



Phenotype networks reveal differences between practice and courtship displays in swallow-tailed manakins

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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 challenge in behavioural ecology, since sexual behaviours can present a diverse set of evolutionary implications. Here, we used phenotype networks to evaluate displays of the swallow-tailed manakin, *Chiroxiphia caudata* (Passeriformes: Pipridae) in two social contexts: (1) practice displays and (2) courtship displays. We built three-modality and two-modality phenotype networks using sound, motor and colour traits extracted from audio and video recordings and plumage. We hypothesized that networks in both contexts would be modular, as a consequence of a higher degeneracy within than between traits in each modality, as traits are produced by different physiological mechanisms. We collected data from a population in an Atlantic Forest remnant in southern Brazil during three breeding seasons (October–March 2015–2018). We found that practice networks had higher modularity than courtship networks, which was the 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 their capability of adjusting performances depending on female responses and preferences. 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.

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Understanding animal communication dynamics, and translating multiple signals into information or function, is a challenge in the face of the variety and complexity of animal displays. Due to this complexity in movements, sounds and other display signals, many behavioural 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 communication by female partners (e.g. Doucet & Montgomerie, 2003; Gibson & Uetz, 2008; Girard, Elias, & Kasumovic, 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 structure (the combination of behaviour organization and expression in the environment, resulting in the signal itself as perceived by a receptor, e.g. the vibration of foreleg brushes) and function (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 & 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;

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Whitacre & Bender, 2010). For example, research with birds-of-paradise has shown that the extreme and extensive phenotypic and behavioural radiation of the family is likely a consequence of the integration of visual and acoustic elements performed during courtship (Ligon et al., 2018). As the whole family presents strong correlations between elements, it is likely that selection has favoured their integration across time (Ligon et al., 2018). Other remarkable studies come from the field of genetics, where genes work as signals. Many organisms present identical copies of a given gene or structurally different genes performing the same function (Mason, 2014), such as MEF2 genes regulating the development of heart complexity in invertebrates and vertebrates. While in the fruit fly (*Drosophila* sp.) the single copy of MEF2 is vital for the expression of proteins (Ranganayakulu et al., 1995; Wagner, 2008), vertebrates have four copies, enabling them to acquire innovations in complexity via mutations. Thus, when deactivating one of them, the MEF2c, only a subset of contractile proteins is eliminated, while the other copies can still maintain partial heart functions (e.g. the MEF2c, Black & Olson, 1998; Wagner, 2008). These examples illustrate how presenting more than one signal with the same function in a given context can lead to extreme evolutionary divergence. 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), signallers will adjust displays to increase signal efficiency, such as detectability or information transmission (Chapman, Morrell, & Krause, 2009; Cole & Endler, 2015; Sicsú, Manica, Maia, & Macedo, 2013). Such context-dependent differences can thus modify display evolution as each context can present differential selective pressures (Rosenthal et al., 2018). For example, if a signal is presented while the individual is at risk of predation, it can be shaped by natural selection; if the signal is presented during a dispute between same-sex individuals, it can be shaped by intrasexual selection; and if it is presented in a courtship context, it can be shaped by intersexual selection.

Phenotype networks (where nodes represent behavioural traits, and edges represent the correlation between traits) are valuable tools in animal behaviour studies as they favour the interpretation of multiple signals relationships (Hebets et al., 2016). These networks (1) provide clear visualization of the integrational properties of displays (Hebets et al., 2016; Magwene, 2001; Patricelli & Hebets, 2016) and (2) allow us to visualize and interpret important elements of biological systems (Chen & Crilly, 2014), such as redundancy (the degree that structurally identical signals transmit the same information consistently; Hebets et al., 2016; Partan & Marler, 1999), degeneracy (the degree that structurally different signals transmit similar information in certain contexts; 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 Neotropical lekking birds known for their intriguing reproductive behaviour. Males of this species gather in display courts where they expose their ornaments in a ritualized dance for females (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 (tail feathers), exuberant coloration (reddish crown, blue body and black wings and head), vocalizations and flight acrobatics (Foster,

1981; Ribeiro, Guaraldo, Macedo, & Manica, 2019; Schaedler, Ribeiro, Guaraldo, & Manica, 2019). 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; Ribeiro et al., 2019). To end the jump display, the dominant male (or 'alpha male', i.e. the individual that participates in all displays, defends the court and copulates with females) hovers in the air while producing a strident vocalization and mechanical sounds, while the other males remain silent in the perch, usually performing a bow (Foster, 1981; Schaedler et al., 2019). The jump display may be followed by a solo precopulatory display by the alpha male, after which, if successful, he will copulate with the female (Foster, 1981).

Another interesting aspect of their lekking behaviour is that juveniles, 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 (Foster, 1981; L. M. Schaedler, personal observation). The practice jump display consists of the same sounds and flight elements performed for females (Ribeiro et al., 2019), but they are often shorter in duration and may include slight variations in acrobatics (as changing the cartwheel flight direction; L. M. Schaedler, personal observation). One hypothesis suggests that practice displays enable males to improve their performance and match their movements to those of their displaying partners, a characteristic thought to influence female choice. Such matching was shown for duetting behaviour in the genus *Chiroxiphia* (Trainer, McDonald, & Learn, 2002). Thus, it is fundamental to understand whether and how signals vary between practice and courtship displays, as it is likely that interactions between signals will be perfected during practice and, in the future, will influence female mate choice, an important process guiding the evolution of ornaments.

To understand the complexity of swallow-tailed manakins' sexual signals and how they might be shaped by selective pressures, it is important to evaluate the interaction between the three modalities (sound, motor and plumage colour). In our study, we evaluated these interactions using phenotype networks in two contexts of the jump display: during practice 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 (presence of modularity) as a consequence of a higher degeneracy (i.e. overlapping functions but not structures) within than between traits in each modality, as traits are produced by different physiological mechanisms. Our second hypothesis was 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 the ability to perform manoeuvres, and that practice networks would have fewer and weaker correlations between traits (lower modularity and degeneracy), reflecting higher variation in displays due to juvenile males' lack of experience.

METHODS

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, <https://www.britannica.com/science/Koppen-climate-classification>).

Field Data Collection

We found display perches by searching for vocalizing swallow-tailed manakins and confirmed the locations through sightings of displays. Males displayed in a total of three courts (one court had only one display perch, while the other courts had two and three display perches, respectively). We captured individuals three times per 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, permit: 1195110) and plastic coloured bands for individual identification. To describe male's plumage and infer age, we categorized plumage stage of males on a scale from 1 to 9 (according to Mallet-Rodrigues & Dutra, 2012). The first four stages represent first-year and second-year males, with juvenile and formative plumages, mostly green and without blue feathers. Stages 5–8 represent third-year and fourth-year males, with predefinitive plumage in which blue and black feathers progressively increase and green feathers are lost. Stage 9 represents males that are at least 4 years old and have

definitive plumage, with a red crown, blue body and black head and wings. We did not include these plumage stages in our analyses and used them only to generally describe males participating in displays. For feather coloration analysis, we collected four to five dorsal and crown feathers using tweezers (following protocol adapted from Sicsú et al., 2013). To quantify practice and courtship displays, we filmed and recorded display perches during ~5 h, three to five times per week. For video recordings, we used Sony HDR-CX290 cameras fixed to a tripod placed 5 m from the perch. For audio recording vocalizations, we used a Marantz PMD661 digital recorder and a Sennheiser ME67 microphone using 44.1 Hz sampling rate and 24-bit resolution. We identified individuals based on the combination of coloured leg bands.

Extraction of Sound, Motor and Colour Traits

For each individual we extracted five sound, seven motor and nine colour traits (Table 1). Sound and motor traits of each male were always taken at the same time point in a display, while colour 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, U.S.A.; <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

Table 1
Phenotypic traits from three modalities extracted from individuals in two contexts, practice and courtship displays

Modality	Trait	Abbreviation	Description	Mean ± SD of practice displays ^a	Mean ± SD 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 frequencies	4614±1507 Hz	4022±406 Hz
Sound	Centre frequency	CFrq	The frequency dividing the selection in two intervals of equal energy	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 the 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 take-off to landing in the perch, divided by number of males performing the display	69±60 mm	55±15 mm
Motor	Cartwheel duration	CwDur	Time interval a male took to fly the cartwheel distance, standardized by number of males performing the display	0.52±0.20 s	0.73±0.24 s
Motor	Cartwheel speed	CwSpd	The ratio between the cartwheel distance and duration, standardized by number of males performing the display	95±70 mm/s	65±13 mm/s
Colour	Crown average brightness	CBri	Mean relative reflectance over the entire spectral range, on crown feathers	7.84±4%	7.37±2.3%
Colour	Crown UV chroma	CUv	Proportion of light reflected in the UV colour range (300–400 nm) in relation to the entire range (330–700 nm), on crown feathers	0.13±0.07	0.12±0.08
Colour	Crown yellow chroma	CYl	Proportion of light reflected in the yellow colour range (550–625 nm) in relation to the entire range (330–700 nm), on crown feathers	0.26±0.03	0.26±0.04
Colour	Crown red chroma	CRd	Proportion of light reflected in the red colour range (605–700 nm) in relation to the entire range (330–700 nm), on crown feathers	0.51±0.13	0.53±0.16
Colour	Back average brightness	BBri	Mean relative reflectance over the entire spectral range, on back feathers	7.79±2%	9.8±2%
Colour	Back UV chroma	BUv	Proportion of light reflected in the UV colour range (300–400 nm) in relation to the entire range (330–700 nm), on back feathers	0.26±0.03	0.31±0.02
Colour	Back blue chroma	BBl	Proportion of light reflected in the blue colour range (400–510 nm) in relation to the entire range (330–700 nm), on back feathers	0.24±0.04	0.29±0.02
Colour	Back green chroma	BGr	Proportion of light reflected in the green colour range (510–605 nm) in relation to the entire range (330–700 nm), on back feathers	0.29±0.03	0.24±0.02
Colour	Back hue	BHue	Wavelength of peak reflectance, on back feathers	497±69 nm	395±72 nm

Statistical comparisons between the practice and courtship displays are presented as results of a principal components analyses in Tables 2 and 3.

^a $N = 11$ individuals for colour traits, $N = 21$ individuals for sound and motor traits.

^b $N = 9$ individuals for colour traits, $N = 16$ individuals for sound and motor traits.

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 (Appendix, Fig. A1; Podos, 1997; Zollinger, Podos, Nemeth, Goller, & Brumm, 2012). We obtained centre (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 ($r \geq 0.97$, $P < 0.0001$; Appendix, Table A1).

We used Windows Movie Maker (v.2012 Microsoft Corporation, Redmond, WA, U.S.A.) to select print screens of displays. Using ImageJ (National Institutes of Health, Bethesda, MD, U.S.A., <http://rsbweb.nih.gov/ij/>), we extracted the following motor traits (described in Table 1) from print screens as described in Ribeiro et al.'s (2019) 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) (Fig. 1). We standardized cartwheel motor traits (CwDt, CwDur, CwSpd) by dividing them by the number of males performing the display during the extraction of trait values. We applied this correction because we found that number of males influences CwDt, CwDur and CwSpd in courtship displays, although not in practice displays (Appendix, Table A2). We scaled all measures relative to the perch width, which we measured in the field with a calliper. 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 (identified by dividing each display in three parts of equal length and sampling pulses on their central position).

We extracted feather reflectance using SpectraSuite software and an Ocean Optics USB4000 spectrometer (Ocean Optics, Dunedin, FL, U.S.A.), attached to a PX-2 pulsed xenon light source and placed the probe at 90° to the feather. We used 10 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). All sound, motor and colour traits were measured by the first author.

Statistical Analyses

In total, we built four phenotype networks. In two networks, we used traits from three modalities (3M), sound, motor and colour, for (1) displays performed to other males (hereafter '3M practice networks') and for (2) displays performed to females (hereafter '3M courtship networks'). In the other two networks, we used traits from two modalities (2M), sound and motor, for (1) displays performed to other males (hereafter '2M practice networks') and for (2) displays performed to females (hereafter '2M courtship networks'). To increase our sample size, we did not use colour in these networks since for many individuals we did not have plumage samples from the same breeding season when they were audio- or videorecorded.

We followed the protocol provided by Wilkins et al. (2015) to build networks, as follows. In networks, each node represents one sound, motor or colour 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 data set 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% confidence interval (CI) did not overlap zero. To access traits degeneracy, we calculated average correlation strength ($|\text{avg cor}|$, the mean of the absolute trait-pair correlations) and network density ($|\text{net dens}|$, the number of significant correlations, after bootstrap



Figure 1. 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 speed (FSpd) and cartwheel speed (CwSpd) as a measure of distance per duration. See Table 1 for a detailed description of motor traits.

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 coefficient differs from the commonly used metric of modularity in systems approaches (Olesen et al., 2007) because it tests the connectivity of nodes in groups defined a priori, instead of identifying groups after the network is generated. Thus, r_d measures whether 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 indicates that there are no correlations between different modalities and a value of -1 indicates that all traits from different modalities are correlated. We calculated the probability of finding our observed assortativity in a distribution of assortativity values generated from 1000 permuted networks (significance level = 0.05). We randomized trait modalities on the newly generated networks while maintaining the number of traits that belonged to each modality (five for sound, seven for motor and nine for colour). If networks are degenerate and modular, we expected to find high $|\text{avg cor}|$ and $|\text{net dens}|$, and significant r_d . All network 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 also tested whether and how display traits varied between the practice and courtship contexts. We used traits included in both 3M and 2M networks and compared contexts 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 the principal component analysis (PCAs, 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 greater than 1 (PC1 to PC5 for 3M, and PC1 to PC4 for 2M display networks) to perform t tests and to colour networks based on the higher value of loading for each trait (see Results, Figs. 2, 3). 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', Torchio, 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; Torchio, 2018).

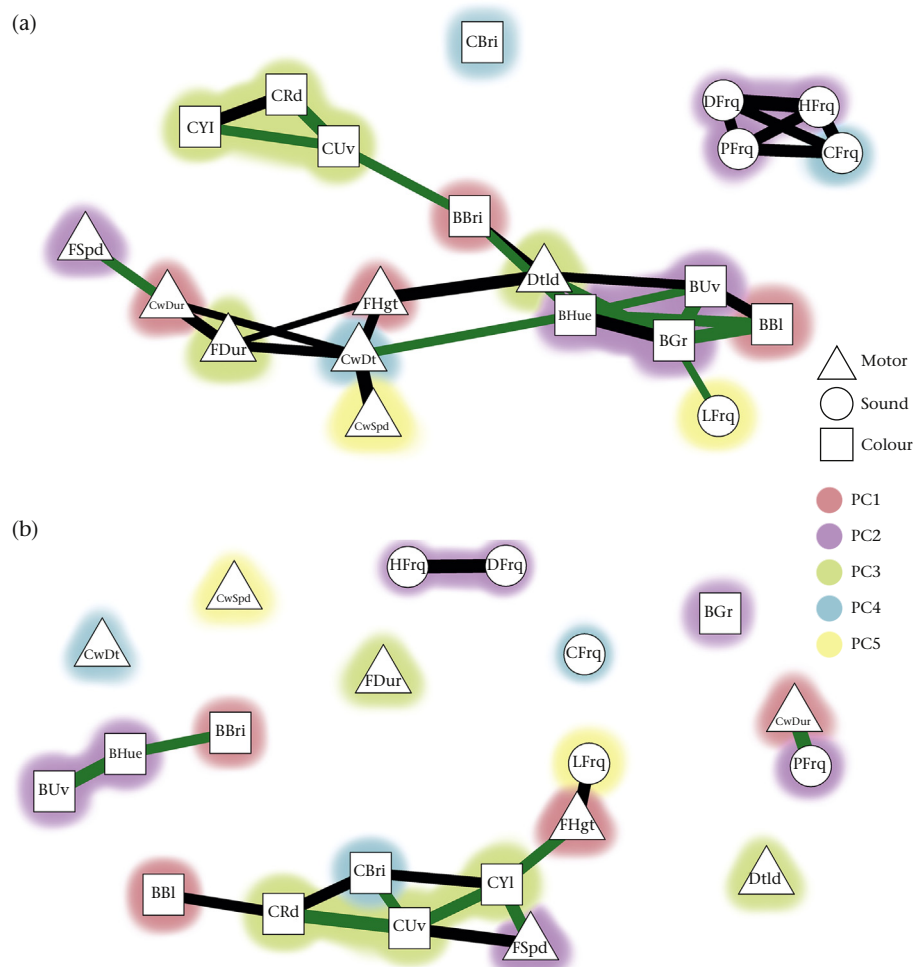


Figure 2. Phenotype networks of traits of three modalities (3M) in (a) practice and (b) courtship displays. Each node represents traits extracted from displays and male plumage colorations, and edges represent correlations between them. Edge width indicates the degree of correlation, and green edges represent negative correlations. Colours represent the principal component (PC) for which traits had higher loadings. One PC analysis was performed for both networks. Both networks present strong correlations between traits. Practice networks (a) were more modular as traits within modalities were highly connected.

2018). In each network included in PCA analyses, we scored completely different individuals between contexts.

Ethical Note

All procedures involving animals in this study were performed in accordance with the ethical standards of the research institute and sampling sites where the study was conducted (Animal Experiment Ethics Committee of Universidade Federal do Paraná permit no. 23075.028690/2014-05, Sistema de Autorização e Informação em Biodiversidade: permit no. 44439, Centro Nacional de Pesquisa e Conservação de Aves Silvestres: permit no. 1195110).

Birds were captured three times per week, during 0600–1200 hours, using mist nets placed approximately 10 m from perches, under shaded environment. We checked mist nets every 20 min to decrease individual distress. In cases when it rained, mist nets were closed immediately and capture ceased until climate was favourable. We banded birds with numbered metallic bands provided by the Brazilian banding agency (CEMAVE/ICMBio) and plastic coloured bands for individual identification. We followed the CEMAVE/ICMBio protocol for banding and used the recommended band size for the species. We collected four to five dorsal and crown feathers using tweezers, which had no short-term or long-term effects on individuals.

RESULTS

Three-modality Networks: Sound, Motor and Colour Traits

To build 3M practice and courtship display networks, we used data from 11 and nine males, respectively, belonging to three different display courts. We found large variation in plumage stages of males performing practice displays, ranging from individuals at stage 3 (formative) to stage 9 (definitive). In courtship display networks, only one male was at plumage state 7 (predefinitive), 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 male present during practice displays, while in courtship displays there were three alpha males.

In the 3M practice network (Fig. 2a), we found $|\text{avg cor}| = 0.75$, $|\text{net dens}| = 0.15$ and $r_d = 0.73 \pm 0.07$, which was greater than expected by chance ($P = 0$; Appendix, Fig. A2). These results indicate modularity and degeneracy in spite of low network density, meaning that even when there were few actual connections between all the potential ones, these were strongly correlated. We found two groups, one including only sound traits and the other including all three modalities, although traits from different modalities were connected by few correlations (Fig. 2a). Only one trait (crown average brightness) was isolated/uncorrelated. In the 3M

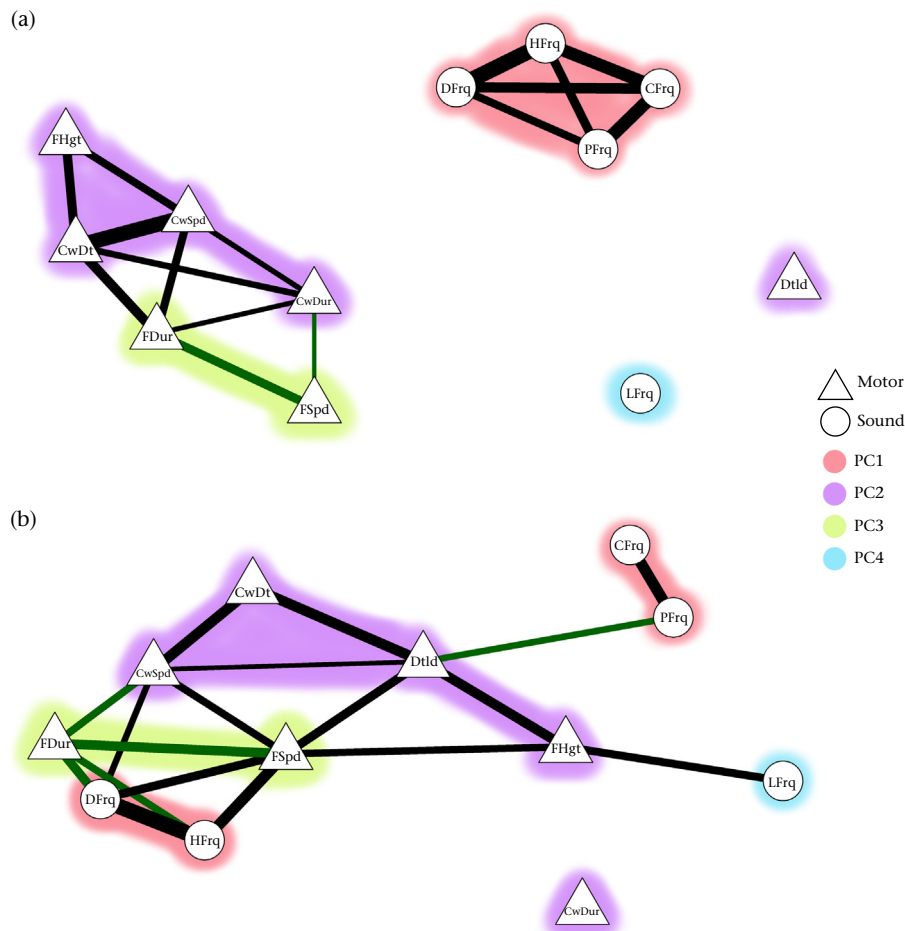


Figure 3. Phenotype networks of traits of two modalities (2M) in (a) practice and (b) courtship displays. Each node represents traits extracted from displays, and edges represent correlations between them. Edge width indicates the degree of correlation, and green edges represent negative correlations. Colours represent the principal component (PC) for which traits had larger loadings. One PC analysis was performed for both networks. By removing colour traits, the practice network (a) showed increased modularity as it lost all connections between modalities. The courtship network (b) was not significantly modular.

courtship network (Fig. 2b), $|\text{avg cor}| = 0.79$, $|\text{net dens}| = 0.06$ and $r_d = 0.27 \pm 0.15$, which was greater than expected by chance ($P = 0.02$; Appendix, Fig. A2). The courtship network included four groups, two of which included traits of only one modality (sound or motor), while two groups were composed by two or three modalities (Fig. 2a). Six traits were isolated and uncorrelated to any other traits. When comparing 3M practice and courtship networks, we found low Jaccard similarity ($J = 0.15$), highlighting that the networks differed in terms of which traits were correlated with each other.

The cumulative proportion of variation explained by PC1–PC5 was 0.88 (individual proportions of 0.32, 0.24, 0.16, 0.10 and 0.06, respectively). We found significant differences between practice and courtship displays for PC1 ($t_{7,7} = 3.56$, $P = 0.007$, $d = 1.96$), and a marginal statistical difference for PC2, supported by a large effect size ($t_{8,3} = 2.16$, $P = 0.06$, $d = 1.18$). PC1 separated back brightness and blue chroma, flight height and cartwheel duration (Table 2), which were larger in courtship displays (Table 1). PC2 separated most sound (high, delta and peak frequency), back colour traits (back green and ultraviolet (UV) chroma and hue) and flight speed. Males performing practice displays had larger values for the majority of traits, with exception of back UV chroma and flight speed (Table 1). We did not find significant differences between practice and courtship displays for PC3–PC5 (PC3: $t_{10,9} = 0.34$, $P = 0.73$, $d = 0.18$; PC4: $t_{10,9} = -0.64$, $P = 0.53$, $d = -0.33$; PC5: $t_{9,2} = -0.07$, $P = 0.94$, $d = -0.38$). PC3 effectively separated most crown colour traits and some motor traits, while PC4 and PC5 separated some sound and motor traits (Table 2).

Two-modality Networks: Sound and Motor Traits

After removing colour 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 (predefinitive), while others were adult males with plumage stage 9 (definitive). There were three alpha males in practice displays and four alpha males in courtship displays.

Table 2
Loadings of the first five principal components of sound, motor and colour traits of individuals included in 3M practice and courtship displays networks

TRAIT	PC1	PC2	PC3	PC4	PC5
LFRQ	0.08	0.08	0.21	-0.24	-0.39
HFRQ	0.26	-0.27	0.06	-0.21	0.17
DFRQ	0.25	-0.28	0.03	-0.18	0.22
CFRQ	0.22	-0.28	0.05	-0.29	0.06
PFRQ	0.18	-0.31	0.03	-0.30	0.14
FHGT	0.33	0.09	-0.21	0.06	-0.15
FDUR	0.27	0.00	-0.32	0.14	-0.30
FSPD	0.06	-0.29	0.01	0.25	0.02
DTID	0.27	-0.17	-0.28	0.12	-0.09
CWDT	-0.01	-0.27	-0.08	0.46	-0.04
CWDUR	0.29	-0.13	-0.22	-0.02	-0.06
CWSPD	-0.21	-0.16	0.23	0.19	0.41
CBRI	-0.07	0.13	0.11	-0.47	-0.25
CUV	0.12	-0.08	0.47	0.10	-0.17
CYL	-0.14	0.15	-0.36	-0.22	0.31
CRD	-0.16	0.06	-0.44	-0.19	0.17
BBRI	-0.25	-0.18	-0.18	-0.01	-0.40
BUV	-0.24	-0.32	0.00	-0.11	-0.20
BBL	-0.28	-0.25	-0.11	-0.08	0.01
BGR	0.25	0.32	0.07	0.10	0.14
BHUE	0.27	0.28	0.06	0.01	0.13

Bold values indicate the highest loading for each trait.

In 2M practice networks, we found $|\text{avg cor}| = 0.68$, $|\text{net dens}| = 0.24$ and $r_d = 1 \pm 5 \times 10^{-16}$, the highest possible assortativity value reached, which was greater than expected by chance ($P = 0.002$; Appendix, Fig. A2), indicating a modular and degenerate pattern. Two groups separated motor and sound modalities, and only two traits (low frequency and distance to individual) did not correlate with the other traits (Fig. 3b). In 2M courtship networks, we found $|\text{avg cor}| = 0.66$ and $|\text{net dens}| = 0.27$. We found a single group including motor traits in the centre and sound traits at the extremities, while only one trait (cartwheel duration) did not correlate with the other traits (Fig. 3a). However, assortativity was not greater than expected by chance ($r_d = 0.16 \pm 0.19$, $P = 0.1$; Appendix, Fig. A2), meaning that our observed network was not modular. We also found a low Jaccard similarity ($J = 0.18$) between 2M practice and courtship networks, highlighting that 2M networks differed in terms of trait correlations.

The cumulative proportion of variation explained by PC1–PC4 was 0.82 (individual proportions of 0.38, 0.20, 0.15 and 0.09, respectively). We found significant differences between practice and courtship displays for PC3 ($t_{22,3} = 2.99$, $P = 0.006$, $d = 1.17$). PC3 separated flight duration and speed, where flight duration was greater in practice displays and flight speed was greater in courtship displays (Table 3). We did not find significant differences for PC1, PC2 and PC4 (PC1: $t_{13,2} = 1.86$, $P = 0.08$, $d = 0.37$; PC2: $t_{15,4} = 1.44$, $P = 0.16$, $d = 0.56$; PC4: $t_{21,2} = -1.27$, $P = 0.21$, $d = -0.5$). PC1 effectively separated sound traits, with the exception of low frequency, which was the only trait in PC4, and PC2 effectively separated most motor traits.

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 behaviour. Using the swallow-tailed manakin as a model system, we applied this methodology to understand a complex courtship behaviour 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. Males performing courtship displays produced more conspicuous signals, such as longer cartwheels, and higher and faster vertical flights, in comparison to males performing practice displays.

In our study, we expected all networks to be modular and degenerate within modalities, meaning that traits of the same

Table 3
Loadings of the first four principal components of sound and motor traits of individuals included in 2M practice and courtship displays networks

LOADINGS	PC1	PC2	PC3	PC4
LFRQ	0.08	0.06	0.42	-0.62
HFRQ	0.39	0.31	-0.10	0.12
DFRQ	0.38	0.30	-0.16	0.20
CFRQ	0.39	0.30	0.01	-0.06
PFRQ	0.37	0.26	0.05	-0.10
FHGT	0.03	-0.35	-0.20	0.42
FDUR	0.30	-0.25	0.38	0.17
FSPD	0.27	-0.16	-0.36	0.002
DTID	0.34	-0.37	-0.05	9×10^{-5}
CWDT	0.19	-0.38	-0.21	-0.40
CWDUR	0.29	-0.40	0.14	-0.12
CWSPD	-0.08	0.06	-0.64	-0.41

Bold values indicate the highest loading for each trait.

modality would be more correlated than traits of different modalities, but while still showing intermodality degeneracy. With the exception of 2M courtship networks, which presented degeneracy but not modularity, all networks fitted our expected pattern of modularity in which traits within groups represent degenerate signals (Ay, Flack, & Krakauer, 2007). Moreover, the low Jaccard similarities found between practice and courtship networks for 3M and 2M indicate that the networks differ 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, practice displays might not only serve the function of improving display manoeuvres and/or synchrony with other males, but may 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 the hierarchy over the years (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 brightness and blue chroma, flight height and cartwheel duration, which were all greater for courtship displays. We expected back colour traits to be greater for courtship displays, as males performing courtship displays have definitive plumages, in comparison to practice males, which have mostly formative and predefinitive plumages. A greater cartwheel duration in courtship displays can be due to males hovering in the air for longer than practicing males. Thus, even travelling shorter distances, males take longer to reach the end of the line. As to flight height, our results indicate that courting males' higher performance of this manoeuvre is due to experience. Although marginally nonsignificant, we also found a large effect size on other colour, motor and sound traits. These results confirm differences in colour of males between contexts (BGr/BHue > practice, BUv > courtship) and support the hypothesis that courting males are more experienced in performing flight manoeuvres (FSpd > courtship). High, delta and peak frequencies were all greater in practice displays, but also more variable. In 2M networks, flight duration was longer in practice displays and flight speed was faster in courtship displays. This result, as in 3M networks, also supports the hypothesis that courting males' greater experience allows them to perform these manoeuvres, which could be indicative of higher energy investment. Similar results have been found for long-tailed manakins, in which courtship displaying males differed from practising ones in several display manoeuvres, and were also more prone to perform specific manoeuvres, suggesting that experience plays a role in performance (Lukianchuk, 2013).

Although we also found differences between practice and courtship networks, they differed in the opposite direction from what we expected. Practice networks, including mostly individuals at the predefinitive stage or younger, were more modular and degenerate, meaning that correlations between traits were stronger within modalities and, consequently, less variable than in courtship networks. This goes against our hypothesis that younger practising 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, like following a 'formula'. It is also possible that juveniles need first to improve the coordination of traits within each modality to integrate all modalities later. This could potentially explain the modular pattern of practices and the multimodal integration in courtships, and is also consistent with the developmental constraint hypothesis (Trainer

et al., 2002). Although display practising by juveniles has been reported for several different manakin species, both alone and in the presence of other displaying partners (Cárdenas-Posada, Cadena, Blake, & Loïselle, 2017; Durães, 2009; Feng, Katz, Day, Barske, & Schlinger, 2010; Robbins, 1983; Schaedler et al., 2019; Schlinger, Day, & Fusani, 2008; Trainer & McDonald, 1995), we lack studies exploring the learning process of courtship dances in manakins. Studies with cotingas (manakin's sister group Cotingidae) suggest that social learning may be more important than previously thought for some behaviours. A study with bare-throated bellbirds, *Procnias nudicollis*, showed that a juvenile male deprived of social interactions with other conspecifics developed abnormal songs and even learnt the song of another captive species present nearby (Kroodsma et al., 2013). Three-wattled bellbirds, *Procnias tricarunculata*, also show signs of vocal learning, as males from different locations have their own dialect, which can be interchanged among populations (Kroodsma et al., 2013). We believe that, like songs, dances produced during courtship displays also depend on social learning, as the process of dance learning strongly relies on imitation (Laland, Wilkins, & Clayton, 2016). As the context changes and males display to females, traits became dispersed in different groups or even became uncorrelated, suggesting a more variable trait-pair relationship. This can be explained by the male's need to adjust his displays according to female interest. This could be done by performing the dance based on female identity, since females may have different preferences (Ronald, Fernández-Juricic, & Lucas, 2018), or by adjusting the performance during displays depending on the female's immediate response (Patricelli, Uy, Walsh, & Borgia, 2002; Sullivan-Beckers & Hebets, 2011).

It is also worth noting the presence of negative correlations in all networks. In 3M networks, most colour traits showed negative correlations among themselves, which is expected due to their nature (such as back UV and brightness being negatively correlated with back hue; and crown UV, red and yellow chroma). Other negative correlations, such as those between sound/motor and colour traits are not so clear and could be indicative of a shared genetic expression pathway (e.g. correlations between cartwheel distance and back hue, flight height and crown yellow chroma). However, in both 3M and 2M networks, some correlations may be indicative of energetic trade-offs during displays, such as between cartwheel duration and peak frequency, and distance to individual and peak frequency. It is possible that, to maximize some traits, males need to diminish energy investment or mechanical abilities in others (Andersson et al., 2002; Manica, Macedo, Graves, & Podos, 2017; Patricelli & Krakauer, 2009). However, we still lack a deep understanding of physiological and neurological mechanisms for explaining possible links between expressions of these signals.

Conclusion

Our results show how the same set of display traits of swallow-tailed manakins can have different trait-pair relations, and possibly functions, depending on the social context. The higher modularity found in practice networks suggests that traits overlap in function, while modularity patterns of courtship networks suggest the opposite. The low similarities between contexts also suggests different functions for each of them. It is likely that practising is related to improving displaying abilities and dominance hierarchy development within courts, while courtship displays stimulate females for copulation. Differences in practice and courtship displays imply not only that traits' relationships can change according to male experience, as it is likely that juvenile males' display performance is developmentally constrained, but also that context can shape networks. Future studies should focus on the role of practice in learning manoeuvres and court hierarchy establishment, on

what traits are related to female choice in courtship displays and whether and how males 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.

Declarations of Interest

None.

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Appendix

Table A1

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_{18}	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
Centre frequency (kHz)	20	28.4	0.99	<0.0001
Peak frequency (kHz)	20	17.6	0.97	<0.0001

Table A2

Results of linear mixed models of cartwheel distance (CwDt), duration (CwDur) and speed (CwSpd) in relation to number of males (NMs) participating in the display

Context	Model	$b \pm SE$	LRT	df	P
Practice displays	CwDt-NMs	0.02 ± 0.10	0.04	1	0.82
Practice displays	CwDur-NMs	0.04 ± 0.03	1.47	1	0.22
Practice displays	CwSpd-NMs	0.04 ± 0.09	0.16	1	0.68
Courtship displays	CwDt-NMs	0.24 ± 0.02	94.1	1	<0.0001
Courtship displays	CwDur-NMs	0.19 ± 0.01	76.9	1	<0.0001
Courtship displays	CwSpd-NMs	0.09 ± 0.01	41.8	1	<0.0001

The random effect term was the individual nested in display identity. b = angular coefficient; LRT = likelihood ratio test.

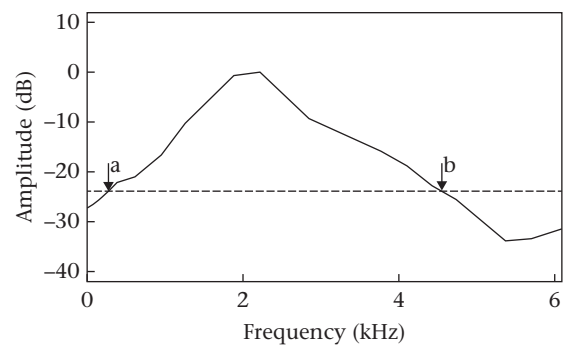


Figure A1. Selection of power spectra used to measure sound traits. The dashed line represents –24 dB criterion for excluding background noise. For each spectrum we measured (a) low frequency (LFrq), (b) high frequency (HFrq) and delta frequency (DFrq, as the difference between high and low frequencies). We obtained centre frequencies (CFrq) and peak frequencies (PFrq) from power spectra using Raven's automatic calculation. See Table 1 for a detailed description of sound traits.

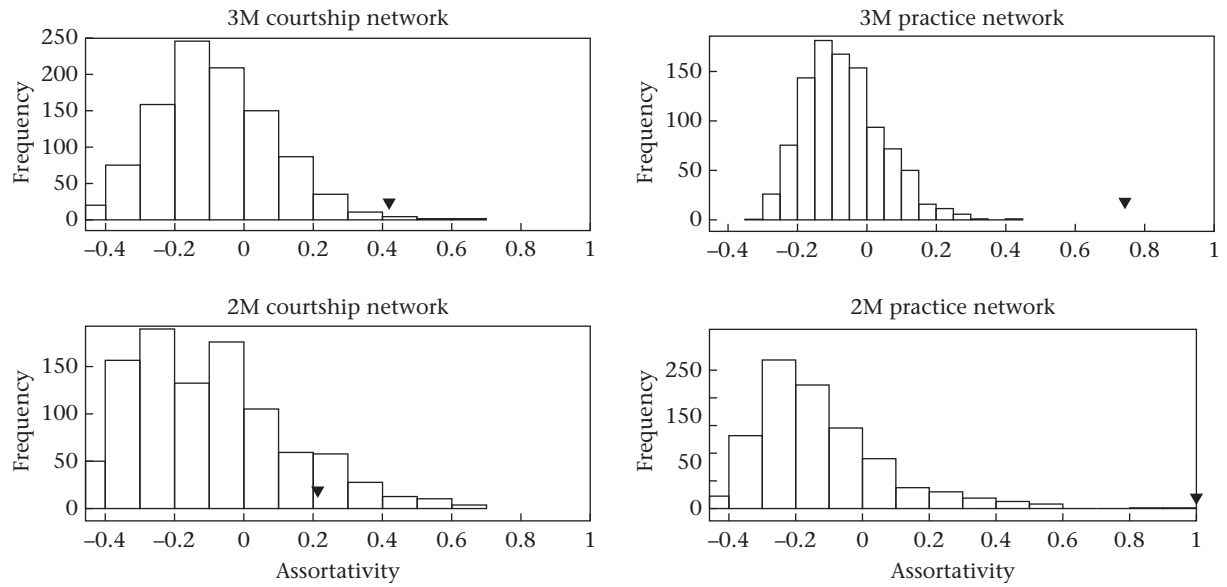


Figure A2. Assortativity values generated from 1000 permuted networks of courtship and practice displays using traits from three modalities (3M) and two modalities (2M). Black triangles correspond to observed values. All networks had an assortativity coefficient greater than expected by chance ($P < 0.05$), with exception of the 2M courtship network ($P = 0.09$).