UNIVERSIDADE FEDERAL DO PARANÁ

LAURA MARIA SCHAEDLER

PHENOTYPIC INTERACTION NETWORKS IN SWALLOW-TAILED MANAKINS (*CHIROXIPHIA CAUDATA*)

CURITIBA

2019

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PHENOTYPIC INTERACTION NETWORKS IN SWALLOW-TAILED MANAKINS (*CHIROXIPHIA CAUDATA*)

Dissertação apresentada ao Programa de Pós-Graduação em Ecologia e Conservação como requisito parcial para obtenção do grau de Mestre em Ecologia e Conservação, Setor de Ciências Biológicas, Universidade Federal do Paraná.

Orientadora: Dra. Lilian Tonelli Manica

CURITIBA

2019

Universidade Federal do Paraná. Sistema de Bibliotecas.
Biblioteca de Ciências Biológicas.
(Giana Mara Seniski Silva – CRB/9 1406)

Schaedler, Laura Maria
Phenotypic interaction networks in Swallow-tailed Manakins (*Chiroxiphia caudata*). / Laura Maria Schaedler. – Curitiba, 2019.
38 p.: il.

Orientador: Lilian Tonelli Manica

Dissertação (mestrado) - Universidade Federal do Paraná, Setor de Ciências Biológicas. Programa de Pós-Graduação em Ecologia e Conservação.

1. Ave - Ecologia 2. Seleção sexual em animais I. Título II. Manica, Lilian Tonelli III. Universidade Federal do Paraná. Setor de Ciências Biológicas. Programa de Pós-Graduação em Ecologia e Conservação.

CDD (22. ed.) 598.823

MINISTÉRIO DA EDUCAÇÃO
SETOR SETOR DE CIENCIAS BIOLOGICAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO ECOLOGIA E
CONSERVAÇÃO - 40001016048P6

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ECOLOGIA E CONSERVAÇÃO da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **LAURA MARIA SCHAEDLER** intitulada: **Phenotypic interaction networks in Swallow-tailed Manakins (Chiroxiphia caudata)**, após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

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Curitiba, 13 de Março de 2019.

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AGRADECIMENTOS

Ainda que o caminho seja longo para que, no Brasil, o sistema de educação e a ciência sejam excelentes e acessíveis a todos, não podemos deixar de celebrar os direitos que conquistamos enquanto população – para que nunca sejam esquecidos e perdidos. É por isso que eu agradeço e valorizo muito as oportunidades concedidas a mim pelo Governo Federal, que me possibilitou:

- Cursar biologia gratuitamente em uma ótima universidade federal, que me introduziu ao mundo da pesquisa científica;
- Receber remuneração durante o mestrado, por meio da bolsa concedida pela CAPES, tornando a pesquisa de base feita por mim e tantos outros colegas possível e valorizada;
- Receber uma formação excelente por meio do Programa de Pós Graduação de Ecologia e Conservação da UFPR, que me apoiou financeiramente para cursar disciplinas dentro e fora da instituição, para apresentar meu trabalho em eventos e para a minha coleta de dados.

Agradeço aos meus professores, dos tempos de escola, da UFPR e do PPG ECO por me ensinarem o fascínio pela natureza e o pensamento crítico, dentro e fora do âmbito científico.

Aos meus pais e à minha avó, meus principais professores, por me ensinarem o respeito e a compaixão pela natureza, pelos animais e pelas pessoas, por incentivarem meus interesses e por se dedicarem tanto a mim.

Ao Luiz, que compartilha minhas paixões e angústias, sempre calmo e carinhoso e me ensina todos os dias o amor, o companheirismo e a importância de concretizarmos nossas ideias.

Ao Pedro, por tornar possível toda a coleta de dados, mas principalmente pelo exemplo de amizade que levarei para a vida e por sempre tornar as coisas mais divertidas.

À minha orientadora, Lilian, por me apresentar à área do comportamento animal e me dar a oportunidade de trabalhar com esse tema e, juntamente com o André, pelo apoio logístico e intelectual.

Aos meus colegas de laboratório e de departamento e a todos os meus amigos, por ouvirem e contribuírem com ideias sobre o trabalho, e por todos os momentos de descontração.

Ao Instituto Ambiental do Paraná, CEMAVE, e SANEPAR pelas licenças de pesquisa.

À Animal Behavior Society, ao CNPq e aos colaboradores do nosso projeto de financiamento coletivo "Desvendando a dança do tangará" pelo apoio financeiro e ao Laboratório de Comportamento Animal da Universidade de Brasília pelo empréstimo do espectrofotômetro.

Aos participantes do Manakin Genomics Research Coordination Network, pela oportunidade de conhecer os pesquisadores por trás dos artigos que sempre li e admirei e poder trabalhar com eles.

Aos meus amados animais de estimação que já se foram ou que ainda estão, cujos nomes vou omitir pois ocupariam mais metade da folha, por me mostrarem que fazemos parte de um todo e que nossos sentimentos não são exclusivos da nossa espécie. Obrigada por mostrarem o valor da empatia e do amor na forma mais pura que se pode encontrar.

E, por fim, meus mais sinceros agradecimentos à todas as mulheres que durante a história lutaram, e diariamente lutam, para que possamos exercer nosso direito de ocupar as universidades (e onde mais quisermos) e provar que somos capazes sim de fazer ciência.

The lens of our own self-interest not only frequently distorts what we see when we look at other animals, it also in important ways determines what we do not see, what we are blind to.

Marlene Zuk

RESUMO

Compreender exibições de corte multimodais complexas em termos das propriedades integrativas dos traços sexuais, suas funções e como mudam em contextos diferentes é um desafio necessário na ecologia comportamental, visto que comportamentos sexuais podem apresentar uma diversa gama de implicações evolutivas. Aqui, nós usamos redes de interação fenotípicas para avaliar as exibições do tangará (*Chiroxiphia caudata*, Passeriformes: Pipridae) em dois contextos sociais: exibições de corte e de treino. Nós hipotetizamos alta modularidade e redundância (*degeneracy*) entre traços da mesma modalidade para todas as redes devido à prováveis limitações fisiológicas, mas hipotetizamos correlações menores entre os traços para exibições de treino, refletindo a inexperiência dos machos jovens em treino em comparação aos machos adultos cortejando fêmeas. Construímos redes fenotípicas de duas e três modalidades utilizando traços de som, movimento e cor extraídos de gravações de áudio e vídeo e da plumagem. Os dados foram coletados de uma população em um remanescente de Mata Atlântica no sul do Brasil, em três estações reprodutivas (de outubro a março de 2015 – 2018). Nós encontramos que redes de treino possuem maior modularidade e redundância (*degeneracy*) em comparação às redes de corte, ao contrário do esperado. Os padrões das redes de treino sugerem que machos jovens produzem uma exibição estereotipada devido a limitações no seu desenvolvimento, enquanto a maior variabilidade entre os traços para machos adultos pode indicar a capacidade de ajustar as performances dependendo da resposta e preferência das fêmeas. Nosso estudo ressalta como diferentes contextos sociais podem alterar a relação entre traços e também fornece direções futuras em relação à quais traços devem ser explorados para desvendar a função desta exibição complexa.

Palavras-chave: exibição multimodal, treino, corte, redundância, modularidade, função, seleção sexual, Pipridae.

ABSTRACT

Understanding complex multimodal courtship displays in terms of the integrational properties of sexual traits, their functions, and how they change in different contexts is a required challenge in behavioral ecology, since sexual behaviors can present a diverse set of evolutionary implications. Here, we used phenotypic interaction networks to evaluate displays of the Swallow-tailed Manakin (*Chiroxiphia caudata*, Passeriformes: Pipridae) in two social contexts: (i) practice and (ii) courtship displays. We hypothesized high modularity and degeneracy between same-modality traits for all networks due to physiological constraints, but relatively weaker trait-correlations for practice displays, reflecting inexperience of practicing juvenile males in relation to adult males performing to females. We built three and two modality phenotype networks using sound, motor and color traits extracted from audio and video recordings and plumage. We collected data from a population in an Atlantic Forest remnant in south Brazil, in three breeding seasons (from October to March 2015-2018). We found that practice networks had higher modularity and degeneracy in comparison to courtship networks, in opposite of what we expected. The constrained patterns of practice networks suggest that juvenile males perform a strict stereotypical display due to developmental constraints, while the higher variability between traits for adult males may indicate capability of adjusting performances depending on female response and preference. Our study sheds light on how different social contexts can alter the relation between traits and also provides future directions for what traits should be explored to unravel this complex display function.

Keywords: multimodal display, practice, courtship, degeneracy, modularity, function, sexual selection, Pipridae.

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Phenotypic interaction networks in Swallow-tailed Manakins (*Chiroxiphia caudata*)*

*This dissertation has been formatted as a manuscript according to the guidelines of the Animal Behaviour journal.

1. INTRODUCTION

Understanding animal communication dynamics, and translating multiple signals into information, is a research challenge in face of the variety and complexity of animals' displays. Due to this complexity in movements, sounds, and other display's signals, many behavioral studies have evaluated communication unimodally, relating one signal to certain functions. However, communication usually occurs through multiple signals, sometimes synchronously, integrating various modalities, such as vision and hearing (Hebets et al., 2016; Partan & Marler, 1999). In the field of sexual selection, several studies have shown that multimodal communication is preferred over unimodal by female partners (e.g. Doucet and Montgomerie 2003; Gibson and Uetz 2008; Girard et al. 2015). One interesting example are wolf spiders (*Schizocosa crassipes*), whose females prefer to mate with males that exhibit visual signals (foreleg brushes) coupled with vibrational signals (produced through stridulation), in comparison to just one of these signals (Stafstrom & Hebets, 2013). Such studies shed light on the relationship of structures (the signal itself, i.e. its expression on the environment, e.g. the vibration of foreleg brushes) and functions (e.g. attraction of reproductive partners), as well as the importance of their interaction on message transmission effectiveness (Hebets et al., 2016; Wilkins, Shizuka, Joseph, Hubbard, & Safran, 2015).

It is important to understand how signals interact with each other, as they can generate a diverse set of evolutionary implications. Each signal, such as a sexual ornament, could reflect a unique or an identical function in comparison to other signals (multiple messages and redundant signal hypotheses, respectively, Møller and Pomiankowski 1993). Thus, when presented together, signals could reinforce the information they are transmitting or even open new possibilities of selection by relaxing and altering the selective pressures that act upon them

(Friston & Price, 2003; Mason, 2014; Whitacre & Bender, 2010). Signals can also be highly dynamic depending on the social and environmental context (Rosenthal, Wilkins, Shizuka, & Hebets, 2018). As environmental features can alter the way a receiver perceives multimodal displays (due to climatic conditions such as light, rain and mist), signalers will adjust displays to increase information transmission (Chapman, Morrell, & Krause, 2009; Cole & Endler, 2015). Such context-dependent differences can thus modify display evolution by changing its functions and differential selective pressures (Rosenthal et al., 2018).

Phenotypic interaction networks (in which nodes represent behavioral traits, and edges represent the correlation between traits), are valuable tools in animal behavior studies as they favor the interpretation of multiple signals relationships (Hebets et al., 2016). They provide i) a clear visualization of displays' integrational properties (Hebets et al., 2016; Magwene, 2001; Patricelli & Hebets, 2016) and ii) introduce important elements of biological systems (Chen & Crilly, 2014), such as redundancy (the degree that same-structure signals transmit the same information; Hebets et al., 2016; Partan & Marler, 1999), degeneracy (the degree that different-structure signals transmit the same information; Hebets et al., 2016) and modularity (the degree of arrangement of signals in modules; Hebets et al., 2016; Olesen, Bascompte, Dupont, & Jordano, 2007). Here we used phenotype networks to understand the courtship display of a Neotropical passerine presenting extravagant multimodal signals, the Swallow-tailed Manakin (*Chiroxiphia caudata* Shaw & Nodder 1793).

Swallow-tailed Manakins are lekking birds in which males gather in display courts, distant within hearing - but not visual - range, where they are accessed by females to expose their ornaments (Foster, 1981; Payne, 1984). In display perches located within courts, Swallow-tailed Manakins expose ornaments by making a curious courtship display: a cooperative dance performed by two to six males to stimulate females for copulation ("cartwheel jump display", Foster 1981). Among signals exhibited during jump displays are the elongated central rectrices, exuberant coloration (reddish crown, blue body, and black wings and head), vocalizations and flight acrobatics (Foster, 1981). The display consists of several males performing acrobatics in turns: males stand in a line at the perch while each individual hovers in a vertical flight, maintaining a short distance to the female, synchronizing flight and vocalization in a movement that resembles a “cartwheel”, and returning to the end of the line where he waits for his turn again (Foster, 1981). To end the jump display, the dominant male (or “alpha male”, i.e. individual that participate in all displays, defends the court and copulates with females) hovers in the air while producing a strident vocalization and mechanical sounds (Foster, 1981; Schaedler, Ribeiro, Guaraldo, & Manica, 2019). The jump display may be followed by a solo precopulatory display by the alpha male, in which if successful will result in copulation (Foster, 1981). Another interesting aspect of their lekking behavior is that mainly juvenile, but also adult males, practice jump displays in the absence of females (Foster, 1981), performing alone or not and, commonly, with other males watching in the same position as a female would (L.S. personal observation, Foster, 1981). One hypothesis suggests that practice displays enable males to improve their performances and match the movements to that of their partners, a characteristic thought to influence female choice, as was shown for duetting behaviour in the genus *Chiroxiphia* (Trainer et al. 2002). Female mate choice, an important process guiding the evolution of ornaments, could thus be influenced by the interaction between display signals.

In order to understand the complexity of sexual signals in Swallow-tailed Manakins, as well as the selective pressures that act upon them, it is important to evaluate the interaction between the three modalities (sound, motor and color). In our study, we evaluated these interactions using phenotype networks in two contexts of the jump displays: during practices and courtship displays for females. We hypothesized that networks in both contexts would be structured in groups of traits belonging mainly to the same modality (high modularity) as a consequence of a higher degeneracy within than between traits in each modality, as traits are produced by different physiological mechanisms. We also hypothesized degeneracy between traits of different modalities since all traits are exhibited during the same behaviour, although at moderate to lower levels. Our third hypothesis is that there are differences between traits of practice and courtship displays, and consequently, differences between the phenotype networks. We expected that traits would be more extreme in courtship displays, reflecting more energy investment and ability to perform maneuvers, and that practice networks would have less and weaker correlations between traits as well as lower modularity, reflecting a higher variation in displays due to lack of experience of juvenile males.

2. METHODS

2.1 Study area

We conducted the study at Mananciais da Serra-Parque Estadual Pico Marumbi Protected Area (25°30'28"S 49°1'30"W), Piraquara, PR, south Brazil, during 2015-2018 breeding seasons (from October to March). The area is within an Atlantic Forest remnant, including mostly Araucaria and Montane rainforests (Reginato & Goldenberg, 2007) and is characterized by humid subtropical climate with hot summers (Cfa climate, according to Köppen’s classification).

2.2 Field data collection

We found display perches by searching for vocalizing Swallow-tailed Manakins and confirmed the locations through displays’ sightings. Males displayed in a total of three courts (one court had only one perch, while the other courts had two and three perches, respectively). We captured individuals three times a week using mist-nets placed approximately 10 m from perches. We banded birds with metallic numbered bands provided by the Brazilian banding agency (CEMAVE/ICMBio, LTM permit: 1195110) and plastic colored bands for individual identification. To describe male’s plumage and infer age, we categorized plumage stage of males from 1 to 9, according to Mallet-Rodrigues and Dutra (2012). The first four stages represent first and second-year males, with juvenile and formative plumages, mostly green and without blue feathers. Stages 5-8 represent third and fourth-year males, with pre-definitive plumage in which blue and black feathers progressively increase while losing green feathers. Stage 9 represents males with at least four years and definitive plumage, with a red crown, blue body and black head and wings. We did not include plumage categorization in our analyses. For feather coloration analysis, we collected from four to five dorsal and crown feathers with a tweezer (following protocol adapted from Sicsú, Manica, Maia, & Macedo, 2013). To sample practice and courtship displays, we filmed and recorded display perches during ~5 h, from three to five times a week. For video recordings, we used Sony HDR-CX290 cameras fixed to a tripod placed at 5 m from the perch. For audio recordings vocalizations, we used a Marantz PMD661 digital recorder and a Sennheiser ME67 microphone using 44.1Hz sampling rate and 24-bit resolution. We identified individuals based on the colored bands combinations.

2.3 Extraction of sound, motor and color traits

For each individual we extracted five sound, seven motor and nine color traits (Table 1). Sound and motor traits of each male were always taken at the same time point in a display, while color traits were taken from feathers collected in the same breeding season as the sound and video recordings. We used Raven Pro 1.4 (Cornell Lab of Ornithology, Ithaca, NY; http://www.birds.cornell.edu/raven) to extract sound traits of the *wah* call, which is the main sound produced by males during displays (Schaedler et al., 2019). To extract all frequency measurements, we used spectrogram and power spectra views generated with Hann window type, discrete Fourier transform (DFT) of 512 samples and 50% overlap. In power spectra, we subtracted 24 dB from the peak amplitude to obtain low (LFrq) and high frequencies (HFrq), excluding background noise while maintaining 99.6% of the acoustic signal (Fig. 1, Podos, 1997; Zollinger, Podos, Nemeth, Goller, & Brumm, 2012). We obtained center (CFrq) and peak frequencies (PFrq) from power spectra using Raven's automatic calculation and calculated delta frequency (DFrq) as the difference between high and low frequencies. We also used audio recordings extracted from display videos in cases when it was not possible to record males with the audio recorder. To confirm that audio file types were providing similar information, we performed Pearson correlations between sound traits extracted from video and audio recordings captured at the same time and distance from the perch from a subset of 20 samples. All sound traits were highly correlated ($p < 0.0001$, $r \geq 0.97$, Table S1).

We used Windows Movie Maker® (©2012 Microsoft Corporation) to select print screens of displays. Using ImageJ, we extracted the following motor traits (described in Table 1) from print screens as described in Ribeiro, Guaraldo, Macedo, & Manica (2019, Fig. 2) protocol: vertical flight height (FHgt), duration (FDur) and speed (FSpd), cartwheel distance (CwDt), duration (CwDur) and speed (CwSpd), and distance to individual attending the display (DtId). We scaled all measures relative to the perch width, which we measured in the field with a caliper. We used the mean of a minimum of three samples for all sound and motor traits for each individual, selecting samples without interruptions from other individuals. To account for possible variation throughout the display (e.g. pulsed sound becomes faster towards the end, Schaedler et al. 2019), we sampled each display's beginning, middle and end (we identified these three display's portions by dividing the display in three parts of equal length).

We extracted feather reflectance using SpectraSuite® software and an Ocean Optics® USB4000 spectrometer, attached to a PX-2 pulsed xenon light source and placed the probe at 90º to the feather. We used ten feathers from the crown and back, taped to a black velvet surface. Reflectance was measured three times for all samples, relative to a WS-1 white standard and the black surface as references. We generated crown and back spectra for each individual using the mean from all three samples, and then extracted standard colorimetric variables (chroma, brightness and hue, Table 1) using the R package *pavo* (Maia, Eliason, Bitton, Doucet, & Shawkey, 2013; R Core Team, 2018).

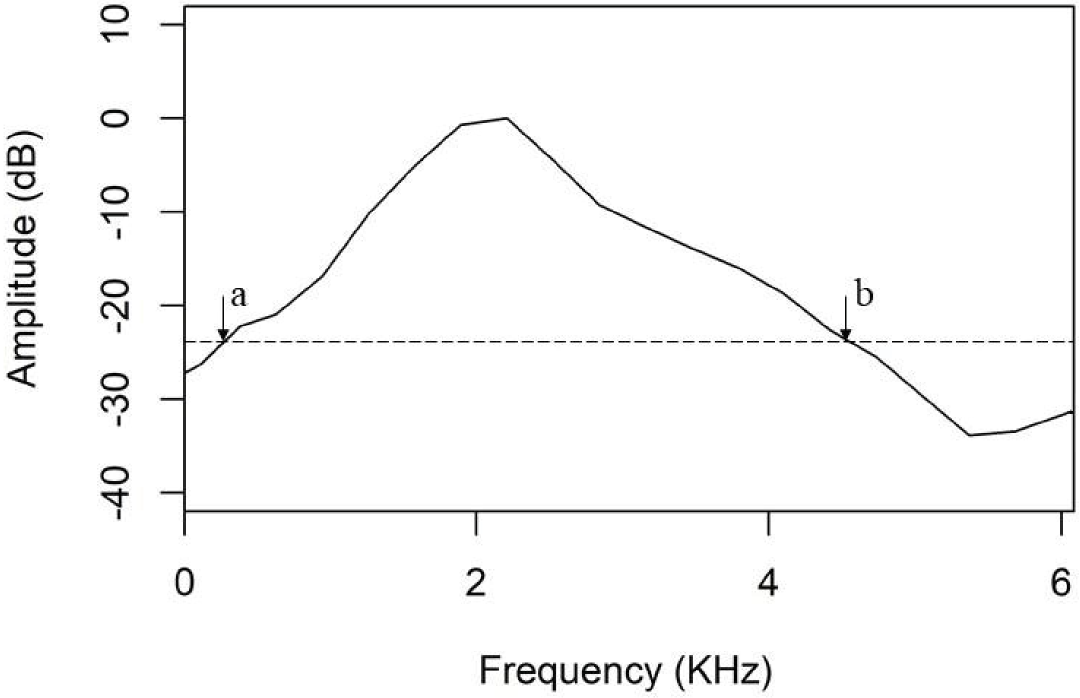

Figure 1. Selection of power spectra used to measure sound traits. The dashed line represents -24 dB criterion for excluding background noise. For each spectrum we measured a) low frequency (LFrq), b) high frequency (HFrq) and delta frequency (DFrq, as the difference between high and low frequencies). We obtained center (CFrq) and peak frequencies (PFrq) from power spectra using Raven's automatic calculation. See Table 1 for a detailed description of sound traits.

Figure 2. Example of a video print screen sample showing methods for extraction of motor traits. For each sample we measured a) vertical flight height (FHgt) and duration (FDur), b) distance to individual (DtId, in this frame, a female, but in practice displays the measure is in relation to a male watching the display) and c) cartwheel distance (CwDt) and duration (CwDur). We also measured vertical flight (FSpd) and cartwheel speed (CwSpd) as a measure of distance per duration. See Table 1 for a detailed description of motor traits.

Table 1. Phenotypic traits from three modalities extracted from individuals in two contexts, practice and courtship displays. Statistical comparisons between these two contexts are presented as results of a Principal Components Analyses in Tables 2 and 3.

Modality	**Trait**	**Initials**	**Description**	**Mean±s.d of practice displays**[a]	**Mean±s.d. of courtship displays**[b]
Sound	Low frequency	LFrq	The lowest vocalization frequency	644±173 Hz	503±96 Hz
Sound	High frequency	HFrq	The highest vocalization frequency	5259±1457 Hz	4525±420 Hz
Sound	Delta frequency	DFrq	The difference between high and low frequency	4614±1507 Hz	4022±406 Hz
Sound	Center frequency	CFrq	The frequency dividing the selection in two intervals of equal energies	2212±237 Hz	2109±61 Hz
Sound	Peak frequency	PFrq	The frequency with the peak energy	2187±290 Hz	2103±18 Hz
Motor	Vertical flight height	FHgt	Distance between the perch and male's beak at the highest point of the vertical flight	137±31 mm	145±21 mm
Motor	Vertical flight duration	FDur	Time interval a male took to reach the highest point of the vertical flight	0.18±0.08 s	0.12±0.02 s
Motor	Vertical flight speed	FSpd	The ratio between the vertical flight height and duration	945±335 mm/s	1261±271 mm/s

Motor	Distance to individual	DtId	Distance between the displaying male, at the highest point of the vertical flight, and the individual that is attending the display (male or female)	125±99 mm	84±49 mm
Motor	Cartwheel distance	CwDt	Distance flown by males from takeoff to landing in the perch	100±88 mm	184±74 mm
Motor	Cartwheel duration	CwDur	Time interval a male took to fly the cartwheel distance	0.7±0.18 s	0.85±0.1 s
Motor	Cartwheel speed	CwSpd	The ratio between the cartwheel distance and duration	133±98 mm/s	214±67 mm/s
Color	Crown average brightness	CBri	Mean relative reflectance over the entire spectral range, on crown feathers	7.84±4 %	7.37±2.3 %
Color	Crown UV chroma	CUv	Proportion of light reflected in the UV color range (300–400 nm) in relation to the entire range (330-700 nm), on crown feathers	0.13±0.07	0.12±0.08
Color	Crown yellow chroma	CYl	Proportion of light reflected in the yellow color range (550–625 nm) in relation to the entire range (330-700 nm), on crown feathers	0.26±0.03	0.26±0.04
Color	Crown red chroma	CRd	Proportion of light reflected in the red color range (605–700 nm) in relation to the entire range (330-700 nm), on crown feathers	0.51±0.13	0.53±0.16

Color	Back average brightness	BBri	Mean relative reflectance over the entire spectral range, on back feathers	7.79±2 %	9.8±2 %
Color	Back UV chroma	BUv	Proportion of light reflected in the UV color range (300–400 nm) in relation to the entire range (330-700 nm), on back feathers	0.26±0.03	0.31±0.02
Color	Back blue chroma	BBl	Proportion of light reflected in the blue color range (400–510 nm) in relation to the entire range (330-700 nm), on back feathers	0.24±0.04	0.29±0.02
Color	Back green chroma	BGr	Proportion of light reflected in the green color range (510–605 nm) in relation to the entire range (330-700 nm), on back feathers	0.29±0.03	0.24±0.02
Color	Back hue	BHue	Wavelength of peak reflectance, on back feathers	497±69 nm	395±72 nm

[a] $N = 11$ individuals for color traits and 21 for sound and motor traits.

[b] $N = 9$ individuals for color traits and 16 for sound and motor traits.

2.4 Statistical analyses

In total, we built four phenotype networks. In two networks, we used traits from three modalities (3M), sound, motor and color, for i) displays performed to other males (hereafter, "3M practice networks") and for ii) displays performed to females (hereafter, "3M courtship networks"). In the other two networks, we used traits from two modalities (2M), sound and motor, for i) displays performed to other males (hereafter, "2M practice networks") and for ii) displays performed to females (hereafter "2M courtship networks"). We did not use color in these networks to increase our sample size, since for many individuals we did not have plumage samples from the same breeding season when they were audio or video recorded.

We followed the protocol provided by Wilkins et al. (2015) to build networks, as follows. In networks, each node represents one sound, motor or color trait (calculated as the mean of all samples), while edges represent values of Spearman trait-pair correlations (Epskamp, Cramer, Waldorp, Schmittmann, & Borsboom, 2012). To discard incidental correlations, we used a bootstrap analysis in which we resampled our original dataset 10,000 times and calculated new correlations for each trait-pair. We maintained the trait-pair correlation in the network if the bootstrap's 95% CI did not overlap zero. To access traits degeneracy, we calculated average correlation strength (|avg cor|, the mean of the absolute trait-pair correlations) and network density (|net dens|, the number of significant correlations, according to bootstrap analysis, divided by the number of total correlations). To access modularity, we used the assortativity coefficient ($r_{d;}$ Farine, 2014; Newman, 2003). The r_d differs from the commonly used metric of modularity in systems approaches (Olesen et al., 2007) because it tests the connectivity of nodes in groups *a priori* defined, instead of identifying groups after the network is generated. Thus, r_d measures if nodes are more connected to nodes of the same modality or to nodes of different modalities (Farine, 2014; Newman, 2003). An assortativity value of 1 means there are no correlations between different modalities and 0 that all traits from different modalities are correlated. If networks are degenerate and with high modularity, we expect to find high |avg cor|, |net dens| e r_d. We calculated the probability of finding our observed assortativity in a distribution of assortativity values generated from

1,000 permuted networks. We randomized trait's modalities on the new generated networks while maintaining the number of traits that belong to each modality (five for sound, seven for motor and nine for color). All statistical analyses were performed in R (R Core Team, 2018) using personalized functions provided by Wilkins et al. (2015). We assessed differences between practice and courtship networks by calculating the Jaccard similarity index, adapting the index to use significant correlations instead of species (Rosenthal et al., 2018). We calculated Jaccard similarity (function vegdist, R package vegan, Oksanen et al., 2017) dividing the number of significant correlations shared by both networks by the total number of significant correlations in both networks. The index varies from 0 (completely dissimilar) to 1 (completely similar).

We tested for differences between practice and courtship display traits included in both 3M and 2M networks using t-tests (R package stats, R Core Team, 2018). For this analysis we considered female presence as the predictor variable and principal components (PCs) extracted from PCAs (Principal Component Analysis, function prcomp, R package stats, R Core Team, 2018) as response variables. We performed one PCA for 3M networks and one for 2M networks, containing all trait values from both practice and courtship displays. We selected PCs with eigenvalues larger than 1 (PC1 to PC5 for 3M, and PC1 to PC4 for 2M display networks) to perform t-tests and to color networks based on the higher value of loading for each trait (Fig. 3 and 4). For t-tests, we adopted a significance level of 0.05 and calculated the effect size using Cohen's d (function cohen.d, R package effsize, Torchiano, 2018). We considered effect sizes of $d < 0.2$ as negligible, $d < 0.5$ as small, $d < 0.8$ as medium and $d \geq 0.8$ as large (Cohen, 1992; Torchiano, 2018). In each network included in PCA analyses, we scored completely different individuals between contexts.

3. RESULTS

3.1 Three-modalities networks: sound, motor and color traits

We used data from 11 and 9 males to build 3M practice and courtship displays networks, respectively, in both cases belonging to three different display courts. We found a large variation in male plumage stages performing practice displays, where there were individuals at stages 3 (formative) to 9 (definitive). In courtship display networks, only one male was at plumage state 7 (pre-definitive), while others were adult males at plumage stage 9 (definitive). Alpha males, identified as such by copulation events, were present in both contexts and were all in definitive plumage. There was only one alpha present during practice displays, while in courtship displays there were 3.

In the 3M practice network (Fig 3b), we found |avg cor| = 0.75, |net dens| = 0.15 and $r_d = 0.74 \pm 0.07$, greater than expected by chance ($p = 0$, Fig S1). These results indicate high degeneracy (in spite of low values of network density) and high modularity. We found two groups, one of them including only sound traits, and the other including all three modalities but connected by few correlations (Fig 3b). Only two traits were isolated/uncorrelated. In the 3M courtship network (Fig 3a), |avg cor| was similar to the practice network (0.78), but |net dens| and r_d were lower (0.07 and $r_d = 0.49 \pm 0.15$, respectively), the last also greater than expected by chance ($p = 0.001$, Fig S1), also indicating high degeneracy, but moderate modularity. The courtship network included four groups, three of which included traits of only one modality (sound, motor or color), while one was composed by the three modalities but mainly color (Fig 3a). Five traits were isolated and uncorrelated to any other traits. When comparing 3M practice and courtship networks, we found low Jaccard similarity (J = 0.17), highlighting that the networks are different in terms of what traits correlate to each other.

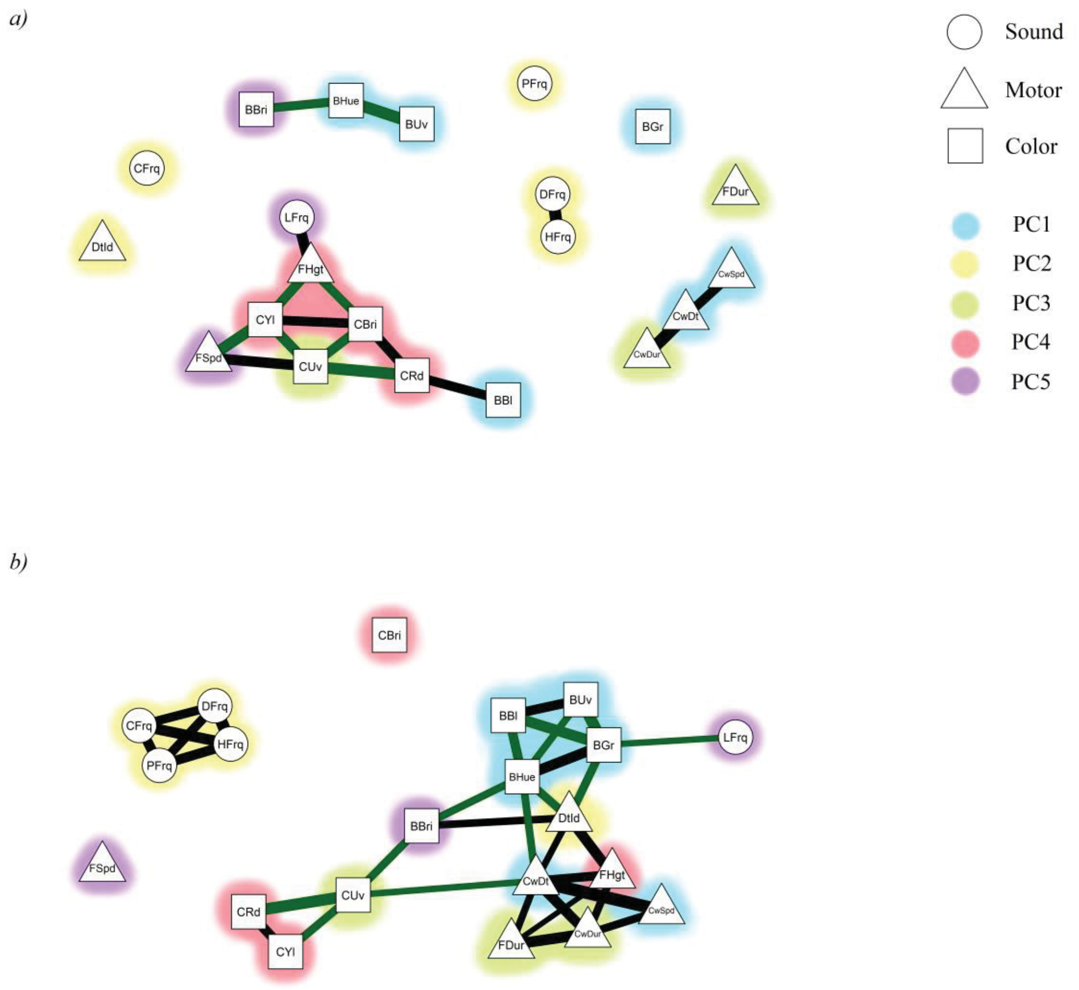

Figure 3. Phenotype networks of traits of three modalities (3M) in courtship (*a*) and practice displays (*b*). Each node represents traits extracted from displays and male plumages, and edges represent correlations between them. Edge's width increases with correlation degree and green edges represent negative correlations. Colors represent the PC for which traits had higher loadings. One PCA was performed for both networks.

The cumulative proportion of explanation of PC1 to PC5 was 0.87 (individual proportions of 0.32, 0.24, 0.14, 0.11 and 0.06, respectively). We found significant differences between practice and courtship displays regarding PC1 ($t_{12.8} = 6.96$, $p < 0.001$, $d = 3.52$). PC1 effectively separated most back color and cartwheel traits (Table 2). Males of practice displays presented larger values of back green chroma and hue (Table 1), while males of courtship displays presented larger values of back UV and blue chroma, and cartwheel distance and speed (Table 1). We did not find differences regarding PC2 to PC5 ($t_{8.2} = 0.74$, p

$= 0.47$, $d = 0.4$; $t_{8.7} = 0.68$, $p = 0.5$, $d = 0.37$; $t_{13.7} = -0.51$, $p = 0.61$, $d = -0.25$; and $t_{9.3} = -0.14$, $p = 0.88$, $d = -0.08$, respectively) for data in practice and courtship displays. PC2 effectively separated most sound traits, while PC3-PC5 separated some motor and color traits, with low frequency also scoring higher in PC5 (Table 2).

Table 2. Loadings of the first five principal components of sound, motor and color traits of individuals included in 3M practice and courtship displays networks. Shaded cells indicate the highest loading for each trait.

Trait	PC1	PC2	PC3	PC4	PC5
LFrq	0.16	0.06	-0.23	-0.10	-0.33
HFrq	0.002	0.40	0.01	-0.25	0.12
DFrq	-0.02	0.40	0.04	-0.24	0.16
CFrq	-0.03	0.38	-0.04	-0.32	0.01
PFrq	-0.07	0.35	-0.05	-0.33	0.10
FHgt	-0.23	0.15	0.15	0.30	-0.07
FDur	0.06	0.14	0.47	-0.04	-0.36
FSpd	-0.18	-0.01	-0.30	0.25	0.50
DtId	-0.19	0.24	0.08	0.17	3x10^{-3}
CwDt	-0.32	0.08	0.17	0.10	0.03
CwDur	-0.24	0.08	0.38	0.14	-0.16
CwSpd	-0.33	0.08	0.07	0.06	0.09
CBri	0.11	-0.13	-0.24	-0.30	-0.26
CUv	0.11	0.27	-0.32	0.28	-0.15
CYl	-0.03	-0.30	0.20	-0.32	0.28
CRd	-0.12	-0.28	0.24	-0.34	0.16
BBri	-0.28	-0.15	-0.05	-0.10	-0.42
BUv	-0.33	-0.01	-0.24	-0.12	-0.18
BBl	-0.34	-0.11	-0.14	-0.12	0.04
BGr	0.35	0.04	0.20	0.13	0.11
BHue	0.33	0.07	0.20	0.06	0.08

3.2 Two-modalities networks: sound and motor traits

After removing color traits from our analyses, we increased our sample size of 2M practice and courtship displays to 21 and 16 males, respectively, also belonging to three different display courts. In practice displays there were individuals of all plumage stages from 3 (formative) to 9 (definitive). In courtship displays, two males had plumage stages 6 and 7 (pre-definitive), while others were adult males with plumage stage 9 (definitive). There were 3 and 4 alpha males in practice and courtship displays, respectively.

In 2M practice networks, we found similar values of average correlation (|avg cor| = 0.70) and network density (|net dens| = 0.25) in comparison to 2M courtship networks. Assortativity reached the highest possible value ($r_d = 1 \pm 1x10^{-17}$), greater than expected by chance ($p = 0.003$, Fig S1), indicating a highly modular and degenerate pattern. Two groups separated motor and sound modalities, and only one trait (low frequency, sound modality) does not correlate with others (Fig 4b). In 2M courtship networks, we found only one groups grouping motor traits in the center and sound traits at the tips (Fig 4a). We found similar values of |avg cor| (0.68) and |net dens| (0.24). However, assortativity was not greater than expected by chance ($r_d = 0.21 \pm 0.19$, $p = 0.09$, Fig S1), meaning that the observed pattern can occur without any biological deterministic process, preventing us from interpreting degeneracy and modularity. We also found a low Jaccard similarity (J = 0.23) between 2M practice and courtship networks, similar to that of 3M network analyses, highlighting that 2M networks are also different in terms of traits correlations.

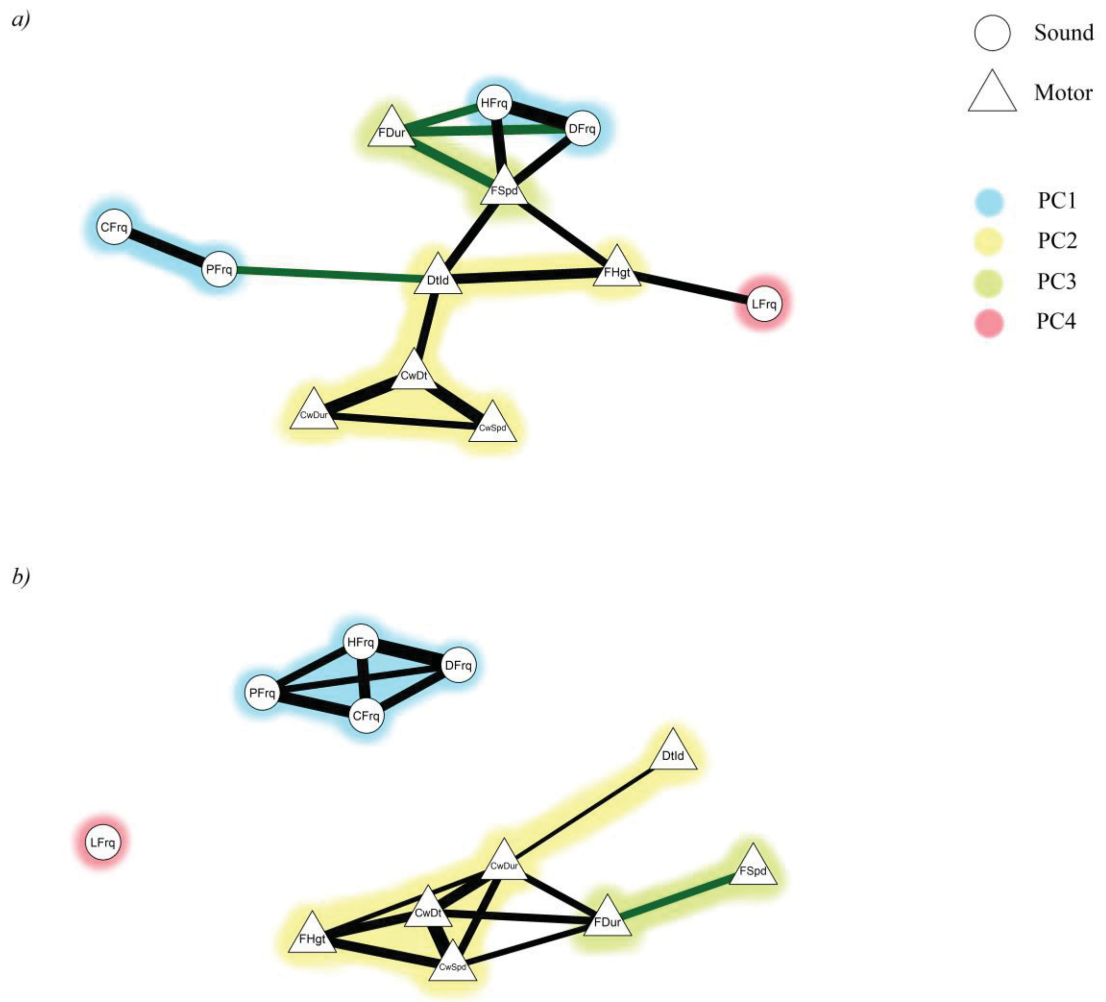

Figure 4. Phenotype networks of traits of two modalities (2M) in courtship (*a*) and practice displays (*b*). Each node represents traits extracted from displays and edges represent correlations between them. Edge's width increases with correlation degree and green edges represent negative correlations. Colors represent the PC for which traits had larger loadings. One PCA was performed for both networks.

The cumulative proportion of explanation of PC1 to PC4 was 0.85 (individual proportions of 0.33, 0.30, 0.13 and 0.09, respectively). We found significant differences between practice and courtship displays regarding PC2 ($t_{23.3} = 5.26$, $p < 0.001$, $d = 2.06$). PC2 effectively separated most motor traits, with exception of flight duration and speed, which are the only traits in PC3 (Table 3). Males of practice displays presented larger values of distance to individual, while males of courtship displays presented larger values of flight height and cartwheel distance, duration and speed (Table 1). We did not find differences regarding PC1, PC3 and PC4 ($t_{14.3} = 0.96$, $p = 0.35$, $d = 0.37$; $t_{17.8} = -1.11$, $p = 0.27$, $d = -0.43$; and $t_{17.3} = 1.07$, $p = 0.29$, $d = -0.43$, respectively). PC1 effectively separated sound traits, with exception of low frequency which is the only trait in PC4.

Table 3. Loadings of the first four principal components of sound and motor traits of individuals included in for 2M practice and courtship displays networks. Shaded cells indicate the highest loading for each trait.

Loadings	**PC1**	**PC2**	**PC3**	**PC4**
LFrq	0.15	0.03	-0.20	-0.75
HFrq	0.48	-0.13	0.09	0.21
DFrq	0.45	-0.13	0.12	0.32
CFrq	0.49	-0.15	0.06	-0.10
PFrq	0.44	-0.16	0.04	-0.17
FHgt	-0.05	-0.39	-0.13	-0.2
FDur	0.08	-0.13	-0.65	0.18
FSpd	-0.07	-0.16	0.62	-0.16
DtId	0.04	-0.27	-0.21	-0.26
CwDt	-0.19	-0.49	0.11	0.01
CwDur	-0.15	-0.44	-0.17	0.27
CwSpd	-0.19	-0.47	0.12	-0.05

4. DISCUSSION

Understanding the production mechanisms and functions of animal multimodal displays is a challenge due to the quantity of information exhibited concomitantly. The phenotype network approach enables a clearer interpretation of what happens to multiple signals during a single behavior. Using the Swallow-tailed Manakin as a model system, we applied this methodology to understand a complex courtship behavior occurring in two distinct social contexts, practice and courtship displays. We found that differences in traits related to the social context can change networks properties of modularity and degeneracy, suggesting different functions between contexts.

In our study, we expected all networks to be highly modular and degenerate within modalities, meaning that traits of the same modality would be more correlated than traits of different modalities, but while still showing moderate levels of inter-modality degeneracy. Both 3M and 2M practice networks fit this pattern, showing a modular network in which it is likely that traits within groups represent degenerate signals (Ay, Flack, & Krakauer, 2007). The 3M courtship network also showed high levels of degeneracy, however, with moderate modularity, while 2M courtship network is probably un-deterministic. Moreover, the low Jaccard similarities between practice and courtship networks for both 3M and 2M indicate that the networks are different in terms of which trait-pair correlations are important, suggesting different functions for the two social contexts. While courtship displays are important for female choice, practices could not only serve the function of improving display maneuvers and/or synchrony with other males, but also influence male-male competition and promote dominance hierarchy establishment within a court. For Long-tailed Manakins *Chiroxiphia linearis*, male age and intrasexual interactions can enhance males chances of rising in hierarchy over the years (K. C. Lukianchuk & Doucet, 2014; Mcdonald, 2007). The same could be true for Swallow-tailed Manakins since there is a hierarchical social structure within courts (Brodt, Della-Flora, & Cáceres, 2014; Foster, 1981).

Differences between practice and courtship displays were mostly explained by more extreme traits in the latter. In 3M networks, differences were mainly on back coloration and motor traits of cartwheel distance and speed. Back green chroma and hue were larger in practice displays, which was expected as males are younger and mostly at formative and pre-definitive plumages, in comparison to adult males at definitive plumages (larger back UV and blue chroma) in courtship displays. Differences in cartwheel distance, which were larger in courtship displays, are probably due to the higher number of males that participate in these displays, so that individuals must travel a longer distance to reach the end of the line (Fig 2). However, even with more individuals participating, males in courtship displays still had a higher cartwheel speed (Table 1) than males in practice, indicating more ability to perform this maneuver. In 2M networks, the differences were regarding flight height, cartwheel distance, duration and speed, and distance to individual. Only distance to individual was larger in practice displays, meaning that in this context males tend to be more far apart from who is watching during the vertical flight. It is possible that proximity to female during courtship is important to female stimulation. The first four traits were larger in courtship displays, also supporting the hypothesis of improved ability of performing and higher energy investment. Similar results have been found for Long-tailed Manakins, in which courtship displaying males differed from practicing ones in several display maneuvers, and were also more prone to perform specific maneuvers, suggesting experience plays a role in performing (K. Lukianchuk, 2013).

Although we also found differences between practice and courtship networks, they differed to the opposite direction of what we expected. Practice networks are more modular and degenerate, meaning that the relation between traits is less variable than in courtship networks. This goes against our hypothesis that younger practicing males would have more variable displays due to the lack of experience. Instead, it opens the possibility that males have a developmental constraint and are unable to vary their performances out of the strict stereotypical display, much as following a “formula”. This is supported by the pattern of grouped correlations between traits, suggesting a restriction in the possibility of variation (Andersson et al., 2002). As the context changes and males display to females, traits became dispersed in different groups or even uncorrelated at all, suggesting a more variable trait-pair relationship. This can be explained by male’s need of adjusting displays according to female interest. This could be done by performing the dance depending on female identity, since they may have different preferences (Ronald, Fernández-Juricic, & Lucas, 2018), or by adjusting the performance during displays depending on female immediate response (Patricelli, Uy, Walsh, & Borgia, 2002; Sullivan-Beckers & Hebets, 2011).

When comparing 2M and 3M practice networks, the correlation patterns of most sound and motor traits are maintained even with the removal of color, supporting the hypothesis that juveniles have a developmental constraint in performing displays. Interestingly, when comparing 2M and 3M courtship networks, we can clearly see that the inclusion of color traits is responsible for network modularity. Without these traits, the resulting pattern cannot be distinguished from randomness. The importance of color traits in generating this pattern may be due to the nature of the trait itself, which is less variable between individuals than sound and motor traits. Thus, when removing color from the network, we significantly reduce network's modularity, relaxing the relations between sound and motor traits.

We also found some negative correlations in all networks. In 3M networks, most brightness and chroma color traits show negative correlations with hue, which is expected due to their nature (such as larger values of back blue and UV chroma correlated with smaller values of back hue, Fig 3a, b). Other negative correlations, such as those between sound/motor and color traits are not so clear and could be indicative of a shared genetic expression pathway. However, in 2M networks some correlations may be indicative of energetic tradeoffs during displays, such as flight duration with high and delta sound frequencies, and distance to individual with peak frequency (Fig 4a). It is possible that to maximize some traits males need to diminish energy investment in others (Andersson et al., 2002; Manica, Macedo, Graves, & Podos, 2017; Patricelli & Krakauer, 2009).

5. CONCLUSION

Our results showed how the same set of traits can have different trait-pair relations, and possibly functions, depending on the social context. The moderate to high levels of modularity and high degeneracy found in networks suggest that traits overlap in function. It is likely that practicing is related to improving displaying abilities and dominance hierarchy development within courts, while courtship displays inform females of different aspects of males, such as quality. While the differences in practice and courtship displays imply that traits can change according to male experience, the lack of experience of juvenile males is also related to a less variable trait-pair relationship, suggesting the existence of a developmental constraint in displaying. Future studies should focus on the role of practices in learning maneuvers and court hierarchy establishment, on what traits are related to female choice in courtship displays and if and how males can change these traits during displays according to female response. Thus, our study opens new possibilities for understanding sexual selection in this species, and also guides future studies in terms of what traits should be explored to unravel the complex display functions of Swallow-tailed Manakins.

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7. APPENDIX

Table S1. Pearson correlation results for all sound traits extracted from audio and video recording samples made at the same time and distance from perch.

Trait	***n***	***t****	***r***	***p***
Low frequency (kHz)	20	63.4	0.99	< 0.0001
High frequency (kHz)	20	19.9	0.98	< 0.0001
Delta frequency (kHz)	20	17.4	0.97	< 0.0001
Center frequency (kHz)	20	28.4	0.99	< 0.0001
Peak frequency (kHz)	20	17.6	0.97	< 0.0001

* df = 18 for all traits.

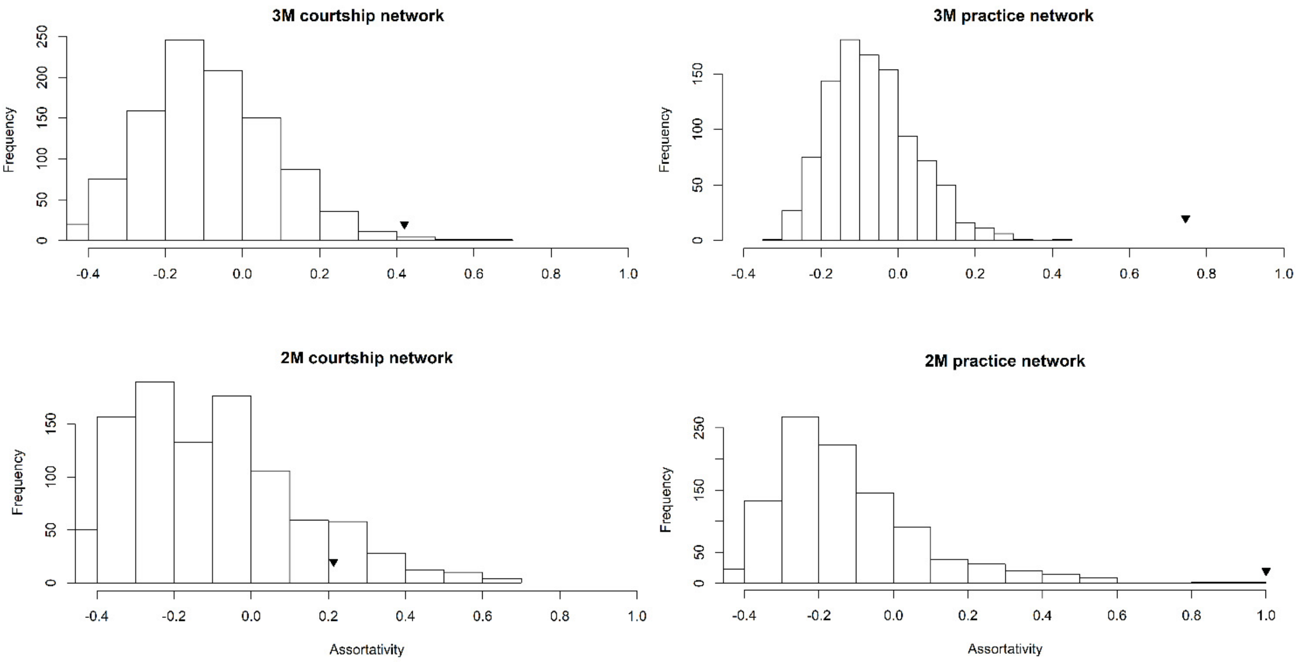

Figure S1. Assortativity values generated from 1,000 permuted networks of 3M courtship and practice displays and 2M courtship and practice displays. The observed values correspond to the black triangles. All networks had assortativity coefficient greater than expected by chance ($p < 0.05$), with exception of the 2M courtship network ($p = 0.09$).