Phenotypic integration and modularity evolve along with plumage coloration across populations in a widespread songbird

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Teaser Text:

Traits often do not evolve independently. Rather, sets of traits can evolve together or separately. How do these patterns of integration and modularity evolve as populations diverge? In this study, we examined plumage color of over 2,000 barn swallows from across their widespread breeding range. Correlations among color patches differed based on which patches are evolving: populations with darker breast, belly, or vent exhibited greater phenotypic integration or overall correlation, but in populations with darker throat color, this patch evolved as a separate module. This provides key insights into the evolution of plumage color differences among closely related populations.

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Abstract:

Patterns of interdependence among traits such as phenotypic integration and modularity can have profound evolutionary consequences. Despite their importance in evolutionary theory, large-scale empirical evidence for processes that lead to population divergence in phenotypic integration and modularity across a species range remains rare. Here, we analyze patterns of correlations between plumage color traits sampled from many populations across the global range of the barn swallow (*Hirundo rustica*). Population divergence in phenotypic integration is associated with both genomic divergence and the evolution of ventral coloration. We also demonstrate that throat coloration acts as a separate module from other ventral plumage patches, and populations with darker throat color exhibit increased modularity. These results provide rare empirical evidence to support the theory that selection on one set of traits can lead to the evolution of modularity. Our study takes a unique, comparative approach that links phenotypic integration and modularity to both phenotypic and genomic divergence among populations within a species known to experience divergent sexual selection pressures. Finally, our study offers insight into the evolution of phenotypic integration and modularity in the context of animal coloration, as well as the structure and function of complex signal traits in general.

Keywords:
modularity, phenotypic integration, complex traits, sexual signaling, phenotype networks, plumage coloration

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Introduction

Phenotypic traits do not evolve in isolation: genetic, developmental, physiological, or functional correlations can act as constraints that affect the direction and magnitude of evolutionary change in response to selection (Lande and Arnold 1983). A large body of work has presented two related concepts: *phenotypic integration* and *modularity*, which together describe the *complex trait architecture* of a population of organisms (Klingenberg 2008; Goswami et al. 2014; Felice et al. 2018; Svensson et al. 2021). Phenotypic integration describes the degree to which traits are correlated, as a result of underlying genetics, development, or shared function (Pigliucci 2003; Armbruster et al. 2014). Modularity describes the degree to which different clusters of phenotypes, termed modules, can function and/or evolve separately from other phenotypes (Wagner et al. 2007; Melo et al. 2016). The combination of phenotypic integration and modularity is thought to fundamentally affect evolvability of traits. For example, increase in phenotypic integration, or high level of correlation among traits, may ensure that functionally interdependent traits evolve together. Meanwhile, increased modularity may mean that sets of traits that are found in different modules will evolve independently. Importantly, the relationships between integration and modularity can vary, as integration can occur within or across modules to shape the architecture of complex traits.

The study of phenotypic integration and modularity has been central to the study of morphological evolution for decades, revealing key genetic and developmental mechanisms as well as modes of selection that generate complex trait architecture (Young and Badyaev 2006; Mallarino et al. 2011; Pavlicev et al. 2011; Melo and Marroig 2015; Do O and Whitlock 2023). More recently, these approaches have become increasingly prevalent in other fields such as the study of complex sexual signal traits (Hebets et al. 2016), including avian plumage signals (Badyaev 2004), combination of visual and acoustic signals in birds (Wilkins et al. 2015), and sexual displays in spiders (Rosenthal et al. 2018). The potential importance of signal evolution on reproductive isolation (Maan and Seehausen 2011; Schield et al. 2024) suggests that the architecture of complex signals could have profound consequences on larger evolutionary processes.

Much research has focused on the role of selection in shaping the evolution of phenotypic integration and modularity. Theory suggests that selection can affect not only the evolution of traits but also the correlations between traits. For example, correlational selection can promote the evolution of phenotypic integration (Lande 1980; Cheverud 1984; Svensson et al. 2021), while divergent selection or a combination of directional selection and stabilizing selection acting on different modules can drive the evolution of modularity (Wagner 1996; Wagner et al. 2007; Melo and Marroig 2015). However, much of this work has been restricted to theoretical models or comparative studies between species, and few studies address the potential role of selection on variation in complex trait architecture among natural populations (Eroukhmanoff et al. 2009; Assis et al. 2016). Populations could potentially vary in: the degree of phenotypic integration (Figure 1A); both integration and modularity (Fig. 1B); or the composition of modules (Fig. 1C). Patterns of phenotypic integration and modularity could also be highly conserved (Young and Badyaev 2006; Porto et al. 2009), resulting in little to no variation across populations (Fig. 1D). Inferring the role of selection on variation in complex trait architecture across populations is critical for understanding phenotypic evolution, yet difficult because it

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requires measurements of multiple traits from many individuals, along with genomic data and information on trait function across multiple populations.

Patterns of variation in complex trait architecture across populations could have important consequences for evolutionary divergence and speciation. For example, integration and modularity may constrain evolution along particular trait axes, limiting divergence along axes that are highly integrated, while promoting divergence between traits contained in different modules (Pigliucci 2003; Melo et al. 2016). Such patterns are prevalent across species: for example, mammalian skulls exhibit highly conserved patterns of trait covariance, suggesting that integration limits morphological diversification (Young and Badyaev 2006; Porto et al. 2009), whereas insect clades with higher levels of developmental modularity have higher rates of morphological divergence in the fossil record (Yang 2001). Within species, patterns of divergence in trait architecture may accompany divergence in the traits themselves. For example, population divergence in covariance of wing morphological traits in four different species of *Calopteryx* damselflies is correlated with divergence in the size of melanin patches on the wing (Eroukhmanoff et al. 2009). Overall, existing evidence demonstrates that the architecture of complex morphological traits can evolve across populations, with likely effects on phenotype divergence and the process of speciation.

We address several important gaps in our understanding of the evolution of complex trait architecture under population divergence using avian plumage coloration as a model. The colors and patterns of avian plumage can serve various functions, including as a signal involved in sexual selection (Hill and McGraw 2006; Cuthill et al. 2017; Mason and Bowie 2020). We investigate how plumage color traits among multiple patches may evolve together as integrated units or evolve independently as separate modules. While previous studies have documented patterns of integration and modularity in bird coloration both within populations (Wilkins et al. 2015) and between species (Badyaev and Young 2004), no study has tracked changes in the trait architecture of coloration across diverging populations. Here, we accomplish this by taking advantage of extensive, worldwide phenotypic and genomic sampling of barn swallows (*Hirundo rustica*).

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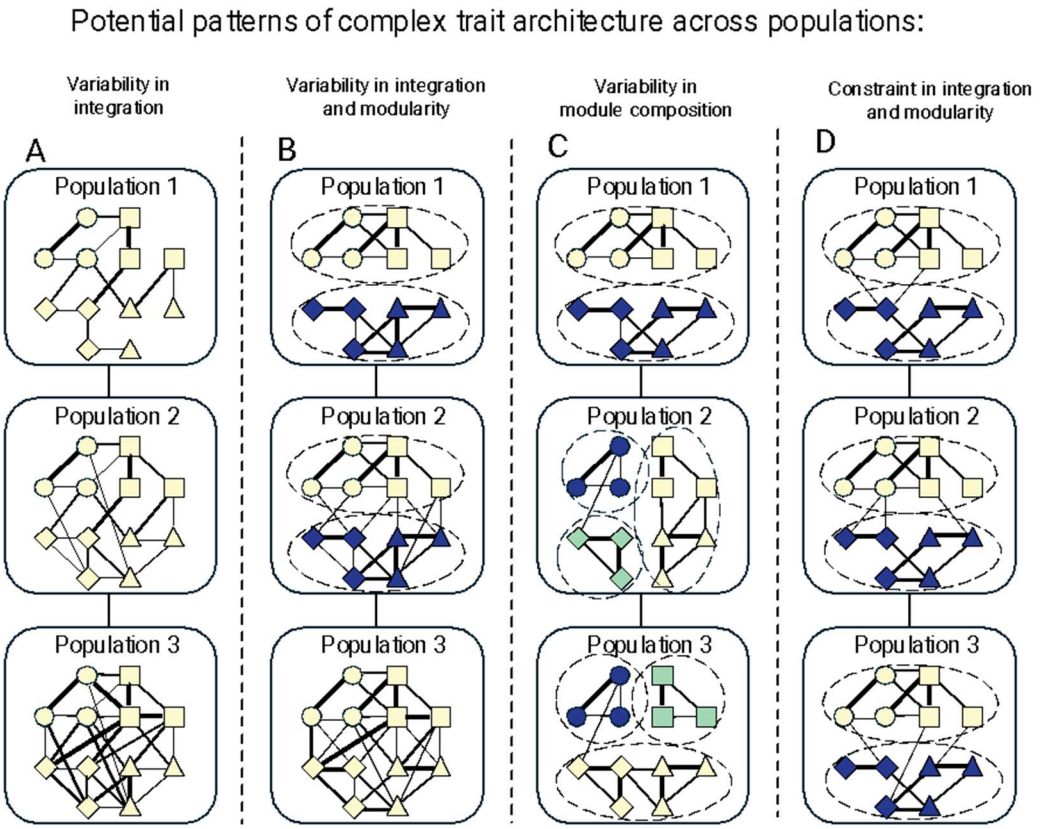


Figure 1: Phenotype network plots demonstrating how complex trait architecture may vary across populations. Nodes represent different trait measurements, and nodes of the same shape represent measurements of shared traits (e.g., different dimensions of skull morphology or different components of signaling traits). Edges represent correlations between trait measurements above a certain threshold, with edge widths representing strengths of correlations. Each network inside a rounded square characterizes the pattern of trait correlations in a population. When applicable, modules are shown by dashed ellipses and node colors. (A) Populations may differ in the degree of overall phenotypic integration, represented here by differences in the number of edges (B) Populations may vary in the degree of integration and modularity. (C) Populations may vary in the configuration of modules, for example if all populations exhibit discrete modules but module compositions differ. (D) Finally, populations may be highly consistent in the degree of integration, modularity, and configuration of modules.

Alt text: A group of social network plots showing different patterns of connectivity representing variations in phenotypic integration and modularity.

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Barn swallows are particularly well-suited for exploring the effects of divergent selection on complex trait architecture because they have been the subject of numerous studies of selection on multiple plumage traits across much of their broad, Holarctic breeding range. They comprise six closely related, yet phenotypically divergent subspecies (Turner 2006; Dor et al. 2010), and previous studies collectively show that sexual selection acts on several different signal traits such as tail length, ventral coloration, and throat coloration, and the strength of selection on these traits differs across populations (Scordato and Safran 2014; Safran et al. 2016b; Wilkins et al. 2016; Romano et al. 2017). Ventral color varies between males and females, and distinctiveness of different color patches varies between populations: in some populations, the throat patch is outlined by a band of black feathers (See Fig. 2: i.e., *H. r. rustica*, *transitiva*, and *savignii*), and in others, there is a broken or absent black band separating the throat from the rest of the ventral surface (i.e., *H. r. erythrogaster*, *tytleri*, and *gutturalis*). Biochemical analyses suggest that this variation (Figure 1) is caused by differences in melanin (phaeomelanin and eumelanin) deposition (McGraw et al. 2004). Population-level variation in plumage color is associated with population genetic differentiation (Safran et al. 2016a). Genetic loci underlying ventral plumage color are associated with reduction in gene flow when populations have overlapping spatial distributions (Schield et al. 2024), suggesting that plumage color plays a role in population divergence and reproductive isolation (Safran et al. 2016a; Romano et al. 2017; Scordato et al. 2020; Schield et al. 2021). Importantly, in populations with stronger evidence for selection on ventral and throat color, darker plumage in those patches is associated with increased fitness (McGraw et al. 2004; Safran et al. 2005; Hasegawa et al. 2010; Wilkins et al. 2016; Liu et al. 2018; Hund et al. 2020, 2021).

We investigated whether trait architecture in ventral plumage coloration is associated with evolutionary divergence in color across populations. First, we determined whether phenotypic integration among ventral coloration traits covaries with phenotypic and genomic divergence between populations. Second, we determined whether different combinations of plumage patches evolve as different modules, and whether the degree of modularity among plumage patches is associated with phenotypic divergence. Our results show that whether ventral plumage patches become more correlated with each other (variability in integration: Figure 1A) or evolve independently (variability in modularity Figure 1B) depends on which plumage patch is under selection: selection for darker color in breast, belly, and vent is associated with increased phenotypic integration across all patches in both sexes, while darker throat coloration is associated with increased modularity among patches in males only. In short, ventral plumage patches don't merely evolve independently: the degree to which they evolve independently depends on the target of selection. Together, these results highlight multiple evolutionary trajectories for complex trait evolution and provide important insights on how complex trait architecture is associated with rapid phenotypic differentiation among closely related populations.

Materials and Methods

Field Work: Barn swallows were captured using mist nets as part of long-term studies in the Czech Republic, England, Israel, Romania, Taiwan, Turkey, and the US States of Colorado and New York. Additional samples were collected from numerous international sampling expeditions (in Russia, the mainland of China, Morocco, and Egypt), spanning the entire breeding range of

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barn swallows. Birds were banded with USGS or locally mandated aluminum rings, standard morphological measures were taken, and a set of ventral feather samples were taken to quantify color, following previous methods (Safran et al. 2010; Wilkins et al. 2015). All research was associated with University of Colorado, IACUC protocol #1303 and in collaboration with on the ground researchers throughout the world.

Plumage Color: We measured three different components of color (hue, chroma, brightness) of 5-10 feathers taken from four different patches (throat, breast, belly, and vent) from 2,057 individual barn swallows sampled from 28 populations spanning the Northern Hemisphere for which we had all plumage color measurements for at least 12 individuals of each sex (Fig. 2A; Supplemental Table 1). Hue and chroma represent different aspects of chromatic perception and brightness is a metric of achromatic perception. Previous studies show that variations in different aspects of color correlate with different mechanisms of color production, and that these correlations are variable across species and patches (Butler et al. 2011). Thus, while some color components may be tightly correlated within or across patches in this species, examining the flexibility or stability of these correlations across populations informs how the complex architecture of plumage coloration diverges along with trait values.

The ventral plumage of barn swallows can range from light (high brightness, low chroma, more pale) to dark (low brightness, high chroma, more brown) color (Safran and McGraw 2004). Previous work showed that melanin-based ventral plumage color is associated with around 18% - 20% additive genetic variation (Hubbard et al. 2015). Additionally, features of the environment during early development predict variation in melanin-based coloration, including maternal effects and the nest environment in which nestlings are cared for by both parents (Hubbard et al. 2015). However, in a larger comparative analysis, features of the environment including variation in and mean temperatures as well as elevation are not predictive of pairwise differences in plumage color (Safran et al. 2016a). Thus, whereas the nest environment influences melanin deposition within populations, large-scale environmental differences do not explain population-level differentiation in plumage color overall.

Following (Safran et al. 2010), reflectance spectra were collected using SpectraSuite software (Ocean Optics v2.0.151) in combination with an Ocean Optics USB4000 spectrometer (range 200-1100nm), using a PX-2 pulsed xenon light source and a fiber optic probe angled perpendicular to the feather surface, at a distance that illuminated 2.5mm diameter feather area. We accounted for measurement error by averaging three separate measurements (lifting and re-placing the probe each time), each consisting of 20 spectrometric scans. From raw spectra, we then calculated average brightness, hue, and red chroma for each of the four ventral patches. Average brightness is the average percent reflectance from 300-700nm; hue is the wavelength between 550 and 700nm where the reflectance curve is steepest; and chroma is the proportion of light reflected in the red range (600-700nm), relative to the entire range (300-700nm). Previous work shows that ventral plumage in barn swallows do not reflect in the UV range (Safran and McGraw 2004).

Pairwise Genetic Distance: We incorporated a measure of pairwise population differentiation (F_{ST}) in our models for a subset of localities (n = 20) where genomic data were available for a minimum of two individuals from previous studies (Safran et al. 2016a; Scordato et al. 2017;

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Schield et al. 2021). Genomic data were filtered and processed as described in (Schield et al. 2021). Briefly, raw reads were quality trimmed and mapped to the European barn swallow (*Hirundo rustica rustica*) reference genome version 'Chelidonia' (Formenti et al. 2019). We then called SNP variants across the genome using GATK (McKenna et al. 2010) and filtered to retain SNPs with called genotypes in ≥ 60% of individuals and with a minor allele frequency ≥ 0.05. We then calculated genome-wide pairwise Weir and Cockerham's F_{ST} (Weir and Cockerham 1984) between each locality and *H. r. savignii* from Egypt using VCFtools (Danecek et al. 2011), taking the mean weighted F_{ST} as a measure of genetic distance. Because all analyses were performed between a given locality and the Egypt population, these genetic distance measures are all relative to Egypt. We chose Egypt for these comparisons based on previous evidence of this being a distinct, genetically structured population, although these comparisons could in principle be performed using any consistent reference population. Choice of reference population did not affect our statistical analysis of phenotypic integration (see below). Pairwise F_{ST} is shallow between all populations and the Egypt population (mean $F_{ST} = 0.066 \pm 0.03$).

Phenotypic integration: We calculated population-level measures of phenotypic integration in ventral coloration of barn swallows separately for males and females for each population for 28 populations, spanning all six named subspecies. We used hue, chroma, and average brightness measures from throat, breast, belly and vent patches, resulting in 12 measures per individual. We then calculated an index of phenotypic integration (PINT) as the variance of eigenvalues of the correlation matrix (Wagner 1984). We calculated the PINTc index (which corrects for the number of traits and populations sampled) using the {PHENIX} package (Torices and Muñoz-Pajares 2015) in R version 4.2.1. Larger variance indicates that most of the variation is explained along the dominant eigenvector (i.e., PC1). Thus, in a population with a high level of phenotypic integration across all ventral color patches, one color measurement is highly predictive of other color measurements regardless of color patch.

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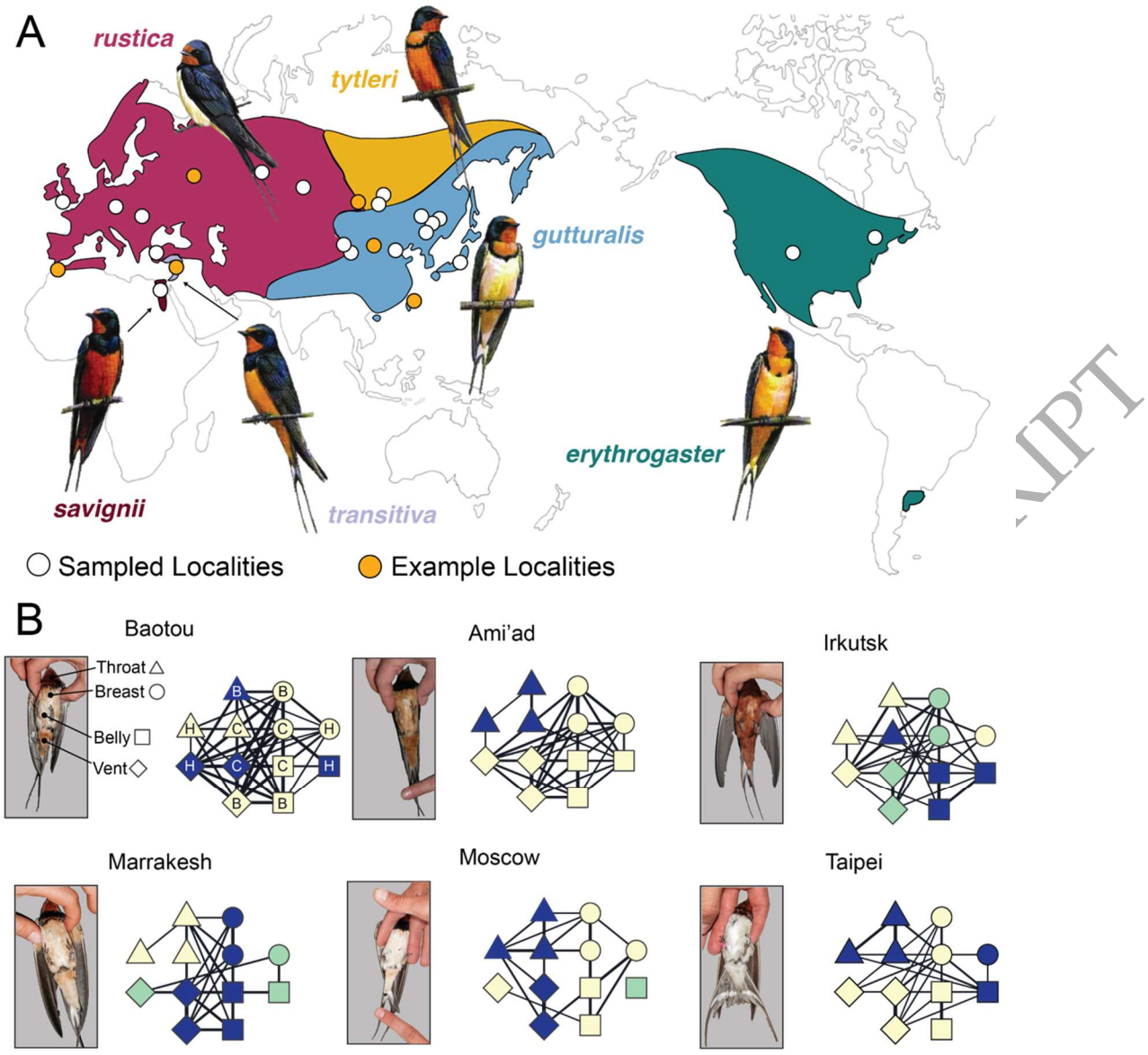


Figure 2: (A) Map of the global distribution of barn swallows and the 28 sampled sites included in the data. (B) Photos of the ventral side of barn swallows from six sites representing variation in average coloration and phenotype networks. Photos and phenotype networks for males are shown. In phenotype networks, the nodes represent 3 color measures from 4 patches sampled from each individual. Node shapes indicate the patch (Throat, Breast, Belly, and Vent), as indicated by the photo of a male from Baotou. The node labels in the network for Baotou indicate the color measures (B: Brightness, C: Chroma, and H: Hue). These nodes are shown in the same position across all networks. Edges are drawn between pairs of traits that have a Spearman's rank correlation > |0.3|, and edge widths are proportional to the correlation coefficients. Node colors represent different modules, as detected by community detection (see Methods). Populations are arranged from highest phenotypic integration in males (Baotou) to lowest in males (Taipei). Networks for males and females of all populations are shown in Supplemental Figures 1 and 2. Barn swallow illustrations by Hilary Burn.

Alt text: Map of the global distribution of barn swallows by subspecies, and example photo and phenotype networks from 6 sites.

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Statistical Analysis of Phenotypic Integration. We used a combination of statistical approaches including linear models, bootstrapping, and phylogenetic multilevel models to investigate the relationships between population average color trait value, sex, genetic relatedness among populations, and phenotypic integration.

Unequal sample sizes between the sexes could bias our comparisons between males and females. Thus, we used 10,000 bootstraps to account for sample size differences between the sexes and to generate confidence intervals for each linear model. In each bootstrap replicate, we randomly selected 12 individuals of each sex as our sample, resulting in equal sample size across all populations. We then fit our statistical model using each bootstrap replicate sample and report means and 95% confidence intervals of the effect of each variable. When the confidence interval does not overlap 0, we treat this as equivalent to statistical significance at $\alpha = 0.05$. We incorporated genetic distances into our models by merging genetic distance data with the plumage data for each of 20 populations (of the total 28) for which we had genetic and phenotypic data.

We first included an interaction effect between plumage color and sex. After finding no interaction effect, we ran final linear models of *PINT.c ~ average color measure + genomic pairwise* F_{ST} *from Egypt + sex* for all four patches. We extracted slope estimates of each term across the 10,000 bootstrap replicates, calculating mean and 95% confidence intervals to assess significance of each variable. To facilitate comparison between the effects of the color and genomic measures, we scaled and centered these variables in these models. We considered a variable to be significant in predicting phenotypic integration if the 95% confidence interval of its slope estimate did not overlap zero. These calculations were conducted using base functions in R. We conducted these analyses for chroma, brightness, and hue, but we focus our discussion on the results for models using chroma for each plumage patch as our predictor variable because it is related to color saturation, which has been shown to be under selection in some populations (Scordato and Safran 2014; Wilkins et al. 2015; Romano et al. 2017). We report the results for brightness and hue in Supplemental Materials.

As an alternative approach, we also used the entire empirical dataset (i.e., not the subsampled bootstrap replicates) to conduct a phylogenetic multilevel model and included a genetic relatedness matrix (grm) as a random effect in the form: PINT.c ~ *average chroma* + *sex* + (1 | gr(population, cov = grm)). We fit this model as a Bayesian Generalized Linear Multilevel Model using the *brm* function in the R package 'brms' (Bürkner 2017). We used default priors and ran four chains of 20,000 iterations with a burn-in period of 1,000 and thinned every 10 steps. While this approach may better control for genetic relatedness across populations, the models are too computationally intensive to conduct on the bootstrap replicates. Thus, we consider this a complementary approach that accounts for genetic relationships among populations in a different way (i.e., using grm instead of F_{ST}) but does not account for unequal sample sizes as we do with bootstrap replicates discussed above.

Finally, we fit the linear models without genomic data to the full 28 population dataset using the bootstrapped dataset to assess the patterns with a larger sample size of populations. In all of our models, we again started by including an interaction effect between color and sex, but finding no interaction effect, we opted to use an additive model (e.g., PINTc ~ Breast Chroma + Sex) given

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the relatively small sample size of populations in our analysis. This approach makes use of our full dataset of plumage color measurements including populations without genomic sampling. Our findings are consistent across all three statistical approaches, providing us with more confidence in our inference.

Modularity analysis. We first identified modules among the plumage traits using a network theoretical approach. We used this analysis to test two non-mutually exclusive hypotheses. First, if modularity represents different production mechanisms of color traits (Butler et al. 2011), then we should observe that chroma, hue, and brightness (or some combination thereof) form different modules. Second, if different plumage patches evolve separately, then throat, breast, belly, and vent color traits (or some combination thereof) would form different modules.

We built phenotype networks by calculating Spearman correlation index (ρ) for each pair of traits, then filtering out weak correlations. We used three different thresholds ($\rho < 0.1$, $\rho < 0.2$, $\rho < 0.3$) to assess the sensitivity of our analyses to filtering. We then used the R package {igraph} (Csardi and Nepusz 2006) to implement the fast greedy network community detection algorithm (Clauset et al. 2004) to our phenotype network of the 12 plumage color traits for each population for each sex. Community detection algorithms assign nodes in a network (here, color traits) into discrete clusters based on their patterns of connectivity, and these node clusters can represent structural modules of traits (Wilkins et al. 2015). We used this information to build a matrix of cluster co-membership where cell value of 1 indicated that the pair of traits were assigned to the same node cluster, and 0 if not. To assess cluster co-membership at the species level, we summed the matrices across all populations to visualize overall patterns, then used hierarchical clustering to identify patterns of consistent clustering of traits across all populations. Finally, we used a hypothesis testing approach using network permutations to assess which sets of traits were found in the same cluster across populations more often than expected by chance. We permuted the cluster assignment for each trait in each population and recalculated the frequencies of cluster co-membership and determined the upper 95% confidence limit of this frequency as the upper end of the probable frequency of co-membership. We then repeated this procedure 1,000 times. We inferred that a set of traits composed a 'module' if they were assigned to the same cluster more often than the upper confidence interval of our permuted networks. We then assessed the robustness of these conclusions by determining if they were consistent across the three different thresholds for network filtering.

Having identified the throat patch as a consistently separate module from the rest of the plumage traits, we sought to test the hypothesis that directional selection on a module can increase modularity—i.e., stronger correlations among traits within in the module under selection, while weakening correlations between traits within and outside the module (Melo and Marroig 2015). To adapt this framework, we first calculated the average edge weights (i.e., average correlation coefficients) for three types of edges: those connecting traits within the throat patch, those connecting traits in the other patches, and those connecting the traits in the throat patch and other patches. Then, we fit a linear model with these average edge weights as the predictor variable, the edge type, population average throat color (chroma), and their interaction as the predictor variables. We predicted that we would see a positive relationship between average edge weight within the throat patch and population throat color, a negative relationship between the average edge weight between the throat and other patches and population throat color, and a significant

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interaction effect, suggesting those slopes are significantly different (Figure 4B). We then repeated this analysis with breast, belly, or vent as the focal patch. We predicted that these focal patches would now show the expected relationship.

Results

Relationships between patch-specific plumage color, genetic divergence and phenotypic integration

Our linear models using 10,000 bootstrapped samples to control for unequal sample size showed that genetic distance had a significant effect on phenotypic integration in all models, while sex was not a significant factor in any model (Table 1; Supplemental Figure 4). In addition, average chroma of breast, belly and vent were significant predictors of phenotypic integration after accounting for genetic distance, but throat chroma was not (Table1; Figure 3 shows breast and throat; patterns for other patches shown in Supplemental Figure 3). Our phylogenetic multilevel models yielded the same results (Supplemental Table 2). We found similar results for breast, belly, and vent brightness, but not hue (Supplemental Table 3). Together, these results suggest that variation in the architecture of ventral coloration in barn swallows is related to both the evolutionary history of population divergence (genetic distance) and the degree of ventral coloration (particularly chroma and brightness) in the breast, belly and vent but not throat. When we repeated the analysis with only the phenotypic and sex data for all 28 populations, we found very similar coefficients for the effect of average chroma on phenotypic integration (Supplemental Table 4).

Patterns of modularity in color traits within and across plumage patches

Our analysis of the consistency of module composition showed that throat color traits are almost always assigned to the same module with each other. Among the breast, belly, vent plumage patches, brightness and chroma were consistently assigned to the same module, but hue was not (Fig. 4A). This result is consistent with the relationships that chroma and brightness of breast, belly, and vent patches show with phenotypic integration (Table 1, Figure 3, Supplemental Figure 1). In females, breast, belly, and vent hue were clustered together as a module, but this pattern was not robust to small changes in the threshold values for filtering network edges (Supplemental Figure 5). Thus, we detected strong support for the hypothesis that one patch (throat) forms a discrete module, and some support for the hypothesis that different measures of color with different production mechanisms (hue vs. brightness and chroma) form different modules.

Throat patch increasingly evolves as a separate module in populations with darker throat color

Having found evidence that the throat patch generally acts as a separate module, we tested whether this modularity becomes more pronounced in association with the evolution of throat color, as would be predicted by the corridor selection model (Melo and Marroig 2015; see Methods). These predictions were supported in males (Fig. 4C: linear model interaction effect: $t = 2.33$, $P = 0.022$), but not females (Fig. 4D: linear model interaction effect: $t = -0.58$, $P = 0.56$), nor for breast, belly, or vent plumage patches (Supplemental Table 5). However, this interaction effect was not significant when using the reduced dataset of populations with genomic information (N = 20), regardless of whether or not we include genomic relatedness. Thus, this pattern seems to be more sensitive to sample size compared to the patterns of phenotypic

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integration. We did not see this pattern when we examine how correlations of color traits within other patches vary across populations with different average breast, belly, or vent coloration (Supplemental Figure 6).

Table 1: The average estimate of the effect of genomic distance, population-level average color (chroma) of different patches, and sex on phenotypic integration ($PINT_c$) for 20 populations for which we had both genomic and phenotypic data. The values represent the average and 95% confidence intervals of the effect over 10,000 bootstraps with equal sample size across populations. The effects where the 95% confidence interval does not overlap zero are highlighted in bold.

Patch	**Estimate for F_{ST} effect (lower CI, upper CI)**	**Estimate of color effect (lower CI, upper CI)**	**Estimate of sex effect (lower CI, upper CI)**
Throat	**0.16 (0.02, 0.30)**	0.01 (-0.14, 0.16)	0.15 (-0.13, 0.43)
Breast	**0.20 (0.06, 0.33)**	**0.23 (0.08, 0.38)**	0.10 (-0.17, 0.38)
Belly	**0.22 (0.09, 0.35)**	**0.22 (0.08, 0.37)**	0.09 (-0.18, 0.37)
Vent	**0.19 (0.05, 0.32)**	**0.24 (0.10, 0.38)**	0.08 (-0.19, 0.36)

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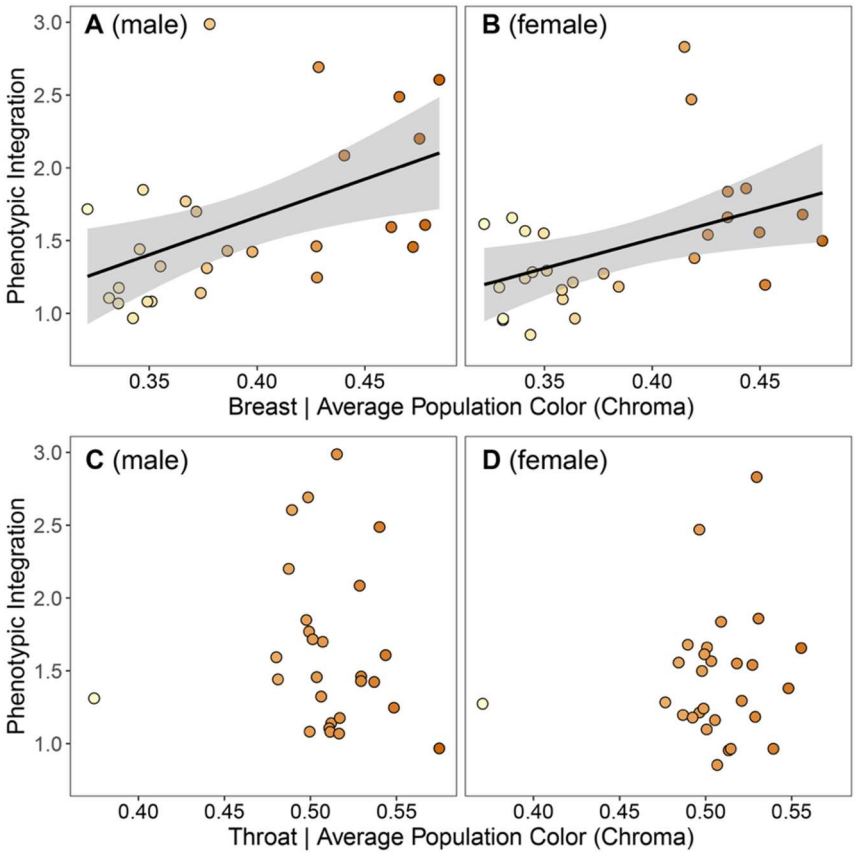


Figure 3: Relationship between phenotypic integration index and population-average breast (A,B) and throat (C,D) chroma, using all 28 populations for which we had plumage data (see Supplemental Table 4 for model outputs). Relationships for males(A,C) and females (B,D) are presented separately in each row. The color of the dots represents the average coloration of the patch in each population. Linear regression lines are shown for each plot where relationships are statistically significant.

Alt text: Graphs comparing the relationships between average population color of different plumage patches (breast, throat) and phenotypic integration, with linear regression fit shown for significant relationships.

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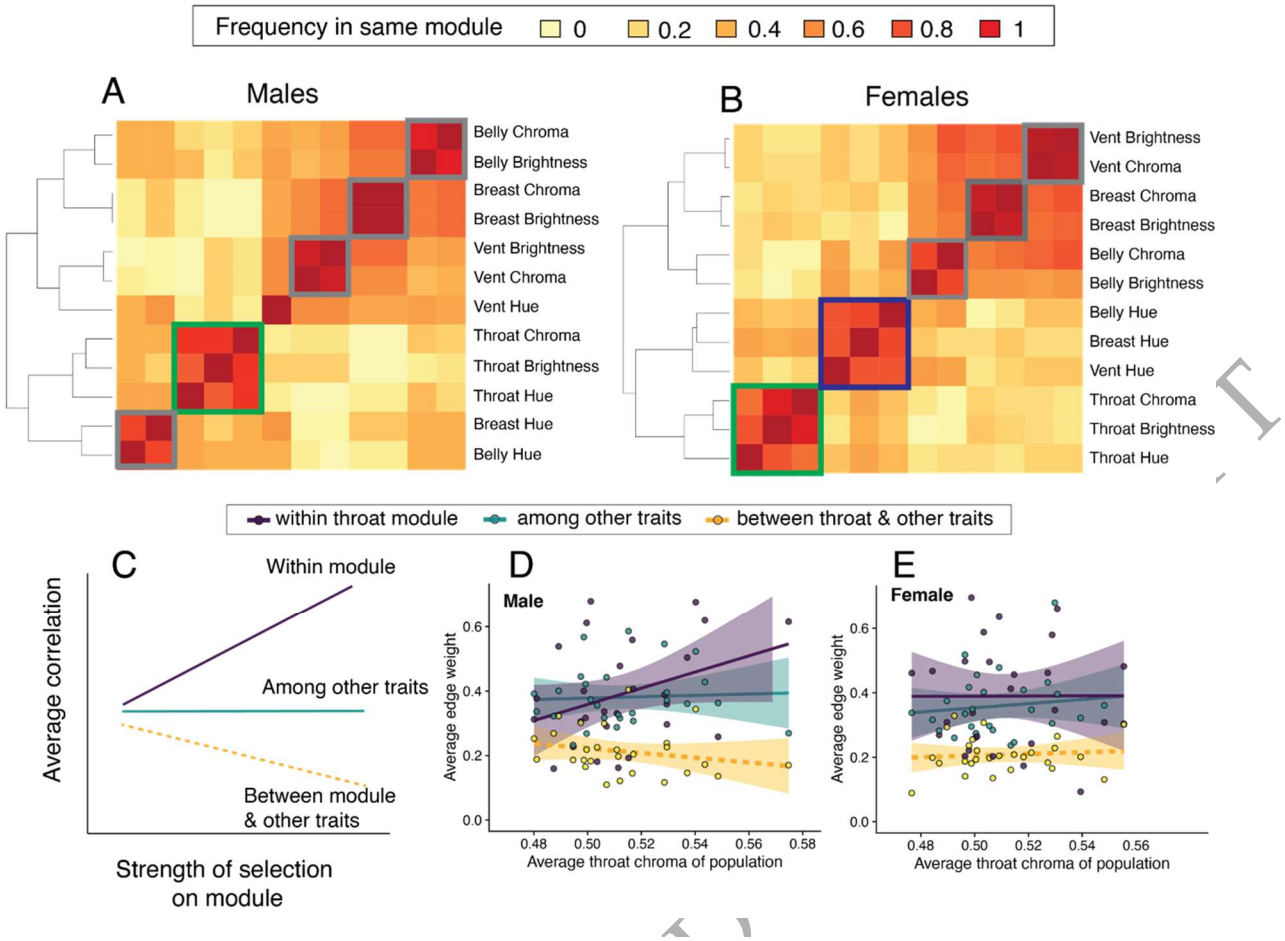


Figure 4: Analysis of modularity of plumage coloration. Heatmap of module co-membership of each pair of traits across all populations with thick borders indicating statistically significant modules (A,B) show that throat color traits (green border) cluster together as a separate module in both sexes. Brightness and chroma are significantly clustered in all patches. Additionally, breast, belly, and vent hue are clustered into a module in females, but this pattern is sensitive to the threshold for filtering edges in the phenotype network (Supplemental Materials). The matrix is symmetrical (rows and columns are the same), and ordering of rows/columns of the heatmap matrix is based on hierarchical clustering (dendrogram shown on left). The color represents the proportion of populations in which each pair of traits are assigned to the same cluster in the phenotype network. Predictions based on existing theory (Melo and Marroig 2015) suggesting that, when traits in one module are under directional selection, the average correlation should increase within the module under selection and correlations with traits outside the module should decrease(C). These predictions are supported for evolution of throat coloration among males (D), as within-throat correlation increases with darker throat while the between-module correlation decreases. However, these predictions are not supported for females (E).

Alt text: Heatmaps showing the patterns of modularity in plumage coloration for males and females, and graphs showing the relationships between throat color and average correlations within the throat module versus other traits.

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Discussion

Our results suggest that evolution of coloration of a plumage patch is accompanied by changes in patterns of correlations in color measures within and across patches, reflecting a reorganization in the structure of plumage patterns as a whole. However, this relationship is different depending on the color patch. Darker color in some patches (e.g., breast, belly, vent) is associated with greater phenotypic integration, as illustrated in Figure 1A. In contrast, darker throat coloration is associated with greater modularity, i.e., weaker correlations with other color traits, as illustrated in Figure 1B. Moreover, this relationship was sex-dependent—the evolution of darker throat color leads to greater modularity in ventral coloration only for males. These patterns arise across divergent populations of the same species, and both genetic and phenotypic differences together explain variation in complex trait architecture.

There is remarkable congruence between our results and the recent evidence for how simple genetic mechanisms allow throat coloration to evolve as a separate module when it is under selection. For example, in a pair of North American warblers that frequently form hybrids (*Vermivora chrysoptera* and *V. cyanoptera*), a single SNP in the Agouti Signaling Protein (ASIP) locus strongly explains variation in throat coloration--the most clearly distinguishable plumage feature between the species (Toews et al. 2016). Similarly, throat color polymorphism within three species of wheatears (genus *Oenanthe*) is controlled by variation in the ASIP locus (in this case, via insertion of a transposable element), and introgression of this gene explains convergence in throat color between species (Lutgen et al. 2025). In this case, there seems to be differential selection on throat and mantle (back) coloration. In contrast to these cases of throat color evolution, the divergence in coloration between two North American warblers (*Setophaga townsendii* and *S. occidentalis*) involves correlated evolution between the crown, cheek, flank and breast coloration (though interestingly, not throat bib), which were all associated with a sequence changes in one gene block associated with ASIP (Wang et al. 2020). Thus, the emerging picture of genome architecture underpinning avian color evolution during divergence is strikingly compatible with our finding that, when under selection, throat coloration evolves as a separate module while breast coloration evolution is associated with integration between color patches.

Barn swallows are a particularly illustrative system for understanding the relationships between integration, modularity and color evolution because a long record of empirical studies show complex and divergent patterns of sexual selection across populations and subspecies (Scordato and Safran 2014; Romano et al. 2017; Lotem et al. 2022), and that this results in restriction of gene flow (Schield et al. 2024). Population divergence in phenotypic integration and modularity of ventral coloration in barn swallows are likely driven by both selection and neutral processes, including genetic drift and changes in genome architecture. Differences in phenotypic integration between populations are predicted by both genetic distance and population differences in average coloration (specifically chroma and brightness) of breast, belly, and vent. However, we note that the significant contribution of genetic distance to phenotypic integration may also include effects of selection on genomic divergence (i.e., isolation-by-adaptation) because up to 42% of genomic variation is attributable to traits under selection, such as wing length and ventral coloration (Safran et al. 2016a). Moreover, recent analysis shows that genetic loci associated with breast

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coloration show clear signatures of divergent selection between subspecies (Schield et al. 2024). Thus, we suggest that our analysis may underestimate the effect of selection on phenotypic integration. In addition, the difference in the effect of sex on patterns of phenotypic integration versus modularity is interesting: we detected no effect of sex on phenotypic integration, but the relationship between throat coloration and modularity was only apparent among males. We do not yet know the cause of such differences, but possibilities could include differences in strength of sexual selection, or differences in developmental or environmental influences on expression of aspects of plumage color. At this point, our evidence for the role of sexual selection on shaping patterns of integration and modularity of plumage coloration remains indirect because targets of sexual selection in many of our focal populations are unknown. If this increase in modularity—i.e., relative increase in correlation only within the throat patch concurrent with weaker correlation between throat color and other patches—does turn out to be driven by stronger selection on throat coloration, then this could provide empirical support for the role of directional selection on the evolution of modularity (Melo and Marroig 2015). We suggest that our results provide a basis for new avenues of exploration for the evolution of phenotypic integration and modularity in the context of sexual selection and population divergence.

This study makes new contributions to the study of evolution of structure and function of complex signal traits. Work on phenotypic integration of sexual ornamentation has existed began decades ago (e.g., plumage color in birds: Badyaev and Hill 2000, Badyaev and Young 2004; coloration and anti-predator behavior in snakes: Brodie 1992; colorful eyespots in butterflies, Beldade et al. 2002). However, there has been renewed interest in applying a systems approach to animal communication in recent years with the advance of network analysis in broader areas of biology (Hebets et al. 2016). Network-based analyses have been used to summarize and compare the structure and function of multi-component and multi-modal signals in many taxa including birds (Wilkins et al. 2015; Schaedler et al. 2021), treefrogs (Reichert and Höbel 2018), spiders (Rosenthal et al. 2018), and butterflies (Chotard et al. 2023). To date, much of these studies have focused on the functional consequences of signal architecture: for example, whether phenotypic integration across multiple modalities may improve robustness of communication systems, or whether modularity in signal traits can facilitate communication of different information to signal recipients. Our findings suggest that signal function and resulting selection could in turn shape the architecture of complex signal traits.

Finally, this study opens the door to a new, large-scale comparative framework for investigating population divergence in complex signal. Such work requires intensive field work and an international network of collaborators for wide-spread species, but such studies could lead to a greater understanding for how the evolution of traits are accompanied by changes in complex trait architecture. This may be fruitful for the study of animal coloration because it intersects many major themes in evolutionary biology, including function, sensory systems, genetic mechanisms, and speciation (Hubbard et al. 2010; Cuthill et al. 2017; San-Jose and Roulin 2017; Delhey et al. 2023).

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Supplementary material: Supplementary material is available online at *Evolution*.

Data availability:

Associated data and R code are available on Dryad: https://doi.org/10.5061/dryad.vt4b8gv85

Author contributions:

Conceptualization: DS, MRW, RJS; Data curation: MRW, DRS, ESCS, YL, RJS; Formal Analysis: DS, MRW, DRS; Funding Acquisition: RJS, MRW, DRS, ESCS, DS; Investigation: MRW, DRS, ESCS, YL, RJS, DS; Methodology: MRW, DRS, ESCS, YL, RJS; Project Administration: MRW, DRS, ESCS, YL, RJS; Resources: MRW, DRS, ESCS, YL, RJS; Supervision: RJS, DS; Validation: MRW, DS; Visualization: MRW, DS; Writing – original draft: DS, MRW, RJS; Writing – review & editing: DS, MRW, DRS, ESCS, YL, RJS

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Conflict of interest:

The authors declare no conflict of interest

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