Testosterone-mediated behavior shapes the emergent properties of social networks

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Abstract

1. Social networks can vary in their organization and dynamics, with implications for ecological and evolutionary processes. Understanding the mechanisms that drive these dynamics requires integrating individual-level biology with comparisons across multiple social networks.
2. Testosterone is a key regulator of vertebrate social behavior. Previous studies have demonstrated that testosterone can influence how individuals interact with social partners. Although the effects of testosterone on individual behaviors are well established, no study has examined whether hormone-mediated behavior can scale up to shape the emergent structure of social groups.
3. We tested this hypothesis in the wire-tailed manakin, a bird species in which the males cooperate at specialized courtship sites known as leks. We used an automated proximity system to longitudinally monitor multiple leks and quantify the social network interactions at each site. Our analysis examines three emergent properties of the social networks that are expected to enhance the benefits of cooperative behavior: social specialization (the exclusivity of relationships), network stability (the overall persistence of partnerships through time), and behavioral assortment (the tendency for like to associate with like).
4. The manakin leks with higher-testosterone dominant males were less specialized, less stable, and had more negative behavioral assortment, after accounting for other factors. These results support our main hypothesis that individual-level hormone physiology can predict group-level network dynamics. We also observed that larger leks with more

interacting individuals had more positive behavioral assortment. This suggests that small groups may constrain processes of homophily and behavior-matching.

5. Overall, these results provide evidence that hormones can act as an extended phenotype that shapes the broader architecture of social groups. High-testosterone groups exhibit collective behaviors that are predicted to impede the evolution of cooperation.

Keywords: androgens, behavioral endocrinology, collective behavior, cooperation, dynamic networks, social networks, testosterone

Introduction

Behavioral interactions are the foundation of social network structures that can vary through time, among populations, and among species. Network structures play an important role in many ecological and evolutionary processes, including the spread of diseases (Sah *et al.* 2018), the transmission of information and resources (Aplin *et al.* 2012), and selection on individual behavior (Ohtsuki *et al.* 2006). A major challenge is understanding how individual-level factors, such as physiological and behavioral mechanisms, scale up to drive the emergent structural properties of social groups (Krause & Ruxton 2002). Linking these two levels of analysis is difficult because it requires integrating individual-level data with repeated measures of entire social groups (Sah *et al.* 2017).

Here, we use a comparison of multiple social networks through time to investigate the hypothesis that testosterone destabilizes social structure. Testosterone is a steroid hormone that is well known for its influence on the social behavior of individuals (Wingfield *et al.* 1990; Oyegbile & Marler 2005; Adkins-Regan 2005; Goymann 2009; Fuxjager *et al.* 2010; Eisenegger *et al.* 2011). These hormone-signaling pathways are essential for the development and expression of complex behavioral phenotypes (Cohen *et al.* 2012). Recent work has shown that testosterone can influence the cooperative behavior of individuals (Boksem *et al.* 2013; Ryder *et al.* 2020). We consequently hypothesize that hormone-mediated behaviors may also shape higher-order social network structure.

To investigate this hypothesis, we studied the wire-tailed manakin (*Pipra filicauda*), a bird species in which the males engage in cooperative display coalitions at specialized courtship sites known as leks. In *P. filicauda*, males can represent two status classes: dominant, territory-holding males, and subordinate, “floater” males who must acquire a territory on a lek before they

can mate (Heindl 2002; Ryder *et al.* 2011a). Cooperative coalitions occur between two unrelated
70 males, most commonly a territory-owner and a floater (Ryder *et al.* 2011a). A hallmark of reproductive cooperation is that, on average, both partners gain some benefit, although the
72 magnitude of these benefits need not be equal (e.g., partners can receive delayed benefits; DuVal 2007; Díaz-Muñoz *et al.* 2014). Consistent with this definition, long-term studies of *P. filicauda*
74 have shown that coalition partnerships are mutually beneficial. First, subordinate males with more social partners are more likely to acquire a territory in subsequent years (Ryder *et al.*
76 2008), similar to other cooperative systems (McDonald 2007). Second, the territory-holders with more social partners sire more offspring, as compared to their direct competitors (Ryder *et al.*
78 2009).

It is impossible to manipulate long-term social relationships in the wild to experimentally
80 test the association between sociality and fitness. Nevertheless, further evidence also supports the hypothesis that partnerships are beneficial in *P. filicauda*. For example, when a male has a social
82 partner present, he displays at a higher frequency and for a longer period of time (Ryder *et al.* 2011a; Vernasco *et al.* 2020). Given that these conspicuous displays are often a target of female
84 mate choice, this provides a mechanism by which coalitions may increase male attractiveness and subsequent reproductive success (Lank & Smith 1992; Trainer & McDonald 1995; Durães *et*
86 *al.* 2009). Territory-holders also use calls to solicit visitation by coalition partners (Schwartz & Snow 1978; Ryder and Vernasco, personal observations), a behavior that would be unlikely if
88 these partnerships were not directly beneficial. Finally, territory-holders who visit and perform displays with males on neighboring territories may also benefit, as a result of reciprocity and
90 behavioral contagion (Dakin & Ryder 2018).

Two features of the manakin leks make them especially well-suited to study the relationship between hormones and group-level social structure. First, we recently established that testosterone has status-specific effects on the coalition behavior of individual male wire-tailed manakins (Ryder *et al.* 2020). Second, these coalition partnerships among males form the basis of complex and dynamic social networks (Dakin & Ryder 2018). Given this background, we sought to test whether testosterone and its effects on male behavioral phenotype could drive the emergent properties of the social network that facilitate cooperation. Previous theoretical studies have shown that cooperation in social networks is favored when individuals interact with a limited set of familiar partners, when these relationships are temporally stable, and when social relationships are reciprocated by immediate neighbors in the group (Trivers 1971; Roberts & Sherratt 1998; Ohtsuki *et al.* 2006). Accordingly, we used repeated measures of the manakin networks to analyze whether testosterone was related to three emergent properties of the social network, described below (Fig. 1).

The first property, social specialization, seeks to capture the exclusivity of the relationships between coalition partners. In the context of manakin behavior, highly specialized networks exhibit a high frequency of exclusive social partnerships between males of different status classes, as illustrated in Fig. 1A. In other species, social specialization has been found to maximize the quality and coordination of different types of behaviors (Jehn & Shah 1997; Laskowski *et al.* 2016). In manakins, we expect specialization to improve the signal quality of male-male courtship displays (e.g., as a result of improved behavioral coordination). Greater specialization is also expected to minimize conflict over mating and territorial ascension opportunities (Schjelderup-Ebbe 1922; McDonald 1993).

The second property, network stability (Fig. 1B), quantifies the average persistence of social partnerships through time (Poisot *et al.* 2012; Dakin & Ryder 2020). Coalition partnerships require the coordination of complex behaviors, and previous empirical work indicates that longer partnership tenure has a positive effect on display coordination (Trainer & McDonald 1995; Trainer *et al.* 2002). Greater temporal stability of partnerships also increases the opportunity for sustained reciprocity within the network (Trivers 1971; Roberts & Sherratt 1998; Croft *et al.* 2006).

The third property, behavioral assortment (Fig. 1C), captures the extent to which males interact with partners who express similar behaviors (i.e., is like associated with like? (Croft *et al.* 2006; Farine 2014)). At the proximate level, positive assortment may represent the outcome of generalized reciprocity and/or partner choice (Fowler & Christakis 2010; Dakin & Ryder 2018). At the ultimate level, positive assortment has also been shown to promote the evolution of cooperation (Ohtsuki *et al.* 2006). To quantify the overall behavioral assortment of the manakin networks, we used a composite measure that averaged the assortativity of "strength" (a male's frequency of daily social interactions) with that of "degree" (his daily number of social partnerships; (Dakin & Ryder 2018)).

As a potential predictor of specialization, stability, and assortment, we quantified the collective testosterone of the manakin leks (Fig. 2; (Akinola *et al.* 2016)). Because hormone-behavior relationships are status-specific in manakins and many other species (Eisenegger *et al.* 2011; Boksem *et al.* 2013; Ryder *et al.* 2020), we analyzed collective testosterone for each of the two status classes separately. High testosterone levels are associated with reduced cooperative behavior in dominant males, but increased cooperative behavior in subordinate males (Ryder *et al.* 2020). We consequently predicted that the collective testosterone for territorial males would

be negatively associated with specialization, stability, and assortment, whereas the collective testosterone for floater males would be positively associated with these three emergent properties.

Materials and Methods

Study Population

We studied wire-tailed manakins (*Pipra filicauda*) at the Tiputini Biodiversity Station in Orellana Province, Ecuador (0º 38' S, 76º 08' W). This population of *P. filicauda* has been studied and individuals color-banded annually since 2002 (Ryder *et al.* 2008, 2009). The present study was conducted on 11 leks during peak breeding activity (December-March) across three field seasons: 2015-16, 2016-17 and 2017-18 (Ryder *et al.* 2020). All research was 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).

Testosterone Assessment

Male manakins were captured using mist-nets on the display leks as described in (Ryder *et al.* 2020). We deployed up to 16 mist-nets simultaneously at a given lek, with the goal of capturing each male up to three times per field season. Each mist-net was checked on a 30-minute schedule (Vernasco *et al.* 2019; Ryder *et al.* 2020). The amount of time a male spends in the net has a subtle, but significant, effect on his circulating testosterone (Vernasco et al. 2019). Therefore, we used video monitoring to determine the duration of time that each bird was in the mist-net prior to blood sampling, so that it could be accounted for in further analyses (mean = 17.5 minutes, SD = 10.3 minutes, range = 1–72 minutes). Following removal of a male from the mist-net, a small

blood sample (< 125uL) was taken from the brachial vein and stored on ice prior to being centrifuged at 10,000 rpm for 5 min, as described in previous studies (Ryder *et al.* 2011b; Vernasco *et al.* 2019; Ryder *et al.* 2020). Plasma volume was measured to the nearest 0.25 ul and stored in 0.75 ml of 100% ethanol (Goymann *et al.* 2007). Testosterone was double extracted from the plasma using dichloromethane. Following extraction, direct radioimmunoassay was used to measure the total plasma androgen concentration (ng/ml) adjusted by the extraction efficiency and plasma volume of each sample (Eikenaar *et al.* 2011; Ryder *et al.* 2011b). Hormone assays were conducted annually, and the detection limits were 0.12, 0.08, and 0.09 ng/ml for 2015-16, 2016-17 and 2017-18, respectively; any sample that fell below the assay-specific limit of detection was assigned that limit as its testosterone concentration as a most conservative estimate. The extraction efficiency for all samples was between 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%.

Behavioral Recording

We used an automated data-logging system to monitor male-male interactions on the display territories of 11 different leks (Ryder *et al.* 2012; Dakin & Ryder 2018; Ryder *et al.* 2020). The territories on these leks are specifically used for courtship displays and male-male social interactions. At the beginning of each field season, male manakins were outfitted with coded nano-tags (NTQB-2, Lotek Wireless; 0.35 g). The tags transmitted a unique VHF signal ping once per 20 s for three months. In total, 296 tag deployments were performed on 180 individuals (mean 1.7 field seasons per male ± SD 0.7), 178 of whom also had hormone data (mean number of hormone samples per male = 3 ± SD 1.5). Approximately 10 days (± SD 7) after tagging and

sampling was completed at a given lek, a proximity data-logger (SRX-DL800, Lotek Wireless) was deployed within each display territory at the lek to record tagged males within a detection radius of 30 m (a distance that corresponds to the typical diameter of a manakin display territory (Heindl 2002; Dakin & Ryder 2018)).

Proximity recording sessions ran from 06:00 to 16:00 for ~6 consecutive days (± SD 1 day) and were performed ~3 times per field season at a given lek. Occasionally, the length of a recording session was extended due to extenuating circumstances such as inclement weather. The recording sessions were scheduled to be distributed evenly throughout each field season at each lek, to minimize any confounding seasonal effects. Each recording session represents an observation of the social network at a given lek. In total, we conducted 86 recording sessions (29,760 data-logger hours) representing repeated measures of the social activity at 11 different leks over the course of three field seasons (see Fig. S1 in the supplement for additional details on the sampling regime).

Prior to data-logger deployment, each territory was also observed on non-recording days to identify the territory-holder based on his color-bands, following previous studies (Ryder *et al.* 2008, 2009). These status assignments were subsequently verified in the proximity data.

Data Processing and Statistical Analysis

All computational and statistical analyses were performed in R (R Core Team 2018). Network illustrations were made using the igraph package (Csardi & coathors 2018).

Social Interactions

Social networks were constructed by first defining interactions between two males that occurred on the display territories. To do this, a computational algorithm was used to identify joint detections, wherein two males were located at the same display territory within a pre-defined spatial and temporal threshold (Ryder *et al.* 2008, 2012; Dakin & Ryder 2018). For the temporal threshold, the two males had to occur < 45 s apart. This temporal threshold was chosen to allow for the fact that each tag pinged with a 20 second pulse rate, such that overlapping individuals could have up to a 40 s gap between their respective pings. For the spatial threshold, the two males had to have a difference in received signal strength values (Δ RSSI) < 10. This threshold corresponded to a typical distance < 5 m apart in a ground-truthing experiment (Dakin & Ryder 2018). Hence, according to our definition, a social interaction is initiated only after males come within this approximate spatial threshold. We chose this spatial threshold because it is close enough to permit visual and acoustic contact during typical social behaviors, such as those described in (Schwartz & Snow 1978). Any repeated co-occurrence of the two males within 5 minutes was considered to be part of the same social interaction, but after a gap of ≥ 5 minutes, it was considered to be a new interaction between those two males. The average duration of social interactions defined by this method was 5.1 min ± SD 13.3, 8.8 min ± SD 20.1, and 7.0 min ± SD 20.5 in the three respective field seasons, further indicating that these were sustained social interactions, rather than random encounters.

In total, we identified 36,885 social interactions over the three field seasons of study. These interactions were used to define a weighted social network for each lek recording session. The nodes in the network were the individual males, and the edges were weighted by the number of social interactions between each pair of males. An additional validation study confirmed that

the social interactions defined by this method corresponded to male-male partnerships that were directly observed (Ryder *et al.* 2012).

Pre-network Permutation Analysis

Two broad classes of methods have been described for building social networks: (i) networks that are built based purely on the proximity of animals unrelated to their behavioral context ("gambit of the group"), and (ii) networks that are built based on specific behavioral criteria that are directly observed (Franks *et al*. 2010; Farine 2015). Although the interactions in our study were not directly observed, they were recorded at specialized display perches that have a known function in male-male reproductive coalitions (Ryder *et al*. 2011a). Hence, they do not qualify as the gambit of the group. To further demonstrate this point, we performed a pre-network permutation of the raw data to validate that most of the observed network edges occurred more often than expected by chance, even within lek recording sessions (Farine *et al*. 2017). This analysis preserved key features of the data including male visit rates to specific territories within each recording session; it is described in detail in the supplement and Fig. S2. The results demonstrated that 95% of the observed network edges had a greater edge weight than expected under stringent permutation conditions. This provides additional validation to our approach, because it indicates that the observed network edges were nearly always preferred, even relative to other possible interactions within the same lek. These results are also consistent with a previous validation study indicating that these methods capture male-male coalition partnerships that are directly observed (Ryder *et al*. 2012).

Although pre-network data permutations are sometimes used to derive adjusted association indices, or to prune networks prior to further analysis, we did not take this approach

for several reasons. First, the statistical rarity of a relationship in our system does not *a priori* define the importance of any one social interaction, especially given that all of the interactions occurred on display territories with specialized function. A single interaction between two rarely interacting males may have been highly consequential (e.g., if one of those males had a highly influential hormone-behavioral phenotype). Conversely, a single interaction between two frequently interacting males may have been relatively unimportant. It would be unwarranted to assume that partnership rarity indicates the importance of any single interaction in this context. Second, and perhaps more importantly, the goal of this study was a comparison across multiple networks. Some networks are genuinely less preferential, and more random, than others. If we modified the network edges based on permutation-based indices, it would disproportionately prune the truly random networks, introducing a source of bias that would be contrary to our main goal. Hence, all further analyses are based on networks where the edge weights are given by the observed number of interactions, as this method is most appropriate for our study system and aims.

Social Specialization

To quantify social specialization, we sought a metric that would capture the extent to which a network was partitioned into exclusive social relationships (as opposed to a network made up of non-specific or non-exclusive partnerships). To do this, we used a network metric of specialization that is commonly used in community ecology called H_2' (Blüthgen *et al.* 2006). An advantage of H_2' is that it is standardized against a theoretical maximum, based on the overall activity levels of different nodes and Shannon entropy (Blüthgen *et al.* 2006); this makes it possible to compare the extent of specialization across different bipartite networks in a

standardized way. To apply this metric to our manakin data, we converted each lek social network into its bipartite adjacency matrix (Fig. 1A), with floaters along one axis, and territory-holders on the other, and then calculated social specialization as H_2' using the bipartite package (Dormann *et al.* 2019). Higher values of specialization indicate that the network is made up of more exclusive relationships, as illustrated in Fig. 1A. We chose to focus on floater-territorial specialization because these two classes are well-defined and floater-territorial partnerships tend to be the most common in this species (Fig. S3 and (Ryder *et al.* 2011a)).

Network Stability

To quantify the stability of social relationships, we compared each lek social network from a given recording session to its subsequent recording session within the same field season (Fig. 1B). Network stability was then calculated as the number of male-male partnerships (network edges) shared by both time points divided by the number of partnerships at either time point (Dakin & Ryder 2020). Higher values of stability indicate greater persistence of social relationships within the network, independent of any changes in the representation of particular males (nodes) (Poisot *et al.* 2012). To focus this measure on the persistence of strong relationships, we computed network stability of partnerships that occurred at least six times (i.e., at least once per day, on average) following (Dakin & Ryder 2020). Previous work using this metric shows that the wire-tailed manakin social networks are more stable than expected by chance (Dakin & Ryder 2020).

Behavioral Assortment

298 Assortment refers to the extent to which individuals associate with similar partners (Fig. 1C); it can be due to partner choice (homophily), shared environments, or the social transmission of
300 behavior (Dakin & Ryder 2018). Assortment was quantified using Newman's assortativity, which is a correlation coefficient for the statistical association among linked nodes within a
302 network. It ranges from –1 (a negative association), through 0 (no association), and up to +1 (a positive association). To quantify the assortment of social behaviors, we first determined the
304 daily frequency of two behaviors for each male: his number of social interactions per day (strength), and his number of unique social partnerships per day (degree). Strength and degree
306 are both repeatable measures of a male's social behavior in our study population (Dakin & Ryder 2018). We used the average log-transformed values of each male's strength and degree within
308 the recording session, and then calculated the assortativity coefficient for the entire social network using the algorithm for weighted networks in the assortnet package (Farine 2016).
310 Because assortativity values for strength and degree were highly correlated (Pearson's $r = 0.78$, $p < 0.0001$, $n = 86$ networks), we took the average of these two values as the measure of overall
312 behavioral assortment within the social network. Note that we used log-transformed values of strength and degree because these two variables are strongly positively skewed (Dakin & Ryder
314 2018; Ryder *et al.* 2020), and assortativity is based on a Pearson's correlation. Finally, we also computed the assortativity of the two discrete status classes (floater and territory-holder), to
316 ensure that our analysis of behavioral assortment was not solely driven by status-assortment.

318

320 ***Collective Testosterone***

To understand how hormones might predict network properties, we derived a measure of
322 collective testosterone of each social network. This was based on the hormonal trait that was the best predictor of cooperative behavior in our previous study, referred to as "mean T" (Ryder *et*
324 *al.* 2020). A male's mean T is his average residual circulating testosterone. It is calculated using a linear regression of log-transformed testosterone to statistically account for the effects of field
326 season, Julian date, time of day when captured, and duration of restraint, all of which may influence point estimates of a male's baseline hormone level (Vernasco *et al.* 2019). Hence,
328 mean T represents a standardized measure of a male's circulating testosterone, independent of his capture conditions (Fig. 2A). Next, to determine collective testosterone, we took the average
330 mean T for each social network, weighted by the interaction frequency (strength) of the males within the network (Fig. 2B). Collective testosterone is thus a group-level characteristic that is
332 weighted towards the males that made the greatest contribution to group social structure. In other words, networks with low collective testosterone are made up of mostly low-testosterone
334 individuals, whereas networks with high collective testosterone are made up of mostly high-testosterone individuals. We calculated collective testosterone separately for each status class,
336 because the effects of hormones on social behavior are status-dependent (Eisenegger *et al.* 2011; Boksem *et al.* 2013; Ryder *et al.* 2020).

338

Statistical Analysis

340 To evaluate the hypothesis that collective testosterone predicts network properties, we analyzed mixed-effects models of the social network properties in the lme4 package (Bates *et al.* 2018).
342 The three response variables were social specialization, network stability, and behavioral

assortment. We used Akaike's Information Criterion (AIC) to compare four candidate models for each response variable, as follows: (1) collective testosterone of territory-holders + collective testosterone of floaters; (2) collective testosterone of territory-holders; (3) collective testosterone of floaters, and (4) no testosterone predictors. All of the models included additional fixed effects to account for field season (a categorical variable with three levels), the average Julian date of the recording session, the average number of recorded hours per territory, and the size of the social network (number of individuals), as well as a random effect of lek to account for repeated measures.

Model comparison was performed on models fit with maximum likelihood, and the best-fit models were re-fit using restricted estimation of maximum likelihood (REML) to derive parameter estimates (Zuur *et al.* 2009). We used the lmerTest package to compute p-values for parameter estimates in the mixed-effects models based on Satterthwaite's method (Kuznetsova *et al.* 2018). We also report Nakagawa and Schielzeth's R^2_{LMM} values as an estimate of effect size (Nakagawa & Schielzeth 2013). We verified that all models met the assumptions of linear regression analyses. First, we checked that the Pearson residuals met the assumption of normality. We visually inspected the partial residual plots for each fixed and random effect, to confirm that there were no outliers or departures from the assumption of homoscedasticity. To check for multicollinearity, we used the performance package (Lüdecke *et al.* 2019) to calculate variance inflation factors (VIFs), and we verified that all VIFs were between 1 – 1.8.

Sample Sizes and Exclusions

In two field seasons (2016-17 and 2017-18), we performed an experiment as part of a separate study to test the influence of transiently-elevated testosterone on individuals (n = 5 individuals in

366 2016-17 and n = 4 in 2017-18 (Ryder *et al.* 2020)). The results of that experiment demonstrated that elevated testosterone caused a temporary decrease in the frequency and the number of social
368 partnerships in the altered males (Ryder *et al.* 2020). It is important to note that this experiment was not designed to test emergent properties at the network level, because it was conducted on a
370 limited scale with only one or two individuals temporarily altered within each lek. We therefore excluded the 6 post-manipulation networks from the main analysis in this study. We verified that
372 when we included these manipulated leks, all of our main conclusions were unchanged.

After excluding the 6 post-manipulation observations, 80 of the original 86 recording
374 sessions remained. Table 1 summarizes the sample sizes for the network-level analyses. In 10 cases, specialization could not be calculated because the bipartite network did have sufficient
376 data to determine H_2'. Stability could not be calculated in 26 cases when either the recording session occurred at the end of a field season, or there were insufficient partnerships that met the
378 criteria for the stability calculation.

380 ***Node-label Permutation Analysis***

To evaluate the possibility that our results could be influenced by other properties of the leks that
382 were independent of hormonal traits, we also performed a statistical permutation of the post-network data (Farine *et al.* 2017). The purpose of this analysis was to verify that the results were
384 driven by (and sensitive to) the relative contribution and social position of different males. To do this, we performed a statistical permutation that randomized the node labels (male IDs) within
386 each of the social networks, retaining network topology, and leaving each male's testosterone traits unchanged. Hence, this analysis preserved which males were present in which recording
388 session, but it randomized the relative position and contribution of each male. After generating

1,000 of these node-label permutation datasets, we recalculated specialization, stability, and assortment, and then refit the top models from our mixed-model analysis. We then compared the slope estimates from the observed data to those derived from 1,000 node-label permutations. As a one-sided p-value, we calculated the proportion of slope estimates from the node-label permutations that were more negative than the corresponding estimate from the observed data.

Results

The manakin social networks had 14 males on average, but there was considerable variation among leks ranging from 3 to 43 individuals (Fig. S1). Additional descriptive statistics are provided in Table 1. Although network size has a lognormal distribution (Fig. S1), all other emergent properties of the social structure were approximately normally distributed (Fig. S4).

Table 1. Descriptive statistics for manakin social networks. Means and standard deviations (SD) are provided for each field season. The bottom row provides sample sizes for the number of social networks analyzed (n_{obs}) at each lek (N_{lek}). Additional data is provided in the supplement in Fig. S1 and Fig. S4.

Field season	Network size (# birds)	Social specialization	Network stability	Behavioral assortment	Collective T (terr.)	Collective T (floa.)
15-16	14.5 (8.9)	0.50 (0.22)	0.43 (0.25)	0.01 (0.36)	0.44 (0.42)	–0.31 (0.56)
16-17	17.8 (10.5)	0.30 (0.24)	0.47 (0.20)	0.06 (0.30)	0.49 (0.24)	–0.22 (0.37)
17-18	10.3 (4.5)	0.42 (0.30)	0.42 (0.27)	0.02 (0.37)	0.43 (0.40)	–0.70 (0.46)
Sample size	n_{obs} = 80 N_{lek} = 11	n_{obs} = 70 N_{lek} = 11	n_{obs} = 54 N_{lek} = 11	n_{obs} = 80 N_{lek} = 11	n_{obs} = 80 N_{lek} = 11	n_{obs} = 80 N_{lek} = 11

We found that the collective testosterone of territorial males could predict all three emergent properties of the networks (specialization, stability, and assortment). The leks with a greater contribution from high-testosterone territorial males were less specialized, less stable, and more

negatively assorted (Fig. 3; all $p \leq 0.03$ in mixed-effects models). The slope coefficients for these three relationships were also all greater than expected under a node-label permutation (inset panels, Fig. 3; all $p < 0.02$). Model selection results for the observed data indicated considerable uncertainty in the best-fit model for each of the three network properties analyzed (Table S1). However, the collective testosterone of territory-holders was a significant predictor in all of the best-supported models (Table S2). In contrast, the collective testosterone of floater males was not a significant predictor of network properties in any of the best-fit models (Table S2).

We did not detect any significant effects of Julian date on network properties within our study period, but we did observe some significant year-to-year differences (e.g., social specialization and behavioral assortment in Table S2). Assortment was the only property that was significantly related to network size and recording effort, but in opposite ways (Table S2). All else being equal, behavioral assortment was more positive in larger networks, but it was more negative in networks that had longer recording sessions.

$R^2_{LMM(m)}$ provides an estimate of the proportion of variance explained by the fixed effects in a model (Table S1). The $R^2_{LMM(m)}$ for assortment in the best-fit model was 0.52. This indicates that around 52% the variation in behavioral assortment could be explained by the combined associations with collective testosterone, network size, sampling effort, and annual variation (Table S2). The $R^2_{LMM(m)}$ for stability was 0.13, indicating that collective testosterone could explain about 13% the variation in that metric (Table S2). Finally, for specialization, the $R^2_{LMM(m)}$ indicated that the combined effects of testosterone and year-to-year variation together explained about 20% of the variation (Table S2).

Discussion

The collective testosterone of dominant, territory-holding males was associated with multiple features of the social network (Fig. 3). This indicates that the effects of testosterone on dominant males may represent an extended phenotype with the power to shape emergent social structure (Dawkins 1982). In contrast, the collective testosterone of floater males was not significantly associated with any of the emergent network properties (Table S2).

How can the relationship between hormone levels and network properties be explained in terms of individual mechanisms? Given that testosterone has antagonistic effects on the cooperative behavior of territorial males (Ryder *et al.* 2020), we hypothesize that the behavior of high-testosterone individuals causes several features of the social organization to break down. Our previous results showed that high-testosterone dominant individuals have a reduced ability to attract and maintain coalition partners (Ryder *et al.* 2020). We propose that this weakening of coalition bonds may cause floater males to prospect elsewhere for new partnerships, negatively affecting both the stability and specialization of the lek network as a whole. Likewise, high-testosterone dominant males may inhibit the processes of social contagion, reciprocity, and/or behavioral matching that cause positive behavioral assortment (Dakin & Ryder 2018).

We included several additional parameters in our analysis to account for network size and sampling effort. Although not one of our main hypotheses, we noted that network size was positively associated with behavioral assortment (Table S2). This result indicates that males are more likely to associate with behaviorally-similar partners in larger leks, whereas they are more likely to associate with dissimilar partners in smaller leks. Effects of group size on assortment have been noted in a few other studies, although the form of this relationship varies (Griffiths & Magurran 1997; Ilmarinen *et al.* 2017; McDonald *et al.* 2017). In manakins and other lekking

systems, larger leks are known to have heightened display activity and higher female visitation rates (Lank & Smith 1992; Durães *et al.* 2009). This raises the possibility that social facilitation and heightened activity may be associated with increased homophily and/or behavioral matching (either through partner choice, contagion, or reciprocity). Another plausible explanation is that smaller networks may constrain these behavioral processes, by making it more difficult to find or match a suitable partner. This hypothesis could be explored in experiments on captive systems and simulation models.

Behavioral assortment was also more positive in networks that were recorded for less time in our study. Recording time was designed to be approximately even among leks, with the most common reason for departure being inclement weather that extended the recording session (Fig. S1). Therefore, we speculate that inclement weather may have reduced the amount of behavioral matching (assortment) while also affecting recording time. Although we did not collect data on weather at the leks as this was not our main goal, the question of how inclement weather affects group-level social dynamics is an interesting one that merits further study.

Conducting experimental tests of causation at the level of whole social networks remains a major challenge in ecology (Pinter-Wollman *et al.* 2014; James *et al.* 2009). Although our node-label permutation analysis provides evidence that our results are not merely due to structural differences among networks, we cannot rule out the possibility that high-testosterone individuals chose to participate in certain networks due to other factors that may also influence the emergent properties of the network (e.g., environmental quality and/or female activity). It is important to note that in this system, as in many other wild animals, controlled experimental manipulations of the broader social network structure are not yet possible (Zyphur *et al.* 2009; Akinola *et al.* 2016). Nevertheless, our data here indicate that the increased prevalence of

dominant, high-testosterone individuals can predict changes to higher-order social structure.
480 These findings establish that hormone-behavior relationships are not limited to one individual, but instead hormones may act as extended phenotypes that have population-level consequences
482 (McClintock 1981; Dawkins 1982; Robinson 1992). Taken together with previous studies, we propose that testosterone can alter social network dynamics in ways that impede the evolution of
484 cooperation.

Figure Legends

Figure 1. Example social networks illustrating social specialization, network stability, and behavioral assortment. (A) Social specialization is measured using the bipartite form of the social network. In the example on the left, the coalitions between floater males and territory-holders are poorly partitioned, creating a network with a relatively low specialization. On the right, there is greater partitioning, such that each floater male maintains a smaller number of partnerships with specific territory-holding males. (B) Network stability is measured by evaluating the persistence of partnerships from one recording session (t_1) to the next (t_2). The example at the top of (B) has a lower stability than the example on the bottom. (C) Behavioral assortment measures the tendency of like to associate with like. In (C), nodes are shaded to indicate a continuous gradient of daily behavior (in this case, each male's average daily strength is shown). In the example network on the left, the males tend to associate with behaviorally dissimilar partners, yielding a negative behavioral assortment. In the example on the right, the males tend to associate with behaviorally similar partners, yielding a positive assortment. See Table 1 for additional data.

Figure 2. Collective testosterone of manakin social networks. (A) Circulating testosterone varies among individual males. This graph shows repeated measures from 210 individual male manakins, sorted along the x-axis by mean T, a standardized measure of a male's average testosterone level (Ryder *et al.* 2020). Opacity is used to denote the two male status classes (with subordinate floaters colored semi-transparent, and dominant territory-holders colored opaque). Color ramping is used to denote each individual's hormone phenotype. (B-D) These data were

508 used to define collective testosterone in the present study. Node colors indicate an individual's mean T following the scale in (A). The collective testosterone of each social network is
510 calculated as the average of the individual hormone phenotypes, weighted by strength, and is shown below each example. Collective testosterone was determined for each status class
512 separately. See Table 1 for additional data.

514 **Figure 3. Collective testosterone predicts the structure of the social network.** The networks with higher-testosterone dominant individuals were less specialized, less stable over time, and
516 had more negative behavioral assortment. Each plot shows the partial residuals from a statistical analysis that also accounts for field season, the average Julian date of the recording session, the
518 number of recording hours, network size, and lek identity. Because floater and territorial males can differ in behavior, the analysis of behavioral assortment also accounted for status assortment
520 within each network. Different symbols are used to indicate repeated measures of 11 different leks, colored according the collective testosterone scale in Fig. 2. Inset panels show the results of
522 the node-label permutation tests. In each case, the slope in the best-fit model (solid vertical line) is significantly more negative than expected based on the distribution of permuted estimates
524 (grey distribution). See Tables S1-S2 for additional data.

Acknowledgments

We thank Camilo Alfonso, Brian Evans, David and Consuelo Romo, Kelly Swing, Diego Mosquera, Gabriela Vinueza, and Tiputini Biodiversity Station of the Universidad San Francisco de Quito. Funding was provided by the National Science Foundation (NSF) IOS 1353085 and the Smithsonian Migratory Bird Center.

Author Contributions

RD, ITM, BMH, and TBR designed the study. ITM, BMH, BJV, and TBR collected the data. RD, ITM, and TBR analyzed the data. RD and TBR wrote the manuscript. All authors edited the manuscript.

Declaration of Interests

The authors declare no competing interests.

Data Availability

All data and R scripts necessary to reproduce this study are available for download at: https://figshare.com/s/13a311662fee686fa4f3

Supporting Information

Supplemental text, Figures S1-S4, and Table S1-S2 in the attached PDF

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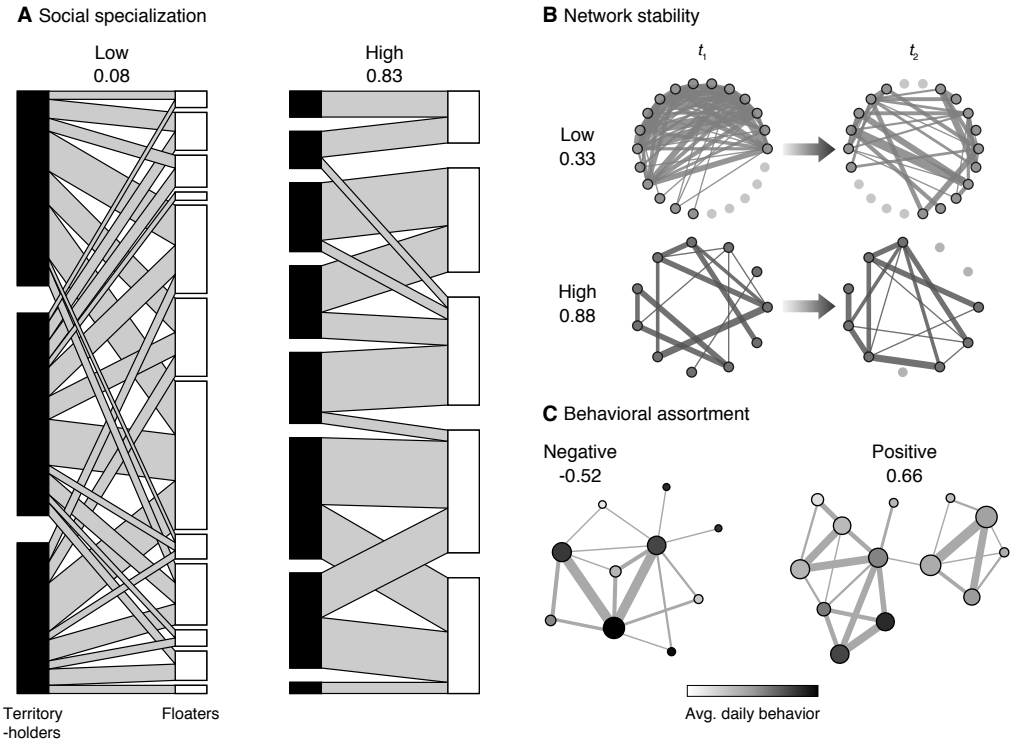
Figure 1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Low
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High
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Territory
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B Network stability
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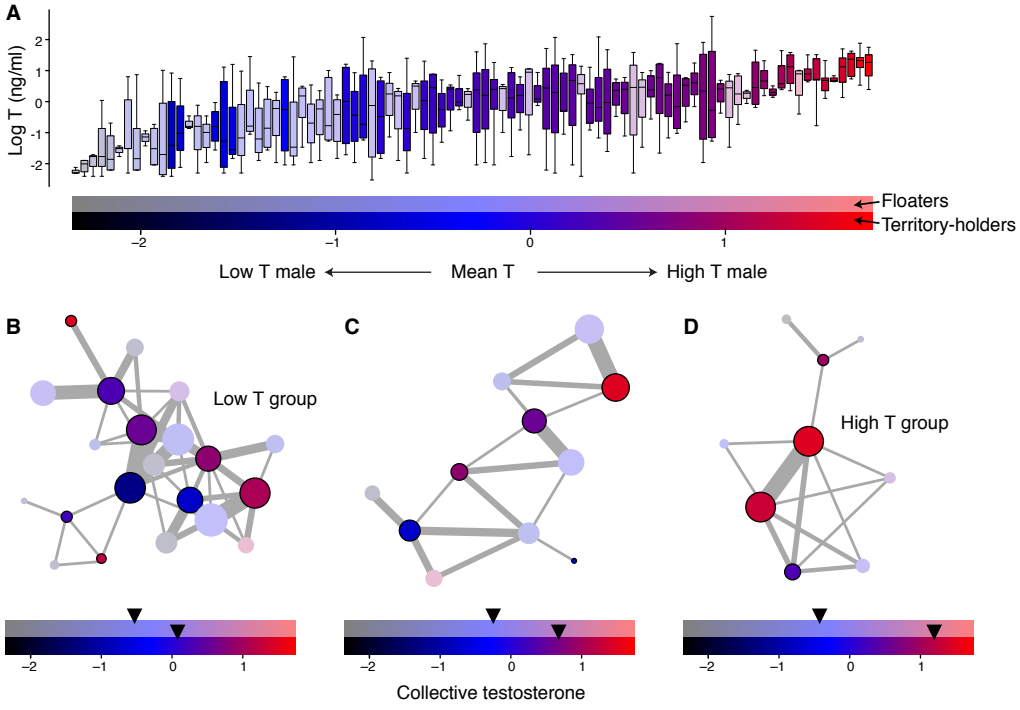
C Behavioral assortment
Negative
-0.52
Positive
0.66
Avg. daily behavior

Figure 2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B
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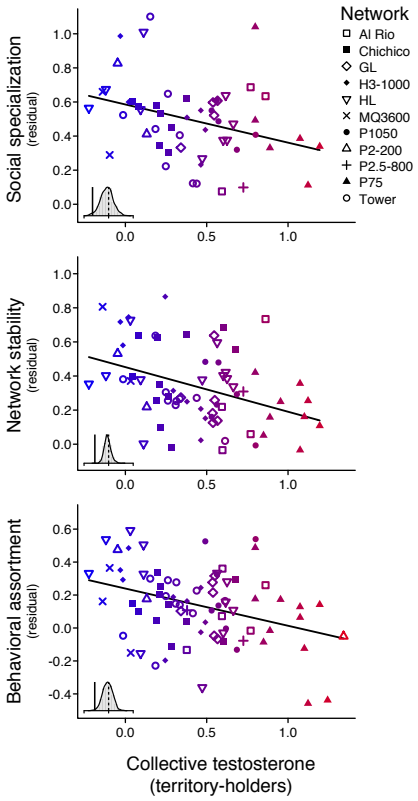
High T group
Collective testosterone

Figure 3


Network
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P2.5-800
P75
Tower
Social specialization
(residual)
Network stability
(residual)
Behavioral assortment
(residual)
Collective testosterone
(territory-holders)