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Extended hormone-phenotypes shape the structure, stability, and assortment of social networks

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Highlights (max 86 characters each)
- Testosterone predicts emergent social network properties in a cooperative bird
- High-testosterone groups exhibit collective behaviors that destabilize cooperation
- These effects are attributed to the dominant territory-holding males

Summary

Testosterone is a key regulator of vertebrate social behavior. As such, testosterone can mediate and respond to social interaction dynamics [1–3]. Although experiments have demonstrated that testosterone signaling pathways can influence aggression and cooperation [2,4,5], no study has examined the links between hormone levels, behavioral phenotypes, and emergent properties of the social network. In other words, how do mechanisms underlying an individual's social behavior scale-up to influence the emergent properties of the network [6]? Here, we address this question by studying wire-tailed manakins, a species of bird in which males cooperate to court females at sites known as leks [7,8]. We used an automated proximity system to monitor multiple leks and measure the social network at each lek repeatedly through time. We also quantified the testosterone phenotype of all individuals in the lek. Our analysis examines how the collective hormone phenotype of individuals within the social network affects three emergent properties: social specialization (the exclusivity of social relationships), network stability (the persistence of partnerships through time), and behavioral assortment (like associating with like). These three properties are expected to enhance the benefits of cooperation [9–11]. We found that the manakin social networks with high-testosterone dominant individuals were less specialized, less stable, and had more negative behavioral assortment. Combined with our previous experiments [12], these results provide evidence that the extended hormone-phenotypes of individuals can shape the broader social architecture of animal groups.

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

Results and Discussion

44 Testosterone signalling pathways can both mediate and respond to dynamic social environments in a diversity of vertebrates [5,13]. For example, hormone-signaling pathways at the level of the

46 individual are essential for the development and expression of complex behavioral phenotypes [14], which are hypothesized to shape social interaction dynamics and higher order social

48 network structure. Testing this hypothesis is challenging, because it requires linking fine-scale measures of behavior and physiology with broad-scale measurements that characterize the

50 collective behavior of the group [15].

Here, we leverage a large and comprehensive dataset on the circulating hormone levels,

52 behavioral phenotypes, and social networks of a cooperative bird, the wire-tailed manakin (*Pipra filicauda*), to test the hypothesis that testosterone-mediated behavior drives emergent social

54 structure. Male wire-tailed manakins form cooperative display coalitions on sites known as leks to attract females [16]. These coalition partnerships are essential for male fitness [7,8] and form

56 the basis of complex and dynamic social networks [7,17,18]. The social networks of wire-tailed manakins can also exhibit temporal variation in structure [19]. This is important because the

58 emergent properties of the social network are theoretically predicted to influence the costs and benefits of cooperation, and hence shape selection on individual behavior. For example,

60 cooperation in social networks is favored when individuals interact with a limited set of familiar partners, when these relationships are temporally stable, and when helping is reciprocated by

62 immediate neighbors in the group [9–11].

Can hormone-phenotypes at the individual level scale-up to influence these processes?

64 We have previously shown that male manakins differ in their circulating testosterone levels, with some individuals have consistently higher testosterone than others (Fig. 1A). Moreover, variation

66 in testosterone level can also explain repeatable, among-individual differences in the frequency and number of social partnerships [12]. As observed in other vertebrates [5,20], these hormone-

68 behavior relationships are status-specific in wire-tailed manakins. Among dominant, territory-holding males, high testosterone levels are associated with reduced cooperative behavior [12].

70 However, in the subordinate, "floater" males, high testosterone is associated with increased cooperative behavior and subsequent territory acquisition [12], both of which are essential for

72 reproductive fitness [7,21].

In this study, we used an automated proximity data-logging system to record over 36,000

74 unique male-male social interactions. These data allowed us to analyze repeated measures of the weighted social networks at 11 different leks where manakin coalitions occur. A recent study

76 showed that the collective hormonal profile of human social groups can predict group success at social tasks [22]. Hence, we similarly defined "collective testosterone" for manakin groups as the

78 weighted average testosterone phenotype of the individuals comprising each lek social network, whereby each male's contribution was weighted by his social activity (i.e., frequency of

80 cooperative interactions or "strength"). Because hormone-behavior relationships are status-specific [12], collective testosterone was calculated for each of the two status classes separately.

82 As illustrated in Fig. 1B, this measure ranks social networks on a scale ranging from those made up of mostly low-testosterone individuals, to those made up of mostly high-testosterone

84 individuals.

We evaluated whether collective testosterone was associated with three emergent

86 properties of social networks. The first property, social specialization, is a measure of the exclusivity of the relationships between coalition partners. To quantify specialization at the

88 network level, we used the H_2' metric from community ecology, which provides a standardized

ranking of bipartite networks on a scale from highly generalized to highly specialized [23]. In the context of manakin behavior, highly specialized networks exhibit a high frequency of exclusive partnerships between males of different status classes, as illustrated in Fig. S1. In other species, social specialization has been found to maximize the quality and coordination of different types of behaviors [24,25]; in manakins, we expect specialization to improve the signal quality of male-male courtship displays. Greater specialization is also expected to minimize conflict over mating and territorial ascension opportunities [26,27].

The second property, network stability, quantifies the average persistence of social partnerships through time [19,28]. Coalition partnerships require the coordination of complex behaviors, and previous empirical work indicates that longer partnership tenure has a positive effect on display coordination [29,30]. Greater temporal stability of partnerships also increases the opportunity for sustained reciprocity within the network [9,10,31]. The third property, behavioral assortment, captures the extent to which males interact with partners who express similar behaviors (i.e., is like associated with like? [31,32]). At the proximate level, positive assortment may represent the outcome of generalized reciprocity [18,33]. At the ultimate level, positive assortment has also been shown to promote the evolution of cooperation [11]. 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 cooperative interactions) with that of "degree" (his number of cooperative partnerships) [18]. Examples of networks illustrating behavioral assortment are also shown in Fig. S1.

We found that the collective testosterone of territorial males could predict all three properties of the social networks. Specifically, the leks with a greater number of high-testosterone territorial males were less specialized, less stable, and more negatively assorted (Fig. 2; all $p < 0.03$). The coefficients of these relationships were also greater than expected under a null permutation of the data within each social network (inset panels, Fig. 2; all $p < 0.015$). Given that testosterone has antagonistic effects on the cooperative behavior of territorial males [12], we hypothesize that the behavior of high-testosterone individuals causes multiple properties of stable cooperative networks to break down. In contrast, the collective testosterone of the floater males did not predict social network properties in our analysis. Therefore, although testosterone may determine the behavior of both floater and dominant males [12], its effects on dominant males may represent extended phenotype that ultimately determines social structure [34].

How can the relationship between hormone levels and network properties be explained in terms of individual behaviors? Based on our results in previous studies [12,19], the high-testosterone dominant individuals have a reduced ability to attract and maintain stable coalition partners [12]. This weakening of coalition bonds may cause floater males to prospect elsewhere for new partnerships, negatively impacting both the overall specialization and stability of the social network [19]. Likewise, we propose that behavioral assortment becomes more negative in networks with many high-testosterone individuals, because these individuals may inhibit the processes of social contagion, reciprocity, and/or behavioral coordination that contribute to positive assortment [18].

To explore the sensitivity of these results to the composition of individuals within the network [35], we performed a simulation exercise in which we selectively removed males from their social networks in the observed data, and then repeated our analysis. We set the number of individuals removed as a constant proportion of the total number of individuals in the network so that the effect size would be standardized across heterogeneous networks. To evaluate the effect

of gradually increasing the amount of social perturbation, the simulation proceeded by removing less social (i.e., low-strength) individuals first. Although this analysis is not a substitute for experimental tests *in vivo* [22,36], it can provide a good indication of the importance of particular individuals to emergent properties of the network.

We found that removing as few as 10% of the males in either social class (dominant territory-holders or subordinate floaters) eliminated the relationship between stability and collective testosterone. This indicates that stability is highly sensitive to the presence of both status classes of males within the network. In contrast, behavioral assortment was more robust to the removal of individuals: we had to drop 40% of the territory-holders, or 60% of the floaters, to disrupt its association with testosterone. Finally, the sensitivity of social specialization was also status-dependent: we found that removing only a few floaters (10%) decoupled the relationship between specialization and testosterone, whereas 40% of the territorial individuals had to be removed to eliminate this result. Thus, the behavior of the floater males (i.e., initiating partnerships) may play a key role in determining social specialization within the network.

Conducting experimental tests of causation at the level of whole social networks remains a major challenge [35,37]. With the present data, we cannot rule out the possibility that high-testosterone individuals chose to participate in certain networks due to other factors that may also influence emergent network properties (e.g., environmental quality and/or female activity). Nevertheless, our data show that the increased prevalence of dominant individuals with high-testosterone is associated with behavioral dynamics and changes to higher-order social structure that can ultimately destabilize cooperation. These findings establish that hormone-behavior relationships are not limited to one individual, but instead they may act as extended group phenotypes that have population-level consequences [38,39].

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.

174   **Figure Titles and Legends**

176   **Figure 1. Testosterone varies at the level of the social network.** (A) Circulating testosterone
        varies within- and among-individuals [12]. This graph shows repeated measures from 210
178   individual male manakins, sorted along the x-axis by their average testosterone level
        (standardized "mean T"; modified from Ryder et al. [12]). Opacity is used to denote the two
180   male status classes (with subordinate floaters colored semi-transparent, and dominant territory-
        holders colored opaque) and color ramping is used to denote each individual's hormone
182   phenotype. (B-D) Three examples of the social network structure and hormone phenotypes at
        manakin display leks. Each node (circle) represents an individual male who engaged in social
184   interactions at the lek. Node size is scaled to his interaction frequency ("strength"); this
        parameter is also used to weight each male's contribution to collective testosterone. Edge
186   thicknesses are also scaled to the log-transformed interaction frequencies for each observed
        coalition. Node colors indicate an individual's hormone phenotype following the scale in (A).
188   The collective testosterone of the social network is calculated as the average of the individual
        hormone phenotypes within the social network, weighted by strength as a measure of each
190   male's contribution. Because hormone-mediated cooperation is status-specific [12], collective
        testosterone was determined for each status class separately (indicated by the semi-transparent
192   and opaque color scales for floaters and territory-holders, respectively). See Figure S1 for
        additional data.
194

        Figure 2. Collective testosterone predicts the emergent properties of cooperative social
196   **networks.** Lek social networks with greater representation of high-testosterone territorial
        individuals were less specialized (i.e., partnerships were less exclusive), less stable over time,
198   and had more negative behavioral assortment (i.e., individuals tended to associate with more
        dissimilar partners). Each plot shows the partial residuals from a statistical analysis that also
200   accounts for field season, the average Julian date of the recording session, the number of
        recorded hours per territory, and network size (fixed effects), as well as a random effect of lek
202   identity. Because the two status classes differ in behavior [18], the analysis of behavioral
        assortment also accounted for assortment of the status classes within each network as an
204   additional covariate. Different symbols are used to indicate repeated measures of 11 different
        leks networks, and colored according the collective testosterone gradient in Figure 1B. Inset
206   panels show the results of a null randomization test. In each case, the slope in the best-fit model
        (solid vertical line) was significantly more negative than expected in the null permutations of the
208   social networks (grey distribution; dotted vertical line indicates 0; all p-values < 0.015). See
        Tables S1 and S2 for additional data.
210

STAR Methods (note that the headings and structure follow STAR required format)

212

Key Resources Table

214

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental Models: Organisms/Strains		
Pipra filicauda	Tiputini Biodiversity Station; Universidad San Francisco de Quito	0º 38' S, 76º 08' W
Chemicals, Peptides, and Recombinant Proteins		
Rabbit Anti-Testosterone-3	Fitzgerald Industries International, Inc.	CAT# 20R-TRO18w
Software and Algorithms		
R 3.5.1	https://www.r-project.org/	3.5.1
igraph	https://igraph.org/r/	1.2.2
bipartite	https://cran.r-project.org/web/packages/bipartite/	2.13
assortnet	https://cran.r-project.org/web/packages/assortnet/	0.12
lme4	https://cran.r-project.org/web/packages/lme4/	1.1-18-1
lmerTest	https://cran.r-project.org/web/packages/lmerTest/	3.0-1
Deposited Data		
Data and analysis code	Available at: https://figshare.com/s/13a311662fee686fa4f3	<the DOI will be added upon final version>

216   **Contact for Reagent and Resource Sharing**
Further information and requests for resources should be directed to and will be fulfilled by the
218   Lead Contact, T. Brandt Ryder (rydert@si.edu).

220   **Experimental Model and Subject Details**

222   *Study Population*
We studied wire-tailed manakins (*Pipra filicauda*) at the Tiputini Biodiversity Station in
224   Orellana Province, Ecuador (0º 38' S, 76º 08' W). This population of *P. filicauda* has been
studied and individuals color-banded annually since 2002 [7,8]. Wire-tailed manakins are a long-
226   lived species in which the males form cooperative display coalitions to court females on sites
known as leks [16]. There are two male status classes in *P. filicauda*: subordinate non-territorial
228   floater males, and dominant territorial males. Previous research has established that territory
ownership is a prerequisite for mating, and that both floaters and territory-holders benefit from

230 cooperative partnerships [7,8]. Specifically, floaters with more social partners are more likely to inherit a territory, and territory-holders with more partners achieve greater reproductive success
232 [7,8]. The present study was conducted on 11 leks where the males perform their cooperative courtship displays during peak breeding activity (December-March) across three field seasons:
234 2015-16, 2016-17 and 2017-18 [12]. All research was approved by the Smithsonian ACUC (protocols #12-23, 14-25, and 17-11) and the Ecuadorean Ministry of the Environment (MAE-
236 DNB-CM-2015-0008).

Method Details

Testosterone Assay

Male manakins were caught at leks using mist-nets up to three times per field season. Following
242 capture and removal 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
244 [12,21,40]. Plasma volume was measured to the nearest 0.25 ul and stored in 0.75 ml of 100% ethanol [41]. Plasma testosterone was double extracted using dichloromethane. Following
246 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
248 [21,42]. 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
250 assay-specific limit of detection was assigned that limit as its testosterone concentration as a most conservative estimate. As reported in our previous study [12], the extraction efficiency for
252 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
254 was 19.5%.

Behavioral Assay

To quantify social behaviors, we used an automated proximity data-logging system to monitor
258 the activity on the territories of 11 leks [12,18,43]. At the beginning of each field season, males were outfitted with a coded nano-tag (NTQB-2, Lotek Wireless; 0.35 g). The tags transmitted a
260 unique VHF signal ping once per 20 s for three months. In total, 296 tag deployments were performed on 180 individual males, 178 of whom also had hormone data (mean 3 hormone
262 samples per male ± 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
264 within each territory to record tagged males within a detection radius of 30 m (a distance that corresponds to the average diameter of a manakin display territory [16,18]). Proximity recording
266 sessions ran from 06:00 to 16:00 for ~6 consecutive days (± SD 1 day) and were performed ~3 times per season at each lek. Prior to data-logger deployment, each territory was also observed
268 on non-recording days to identify the territory-holder based on his color-bands, following the protocol in previous studies [7,8]. These status assignments were subsequently verified in the
270 proximity data. In total, we conducted 86 recording sessions (29,760 data-logger hours) representing repeated measures of the social activity at 11 different leks.
272 To quantify social interactions in the proximity data, the tag detections were filtered using an spatiotemporal algorithm to identify unique joint detections, when two males were
274 located within a display territory [7,43,18]. A detailed description of the algorithm is provided in [18]. A ground-truthing experiment in that study also confirmed that these joint detections

276   represent occasions when two males were < 5 m apart [18], corresponding to the range required for a typical male-male social display [16]. An additional validation study also confirmed that
278   the social interactions defined by this method corresponded to display coalitions that were directly observed [43]. In total, we identified 36,885 unique social interactions over the three
280   field seasons in this study.

Quantification and Statistical Analysis
282   All quantitative analyses were performed in R [44].
284

Network Analysis
286   We used the igraph package [45] to construct a weighted social network for each lek recording session. The individual males who interacted on the lek were defined as the nodes, and the links
288   (or edges) between them were weighted by the social interaction frequencies. We quantified three emergent network properties for each lek recording session: social specialization, network
290   stability, and behavioral assortment, as described below.

    For specialization, we sought a measure that would capture the extent to which a network
292   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
294   that is commonly used in community ecology to analyze ecological networks, called H_2' [23]. An advantage of H_2' is that it is standardized against a theoretical maximum, based on the
296   overall activity levels of different nodes and Shannon entropy [23]; this makes it possible to compare the extent of specialization across different bipartite networks in a standardized way. To
298   apply this metric to our manakin data, we converted each lek social network into its bipartite adjacency matrix, with floaters along one axis, and territory-holders on the other, and then
300   calculated social specialization as H_2' using the bipartite package [46]. Higher values of this metric indicate that the network is made up of more exclusive relationships, as illustrated in Fig.
302   S1. We chose to focus on floater-territorial specialization because these are by far the most common partnerships in this social system with reproductive benefits to both parties [17].

304   To quantify the stability of social relationships, we compared each lek social network derived from one recording session to that derived from the subsequent recording session within
306   the same field season (if available). Network stability was then calculated as the number of male-male partnerships (network edges) shared by both time points divided by the number of
308   partnerships at either time point (i.e., the intersection divided by the union [19]). Higher values of stability indicate greater persistence of social relationships within the network, independent of
310   any changes in the representation of particular males (nodes) [28]. To reduce the influence of partnerships that occurred only rarely [47], prior to the stability calculation we filtered the data to
312   include only significant edges that occurred more often than expected in 1,000 random permutations of the interaction data, and at least six times during a recording session (i.e., on
314   average, once per day). Our previous work has established that network stability is robust to alternative thresholds for occurrence and that the wire-tailed manakin networks are more stable
316   than expected by chance [19].

    Assortment refers to the extent to which individuals associate with similar partners; it can
318   be due to partner choice (homophily), shared environments, or the social transmission of behavior [18]. Assortment was quantified using Newman's assortativity, which is a correlation
320   coefficient for the statistical association among linked nodes within a network. It ranges from –1 (a negative association), through 0 (no association), and up to +1 (a positive association). To

322 quantify the assortment of cooperative behaviors, we first determined the daily frequency of two behaviors for each male: his number of social interactions (strength), and his number of unique
324 social partnerships (degree) per day [18]. Strength and degree are both repeatable measures of a male's cooperative behavior in our study population [18]. Next, we computed the average log-
326 transformed strength and degree within the recording session for each individual, and then calculated a weighted assortativity coefficient for the entire social network using the assortnet
328 package [48]. Because assortativity values for strength and degree were highly correlated (Pearson's r = 0.78, p < 0.0001, n = 86), we took the average of these two values as the measure
330 of overall behavioral assortment within the social network. Finally, we also computed the assortativity of the two discrete status classes, to ensure that our analysis of behavioral
332 assortment was not solely driven by status-specific patterns of assortment.

Statistical Models
334 Following our previous study [12], we characterized each male's average circulating testosterone after statistically accounting for capture conditions ("mean T" in [12]), including the time of day
336 and duration of time the bird was in the mist-net [40]. This measure of among-individual testosterone variation was a significant predictor of cooperative behavior in our study system,
338 albeit with different relationships within each status class [12]. To quantify collective testosterone, we took the average "mean T" within the social network, weighted by each male's
340 interaction frequency (strength) as a measure of his contribution to the network. We calculated collective testosterone separately for each status class because the effects of hormones on social
342 behavior are status-dependent in this and other species [5,12,20].
344   To evaluate whether collective testosterone could explain emergent social network properties, we fit mixed-effects models using the packages lme4 and lmerTest [49,50]. Each
346 model was fit with a random effect of lek to account for repeated measures (n = 86 measures of 11 leks, except for stability which had n = 60 because stability requires a subsequent recording
348 session). We used Akaike's Information Criterion to compare goodness-of-fit for four candidate models, as follows: (1) collective testosterone of territory-holders + collective testosterone of
350 floaters; (2) collective testosterone of territory-holders; (3) collective testosterone of floaters, and (4) no testosterone predictors. All of the candidate models also included additional fixed effects
352 to account for field season (a categorical variable with three levels), the average Julian date of the recording session, the number of recorded hours per territory, and the size of the social
354 network (number of individuals). Continuous predictors were standardized (mean = 1, SD = 1) prior to being entered into the analysis so that the slope estimates would be comparable with
356 other models. Model selection was performed on models fit with maximum likelihood, and then the best-fit models were refit using restricted estimation of maximum likelihood (REML) to
358 determine p-values [51]. We used the lmerTest package to compute p-values for generalized mixed-effects models based on Satterthwaite's method.
360   In two field seasons (2016-17 and 2017-18), nine of the territory-holders were part of an experiment testing the influence of transiently-elevated testosterone on individual behavior (n =
362 5 individuals in 2016-17 and n = 4 in 2017-18 [12]). The results of that experiment demonstrated that elevated testosterone caused a temporary decrease in the frequency and the number of
364 cooperative partnerships in the altered males, relative to control males [12]. However, it is important to note that this experiment was not designed to test effects at the collective level,
366 because it was conducted on a limited scale whereby only one or two individuals were temporarily altered in only four leks. Therefore, our main analysis here excluded data from

368 recording sessions at manipulated leks. However, we verified that when we repeated our analyses including these manipulated leks, the main conclusions were unchanged. Furthermore,
370 we did not detect any statistically significant effect of the individual hormone manipulation on the three network-level properties (i.e., specialization, stability, and assortment; all $p > 0.16$).
372 Finally and most importantly, controlling for the manipulation also did not affect any of our conclusions about collective testosterone shown in Fig. 2
374

Randomization Test
376 We performed a randomization test using a null model [47] to assess the effect of randomizing each social network, leaving the testosterone data unchanged. To generate the null data, we
378 randomly permuted the node labels (ID labels) within each of the 86 social networks, retaining all other features of the social network. After generating each null dataset, we recalculated the
380 network-level properties (specialization, stability, and assortment), and then refit the top models from our original analysis. We then compared the slope estimates from the observed data to the
382 values obtained from 1,000 of these null permutations.

Perturbation Analysis
384 We performed a social perturbation analysis to determine the sensitivity of our results to the
386 composition of the social network [35]. To do this, we performed six iterations removing an increasing number of either floater or territory-holding males with each iteration (node removal
388 iterations = 10%, 20%, 30%, 40%, 50%, and 60% of the individuals in the relevant status class). The number of individuals removed from each network was rounded, such that it was at least
390 one, but not all of the individuals from that status class. To perform the removals, the males were sorted by strength, and low-strength individuals were removed first. After severing all social ties
392 of the removed individuals, we recalculated the network properties, and refit the top models from our original analysis (Table S1). Then, we compared the beta coefficients for collective
394 testosterone (standardized slope estimates) to those derived from the original data. A result was considered robust to perturbation if two criteria were met: the slope in the removal analysis was
396 significantly different from 0, and it was within the 95% confidence interval of the slope from the original data.
398

Data and Software Availability
400 All data and R scripts necessary to reproduce this study are available for download at: https://figshare.com/s/13a311662fee686fa4f3
402

Supplemental Information
404 Figure S1 and Table S1-S2 in attached PDF

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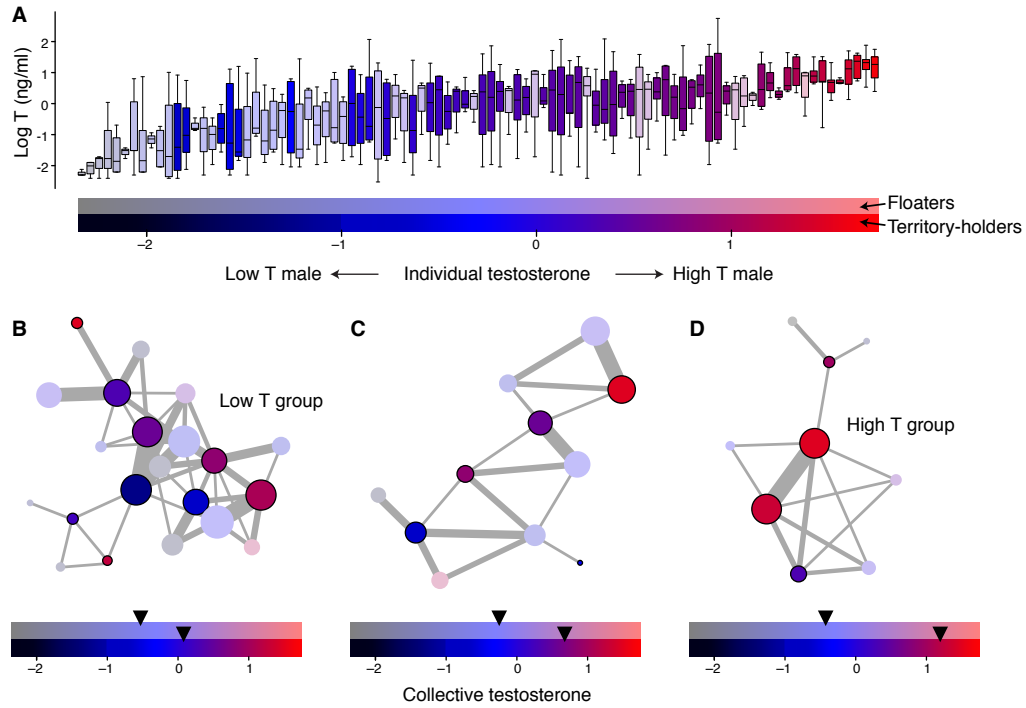
Figure 1

Figure 2