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## Research

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### Author for correspondence:

Iris I. Levin

e-mail: [iris.levin@colorado.edu](mailto:iris.levin@colorado.edu)

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## Animal behaviour

# Stress response, gut microbial diversity and sexual signals correlate with social interactions

Iris I. Levin<sup>1</sup>, David M. Zonana<sup>1</sup>, Bailey K. Fosdick<sup>2</sup>, Se Jin Song<sup>1</sup>, Rob Knight<sup>3</sup> and Rebecca J. Safran<sup>1</sup>

<sup>1</sup>Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309, USA

<sup>2</sup>Department of Statistics, Colorado State University, Fort Collins, CO 80523, USA

<sup>3</sup>Department of Pediatrics, University of California, San Diego, CA 90293, USA

ILL, 0000-0001-8932-5773

Theory predicts that social interactions are dynamically linked to phenotype. Yet because social interactions are difficult to quantify, little is known about the precise details on how interactivity is linked to phenotype. Here, we deployed proximity loggers on North American barn swallows (*Hirundo rustica erythrogastrer*) to examine intercorrelations among social interactions, morphology and features of the phenotype that are sensitive to the social context: stress-induced corticosterone (CORT) and gut microbial diversity. We analysed relationships at two spatial scales of interaction: (i) body contact and (ii) social interactions occurring between 0.1 and 5 m. Network analysis revealed that relationships between social interactions, morphology, CORT and gut microbial diversity varied depending on the sexes of the individuals interacting and the spatial scale of interaction proximity. We found evidence that body contact interactions were related to diversity of socially transmitted microbes and that looser social interactions were related to signalling traits and CORT.

## 1. Introduction

Social interactions are predicted to reflect and respond to variation in phenotypic traits [1,2]. However, we often lack detailed information about social interactivity to fully understand these relationships. Social network analysis is an analytical framework used to characterize social structure at different organizational levels [3]. By quantifying social interactions, we can analyse associations between phenotypic variation and position in the social network.

Interactivity during the breeding season is predicted to correlate with mate selection signals. For example, North American barn swallow females have been shown experimentally to allocate paternity to males with darker ventral plumage [4], suggesting that dark males interact with additional females beyond their social mate. Other aspects of the phenotype might also be correlated with interactivity, particularly traits connected to social stress (glucocorticoid hormones, corticosterone (CORT) or cortisol [5]) and social transmission (parasites, gut microbes [6–8]).

Social interactions, gut microbial diversity and CORT have never been examined simultaneously in the wild despite evidence suggesting coordination among these traits. For example, research in mammalian systems indicates that gut microbes are socially transmitted via close contact [7,8], and that higher CORT levels have been associated with lower microbial diversity [9]. If acquiring a diverse gut microbial community depends on social behaviour, and social behaviour correlates with circulating CORT levels (e.g. [10]), there may be trade-offs between social behaviour, stress physiology and gut microbial diversity.

Here, we fitted barn swallows (*Hirundo rustica erythrogaster*) with proximity loggers (radio devices that communicate directly with one another [11,12]) to characterize barn swallow interaction networks and relate morphology, stress-induced CORT response and gut microbial diversity to network position. We predicted that gut microbial diversity would be correlated with interactions at close proximity, because contact is necessary for microbial transmission, and that morphological signals would be associated with social affiliations at larger proximities, based on the assumption that these proximities are relevant for visual signalling. Therefore, we constructed interaction networks from two spatial proximities (social affiliation (*social network*) and body contact (*contact network*)) to assess the influence of scale of interaction proximity on relationships between interactivity and aspects of phenotype.

## 2. Material and methods

### (a) Study system and proximity loggers

We studied a medium-sized population of barn swallows ( $n = 25$  individuals) in Boulder County, Colorado (40°07'57.6" N, 105°10'40.1" W) during second clutch initiation (19–30 July 2014). We tagged 21 adult birds with Encounternet proximity loggers. Tags turned on 3 days after we started tagging birds and recorded from 6.00 to 9.00 and from 17.00 to 20.00 each day, when birds were most active. Details, including validation tests, of Encounternet tags can be found in Levin *et al.* [11].

### (b) Morphological measurements

We weighed each bird, measured wing and tail streamer length and collected ventral feathers. Details on feather spectrometry and principal component analysis of colour data are in the electronic supplementary material.

### (c) Stress-induced corticosterone assay

Birds were subjected to a standardized restraint (held solitarily for 15 min [13]) prior to blood sampling. Sampling details are in the electronic supplementary material. We used an enzyme immunoassay kit (Enzo Life Sciences, Plymouth Meeting, PA, ADI-900-097) to quantify CORT levels in plasma using a protocol optimized for barn swallows [13]. Standards and samples were run in duplicate and average intra-assay variation was 7.09%.

### (d) Gut microbiome

We extracted DNA from faecal samples and amplified the V4 region of the 16S rRNA gene with Golay barcoded primers 515F and 806R, following the Earth Microbiome Project protocol (<http://earthmicrobiome.org>) [14]. Microbial diversity (Faith's Phylogenetic Diversity) was estimated for each sample as the average across 10 different rarefactions at 5000 reads. Details on gut microbial analyses are in the electronic supplementary material.

### (e) Social and contact networks

We obtained proximity data from 17 of the 21 tags (10 males and seven females) and analysed interactions logged during the first 15 h of logging over 3 days when all tags were operational. We constructed two networks with edges weighted by the number of interactions logged by at least one tag at two spatial proximities: body contact interactions (0.1 m and closer, *contact network*), and all other interactions recorded, corresponding to proximities between 0.1 and 5 m (*social network*) [11]. We split the two networks into male–male (M-M), female–female (F-F) and male–female (M-F) networks because of the potential for

different relationships between morphological and physiological traits and inter- and intrasexual interactions. We used node strength, the sum of all interactions for a focal node, as a measure of interactivity. We visualized networks and calculated node strength using the *igraph* package in R v. 3.2.2 [15].

### (f) Statistical analyses

We examined correlations between and among male and female node strength and traits and between traits themselves (electronic supplementary material, figures S1 and S2). Single-variable regression analyses were performed to quantify the association between sex-specific node strength and morphological and physiological traits. Jackknife resamplings [16] were used to assess robustness and statistical significance was assessed using permutation tests. R code is provided in the electronic supplementary material and the data are archived in Dryad [17].

## 3. Results

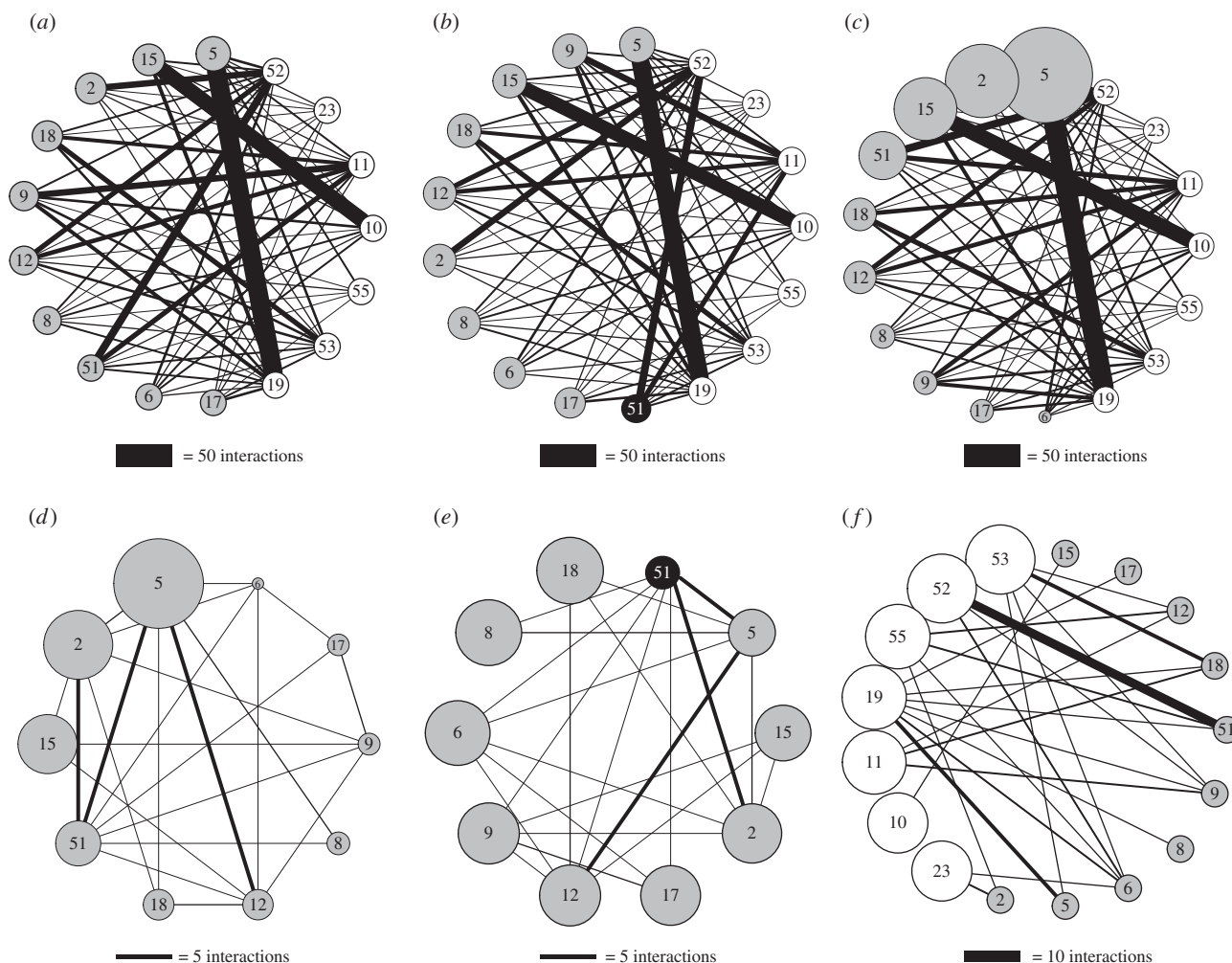
Male ventral colour, tail streamer length and stress-induced CORT were inter-correlated in this sample ( $r = 0.61–0.88$ ); males dark in colour tended to have higher CORT levels and longer streamers. We found positive relationships between male node strength in the male–female social network and male ventral colour, streamer length and CORT response (figure 1 and table 1). Darker, redder males with long tail streamers and greater stress-induced CORT responses were more socially interactive with females.

Stress-induced CORT and gut microbial diversity explained a significant amount of variation in node strength in various contact sub-networks (table 1). CORT was positively associated with male node strength in the male–male network (figure 1*d*). The direction of the relationship between contact node strength and gut microbial diversity differed between the sexes: males with more contacts with other males had lower microbial diversity (figure 1*e*), while females with more contacts with males had higher microbial diversity (figure 1*f*).

## 4. Discussion

Social interactions are hypothesized to underlie the coordination of phenotypic traits [1,2], yet little is known about the fine-scale details of these behaviours. Here, network analyses based on inter-individual interactions at known spatial proximities reveal that the scale of interaction proximity is important for understanding what traits mediate—or correlate with—social behaviour in swallows. As predicted, social interactions between males and females were strongly associated with variation in male morphology (and stress response), while body contact interactions among males and between males and females were associated only with stress reactivity and gut microbial diversity.

Ventral colour is a known mate selection signal in North American barn swallows [4,18]. Experimental darkening of male plumage resulted in increased testosterone levels one week later [19]. Because animals experience their phenotype via interactions with conspecifics, these physiological changes in response to colour manipulation could result from altered social experience. Our findings reported here, where darker males (with longer streamers and higher CORT responses) were more interactive with females, support the hypothesis that social behaviour mediates the physiological changes associated with the colour manipulation.



**Figure 1.** Barn swallow social and contact networks. Grey nodes, males; white nodes, females; black nodes, individuals missing trait values. Node number corresponds to bird ID. Node size is scaled and ordered by trait value. (a–c) Male–female social network of interactions occurring between 0.1 and 5 m ((a) M node size = ventral colour, (b) M node size = tail streamer length and (c) M node size = stress-induced corticosterone). (d,e) Male–male contact network of interactions within 0.1 m ((d) node size = stress-induced corticosterone and (e) node size = gut microbial diversity). (f) Female–male contact network (F node size = gut microbial diversity).

**Table 1.** Regression analyses of social (interactions between 0.1 and 5 m) and contact (interactions within 0.1 m) network node strength on morphology, stress-induced CORT and gut microbial diversity. In cases where jackknife  $p$ -values were  $>0.05$ , only one resampling failed to support the relationship.

network	covariate	$n$	$r^2$	coefficient (jackknife range)	$p$ -value (jackknife range)
male node strength in M-F social network	ventral colour	10	0.47	6.33 (4.03, 7.44)	0.013 (0.007, 0.128)
	tail streamer length	9	0.71	3.84 (2.84, 4.49)	0.003 (0.002, 0.019)
	stress-induced CORT	10	0.48	0.42 (0.26, 0.56)	0.014 ( $<0.001$ , 0.209)
male node strength in M-M contact network	stress-induced CORT	10	0.40	0.10 (0.07, 0.12)	0.045 (0.009, 0.286)
	microbial diversity	9	0.40	−0.85 (−1.03, −0.32)	0.038 (0.008, 0.597)
female node strength in F-M contact network	microbial diversity	7	0.84	1.38 (1.07, 1.37)	0.005 (0.001, 0.11)

Our finding that a male's tail streamer length was predictive of the frequency of social interactions with females may suggest that older males were more interactive. In North American populations of barn swallows, male tail streamers increase with age [20]. North American barn swallow females do not show any preference for longer tail streamers [18]; however, unlike Safran & McGraw [18], we found that tail streamer length and ventral colour were correlated for males in this study.

We found that stress-induced CORT was associated with male network position at both scales. In bird species that show

linear dominance hierarchies, more dominant individuals are more stress-reactive (e.g. [21]). Therefore, it is possible that the more interactive swallows that had higher acute stress responses were socially dominant, consistent with increased access to and interactivity with females. Higher CORT levels might also facilitate gregarious behaviour; a study in captive birds demonstrated that young birds treated with CORT were more interactive in the social network upon nutritional independence [5].

Social transmission of symbiotic microbes is increasingly recognized as a benefit of sociality [22]. Social interactions

influence the composition of gut microbial communities [8,23], and there is evidence that the gut microbiome can alter host social behaviour [22]. Male swallows that were more interactive with other males in the contact network had lower gut microbial diversity and females who had more contacts with males had higher diversity. One explanation involves the positive relationship observed in males between contact interactivity and stress-induced CORT. The gut microbial communities of mice subjected to chronic restraint stress differed from mice not exposed to the stressor [24] and wild squirrels with higher CORT levels had reduced oral microbiome diversity [9]. Therefore, high male–male interactivity and stress could negatively affect male gut microbial diversity. However, increased interactivity between females and males could expose females to more microbes; for example, there is evidence of greater male-to-female microbial transfer during copulation [25].

## 5. Conclusion

This research demonstrates that aspects of morphology, stress physiology and gut microbial diversity are related to interactivity, which has often been inferred but rarely tested,

especially in situations where these traits predict reproductive performance. Our work also reveals that an analysis of different scales of inter-individual connectivity has important implications for understanding the dynamic relationships among social interactions and aspects of phenotype.

**Ethics.** Research was approved by University of Colorado IACUC no. 1303.02.

**Data accessibility.** The datasets supporting this article can be accessed in Dryad (<http://dx.doi.org/10.5061/dryad.3jn35>).

**Authors' contributions.** I.I.L. and R.J.S. designed the study, I.I.L. and D.M.Z. did the fieldwork, and I.I.L. and B.K.F. analysed the network data with input from R.J.S., D.M.Z. and R.K. S.J.S. analysed the microbial data. I.I.L. and R.J.S. wrote the manuscript with input from D.M.Z., B.K.F., R.K. and S.J.S. All authors gave final approval for publication and agree to be accountable for all aspects of the work.

**Competing interests.** We have no competing interests.

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