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Multimodal signaling in the North American barn swallow: A phenotype network approach

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Abstract

Complex signals, involving multiple components within and across modalities, are common in animal communication. However, decomposing complex signals into traits and their interactions remains a fundamental challenge for studies of phenotype evolution. We apply a novel phenotype network approach for studying complex signal evolution in the North American barn swallow (*Hirundo rustica erythrogaster*). We integrate model testing with correlation-based phenotype networks to infer the contributions of female mate choice and male–male competition to the evolution of barn swallow communication. Overall, the best predictors of mate choice were distinct from those for competition, while moderate functional overlap suggests males and females use some of the same traits to assess potential mates and rivals. We interpret model results in the context of a network of traits, and suggest this approach allows researchers a more nuanced view of trait clustering patterns that informs new hypotheses about the evolution of communication systems.

Keywords: sexual selection, multimodal signals, modularity, redundancy, mate choice, competition

1. Introduction

Sexual selection has led to the evolution of a seemingly boundless variety of traits used to assess potential mates and competitors. Striking elaborations in visual, acoustic and chemical signals have intrigued biologists for over a century, leading to hundreds of studies on the function of these signals in communication [1]. Increasingly, it has become clear that animal signals are often complex, incorporating multiple traits across one or more modalities (e.g. visual or acoustic cues) [2–4]. Moreover, recent theory suggests that multicomponent signals are often favored over simple signals [5]. For example, complex signals may arise if redundant signals act as ‘backups’ to ensure signal transmission to intended receivers. Alternatively, non-redundancy of signals would be favored if ‘multiple messages’ are more informative in terms of localizing conspecifics and reinforcing honesty [6,7]. Multicomponent signals may also be beneficial when there are multiple audiences [8]. Because many signals, including birdsong, have dual functions in competition and mating [9], determining which signal components mediate intra- versus intersexual communication is key for understanding the evolutionary processes shaping complex signals.

The complexity of multicomponent signaling systems poses significant logistical hurdles. The classic approach for parsing this complexity has focused on isolating modalities (e.g. observing responses to acoustic signals in the dark), allowing researchers to test for various types of interactions (e.g. dominance, additive or synergistic effects) across modalities [3,10,11]. Similarly, manipulation (rather than isolation) of different signal components within a single modality (e.g. [12]) or across multiple modalities (e.g. [13–15]) can further elucidate signal interactions. However, the sheer number of treatments necessary to assess trait interactions through serial manipulations increases rapidly, even with relatively simple signals [16,17].

In this study, we propose a network-based approach as a complementary tool for understanding biologically relevant signal complexity. We can represent the architecture of complex signal traits as *signal phenotype networks*, in which putative signaling traits are represented as nodes, and edges (links between nodes) indicate strengths of marginal (i.e. not partial) correlations between traits. Recent theory suggests that the correlational structure of complex traits could reflect the evolutionary dynamics that shape phenotypes [18,19]. Network approaches provide the opportunity to apply mathematical tools developed in complex systems research to quantitatively assess signaling architecture and test hypotheses about the evolution of communication systems. In combination with dimensionality reduction and model selection procedures to help circumvent issues of statistical power related to correlational analyses of multiple traits, phenotype networks can

illuminate both the structure and putative function of multicomponent signals and thus represent an important step forward in disentangling the tremendous complexity of animal communication systems. We suggest that combining system-level associational analyses with manipulative experiments offers a way to investigate common structural and functional attributes of animal communication systems.

Here, we use multimodal phenotypic data from the North American barn swallow (*Hirundo rustica erythrogaster*), including morphological, plumage color and song features, to assess trait redundancy, modularity and function. Specifically, we compare the correlation structure of traits that predict paternity and nearest competitor distance to learn how inter- and intrasexual selection influence complex signal architecture. We do this by introducing and applying a novel workflow to test hypotheses about the evolution of complex signal phenotypes: (i) identifying clusters of correlated traits using principal components analysis (PCA), (ii) performing model selection to determine which trait clusters are important predictors of reproductive performance and competitive environment, (iii) developing a phenotype network based on trait correlations to represent the potential for signal redundancy and (iv) integrating model selection results with the phenotype network to assess modularity and function of putative signals across sexual signaling contexts.

(a) Characterizing phenotype networks

Studies of multicomponent signals have thus far focused on testing alternative hypotheses based on signal information content and efficacy (e.g. increased detection) [3], or whether signals function as ‘backups’ or ‘multiple messages’ [10] at the scale of pairs or suites of traits. The phenotype network approach leverages these existing conceptual frameworks and provides a workflow to describe the signal system as a whole and quantify the degree to which different sets of traits play different roles. The architecture of phenotype networks can be described along two axes: *redundancy* and *modularity* (**Figure 1**). Here, we interpret correlated traits as being *structurally* redundant (in a network sense), potentially signaling the same information (about quality, condition or motivation) to receivers. This concept is distinct from *functional* redundancy, wherein two traits elicit the same receiver responses [10]. Structural redundancy can be measured as the density of the phenotype network—i.e. the proportion of pairs of nodes that are significantly correlated.

Modularity is a general term that refers to the degree to which connections occur within versus across clusters. Thus, the assessment of modularity depends on how one defines modules. From a network perspective, modules

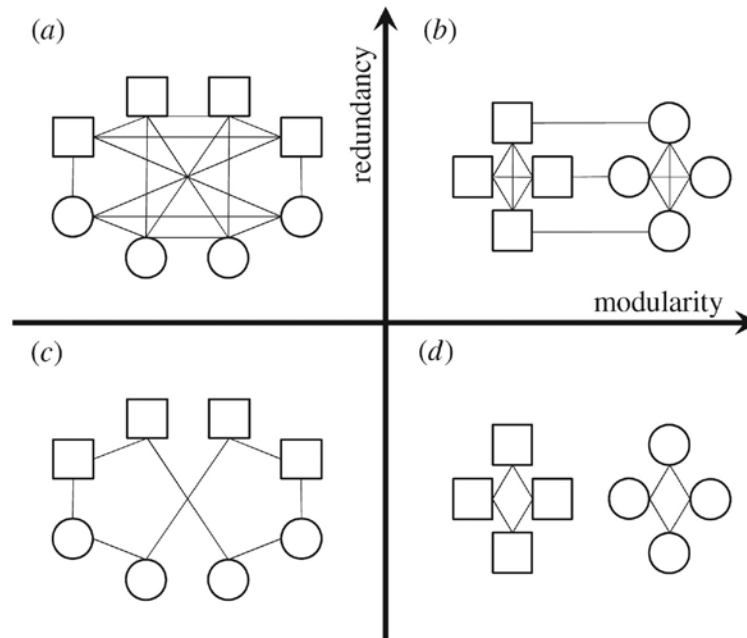


Figure 1. A conceptual diagram showing trait correlations for different patterns of signal redundancy and modularity. Shapes (nodes) represent four different traits in two different modalities (e.g. squares represent morphological features, while circles represent song components). Lines (edges) signify correlations between traits. In *(a,b)*, many traits are correlated (high redundancy), while in *(c,d)*, few traits are correlated (low redundancy). In *(a,c)*, trait correlations occur regardless of modality and are not organized into modules (low modularity), while in *(b,d)*, trait correlations are clustered into modules (high modularity).

are often defined as tightly linked clusters of nodes that are sparsely interconnected [20]. While many approaches exist to detect such clusters (often termed ‘community detection’; reviewed in [21]), these statistical definitions of modules do not lend themselves naturally to biological interpretation. Alternatively, we can define modules *a priori* as nodes of the same trait type—e.g. acoustic, color or morphological traits—and measure modularity as the relative strength of connections within versus across node types. This approach lends itself much more readily for hypothesis testing, and ultimately, comparative analyses across systems.

Empirical studies of the architecture of signal systems will help integrate ongoing behavioral research with the emerging theory on the evolution of complex phenotypes. For example, theory suggests that distributing informational units across multiple weakly correlated clusters, each composed of tightly intercorrelated traits, maximizes information content for receivers ([18]; figure 1*b*). Recent work also suggests that directional selection

operating independently on different trait clusters would promote modularity in complex phenotypes [19]. The same study also suggests that a pattern in which one trait complex is under directional selection while another is under stabilizing selection, would lead to intermediate levels of modularity. Thus, there is emerging theory that predicts intermediate levels of modularity—trait clusters connected by weak correlations—from the perspectives of signal design and sexual selection. These theoretical models do not necessarily make realistic assumptions about animal communication in nature, and empirical studies seldom assess complex signal architecture directly. Thus, there is currently a large gap in our knowledge about how ecology and social evolution shape signaling systems as a whole. Our goal is to offer a new analytical approach to help bridge this gap between theory and empirical studies of signal design across multiple modalities.

2. Material and methods

(a) Study system

The barn swallow, *Hirundo rustica*, is a Holarctic-distributed migratory oscine songbird, comprising six described subspecies. Within some populations of barn swallows, the length of streamers (the outermost tail feathers) plays a key role in mate choice [22–24]. However, studies in North America suggest dark melanin-based plumage color is more important in mate choice among *H. r. erythrogaster* [25–27], although this is less clear for a Canadian population [23,28]. We studied populations of *H. r. erythrogaster* between 2009 and 2012 in Boulder County, CO, USA (latitude 40° 29' 360" N, longitude 105° 169' 390" W). Ten breeding locations were monitored, ranging from 3 to 43 nesting pairs.

(b) Field methods

Each year, barn swallows were captured at the start of breeding using mist nets, banded with USGS metal bands, and given a unique combination of a color band and non-toxic permanent ink pen applied to white spots on tail feathers. Contour feather samples were taken from four areas along a ventral transect for standardized color analysis (see below). During the entire breeding season, we conducted behavioral observations to match banded individuals to nests and thus identify social pair mates. We then monitored reproductive success for all active nests at study sites. Blood samples were taken from adults upon capture and from nestlings on day 12 post-hatching for paternity analyses.

(c) Phenotypic measurements

Our choice of phenotypic measures (**Table 1**) was based on previous work within this species. We measured two morphological features: right-wing length, and maximum length of streamers (the outermost tail feathers), as these are potential indicators of age [22,28,29]. Additionally, we measured feather color for samples collected from four ventral patches (throat, breast, belly, vent), following Safran *et al.* [30]. For each patch, we used a spectrometer to measure average brightness, hue and red chroma (see the supplementary material, appendix S1 for details). We also recorded between 3 and 20 songs (10.67 ± 0.67 s.e.) from 66 males between 5.00 and 13.00 during May–August, 2009, 2011 and 2012. We included only complete songs in our analysis, which comprised a warbling series of syllables not separated by more than 0.2 s and terminated in a harsh trill (the rattle). We extracted 14 measures of song frequency and temporal characteristics, repertoire size and composition (table 1) based on previous work and hypothesized roles in social interactions [31–33]. Based on our preliminary analyses (supplementary material, figure S2), we averaged song parameters across at least five songs from each male. Our final dataset consisted of 50 males with complete morphological, color and song data.

(d) Paternity analyses

Because extra-pair copulation is common in barn swallows [22], we assigned paternity to offspring in each focal male's nest using six polymorphic microsatellite markers. We analyzed allele frequencies and performed paternity exclusions using CERVUS v. 3.0 [34]. None of the six loci differed from Hardy–Weinberg equilibrium. The probability of correctly excluding a focal male as the genetic father was 0.9891, and given a known mother was 0.9991. The mean rate of extra-pair young per nest was 23.3%, comparable to rates found in other barn swallow populations (range: 17.8–34%) [25,35]. For additional color, song and paternity methods, see the supplementary material appendix S1.

(e) Measures of inter- and intrasexual selection

A primary goal was to determine whether different components of the communication system play different roles across signaling contexts, such as mate preferences and intrasexual competition. We used *paternity*, defined as the proportion of genetically determined within-pair offspring sampled within a male's nest on day 12 post-hatching, as our measure of female choice because previous work has shown that females dynamically allocate paternity as a function of changes in phenotype [27].

Table 1. Measured phenotypic traits included in principal components analysis.

<i>Module</i>	<i>Subcat</i>	<i>Trait</i>	<i>Description</i>	<i>Mean (S.E.)</i>
Song traits				
	Time domain			
	WL		warble length—time between peak of first syllable and peak of last syllable before P (sec)	3.08 (0.10)
	PL		P-syllable length—time from beginning to end of P-syllable (sec)	0.31 (0.00)
	RL		rattle length—time between the first and last pulses in the terminal trill (sec)	0.33 (0.01)
	RTmp		rattle tempo—number of rattle pulses/rattle length (Hz)	31.27 (0.25)
	WTmp		warble tempo—number of syllables before P/warble length (Hz)	4.56 (0.06)
	Frequency domain			
	PF W		peak frequency of the warble—frequency at the maximum amplitude in the warble (Hz)	3960.38 (52.07)
	PF P		peak frequency of the P-syllable—frequency at the maximum amplitude in the P-syllable (Hz)	4279.26 (71.12)
	PF R		peak frequency of the rattle—frequency at the maximum amplitude in the central rattle, disregarding the first and last pulse, which have lower frequencies than the primary pulse train in our population (Hz)	5421.30 (93.05)
	FB R		frequency bandwidth of the central rattle—song frequency bandwidth above a -10 dB threshold, relative to peak frequency, averaged across the primary pulse train (Hz)	2694.63 (98.19)
	WE W		Wiener entropy of the warble—ratio of the geometric mean to the arithmetic mean of the warble spectrum (0 = pure tone; 1 = random noise)	0.62 (0.01)
	Repertoire			
	%A		% A-syllables—(a measure of low song complexity) number of A-syllables/total number of syllables; these syllables are simple, resembling contact calls	29.50% (0.01)
	%S		% S-syllables—(a measure of intermediate song complexity) number of S-syllables/total number of syllables; these syllables are 'scratchy' and atonal	9.01% (0.01)
	%T		% T-syllables—(a measure of high song complexity) number of T-syllables/total number of syllables; these syllables are complex, highly frequency modulated, and tonal	3.38% (0.003)
	Rep		repertoire size—cumulative number of unique syllables sampled for a given male	27.53 (0.67)
Morphological traits				
	RWL		right-wing length (mm)	118.48 (0.38)
	TS		tail streamer length—maximum length of the outermost tail feathers (mm)	91.31 (1.03)
Color traits ^a				
	TBri		average brightness—the average per cent reflectance between 300 and 700 nm; lower values darker	T: 17.96 (0.82)
	RBri			R: 28.89 (0.91)
	BBri			B: 28.35 (0.98)
	VBri			V: 21.47 (0.63)
	THue		hue—the wavelength at maximum slope; low values pale/yellowish, high values dark/reddish (nm)	T: 653.38 (3.33)
	RHue			R: 631.03 (3.17)
	BHue			B: 626.18 (4.11)
	VHue			V: 646.12 (3.38)
	TChr		red chroma—the proportion of light reflected in the red color range (600–700 nm); higher values darker	T: 0.5044 (0.01)
	RChr			R: 0.4550 (0.01)
	BChr			B: 0.4490 (0.01)
	VChr			V: 0.4978 (0.01)

a. Each color axis measured for T, throat; R, breast; B, belly; V, vent.

We used the linear distance to the nearest active nest (hereafter *inter-nest distance*) as a measure of intrasexual competition. We defined inter-nest distance as the log-transformed linear distance in centimeters between the focal male's nest and the nearest active nest with a fertile female (and her mate) at the site and day of song recording. Male barn swallows are highly territorial, defending nesting areas within larger breeding sites. Previous work indicates that barn swallows maximize distance between each other [36], preferring nests hidden from neighbors [37]. Moreover, males with more active neighbors had shorter songs which emphasized the rattle, and the length of rattles correlated with circulating testosterone concentrations [31]. These results indicate that nearest neighbor distance is a proxy of intrasexual competition. In our competition analysis, we excluded males whose nearest neighbor was more than 12 m away, as this was a natural break point in the bimodal distribution of neighbor distances (supplementary material, figure S4). Our sample size for male seasonal paternity was 28 and 38 for inter-nest distance.

(f) Exploring function(s) of signal traits

We performed PCA on the 28 phenotypic variables (descriptions: table 1), extracting nine components with eigenvalues greater than one. Extracted components were rotated using the varimax method to maximize differences between orthogonal vectors and facilitate interpretation of these phenotypic axes. Rotated factors were then renamed according to trait loadings (supplementary material, table S1).

We used an information-theoretic approach to determine which signal axes best explained variation in fitness metrics and competitive environment [38,39], as it offers greater power for ranking alternative models and avoids the problem of multiple testing associated with traditional step-wise model selection [39,40]. For each response variable, we specified a global model including all nine phenotypic factors, with site nested in year as random effects. The candidate model set for both analyses included every combination of fixed effects, including a minimal model containing only the random effects and a global intercept term, for a total of 512 models for both response variables. This approach was necessary because we had no *a priori* expectations about which combination of traits explained each response [41], and further justified in that each covariate was identified as a biologically relevant phenotypic axis reflecting different aspects of male quality.

We used model averaging to calculate effect estimates and 95% CIs from models within $2 \Delta AIC_c$ of the best model. General and generalized linear mixed models (LMM and GLMM, respectively) were specified using the 'lme4' package [42] and model averaging was conducted using the 'MuMIn'

package [43] implemented in R v. 3.1.0 [44]. Models were specified as follows: analysis of paternity was a binomial GLMM with the number of trials equal to the number of fledglings in a nest, and inter-nest distance was analyzed using a lognormal LMM.

We report model-averaged parameter estimates and 95% CIs for all factors included in our top model set. Because we have adopted an information-theoretic approach for inference, we emphasize that these should not be interpreted through a null hypothesis-testing perspective [39,45]. We further assess model fit by calculating marginal and conditional R^2 -values (R^2_m and R^2_c), which represent the variance explained by the fixed effects and both the fixed and random effects, respectively, for each well-supported model [46]. Therefore, factors retained in a top model set were the most important for predicting a given response variable, with the index of variable importance (hereafter *importance*, the sum of AIC_c weights of the models that included a factor) acting as a quantitative measure [38]. The inclusion of a random effects model in each analysis additionally allowed us to assess whether phenotypic variables contribute explanatory power after accounting for effects due to differences across breeding sites and the particular year in which data collection took place.

(g) Phenotype network architecture

We generated a phenotype network using all 28 features of phenotype that we measured for individuals in our population. Each edge of this network represents Spearman's ρ correlations. In order to minimize the interpretation of incidental correlations, we discarded any trait-pair correlation if its 95% CI for 100,000 bootstrap permutations overlapped zero. We visualized the phenotype network using the R package 'qgraph' [47]. For an annotated script describing our network analyses and visualizations, see the supplementary material, appendix S2.

We used two metrics to assess overall levels of redundancy: average correlation strength and network density. Average correlation strength was calculated as the mean of the absolute value of the unsigned phenotypic correlation matrix. Network density was calculated as: no. robust edges (correlations with nonzero bootstrapped 95% CIs)/total no. pairwise correlations. To test whether the network of traits predicting paternity and inter-nest distance had different degrees of redundancy from the network as a whole, we randomly selected (without replacement) an equivalent number of nodes for each network ($n = 20$ for paternity; $n = 14$ for inter-nest distance). By iterating this procedure 1000 times, we generated distributions for each redundancy metric. From these distributions, the proportion of observations more extreme than our empirical value was used as a p -value for assessing significance.

We measured modularity as the degree to which correlations are structured based on trait types. The coefficient of assortativity [48] describes the degree to which edges in a network connect nodes of similar type. If trait correlations within modules are stronger than correlations across modules, then assortativity should be greater than the random expectation. Moreover, if traits are weakly correlated across modalities, then assortativity will be less than the maximum possible value, 1, which occurs when there are no connections between modalities. We divided nodes into three types—morphology, color and song—and measured the weighted assortativity coefficient (r_d) using the R package 'assortnet' [49]. We compared this value with the expected level of assortativity in randomized networks generated by permuting the 'node type' across nodes (i.e. node-label permutation, supplementary material, appendix S1). If traits are tightly correlated within modalities and weakly connected across modalities, then the coefficient of assortativity would lie between the random expectation (estimated by permutation) and one.

(h) Integrating structure and function of the signaling system

We further tested whether patterns of connectivity between modalities differed based on functional contexts. Using the model selection procedure as described above, we categorized nodes based on whether they loaded highest on factors important in predicting paternity and/or inter-nest distance. This allows for simultaneous visualization of trait architecture, modality and function in communication.

3. Results

(a) Identifying phenotypic axes

Our PCA of 28 morphological, song and color traits produced nine orthogonal factors with eigenvalues greater than 1, explaining 75% of the cumulative variance in phenotypic traits (supplementary material, appendix S1 and table S1). These factors included three color axes ([Ventral Paleness], [Throat Darkness] and [Ventral Redness]), four song axes ([Song Tonality], [Monotony], [Song Tempo] and [P-Length]) and two multimodal axes ([Complexity/Feather Length] and [Repertoire/ Vent Darkness]). Biological interpretations of factors are provided in **Table 2**.

Table 2. Variables used in model selection.

Predictor variables (rotated principal components of phenotype)	
<i>Variable</i>	<i>Higher value indicates</i>
[Ventral Paleness]	lighter breast, belly and vent
[Song Tonality]	higher pitch, less tonal, narrower frequency bandwidth, with more intermediately complex 'S' syllables
[Throat Darkness]	darker, redder throat
[Monotony]	longer songs, comprised of many simple 'A' syllables
[Complexity/Feather Length]	greater proportion of complex 'T' syllables, and longer wings and tail streamers
[Ventral Redness]	redder breast, belly and vent
[Song Tempo]	faster warble tempo, with shorter, faster rattles
[Repertoire/Vent Darkness]	larger cumulative number of syllables and darker vent
[P-Length]	longer P-syllables
Response variables (metrics of female choice and male–male competition)	
<i>Variable</i>	<i>Description</i>
paternity	proportion of genetic offspring in a focal male's nest; binomial—in logistic regression, number of within-pair young = wins, number of extra-pair young = losses, $n = 28$
inter-nest distance	distance to the nearest nest with a fertile female at the site and day a male was recorded; females were considered fertile if the day of recording was within the range of 7 days before clutch initiation and the day prior to clutch completion; Gaussian after log transformation, $n = 38$

(b) Assessing trait functions

(i) Traits explaining paternity

The best model for paternity included [Complexity/Feather Length], [Song Tempo] and [P-Length], with a model weight of 0.12, indicating low model certainty (see supplementary material, table S2, in appendix S1 for model results). The top model set retained 11 models, including six factors: [Complexity/Feather Length], [Song Tempo], [P-Length], [Ventral Paleness], [Song Tonality] and [Monotony] (*importance* = 1.00, 0.85, 0.55, 0.45, 0.20, 0.18, respectively). Overall, [Complexity/Feather Length] and [Song Tempo] were the best predictors of paternity, while there was moderate support for [P-Length] and [Ventral Paleness]. There was minimal support for [Song Tonality] and [Monotony].

Thus, males with a greater proportion of complex ('T') syllables, longer streamers and wings, faster, shorter warbles, p-syllables and rattles, with darker ventral plumage had higher paternity in their social nests (**Figure 2a**).

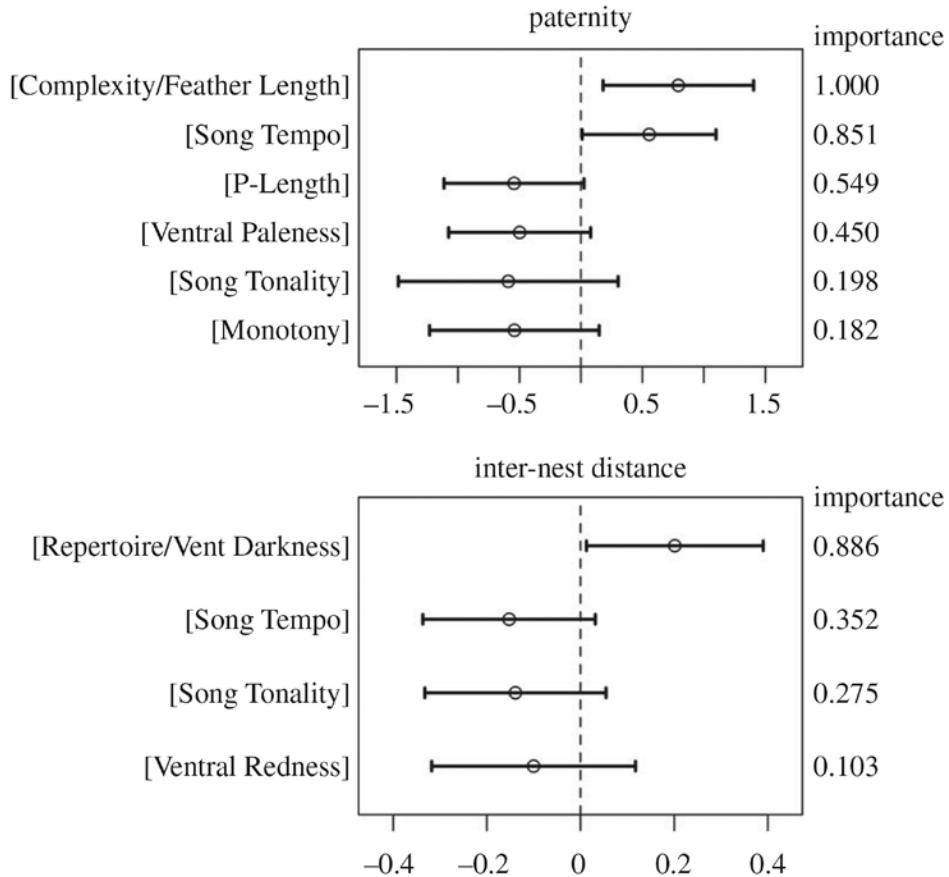


Figure 2. Model-averaged slope estimates and confidence intervals for traits ranked in the top 2 ΔAIC_c for paternity and inter-nest distance. Importance values (sum of AIC_c weights of top models including a factor) are shown outside the right margins.

To a lesser degree, males with less tonal, lower pitched, songs with fewer simple ('A') syllables had higher genetic paternity. R^2_m for the paternity models ranged from 0.18 to 0.37, and R^2_c ranged from 0.24 to 0.55, suggesting that site and year differences did not greatly influence cuckoldry rates (see supplementary material, table S2).

(ii) Traits explaining inter-nest distance

The best model for inter-nest distance contained only [Repertoire/Vent Darkness], with a model weight of 0.28. The top model set contained six models, including four factors: [Repertoire/Vent Darkness], [Song Tempo], [Song Tonality] and [Ventral Redness] (*importance* = 0.89, 0.35, 0.28, 0.10, respectively). All top models, except one, contained [Repertoire/Vent

Darkness], and model-averaged estimates indicated a strong effect (figure 2*b*), with males having larger syllable repertoires and darker vents maintaining a greater distance to nearest neighbor.

Overall, males with greater distance to the nearest active nest tended to have larger repertoires, darker vents, yellow-shifted breast hue, slower, lower pitched, more tonal songs, composed of fewer 'S-syllables', with slower, longer rattles covering a broader frequency bandwidth. Because the random effects model, which did not contain any phenotypic predictors, was included in the top model set, R^2_m values ranged from 0 to 0.17, and R^2_c from 0.28 to 0.55. These higher R^2_c ranges, relative to R^2_m highlight the considerable among-site differences.

(c) Structure of the phenotype network

The structure of the phenotype network in **Figure 3a** represents the overall patterns of correlations between all possible pairs of measured traits. Our redundancy measures for the full network were $|\text{avg corr}| = 0.435$, network density = 0.196. As shown in the supplementary material, figure S5, we found that levels of redundancy for the paternity network did not differ from the full network ($|\text{avg corr}| = 0.435$, $|\text{avg corr}_{\text{permutation}}| = 0.434$, $p = 0.489$; network density = 0.221, network density_{permutation} = 0.197, $p = 0.228$). The same was also true for the inter-nest distance network ($|\text{avg corr}| = 0.389$, $|\text{avg corr}_{\text{permutation}}| = 0.432$, $p = 0.872$; network density = 0.198, network density_{permutation} = 0.199, $p = 0.512$).

The assortativity coefficient for the full network ($r_d = 0.669 \pm 0.028$ jack-knife s.e.) was greater than expected under random assortment (permutation test: $p < 0.001$; supplementary material, appendix S1, text and figure S6), and less than the assortativity value under perfect assortment ($r_d = 1$). There were very similar patterns of significant assortativity for the paternity network ($r_d = 0.669 \pm 0.039$, $r_{d,\text{permutation}} = -0.071$, $p < 0.001$) and inter-nest distance network ($r_d = 0.805 \pm 0.077$, $r_{d,\text{permutation}} = -0.095$, $p = 0.003$) (see the supplementary material, figure S6). Thus, levels of structural modularity and redundancy were similar for all three phenotype networks (figure 3). Moreover, based on significant modularity and some level of redundancy within modules, the overall network most closely resembles figure 1*b*.

(d) Integrating trait functions with signal architecture

Figure 3*b,c* includes only nodes that loaded highest on factors included in the top models for paternity and inter-nest distance, respectively. In addition, node colors are graded by importance (i.e. sum of AIC_c weights of top models including a factor on which a trait loaded highest). Thus, because all

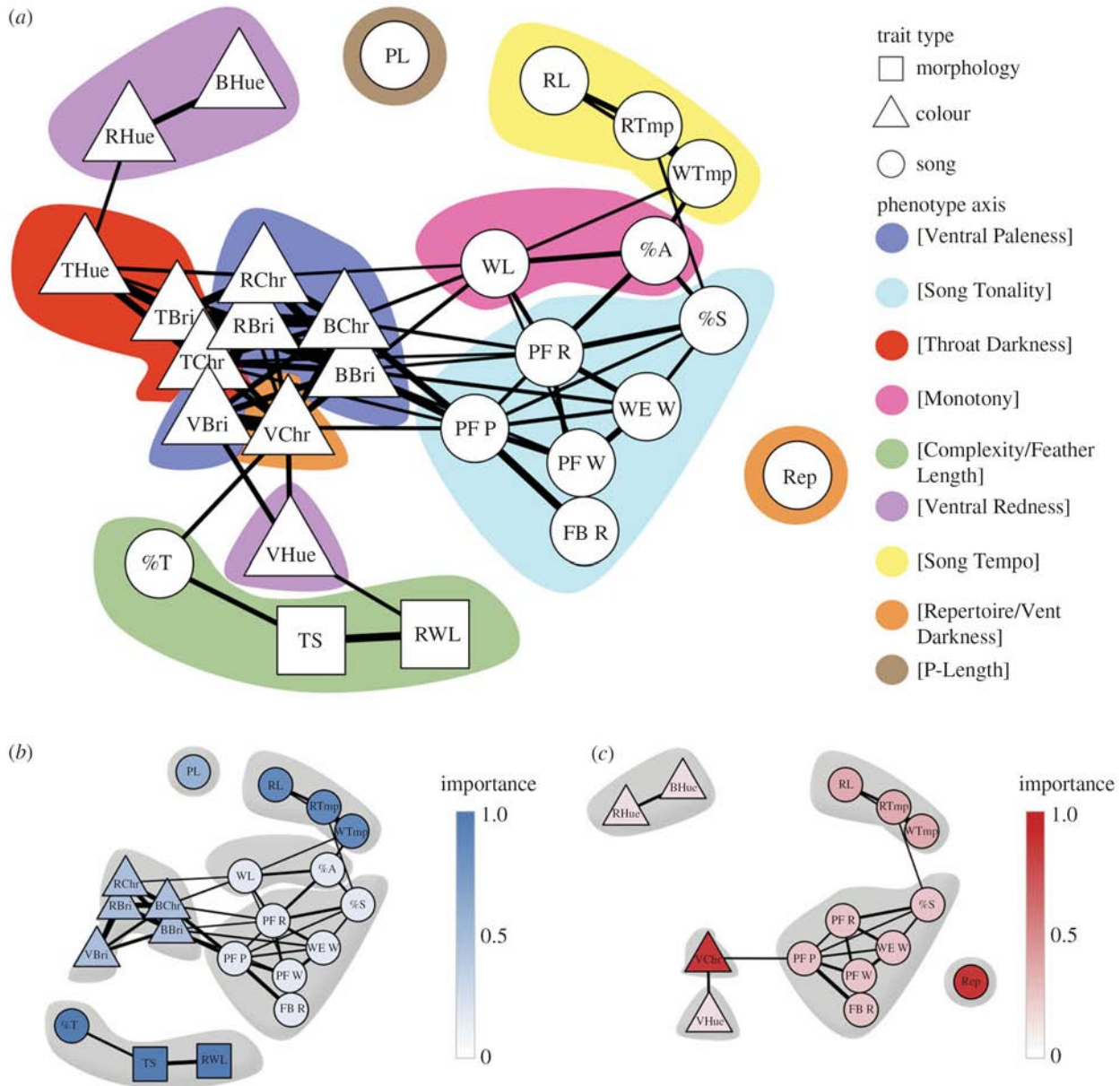


Figure 3. (a) Represents the barn swallow phenotype network, showing Spearman's ρ correlations between measured traits for $n = 50$ males. Node trait types are symbolized by different geometric shapes; the factors on which traits loaded highest are indicated by colored freeform shapes around clusters. To highlight relationships most likely to be biologically relevant, we only include edges that were robust to bootstrap resampling. Thus, isolated nodes did not exhibit robust correlations with any other trait. The thinnest edge represents a correlation of 0.27 (between PF W and WL); the thickest edge is 0.95 (between B Chr and B Bri). Edge lengths do not directly reflect correlation, and spatial orientation is based on a 'spring' algorithm that minimizes edge crossings. (b) Intercorrelations between the subset of traits explaining variation in paternity. These traits loaded highest on factors represented in the top models for this measure of female choice. (c) Only traits explaining variation in inter-nest distance (i.e. male–male competition). Freeform shapes in (a) are colored grey in (b,c) for clarity. Node colors in (b,c) are graded by importance (i.e. sum of AIC_c weights of top models including a factor on which a trait loaded highest). The best predictors of paternity and inter-nest distance were distinct; however, some measures of song pitch and tempo were included in the best models for both of these response variables.

top models of paternity included [Feather Length/Complexity], this factor has an importance value of 1, and its component traits—Right-Wing Length (RWL), Tail Streamer Length (TS) and percentage of complex syllables (%T)—are colored accordingly in figure 3*b*.

Combining our model selection results with the phenotype network provides simultaneous information on signal modality, potential for redundancy and putative function in communication. As shown in figure 3*b,c*, there is some overlap in the traits predicting the two response variables (9/28 nodes = 32%). However, the traits explaining the greatest amount of variation in paternity did not predict variation in inter-nest distance, and vice versa. The cluster of traits comprising [Song Tempo] show the clearest evidence of dual function across inter- and intrasexual contexts, as this factor was the second-best predictor of both paternity and inter-nest distance (figure 2).

4. Discussion

Describing the architecture of a signaling system as a phenotype network has several advantages. First, we can visualize and measure how signal systems are organized based on trait types—e.g. the overall levels of correlations between traits, and how sets of traits cluster. Moreover, by combining this network with an analysis of the function(s) of different sets of traits, we can begin to explore the interplay between the architecture of signal phenotype networks and the evolution of animal communication.

The network approach also allows us to assess the level of modularity of signal systems to test specific hypotheses. For example, the signal phenotype network of our barn swallows is characterized by discrete clusters of traits that are partitioned mostly by trait type: morphological, acoustic and color traits are more closely correlated with each other than to other trait types. This is not surprising, as different trait types are subject to distinct genetic and developmental constraints that make them less likely to be correlated. However, our analyses show that there are also many correlations between node types. Theoretically, such a pattern may emerge when suites of traits are functionally correlated, and this generates selection for genetic correlations, as well [19]. An alternative explanation, proposed by Ay *et al.* [18], is that when signalers and receivers both benefit from enhanced communication, weak correlations among signal modules are favored. The empirical measurements of phenotype networks, combined with development of theory regarding the evolution of complex trait architecture, could motivate future experimental work to test these hypotheses. The phenotype network approach provides a method by which we can quantify 'assortment' by trait type, or by any other criteria. This measure can be compared across systems (e.g. species, populations, sexes), potentially opening the door for new comparative analyses of multicomponent signal systems.

(a) Comparing functions of traits on phenotype networks

In the current study, we focused on the potential roles of traits in mediating social interactions in two different contexts: mate choice (as measured by paternity) and intrasexual competition (as measured by inter-nest distance). Our results indicate that the most important factors predicting paternity and inter-nest distance were different phenotypic PCs—[Complexity/Feather Length] and [Repertoire/Vent Darkness], respectively—comprising unconnected clusters on the phenotype network (figures 2 and 3). Thus, some traits are structurally correlated and share the same function and may perhaps be considered redundant (e.g. the proportion of 'T' syllables and streamer/ wing lengths). By contrast, other traits are uncorrelated and could convey different information, yet share the same function, suggesting that they could be 'multiple messages'. Importantly, a phenotype network perspective clarifies that both types of signal complexity can occur in the same system.

In addition, the second-most important factor for both paternity and inter-nest distance was [Song Tempo], comprising warble tempo, rattle tempo and rattle length, and this cluster of traits was disconnected from other higher ranking PCs (figures 2 and 3). This finding suggests that males and females may use different signals to assess potential mates and competitors, but use a common set of orthogonal signals to reinforce information across both contexts. Lower ranking factors (and their constituent traits) in both signaling contexts likely provide a mixture of additional redundant and non-redundant information, with smaller or less-consistent effects on receivers. Phenotype networks provide a formal method to evaluate how signaling to multiple audiences [8] might shape the architecture of communication systems as a whole. However, experimental manipulations are necessary to clarify functional interactions of different signal clusters to determine whether, for example, ventral darkness and rattle tempo act as 'backup signals' or 'multiple messages' [10] when females choose mates.

The finding that darker males had higher paternity is consistent with several previous studies within this subspecies [26,27,50]. However, our best measure of female phenotypic preference was a trait complex involving tail streamer length, wing length and %T syllables. This result was unexpected, given previous studies showing no relationship between tail streamer length and reproductive success in this subspecies [25,50], and the results of a recent experiment in our study population wherein males with artificially elongated streamers lost paternity in the brood following manipulation [51]. These mixed results with respect to streamer length may have to do with the differences in the overall architecture of signal systems. To better understand the dynamics of female mate choice, combinations of targeted experiments with assessments of the overall phenotype network in which these signals function may be particularly informative.

(b) Traits affecting inter-nest distance

Our analyses identified a single principle component axis, [Repertoire/Vent Darkness], as the best predictor of the nearest competitor distance, our measure of intrasexual selection. Although repertoire size has often been suggested to result from female preference for elaborate songs [52,53], recent work indicates weak support for this hypothesis across birds [54,55]. Our findings are consistent with the idea that overall repertoire size results from intrasexual competition, rather than mate choice [55]. Given the known function of dark coloration in sexual signaling within this subspecies as well as previous experimental and correlational work showing that darker males have greater concentrations of circulating testosterone [26,56], it is not surprising that darker birds should defend larger territories. However, vent chroma loaded highest on the repertoire axis, but loaded nearly as highly on the [Ventral Paleness] axis, which did not predict inter-nest distance. Thus, whether the darkness of vent plumage (on the underside of a male's rump, see the supplementary material, figure S1) is important in male–male interactions is unclear, particularly as this feather patch would often be invisible when perched inside the nesting area.

Greater inter-nest distance was also associated with lower pitch, higher tonality, wider frequency bandwidth, and slower warbles and rattles. These results are consistent with previous studies of *H. r. rustica* in Italy, indicating correlations between pitch, rattle exaggeration and number of competitors [31].

5. Conclusion

In summary, we argue that mapping the results of traditional analyses of trait function onto phenotype networks provides new insights into multicomponent signaling systems. While data reduction techniques such as PCA are focused on creating orthogonal (i.e. statistically independent) variables amenable to statistical analysis, phenotype networks turn the focus on investigating the *patterns* of correlations. A strength of our combined approach is that it provides a robust tool for analyzing both structure and function of complex phenotype associations. Further, the identification of trait correlations and modules among phenotype networks is highly amenable for use in hypothesis testing about the evolutionary ecology of complex signal traits. Specifically, identification of structurally independent trait modules with common functions (equivalent to clusters conveying 'multiple messages') allows for the design of appropriate manipulative experiments to test for functional signaling interactions. We suspect that further progress in network theory will lead to more sophisticated tools to incorporate additional information, such as the strengths of correlations between traits.

Using a phenotype network approach in our barn swallow example allows us to see that traits that are most important in two different contexts (mate choice and intrasexual competition) are generally discrete trait clusters. Moreover, we are able to begin exploring how modules of traits are organized within and across trait types—patterns that are not necessarily obvious based on biological intuition. We hope that further development of such methods across taxonomic groups will facilitate a move towards a common framework for assessing the complexity of signal architecture and function across systems.



Ethics. Our research protocols were approved by the University of Colorado's IACUC (no. 1004.01), Colorado Division of Wildlife and the U.S. Federal Bird Banding Laboratory (no. 23505).

Data accessibility. Original phenotypic data for generating phenotype network graphs and metrics can be found in the supplementary material, appendix S3.

Authors' contributions. M.W. collected data and wrote first draft; D.S. helped solidify the network statistical approach and conceptual framework; M.J. aided in linear modelling and model averaging; J.H. facilitated color data collection and analysis; R.S. aided in all phases of data collection, analysis and interpretation; all authors contributed substantially to revisions.

Competing interests. None.

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