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Biological Sciences
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Testosterone drives status-specific patterns of cooperation and transmission of behavior in a social network

Short title: Testosterone modulates cooperation

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

DATA AVAILABILITY
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: https://figshare.com/s/d36c1a5c822e00abc581

AUTHOR CONTRIBUTIONS
TBR, BMH, and ITM designed research
TBR, RD, BMH, BJV, and ITM performed research
RD, TBR, and BSE analyzed the data
TBR and RD wrote the manuscript
All authors edited the manuscript

CONFLICT OF INTEREST
We have no conflict of interest.

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Abstract

Stable social partnerships require physiological mechanisms that maintain a balance between competition and cooperation. Although the endocrine system is known to facilitate competitive behavior, we know very little about how steroid hormones affect cooperation and the stability of partnerships. Here, we examine how testosterone (T) modulates social behavior in wire-tailed manakins (*Pipra filicauda*), a species in which territorial and non-territorial males form cooperative coalitions within a larger social network. Our approach links repeated hormone sampling and a hormone-manipulation experiment with an automated telemetry system that captured >30,000 male-male social interactions. Using 528 repeated T samples from 209 males, we find that circulating T is repeatable with individual differences accounting for 19% of the total variation, and that among-individual differences in baseline T can explain 17% of the 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.

Significance Statement (max 120 words)

The hormone testosterone is often linked to aggressive behaviors that aid in competition. In cooperative societies, hormone-mediated behavior must be modified to allow stable partnerships. We studied testosterone in the wire-tailed manakin, a gregarious bird whose cooperative 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.

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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 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, 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 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 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-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 (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 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). 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, 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 systems. As a result, the role of steroids in shaping complex behaviors, like cooperation, remain virtually unknown (Soares et al., 2010).

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 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 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 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 repeated sampling design and analytical approaches that enable partitioning of within- and among-individual phenotypic variation, while accounting for the dynamic social environment (Dingemanse and Araya-Ajoy, 2015).

Here, we designed such a study to evaluate how testosterone modulates cooperative 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 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 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 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 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; Dakin and Ryder, preprint in review).

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Our goal in this study was to evaluate the relative contribution of three hormonal mechanisms of social behavior: (i) within- and (ii) among -individual standing variation in circulating testosterone (loop arrows in Fig. 1A); as well as (iii) the hormonal phenotype of a male's social partner(s) as a mechanism of social influence (straight arrows in Fig. 1A). Towards this end, we collected repeated measures of circulating testosterone from a large population of 209 male manakins and verified that hormonal concentrations varied both within and among individuals (Fig. 1C-D). To match these hormone samples to repeated behaviors, we developed an autonomous data-logging system to monitor social behavior and coalition partnerships that 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 daily sociality and the behaviors that underlie stable cooperative partnerships (Fig. 2A). These included a measure of attendance on the display territories (effort); the number of territories visited (dispersal); the total frequency of cooperative interactions with any partner (total strength); the average frequency of cooperation per partner (avg. strength); the total number of unique cooperative partners (degree); and the exclusivity of his partnerships (importance). We consider effort and dispersal to be measures of a male's status-seeking behavior and strength, degree, and importance to be measures of his cooperative tendencies. Because our focus was on hormonal effects on behavior, we took behavioral recordings at a given subset of territories after capturing the birds for blood samples. We also paired this observational approach with a hormone manipulation of territorial males to verify the causal relationship between circulating testosterone and cooperation.

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

Results

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

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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).

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 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 importance. This behavioral phenotype captures the exclusivity of male's partnerships. When floater males had high T relative to their baseline (i.e., more positive residual T), their social 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 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 and maintain higher circulating hormone concentrations.

We next used a hormone manipulation experiment to directly test if T negatively affects 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 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 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 behavior, and whether this change was greater than that observed in the blank control treatment, after correcting for multiple comparisons (Table S5).

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 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 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 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 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 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 and extrinsic factors can influence how T modulates social behavior.

In addition to intrinsic hormone levels, social behavior is also influenced by dynamic 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 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 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 partners (Dakin and Ryder, preprint in review), this analysis tests whether partner T induces behavioral plasticity.

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

Discussion

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

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

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

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

These results suggest that wire-tailed manakin social behavior and its heritable hormonal basis are the result of stabilizing selection, such that intermediate hormone concentrations balance competitive behaviors (for status and dominance) with behaviors that promote stable cooperation. Higher T in floaters facilitates a suite of status-seeking behaviors (Eisenegger et al., 2011), such as territory attendance and visitation (Fig. 3). At the same time, standing variation in floater T, which is on average lower than that of territorial males (Fig. 1), does not antagonize cooperation, and indeed promotes floaters to interact with coalition partners at a higher frequency (Fig. 3). Given that territoriality is a prerequisite for reproductive success and coalition partnerships predict social ascension (Ryder et al., 2008), higher T may maximize 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.

The plasticity in the androgen system may result from a number of potential physiological mechanisms that are not mutually exclusive. One possibility is that T is only antagonistic to cooperative behavior above some threshold that most floater males have not yet reached. The idea of dose-dependent hormonal modulation of cooperative behavior is supported by the fact that manipulated territory-holders with lower T had larger antagonistic effects on cooperation (Fig. 3). A second possibility is direct transcriptional effects, whereby status-specific 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.

Cooperation is an emergent property of repeated interactions in a social network, and these interactions are integral to determining the costs and benefits of cooperative behavior (Ohtsuki et al., 2006). Moreover, each social partner represents a potential extrinsic proximate influence on a focal individual's behavior (Fehl et al., 2011; Rand et al., 2011). Previous work 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

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

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

Materials and Methods

Study System

We studied wire-tailed manakins (*Pipra filicauda*) at the Tiputini Biodiversity Station, Orellana Province, Ecuador (0° 38' S, 76° 08' W, approx. 200 m elevation), a population that has been observed and color-banded since 2002. 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 instead we aimed to sample all males in the population and to recapture as many as possible for up to three repeated hormone samples per season.

Testosterone Assay

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

Social Behavior

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

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

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

Hormone Experiment

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

Baseline and Residual T

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

Analyses of Behavior

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

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

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

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

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

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FIGURE LEGENDS

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.

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.

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 ± 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 (± 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.

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

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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.

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.

SUPPLEMENTAL FIGURES

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).

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. 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.

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.

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.

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. 1

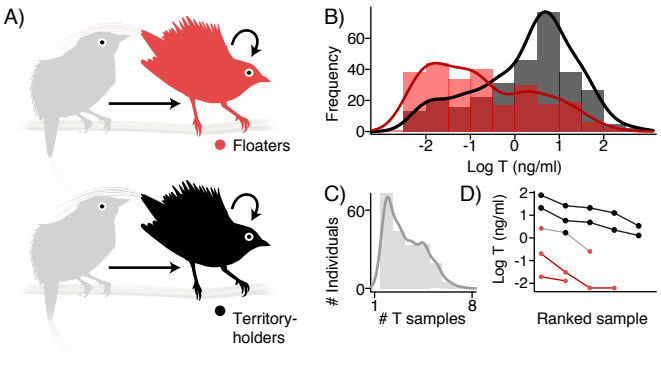

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Fig. 2

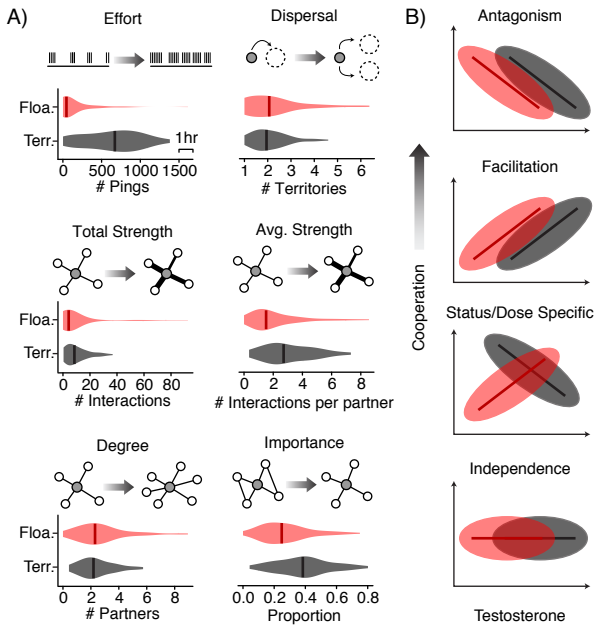

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Fig. 3

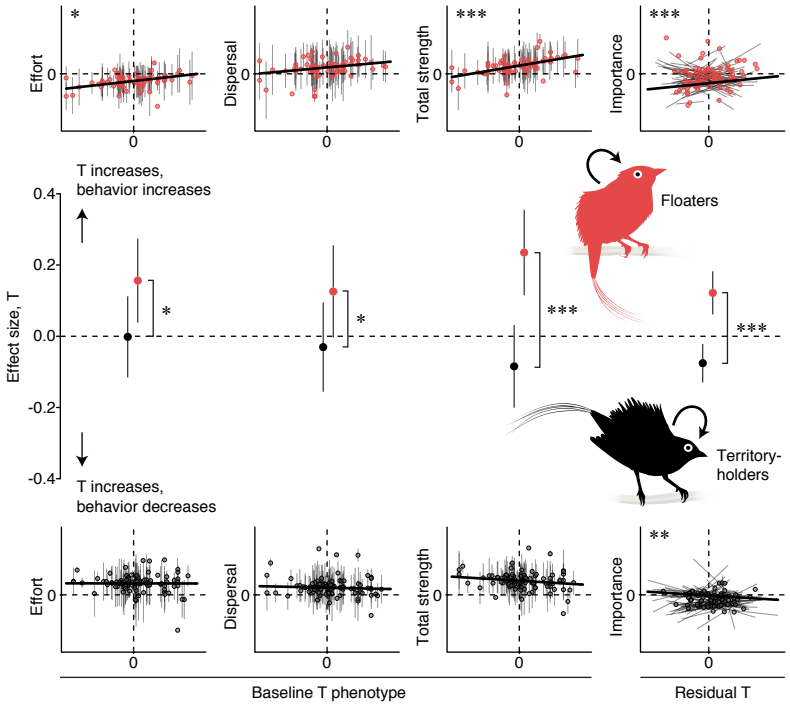

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Fig. 4

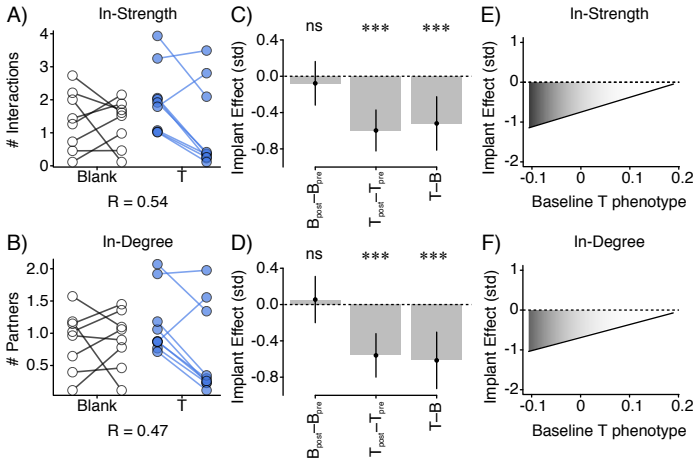

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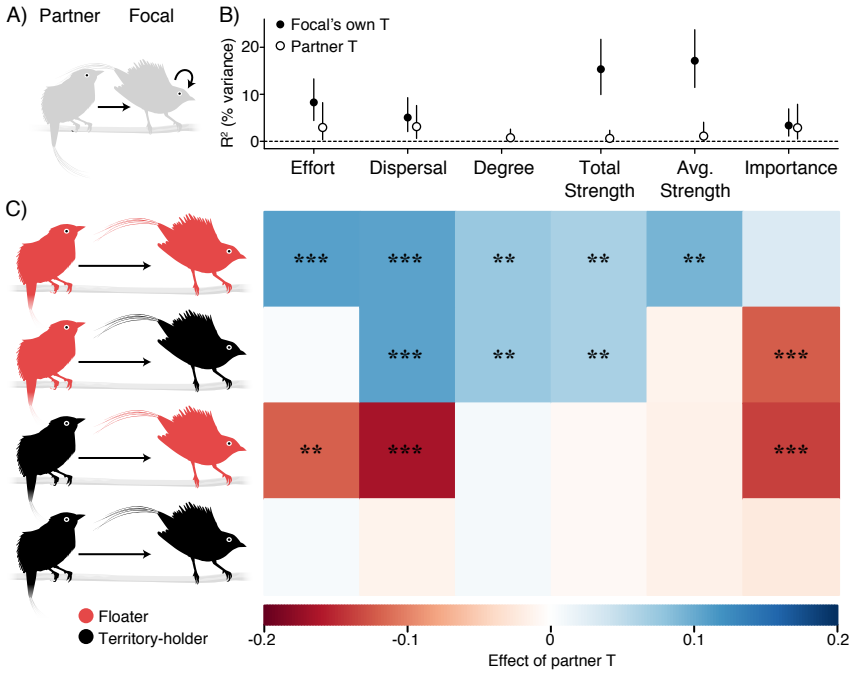
Fig. 5



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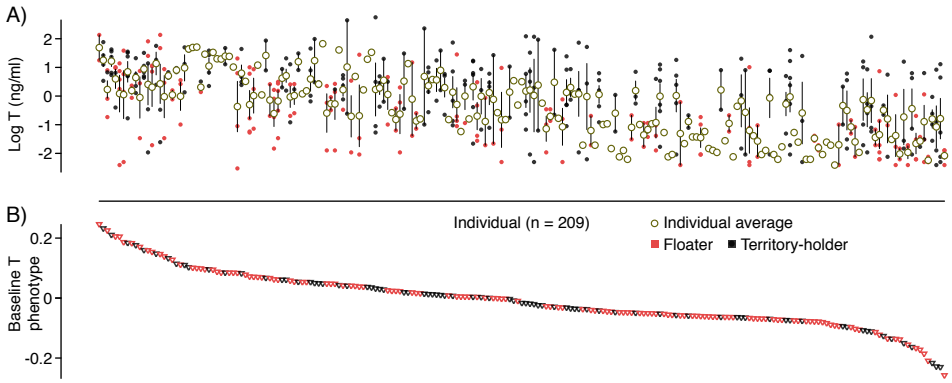

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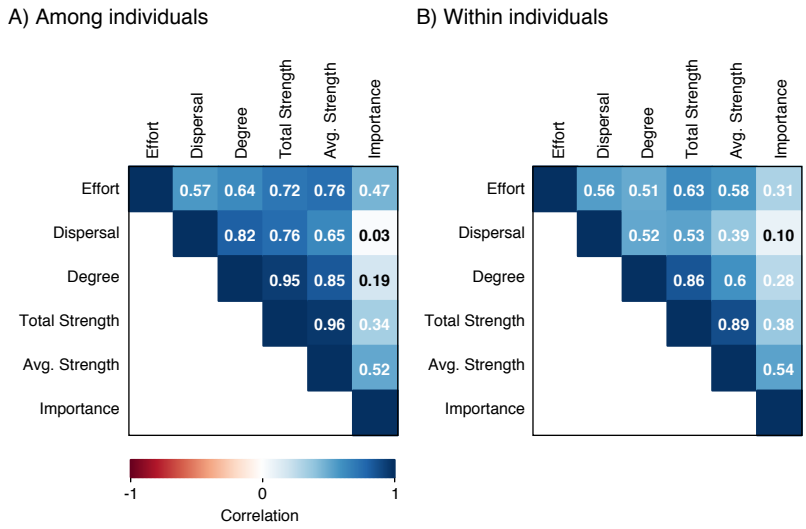
Fig. S2



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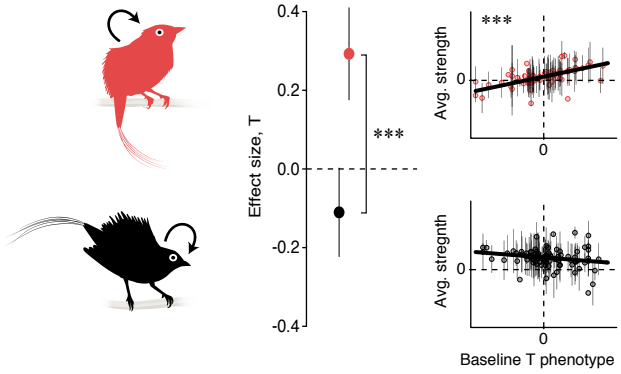

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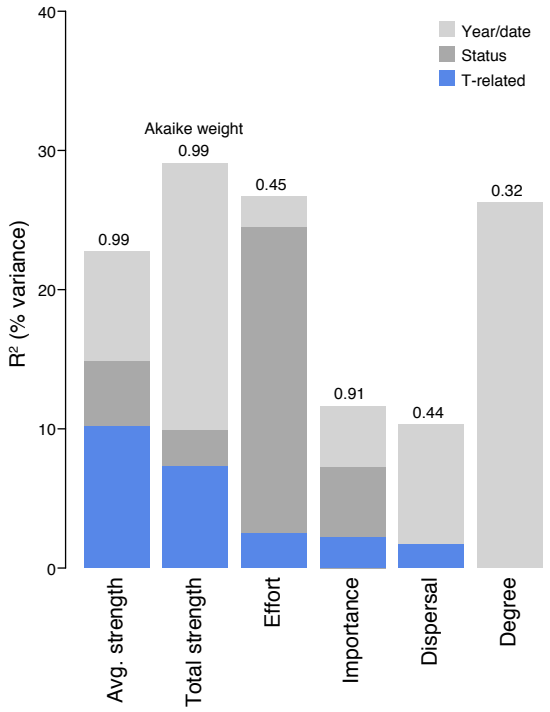

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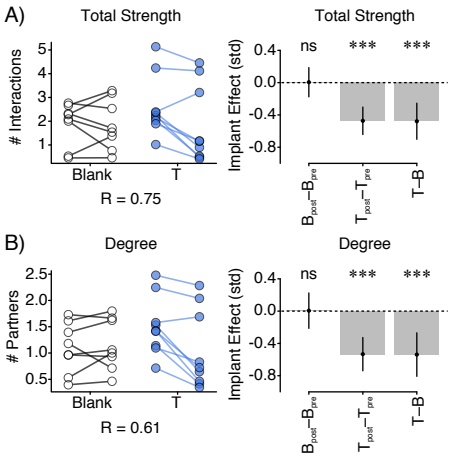
Fig. S5



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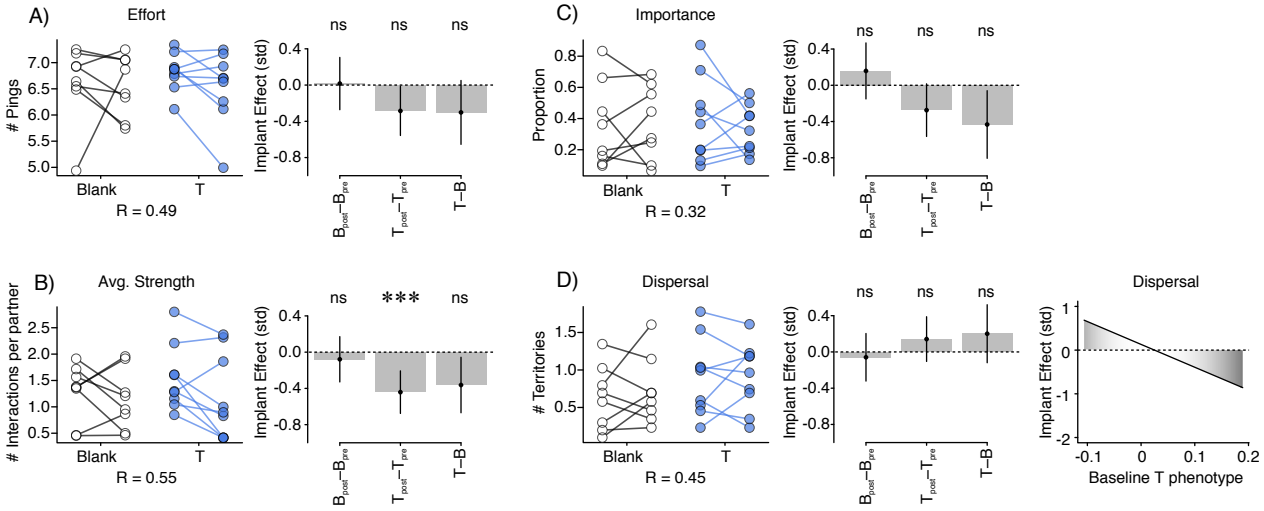

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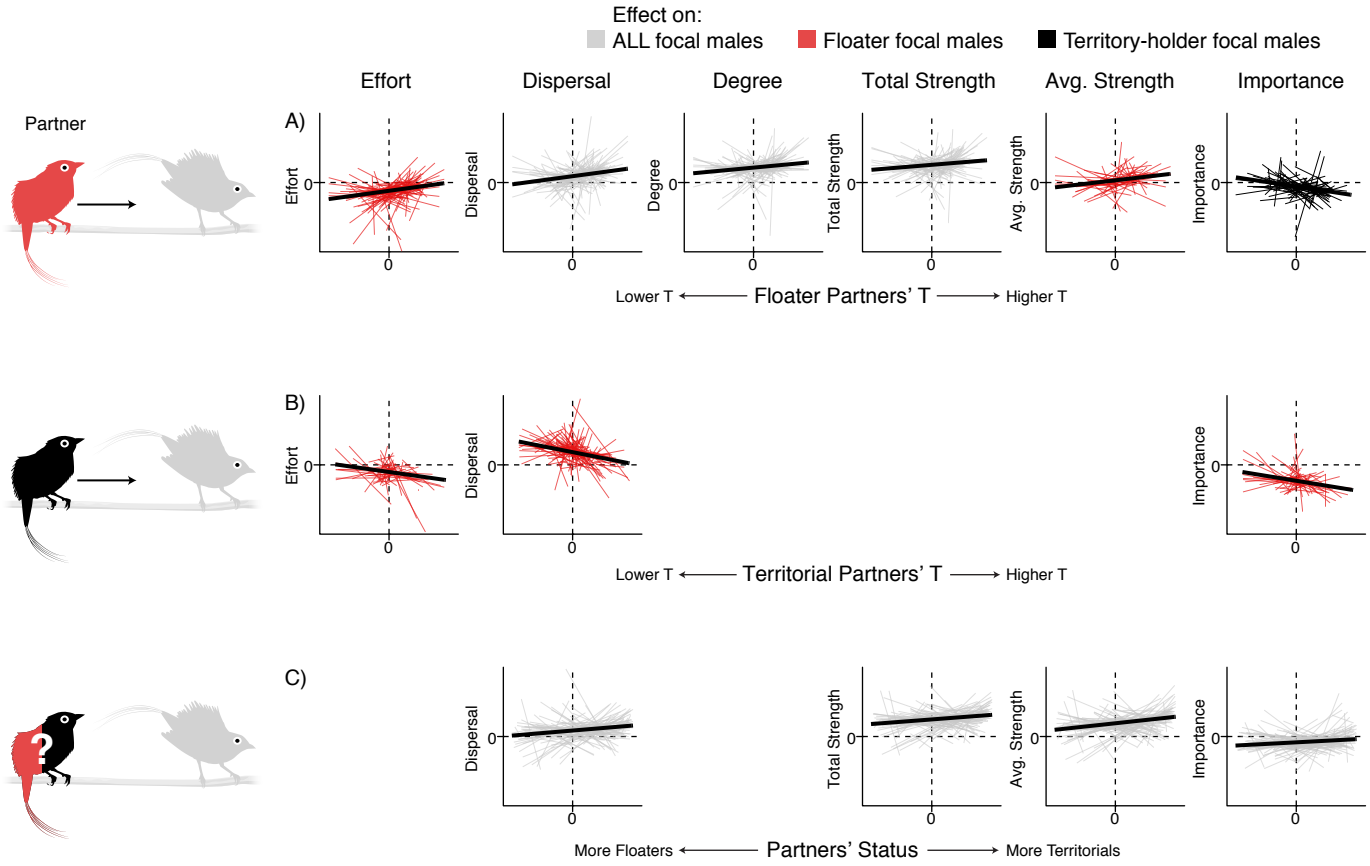

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