males' floater partners during the pre- and post-implant dates (n = 195 measures of the partners of 14 implanted individuals; see Table S10). This analysis followed the same procedure used to analyze the direct effects of the experiment above.

Data Availability

All materials necessary to reproduce the results and figures, 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

References

Adkins-Regan, E. (2005). *Hormones and animal social behavior*. Princeton University Press.

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

Bartoń, K. (2018). *MuMIn: multi-model inference*.

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., Dai, B., Scheipl, F., Grothendieck, G. and Green, P. (2018). *lme4: linear mixed-effects models using "Eigen" and S4*.

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

Boksem, M. A. S., Mehta, P. H., Van den Bergh, B., van Son, V., Trautmann, S. T., Roelofs, K., Smidts, A. and Sanfey, A. G. (2013). Testosterone Inhibits trust but promotes reciprocity. *Psychol Sci* **24**, 2306–2314.

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

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

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 Review of Ecology and Systematics* **29**, 141–177.

Cox, R. M., McGlothlin, J. W. and Bonier, F. (2016). Hormones as mediators of phenotypic and genetic integration: an evolutionary genetics approach. *Integr Comp Biol* **56**, 126–137.

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

De Vries, G. J. and Panzica, G. C. (2006). Sexual differentiation of central vasopressin and vasotocin systems in vertebrates: different mechanisms, similar endpoints. *Neuroscience* **138**, 947–955.

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

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

Eikenaar, C., Whitham, M., Komdeur, J., van der Velde, M. and Moore, I. T. (2011). Endogenous testosterone is not associated with the trade-off between paternal and mating effort. *Behav Ecol* **22**, 601–608.

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

Eisenegger, C., Haushofer, J. and Fehr, E. (2011). The role of testosterone in social interaction. *Trends in Cognitive Sciences* **15**, 263–271.

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

Fuxjager, M. J., Forbes-Lorman, R. M., Coss, D. J., Auger, C. J., Auger, A. P. and Marler, C. A. (2010). Winning territorial disputes selectively enhances androgen sensitivity in neural pathways related to motivation and social aggression. *PNAS* **107**, 12393–12398.

Fuxjager, M. J., Miles, M. C. and Schlinger, B. A. (2018). Evolution of the androgen-induced male phenotype. *J Comp Physiol A* **204**, 81–92.

Goymann, W. (2009). Social modulation of androgens in male birds. *General and Comparative Endocrinology* **163**, 149–157.

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

Grunt, J. A. and Young, W. C. (1952). Differential reactivity of individuals and the response of 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 vertebrate life histories. *Bioessays* **29**, 133–144.

Hau, M. and Goymann, W. (2015). Endocrine mechanisms, behavioral phenotypes and plasticity: 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. *The Condor* **104**, 772–779.

Horton, B. M., Hudson, W. H., Ortlund, E. A., Shirk, S., Thomas, J. W., Young, E. R., Zinzow-Kramer, W. M. and Maney, D. L. (2014). Estrogen receptor α polymorphism in a species with alternative behavioral phenotypes. *PNAS* **111**, 1443–1448.

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

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

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

Kabelik, D., Morrison, J. A. and Goodson, J. L. (2010). Cryptic regulation of vasotocin neuronal activity but Not anatomy by sex steroids and social stimuli in opportunistic desert finches. *BBE* **75**, 71–84.

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

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

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

Kimura, T., Okanoya, K. and Wada, M. (1999). Effect of testosterone on the distribution of
566 vasotocin immunoreactivity in the brain of the zebra finch, *Taeniopygia guttata castanotis*. *Life Sci.* **65**, 1663–1670.

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

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

572 **Lema, S. C.** (2014). Hormones and phenotypic plasticity in an ecological context: linking physiological mechanisms to evolutionary processes. *Integr Comp Biol* **54**, 850–863.

574 **Lynn, S. E., Walker, B. G. and Wingfield, J. C.** (2005). A phylogenetically controlled test of hypotheses for behavioral insensitivity to testosterone in birds. *Hormones and Behavior*
576 **47**, 170–177.

Maruska, K. P. (2015). Social transitions cause rapid behavioral and neuroendocrine changes.
578 *Integr Comp Biol* **55**, 294–306.

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

Maruska, K. P. and Fernald, R. D. (2011). Social regulation of gene expression in the
582 hypothalamic-pituitary-gonadal axis. *Physiology*.

Mills, S. C., Grapputo, A., Jokinen, I., Koskela, E., Mappes, T., Oksanen, T. A. and Poikonen, T.
584 (2009). Testosterone-mediated effects on fitness-related phenotypic traits and fitness. *The American Naturalist* **173**, 475–487.

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

Oliveira, R. F. (2009). Social behavior in context: hormonal modulation of behavioral plasticity
590 and social competence. *Integr Comp Biol* **49**, 423–440.

Peters, A., Astheimer, L. B. and Cockburn, A. (2001). The annual testosterone profile in cooperatively breeding superb fairy-wrens, *Malurus cyaneus*, reflects their extreme infidelity. *Behav Ecol Sociobiol* **50**, 519–527.

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

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

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

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

Roseel, Y., Oberski, D., Byrnes, J., Vanbrabant, L., Savalei, V., Merkle, E., Hallquist, M., Rhemtulla, M., Katsikatsou, M., Barendse, M., et al. (2018). *lavaan: Latent Variable Analysis*.

Rosvall, K. A., Burns, C. M. B., Barske, J., Goodson, J. L., Schlinger, B. A., Sengelaub, D. R. and Ketterson, E. D. (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.

Ryder, T. B., McDonald, D. B., Blake, J. G., Parker, P. G. and Loiselle, B. A. (2008). Social networks in the lek-mating wire-tailed manakin (*Pipra filicauda*). *Proceedings of the Royal Society B* **275**, 1367–1374.

Ryder, T. B., Parker, P. G., Blake, J. G. and Loiselle, B. A. (2009). It takes two to tango: reproductive skew and social correlates of male mating success in a lek-breeding bird. *Proceedings of the Royal Society B* **276**, 2377–2384.

Ryder, T. B., Blake, J. G., Parker, P. G. and Loiselle, B. A. (2011a). The composition, stability, and kinship of reproductive coalitions in a lekking bird. *Behav Ecol* **22**, 282–290.

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

Ryder, T. B., Horton, B. M., Tillaart, M. van den, Morales, J. D. D. and Moore, I. T. (2012). Proximity data-loggers increase the quantity and quality of social network data. *Biology Letters* **8**, 917–920.

Schoech, S. J., Mumme, R. L. and Moore, M. C. (1991). Reproductive endocrinology and mechanisms of breeding inhibition in cooperatively breeding Florida scrub jays (*Aphelocoma c. coerulescens*). *The Condor* **93**, 354–364.

Soares, M. C., Bshary, R., Fusani, L., Goymann, W., Hau, M., Hirschenhauser, K. and Oliveira, R. F. (2010). Hormonal mechanisms of cooperative behaviour. *Philosophical Transactions of the Royal Society B* **365**, 2737–2750.

Taborsky, B. and Oliveira, R. F. (2012). Social competence: an evolutionary approach. *Trends in Ecology & Evolution* **27**, 679–688.

Trivers, R. L. (1971). The evolution of reciprocal altruism. *The Quarterly Review of Biology* **46**, 35–57.

van de Pol, M. and Wright, J. (2009). A simple method for distinguishing within- versus between-subject effects using mixed models. *Animal Behaviour* **77**, 753–758.

van Oers, K., Buchanan, K. L., Thomas, T. E. and Drent, P. J. (2011). Correlated response to selection of testosterone levels and immunocompetence in lines selected for avian personality. *Animal Behaviour* **81**, 1055–1061.

Vernasco, B. J., Horton, B. M., Ryder, T. B. and Moore, I. T. (2018). Sampling baseline androgens in free-living passerines: methodological considerations and solutions. *General and Comparative Endocrinology*.

While, G. M., Isaksson, C., McEvoy, J., Sinn, D. L., Komdeur, J., Wapstra, E. and Groothuis, T. G. G. (2010). Repeatable intra-individual variation in plasma testosterone concentration and its sex-specific link to aggression in a social lizard. *Hormones and Behavior* **58**, 208–213.

Williams, T. D. (2008). Individual variation in endocrine systems: moving beyond the 'tyranny of the Golden Mean.' *Philosophical Transactions of the Royal Society B: Biological Sciences* **363**, 1687–1698.

Wingfield, J. C., Hegner, R. E., Dufty, Alfred M. and Ball, G. F. (1990). The "challenge hypothesis": theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *The American Naturalist* **136**, 829–846.

Wingfield, J. C., Lynn, S. E. and Soma, K. K. (2001). Avoiding the 'costs' of testosterone: ecological bases of hormone-behavior interactions. *BBE* **57**, 239–251.

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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

Additional Information

Supplementary Information see Tables S1-S10 in the attached file

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).

Competing Interests

The authors declare no conflict of interest or competing financial interests.

FIGURE LEGENDS

Fig. 1. We studied how testosterone mediates 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. Testosterone is hypothesized to drive variation in male social behavior directly via his own circulating levels (loop arrows) and indirectly through his partners via social transmission (straight arrows). (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 Table S1-S2 for additional data. (C) We also collected repeated testosterone samples from 210 individuals (inset; n = 524 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 (Table S3). 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 hormone level after accounting 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) Our analysis 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.

Fig. 2. The direct effects of circulating testosterone are status-dependent. (A-C) Hormone-behavior relationships for T-among, defined as the repeatable differences among individuals in circulating testosterone. Floaters with the highest T-among had greater lek attendance (effort), cooperated with other males at higher frequencies (strength), and had more unique cooperative partners (degree), as compared to other floater males. In contrast, territory-holding males with the highest T-among had lower lek attendance. T-among was not significantly related to the frequency of cooperation (strength) or the number of cooperative partners (degree). (D-F) Examining the variation in testosterone within individuals ("T-within"), floaters with the most elevated T-within had a higher cooperation frequency (strength), whereas territory-holding males with the most elevated T-within decreased lek attendance and cooperation (strength and degree). Each scatterplot shows the residuals (y-axis) from the best-fit model after accounting for other predictors; note that all phenotypes were analyzed on a log-scale. Red and black asterisks indicate statistical significance for floaters and territory-holders, respectively; gray asterisks indicate the comparison between status classes (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). The sample size for this analysis was n = 2,565 daily measures of behavior from 165 individuals. To visualize the relationships, the larger opaque points show one average value per male, whereas the smaller semi-transparent points show all residuals. Further details are provided in Tables S4-S6.

Fig. 3. Elevated T is associated with ascension to territorial status. The data points show partial residuals from an analysis that controls for season and capture conditions (n = 524 measures from 210 individuals, including 15 who acquired territories during the study). Different

letters indicate statistically significant Tukey contrasts, after correcting for multiple comparisons. Floater males who acquired a territory the following season had, on average, higher testosterone than floaters who did not acquire a territory. The ascent to established territory ownership is associated with subtle increases in the average testosterone level.

Fig. 4. Experimental elevation of testosterone decreased cooperation in territory-holders. 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 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 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% confidence intervals. Experimental elevation of testosterone decreased the frequency of cooperative interactions (strength) and the number of unique partners (degree), because T post vs. pre and T vs. C were statistically significant ($p < 0.05$) after correcting for multiple comparisons (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). The sample size for this analysis was n = 211 daily measures of 14 individuals. Effect sizes in (B) are standardized to be comparable across the figure. (C) Further analysis indicated that the effect of elevated testosterone on the number of cooperative partnerships, degree, depended on the subject male's initial pre-treatment testosterone level. Specifically, stronger negative effects were observed for territory-holding males with lower pre-implant testosterone. See Tables S7-S8 for additional data.

Fig. 5. Testosterone can facilitate or constrain the social transmission of cooperative behavior to other individuals. A path analysis was used to evaluate whether the direct effect of testosterone (curved arrows) can also explain downstream social contagion (straight arrows) of each behavior, after accounting for other factors. The sample sizes were n = 1,278 and 1,678 measures of behavior for 84 floaters and 80 territory-holders, respectively, as well as the average behavior of their coalition partners the next day. See Table S9 for further details. (A-B) Schematic showing the direct and indirect effects of testosterone in each status class. Solid curved arrows indicate a significant effect of T-among in Fig. 2. Solid straight arrows represent the positive transmission of behavior between males; blue color indicates that this social contagion is mediated by an indirect effect of testosterone. The testosterone-mediated behavior of floater males (A) was significantly associated with the social transmission of cooperation frequency (strength), and to a lesser extent, lek attendance (effort). (B) Indirect effects of testosterone were not observed in unmanipulated territory-holding males, however in the hormone manipulation experiment (C), the testosterone treatment indirectly decreased the cooperative behavior of social partners (both strength and degree; Table S10). All features in (C) follow Fig. 4B.

Fig. 1

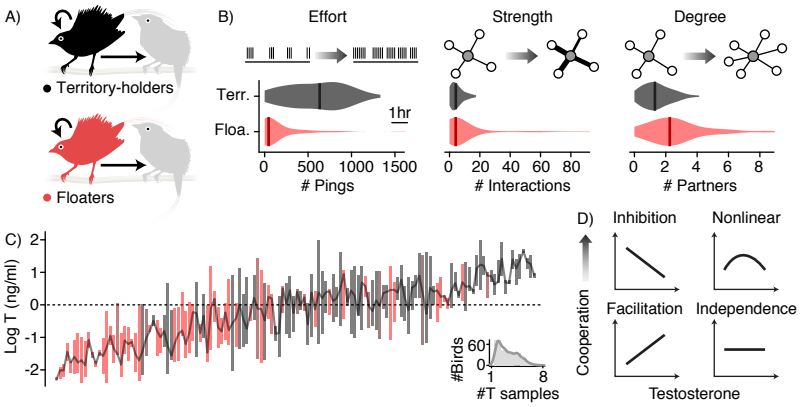

A)
Territory-holders
Floaters
B)
Effort
Terr.
Floa.
1hr
0
500
1000
1500
Pings
Strength
0
20
40
60
80
Interactions
Degree
0
2
4
6
8
Partners
C)
Log T (ng/ml)
2
1
0
-1
-2
#Birds
60
0
1
8
#T samples
D)
Inhibition
Nonlinear
Facilitation
Independence
Cooperation
Testosterone

Fig. 2

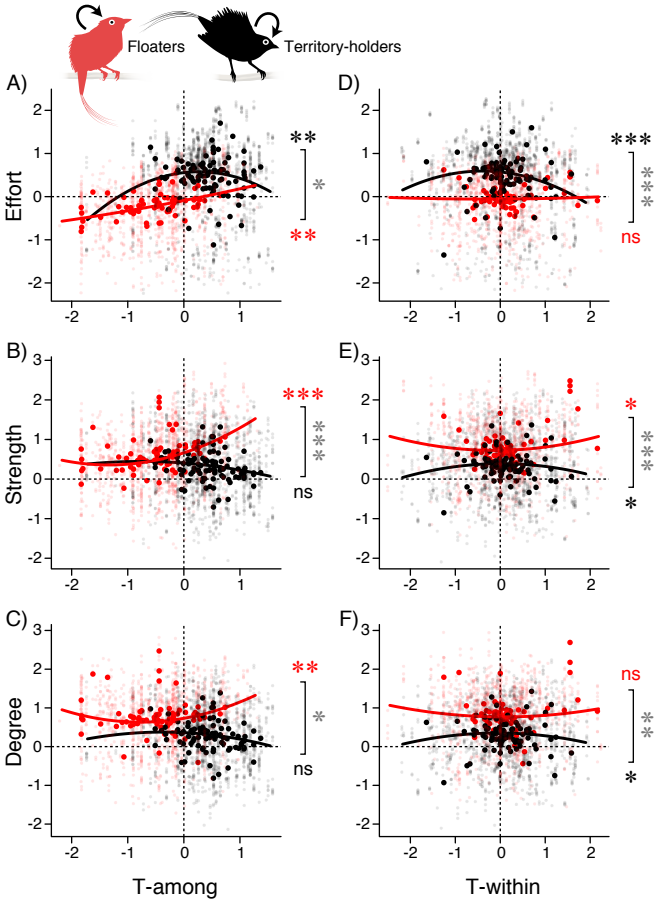

Floaters
Territory-holders
A)
B)
C)
D)
E)
F)
Effort
Strength
Degree
T-among
T-within
ns

Fig. 3

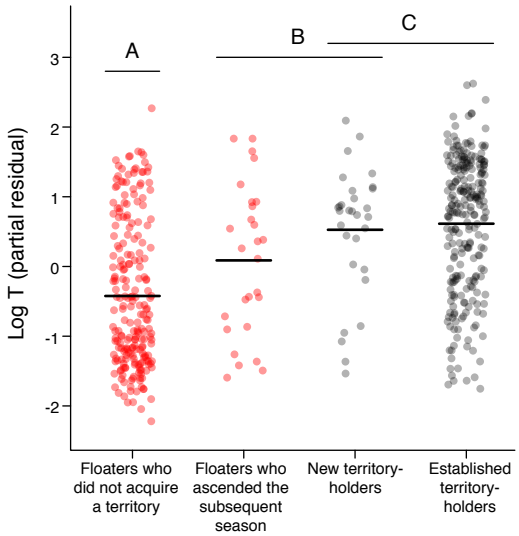

A
B
C
3
2
1
0
-1
-2
Log T (partial residual)
Floaters who did not acquire a territory
Floaters who ascended the subsequent season
New territory-holders
Established territory-holders

Fig. 4

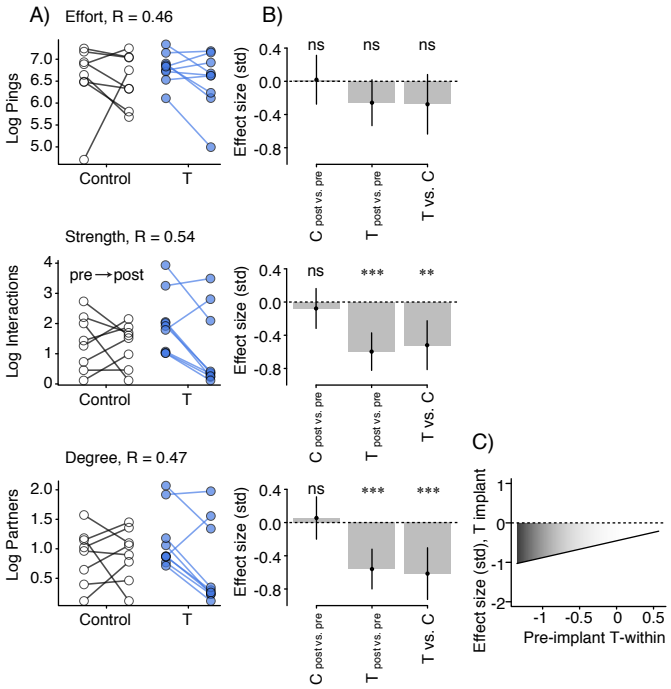
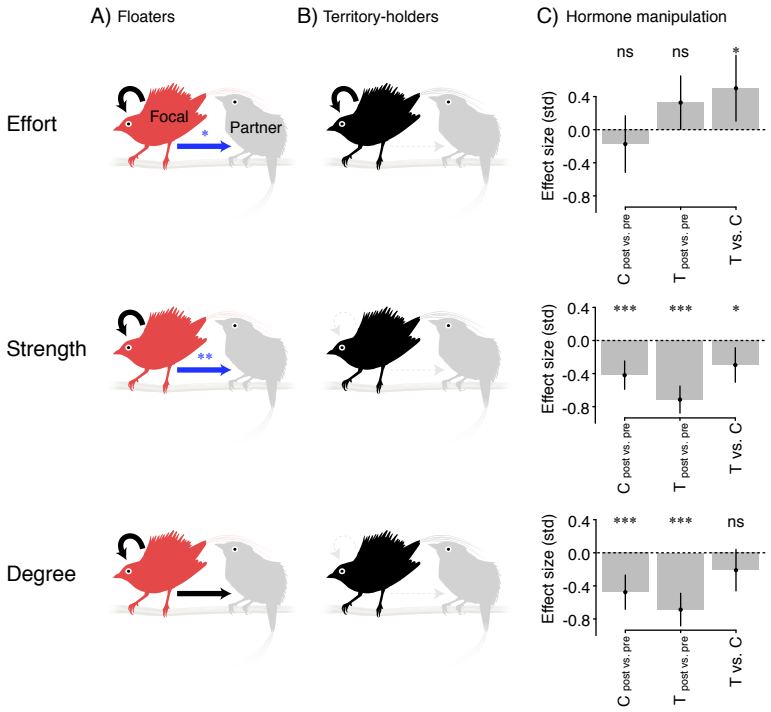

A)
Effort, R = 0.46
Log Pings
7.0
6.5
6.0
5.5
5.0
Control
T
Strength, R = 0.54
Log Interactions
pre→post
4
3
2
1
0
Control
T
Degree, R = 0.47
Log Partners
2.0
1.5
1.0
0.5
Control
T
B)
Effect size (std)
0.4
0.0
-0.4
-0.8
ns
ns
ns
C post vs. pre
T post vs. pre
T vs. C
ns
***
**
C post vs. pre
T post vs. pre
T vs. C
ns
***
***
C post vs. pre
T post vs. pre
T vs. C
C)
Effect size (std), T implant
1
0
-1
-2
-1
-0.5
0
0.5
Pre-implant T-within

Fig. 5


A) Floaters
B) Territory-holders
C) Hormone manipulation
Effort
Strength
Degree
Focal
Partner
*
**
Effect size (std)
0.4
0.0
-0.4
-0.8
ns
ns
*
***
***
*
***
***
ns
C post vs. pre
T post vs. pre
T vs. C