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Genome-wide differentiation in closely related populations: the roles of selection and geographic isolation

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Abstract

Population divergence in geographic isolation is due to a combination of factors. Natural and sexual selection may be important in shaping patterns of population differentiation, a pattern referred to as 'Isolation by Adaptation' (IBA). IBA can be complementary to the well-known pattern of 'Isolation by Distance' (IBD), in which the divergence of closely related populations (via any evolutionary process) is associated with geographic isolation. The barn swallow *Hirundo rustica* complex comprises six closely related subspecies, where divergent sexual selection is associated with phenotypic differentiation among allopatric populations. To investigate the relative contributions of selection and geographic distance to genome-wide differentiation, we compared genotypic and phenotypic variation from 350 barn swallows sampled across eight populations (28 pairwise comparisons) from four different subspecies. We report a draft whole genome sequence for *H. rustica*, to which we aligned a set of 9,493 single nucleotide polymorphisms (SNPs). Using statistical approaches to control for spatial autocorrelation of phenotypic variables and geographic distance, we find that divergence in traits related to migratory behavior and sexual signaling, as well as geographic distance together, explain over 70% of genome-wide divergence among populations. Controlling for IBD, we find 42% of genome-wide divergence is attributable to IBA through pairwise differences in traits related to migratory behavior and sexual signaling alone. By (i) combining these results with prior studies of how selection shapes morphological differentiation and (ii) accounting for spatial autocorrelation, we infer that morphological adaptation plays a large role in shaping population-level differentiation in this group of closely related populations.

Keywords: climate variability, genomic divergence, Genotyping By Sequencing, reproductive isolation, population genetics, speciation

Introduction

Species are thought to arise primarily as populations diverge in spatial isolation and accumulate differences that preclude their reproduction upon secondary contact (Mayr 1963, Coyne and Orr 2004). This has been argued to be particularly true for birds (Price 2008). There is also long-standing interest in the extent to which population divergence can arise or increase in the context of incomplete spatial isolation, coupled with selection against immigrants or hybrids to offset homogenizing genetic exchange ('divergence with gene flow': Nosil et al. 2008, 2009). More generally, spatial isolation is a continuum, and divergence can arise as a consequence of: spatial isolation and genetic drift (isolation by distance, IBD; Wright 1943, Slatkin 1993); spatially varying natural and sexual selection (isolation by adaptation, IBA, or a subset of IBA: isolation by environment, IBE; Rundle and Nosil 2005, Crispo et al 2006, Nosil et al. 2009, Schluter 2001, 2009, Shafer and Wolf 2013); or both (Lande 1980, Shafer and Wolf 2013, Wang and Bradburd 2014). While the contributions of spatial isolation and divergent selection to population differentiation have been research areas in evolutionary biology since the beginning of empirical population genetics (Fisher 1930), only more recently have we begun to characterize genomic divergence by simultaneous tests of IBA and IBD (e.g., Wang and Summers 2010, Wang et al. 2013, Bradburd et al. 2013, Wang and Bradburd 2014).

In comparison to accumulating evidence for the role of ecological adaptation (one form of IBA) and geographic isolation (IBD) in population divergence and speciation (Shafer and Wolf 2013, Wang and Bradburd 2014), there is little empirical data on the contribution of sexual selection to population genetic divergence (e.g., Parchman et al

2013, Baldassarre et al. 2014, Morgans et al. 2014). Adaptation to the local environment may come in the form of sexual selection if competition over mates differs among closely related populations or signal traits reflect variation in the environmental context in which these traits are developed or advertised (Ingleby et al. 2010, Maan and Seehausen 2011, Baldassarre et al. 2013, Safran et al. 2013, Matute, 2014, Seehausen et al. 2014). The lack of such data represents a major gap in our understanding of speciation because divergence in sexual traits is a common form of phenotypic differentiation among populations that may strongly relate to reproductive isolation (e.g., West-Eberhard 1983, Panhuis et al. 2001, Irwin et al. 2001, Ritchie 2007, Curie Network 2012, Safran et al. 2012, Langerhans and Riesch 2013, Seddon et al. 2013). Moreover, previous work has shown that sexual signals can evolve more rapidly than traits related to ecological adaptation (e.g., Kingsolver et al., 2001, Svensson et al. 2006, Kingsolver and Pfennig 2007, Siepielski et al. 2011, Seddon et al. 2013). Collectively, these observations suggest that sexual signals could be an important feature of biological diversification, especially in the early, formative stages of speciation (Kraaijeveld et al. 2011). Here, a testable prediction for a role of sexual selection in divergence is that divergence in aspects of phenotype related to mate selection is positively associated with genetic divergence, either due to increased local adaptation, reduced immigration, or both. This is similar to evidence for ecological speciation, where divergence in traits related to ecological adaptation predicts population genetic differentiation (Shafer and Wolf 2013).

Thus, in its most general form, IBA predicts associations between genomic divergence and differentiation in traits related to both ecology and mating success. Note

that ecological adaptations and mating adaptations are not mutually exclusive, and indeed, may even be related, as sexual signals necessarily evolve in an ecological context (Ingleby et al. 2010, Maan and Seehausen 2011, Baldassarre et al. 2013, Safran et al. 2013, Seehausen et al. 2014). Thus, important questions remain about the relative contributions and likely interactions of sexual selection, natural selection, and geographic distance to genomic differentiation among closely related populations (e.g., van Doorn et al. 2009, Maan and Seehausen 2011, Wagner et al. 2012, Safran et al. 2013). For example, sexual selection has been shown to interact with ecological context in the early stages of population divergence and the formation of premating barriers to reproduction (Scordato et al 2014), yet we know very little about the relative importance of each selective process in the accumulation of biologically relevant genomic differences among populations.

Closely related populations with variation in geographic distance are advantageous for identifying spatial and trait-based predictors of genomic divergence. To address the extent to which sexual and natural selection either interact or singly influence population divergence, we analyzed patterns of genomic divergence as a function of variation in aspects of phenotype known to be involved in mate selection and migration, as well as relevant measures of environmental variation (using elevation data and long-term climate databases), in a widespread, phenotypically divergent, yet young group of subspecies: barn swallows (*Hirundo rustica*). Barn swallows include six sub-species worldwide (Figure 1), with populations varying in: (1) geographic distance from one another, (2) trait combinations with known importance in sexual signaling, (3) breeding and non-breeding environments, and (4) migratory behavior (reviewed in

Scordato and Safran 2014). A recent phylogenetic reconstruction for this group of closely related sub-species indicates that they are monophyletic with respect to other members of the genus *Hirundo* (Dor et al. 2010). Additionally, recent mtDNA, phylogeographic, and microsatellite analyses suggest this group formed rapidly (between 100,000 and 27,000 years ago; Zink et al. 2006) and is not strongly genetically differentiated, despite marked sexual signal and behavioral differentiation among populations (Dor et al. 2010, Dor et al. 2012). Phenotypic and genomic divergence among barn swallow populations is likely due to a combination of selection and drift in the context of variable degrees of geographic isolation. For example, populations in North America and Eurasia have likely diverged without gene flow, whereas evidence suggests that populations in the Middle East have experienced recent historical or ongoing gene flow with populations in Europe (Dor et al. 2010, 2012).

Previous research in barn swallows has demonstrated phenotypic divergence in traits important to both sexual selection (morphological and behavioral sexual signals; e.g., Safran and McGraw 2004, Safran et al. 2005, Vortman et al. 2011, Vortman et al. 2013, Scordato and Safran 2014) and natural selection (traits related to flight, foraging, and migration; reviewed in Turner 2006). One of six well-characterized sub-species within the larger *H. rustica* complex, the European barn swallow (*H.r. rustica*), has been the subject of intense research activity over the last twenty years, with an emphasis on sexual selection (Turner 2006). This work clearly demonstrates that females mate preferentially with long-tailed males, and their offspring experience advantages over those of shorter-tailed males (e.g., Møller 1994). By contrast, in two North American

populations of *H.r. erythrogaster,* males with darker plumage color have greater social and extra-pair mating success (Safran et al. 2005, Safran et al. *in revision*). In the east-Mediterranean distributed *H.r. transitiva,* males with a combination of darker ventral plumage and longer streamer lengths are favored by females through social and extra-pair mating decisions (Vortman et al. 2011, Vortman et al. 2013).

Both experimental and observational data suggest that tail streamer length and ventral color are under varying degrees of sexual selection in different populations of barn swallows, and previous work has defined characteristics of wing shape, such as wing length, as being associated with migratory behavior (Von Ronn et al 2016). We therefore use these traits to examine the roles of sexual and natural selection in contributing to genome-wide divergence among populations of barn swallows. Further, previous work has shown that variation in ventral color (Saino et al 2013, Hubbard et al. 2015) and tail streamer length have heritable components (Møller 1994), indicating that they are subject to evolutionary change and thus relevant aspects of phenotype to investigate in terms of their influence on genome-wide divergence.

Here, we genotyped thousands of SNPs (using genotyping-by-sequencing, GBS) in 354 barn swallows from eight populations, representing four of six subspecies, distributed across the Northern Hemisphere. Additionally, we report a draft whole genome sequence for a male *Hirundo rustica erythrogaster*, which was constructed to ensure that GBS sequences cleanly assembled to a reference and to reduce problems with duplicates/paralogs. We use these population genomic data to address the extent to which population genomic divergence (based on average genome-wide F_{ST}) is associated with divergence in (i) sexual signals, (ii) wing length, (iii) features of climate,

and/or (iv) geographic distance. The first three factors all relate to IBA, while the fourth relates to IBD. A specific objective of this study is to partition variance in pairwise genomic divergence as a function of geographic distance (IBD); population differences in ecology, i.e. climate, (IBA, influenced by natural selection); wing morphology related to flight and migratory behavior (IBA, influenced by natural selection); and differences in morphology related to sexual communication (IBA, influenced by sexual selection).

METHODS

Field Data Collection

We sampled individuals from eight locations representing four sub-species across the barn swallow breeding range (Table 1). The following samples or measures were taken from male barn swallows known to be breeding at each site: 1) a sample of ventral feathers for objective color quantification, 2) length of wing and tail streamers (outer rectrices; see Table 2 for a complete description), and 3) blood samples as a DNA source from each bird (approx 50 µl, stored in 2% SDS lysis buffer). See Figure 1 and Table 1 for sampling location and final sample size information.

Genomics Methods

Genome Assembly. A draft genome was assembled, to provide a resource for future work and to ensure that regions used for GBS cleanly assembled to a reference. A draft genome assembled with moderate to high coverage enables the identification of single-copy portions of the genome, reduces the challenges associated with distinguishing close paralogs from alleles at the same locus, and (depending on quality and scaffold

sizes) can provide context of the genomic location of GBS loci. The DNA for our genome assembly came from a male barn swallow with a well-known reproductive history in our Boulder, Colorado study site (ID 2540-44680). This male was first captured in 2008 and had five successive breeding seasons at the same location (from 2008 through 2012). To generate a draft reference genome we obtained sequences from four lanes of Illumina HiSeq platform, two lanes of 101 base-pair reads from two paired-end libraries and two lanes of 101 base reads from a mate-pair library from Macrogen (www.macrogenusa.com). One paired-end library had an insert size of 176 bp, while a second paired-end library had an insert size of 454 bp. The mate-pair average insert size was 1,458 bp. We obtained a total of 27 Gbp from paired-end library one, and 17.4 Gbp from library two. From the two lanes of mate pair sequence we obtained 43.4 Gbp. After cleaning to remove low quality reads (lower than a base quality of 20 on either end of the read) and common contaminants, 129.4 million pairs of reads remained in library one, 80.0 million pairs in library two, and 95.8 million pairs in the mate pair library remained, for a total of 61.7 Gb of sequence. Based on an estimated genome size of 1.3 Gb (Andrews et al. 2009) this is an average of 47x coverage.

Reads were assembled using SOAPdenovo 2.04, using a K-mer length of 47, an edge coverage cutoff of 3, a k-mer frequency cutoff of 3, and an arcweight filter of 3. Repeats were resolved with reads. Otherwise, parameters were as defaults. After removing short scaffolds (below 1000 bp) we had a total of 100,153 scaffolds, and a total assembly length of 1.1Gb, 85% of the estimated 1.3 Gb genome size (Andrews et al. 2009). The average scaffold length was 11,010 bp, the longest scaffold was 732,517

bp, the N50 38,844bp, and N90 was 3,718. Of this 1.1 Gb of assembled sequence, a total of 1.06 Gb could be conservatively mapped to the *Ficedula albicollis* genome (Ellegren et al. 2012), using blastn with a minimum e-value cutoff of 10^{-80} and a minimum sequence similarity of 80%.

This alignment of our *Hirundo rustica* genome assembly to the most closely-related, well-annotated genome assembly for Collared Flycatcher *Ficedula albicollis* (Ellegren 2012), shows a high degree of sequence conservation. Over 91% of our single-copy, assembled sequence could be uniquely placed onto the genome. Thus, although we do not have a genetic or physical map placing our sequences onto chromosomes, we can provisionally place virtually all of our assembled genome using sequence similarity to existing avian genome sequences. Given the high degree of synteny found across passerines (Backström et al 2008, Ellegren 2013, Kawakami 2014), and among much more distant avian lineages (Derjusheva et al 2004, Zhang 2014), taking advantage of existing related genomes enabled us to infer which markers were likely to be linked on chromosomes. We also identified markers likely to be on autosomes and on the sex-determining Z chromosome. As the barn swallow populations we focus on in this paper exhibit only slight genome wide differentiation (see Results), this draft genome is ideal for use as a reference for aligning the GBS SNPs described below.

Population Genomics. We generated DNA sequence data from 354 individual barn swallows sampled across eight populations (see Table 1). We constructed reduced genomic complexity libraries for each individual using a restriction fragment-based procedure (Parchman et al., 2012; Gompert et al., 2012). We first digested genomic

DNA with two restriction endonucleases (EcoRI and MseI) and ligated double-stranded adaptor oligonucleotides to the digested fragments. These oligonucleotides consisted of the priming sites for Illumina sequencing, followed by eight, nine, or ten base pair barcode sequences that allow for the unique identification of sequences from each individual. This method has been successfully used to generate population genetic data for a large number of projects (Gompert et al. 2012, 2014b, Parchman et al. 2012, 2013, Nosil et al. 2012b, Mandeville et al. 2015); a full version of the protocol is available at dryad (doi:XXX). We used 354 unique barcoded adaptors, which allowed us to sequence all individuals in one Illumina Hiseq sequencing lane. After the restriction and ligation reactions, we pooled all samples and used standard Illumina PCR primers to amplify the barcode-adapted fragments. We separated the amplification products on a 2% agarose gel and excised fragments between approximately 350 and 450 bp in length. We purified these fragments using Qiagen's Qiaquick Gel Extraction Kit (Qiagen Inc.). Concentration and quality of the pooled library was evaluated on an Agilent BioAnalyzer qPCR. Sequencing was performed by the National Center for Genome Research (Santa Fe, NM, USA) with 100 base pair single end reads, and yielding 98,401,301 reads following standard contaminant filtering.

We used a custom Perl script (dryad doi:XXX) to remove bases associated with the barcode and EcoRI cut site for all 98,401,301 sequences and to replace the sequence IDs with individual IDs for each DNA sample. This script also corrected barcodes with potential single or double base mismatches due to sequencing or oligonucleotide synthesis errors. Sequences were then split by individual for further analysis. Four of the individuals had fewer than 2,000 reads and were disregarded,

which left 350 individuals for further analysis. We used *bwa* v 0.7.12 (Burrows-Wheeler Aligner; Li & Durbin 2009) to assemble data for each individual against the barn swallow genome assembly, with an edit distance of 4 and the remaining parameters set as default. This edit distance ensures that, even with 1% sequencing errors typical of Illumina sequencing, distant alleles from divergent subspecies will map to the genome, while nevertheless preventing non-specific alignments. Across all individuals an average of 61.66% of reads assembled, with 97.5% of individuals having 57.9% or greater percentage of reads assembling. We used *samtools* v 1.2 and *bcftools* v 1.2 (Li et al., 2009) to identify variant sites in the assembled sequences and obtain genotype likelihoods for variable sites. To identify variants, we required data for 60% or more individuals. This resulted in 67,773 single nucleotide variants. A complete list of parameters used for assembly and variant calling are available from the authors by request.

We used point estimates of allele frequencies in all 350 individuals to separate this set into 22,328 common variants (minimum minor allele frequency of 5% or greater) that were used for further analysis and 45,445 rare variants that were disregarded. We focused on comparatively common variants because they are more likely to occur in more than one location and contain information about population histories (Gompert et al. 2012). To avoid analyzing SNPs with highly correlated allele frequencies, we randomly sampled a single SNP from each of the 100 bp regions in the assemblies (GBS loci are 100 bases in length). For the resulting final set of 9493 single nucleotide polymorphisms (SNPs), the average coverage depth per individual per site was 1.5× (0.09-3.57, 2.5% and 97.5% quantiles). This kind of low coverage genomic data is

297 appropriate for population-level inferences when analyzed with models that incorporate
298 uncertainty arising from variability in sequencing coverage across individuals and loci
299 (e.g., Neilsen et al. 2011, Gompert et al. 2012, Gompert and Buerkle 2014).

300 ***Population genetic analyses***. We used a Bayesian model to estimate population allele
301 frequencies for each of 9,493 variable nucleotides based on the point estimates of
302 genotypes from *bcftools* (described in Gompert et al., 2012). Genotypes and the
303 population allele frequencies were treated as unknown model parameters, and
304 genotype probabilities and allele frequencies were simultaneously estimated for each
305 sampling locality (separately from estimates for other localities). Importantly, this model
306 incorporates uncertainty from stochastic variation in sequencing coverage depth across
307 individuals and loci into the estimation process, and provides a sound approach for
308 estimating population level parameters for low coverage sequencing approaches.
309 Population allele frequencies serve as prior information, and genotype probabilities are
310 inferred for each locus in each individual. We obtained posterior probabilities for
311 parameters using Markov chain Monte Carlo (MCMC). Each analysis consisted of a
312 single chain iterated for 2,000 steps following a 1,000 step burn-in, with samples
313 retained every other step, yielding 1,000 samples from the posterior distributions. For
314 these data and this simple model, mixing and convergence were clearly evident in plots
315 of MCMC histories.

316 We obtained the mean genotype (scale of 0 to 2) from the posterior distributions
317 for each SNP in each individual. Furthermore, we calculated the mean genotype across
318 all individuals to center the genotypes at each locus before calculating the genetic
319 covariance between the genotype vectors for all pairs of individuals. We summarized

the genetic covariance matrix using principal component analysis (PCA). Because the PCA will reflect all genetic covariances and will be affected by uneven sampling (e.g., 144 samples from Colorado and 16 from Romania), we performed a second PCA in which we randomly down-sampled all localities to the smallest sample size from any locality (N=16). We also obtained mean allele frequencies from the posterior distributions and transformed these to estimates of mean F_{ST} (Hudson 1992) and Nei's D (Nei et al. 1983; Takezaki & Nei 1996) between all pairs of populations. F_{ST} and Nei's D were highly correlated (r=0.996), so we only present results for F_{ST}. We performed the PCA and calculated F_{ST} and Nei's D in R (using *prcomp* and custom functions, R Core Team, 2015).

Phenotypic and Environmental Variables

Our goal was to compare divergence among traits known to be related to sexual signaling (plumage color and streamer lengths) and migratory behavior (i.e., wing length). We also compared aspects of environmental variability known to affect the aerial insect populations that barn swallows prey upon. These variables include elevation and several measures of temperature variation (e.g., Thomsen et al 2016).

Quantifying Feather Color. We collected 5-10 feathers from the throat and breast (upper ventral region) and vent (lower ventral region, below the attachment of tail streamers) and stored them in small envelopes in a dark, dry environment prior to measurement (*following* Safran and McGraw 2004). We assessed the color of these samples by measuring plumage brightness using an Ocean Optics USB4000

spectrometer (Dunedin, FL). Reflectance data were generated relative to a white standard (Ocean Optics WS-1) and a dark standard (all light excluded), and spectra were recorded with the SpectraSuite software package (version 2.0.125, Ocean Optics Inc.). We find no evidence of UV reflectance in the ventral plumage color of barn swallows (Safran and McGraw 2004) and thus used three traditional axes of color for objective measurement of color variation. We used average brightness, which was calculated from three separate measurements of the collected throat and breast feathers, as a representative metric of overall ventral plumage color. Average brightness is a good color metric, as all three traditional axes of color (hue, chroma, and brightness) were previously found to be highly correlated across the ventral region of individual barn swallows (McGraw et al. 2005, Safran and McGraw 2004, Hubbard unpublished data), and brightness is the most variable dimension of color in this region (Hubbard unpublished data). Lower brightness scores (% reflectance) indicate plumage color that appears darker, redder, and more saturated when compared to feathers with higher brightness scores.

Estimates of Phenotypic Divergence. To determine pair-wise distance in phenotypes among environmental and morphological traits, we used an unbiased effect size statistic (ΔP; Safran et al. 2012) to calculate trait distance for each trait in pair-wise comparisons among the 8 sampled populations. ΔP is calculated based upon a joint cumulative distribution function (CDF) from all populations in the data set. Distances were calculated for each pairwise comparison using the population median percentile in the overall CDF. ΔP was developed specifically to analyze phenotypic distance among

366 closely related populations, as it easily accommodates simultaneous comparisons of
367 any number of traits across any number of populations, and is relatively insensitive to
368 unequal variances and sample sizes among populations (Safran et al. 2012). For all
369 analyses, we use the absolute value of pairwise distances.
370
371 ***Climate and Elevation Data*****.** Temperature and elevation data were obtained using the
372 CRUTEM database maintained by the Climatic Research Unit and available at
373 http://www.cru.uea.ac.uk/cru/data/crutem/ge/. Using this database, we downloaded the
374 last 50 years of temperature data from the 3 weather stations closest to each of our
375 sampling sites (see supplementary materials). From these data, we derived the mean,
376 minimum, and coefficient of variation in temperature during the breeding season.
377 Breeding seasons vary among our populations, and, accordingly, we used the following
378 months for climate data collection for each site: Colorado, USA: April-September; New
379 York, USA: May-August; UK: April-August; Israel, January-April; and Czech Republic,
380 Romania, Turkey and Taiwan, April- July. These measurements have been routinely
381 employed in studies of other avian taxa with widespread geographic ranges
382 (Rubenstein and Lovette 2007, Rubenstein 2007, Botero et al. 2009, Botero et al.
383 2014).
384
385 ***Estimates of Geographic Distance.*** Geographic distances between study sites were
386 calculated in the R package geosphere using the Haversine great circle distance
387 between points. This is the shortest “as the crow flies” distance, assuming a spherical
388 earth.

Associations of Geographic Distance, Environmental Context and Phenotype with Population Genomic Divergence.

To assess the degree to which genome-wide divergence is associated with geographic and phenotypic distance, we analyzed correlations between pairwise trait distance and both pairwise geographic and genetic distances (F_{ST}), and assessed their significance (999 permutations) using Mantel tests using the R package 'vegan'. Next, to quantify the relative strength of association among geographic, phenotypic, and genomic variables, accounting for correlations between phenotypic and geographic distance, we used two complementary statistical approaches. First, we applied multiple matrix regression (Wang 2013). This performs multiple regression on distance matrices and uses permutation tests (n = 10,000) to obtain p and R^2 values using the MRM function in the R package 'ecodist' (Wang 2013). In the second approach, we employed constrained redundancy analysis and variance partitioning to analyze the relative contributions of traits related to natural and sexual selection and environmental context in explaining pairwise genetic divergence (Legendre and Fortin 2010) using the R package 'vegan' (Oksanen et al 2015 v. 2.2). Redundancy analysis is a type of constrained ordination that quantifies how much variation in a set of variables is explained by a second set of variables, with the option of conditioning on a third set. This analysis is ideal for correlated matrices, as is often the case with the matrices related to phenotype, genotype, environmental variables and geographic distance among closely related populations (Shafer and Wolf 2013, Wang and Bradford 2014). Using these methods, variance can then be partitioned between the constrained, conditioned, and joint variable sets. Our data were structured in two different formats,

412 depending on the analysis. For Mantel tests, we employed matrices of pairwise
413 differences for each population. For MRM and the variance partitioning analyses, these
414 matrices were transformed into vectors of pairwise differences for each variable and
415 each pair of populations. When the response variable is a single vector (as here, using
416 pairwise differences in mean F_{ST} between populations), variance partitioning is done by
417 partial regression.
418

419 ## Results

420 ### Population Genomics

421 Principal component analysis of genetic covariances between an even sample of
422 individuals from each population (n = 16) revealed clear genomic differences between
423 some localities, but greater genomic similarity among the nearby sampling locations in
424 Czech Republic, Romania, Turkey, and Israel (Figure 2). The first two principal
425 components explained 52% of the genomic variation among individuals in each locality
426 (Figure 2). The separation of populations on PC1 is consistent with the phylogenetic
427 hypothesis (Dor et al. 2010) that separates eastern and western barn swallows, with
428 samples from Israel and Europe recoverable as a distinct lineage from samples in North
429 America (Colorado and New York) and southern Asia (Taiwan). Interestingly, while
430 Israel is considered a distinct subspecies based on phenotype, it cannot be
431 differentiated from European populations based on genomic covariance. PC2 explains a
432 relatively small amount (10.3%) of genetic variation, compared to PC1 (41.9%), and
433 further separates subspecies within the eastern and western clades. The PCA based on
434 all 350 individuals gave considerable weight in the first axis to distinction between the

large sample from North America (N=144 from Colorado and 27 from New York) and samples from elsewhere, with PC1 accounting for 67.9% of the genetic variation. Pairwise F_{ST} ranged from 0.024 to 0.073 (Table 3).

Phenotypic and Environmental Variables

***Phenotypic and environmental divergence*.** Populations differed both in the extent of phenotypic variation and environmental context (Tables 4 and 5). Mean percentiles within a cumulative frequency distribution varied considerably among populations; the distribution of variables measured from each population differed in terms of its placement on the overall cumulative frequency distribution (Table 4). For example, populations in the UK have the longest wing length, whereas populations in Taiwan have the shortest. Populations in North America (Colorado and Ithaca) have the lightest throat color yet the darkest breast color; thus, there is variation in the extent and direction in which color patches differ among sub-species. The spread of percentile values is indicative of the degree of pairwise divergence for each trait or climate variable. For example, of all phenotypic traits, populations exhibited the most extreme differences in wing length, with a range of percentiles from 9.02–91.16. Note that the percentile values for the phenotypic measures are based on multiple individuals within populations (Table 1), whereas the percentile values for the environmental measures are based on one measure within each population. Analyses of variance reveal that all of the phenotypic traits we measured in this study showed statistically significant differences among populations (adjusted R-squared values range from 0.25 to 0.52; Table 5).

458 **Associations of Geographic Distance, Environmental Context and Phenotype with**
459 **Population Genomic Divergence.**

460 ***Geographic Distance.*** Geographic distance between pairs of populations predicted
461 genome-wide divergence, consistent with a model of Isolation by Distance (r = 0.628,
462 Mantel p value < 0.008, Figure 3, Table 3).

463 ***Phenotypic Divergence and Environmental Context***. Distance matrices (based on
464 ΔP) for various features of phenotype, including measures of ventral color, tail and wing
465 length, were positively correlated with genome-wide divergence among pairs of eight
466 geographically isolated populations (Mantel tests; Figure 4, Table 6). None of the
467 pairwise differences in environmental variables (Mantel tests; elevation and various
468 measures of breeding season temperature) were significantly associated with genome-
469 wide divergence among our study populations (Figure 5, Table 6).

470 ***Geographic Distance, Environment, Phenotype.*** Several features of phenotypic
471 divergence also co-varied with geographic distance (Mantel tests; Figure 6, Table 6),
472 whereas environmental and climate features did not (Mantel tests; Figure 7, Table 6).
473 Thus, genome-wide divergence among closely related populations was associated with
474 both geographic and phenotypic trait distance, and the two are sometimes correlated
475 with each other. It is therefore necessary to adequately control for correlations between
476 phenotypic and geographic distance in order to infer the relative significance of IBA and
477 IBD. We did this using two complementary approaches.

478 First, to investigate the associations of specific traits with pairwise, genome-wide
479 F_{ST}, while accounting for correlations among variables in our model (e.g., phenotypes
480 correlated with one another and with geographic distance), we used multiple matrix

481 regression (Wang 2013). We started with a maximal model that included the pairwise
482 distance matrix of F_{ST} values as the response variable and distance matrices (based on
483 ΔP values) for all nine phenotypic and ecological variables as predictors. We then used
484 backwards-stepwise model selection, sequentially deleting the least significant term and
485 rerunning the model, until coefficients for all remaining predictor variables were
486 significantly different from zero. The final multiple matrix regression model included two
487 aspects of phenotype that explained significant variation in pairwise F_{ST} while controlling
488 for spatial autocorrelation: wing length and throat color (full model $F_{2,17}$= 24.95, P =
489 0.002, r-squared = 0.757; parameter estimate and t-test: wing length coefficient = 0.82,
490 T=6.12, P = 0.002, throat color coefficient = 0.22, T = 1.79, P = 0.03, geographic
491 distance coefficient = 0.01, T = 0.09, P = 0.94).

492 We used the significant predictor variables from the multiple matrix regression in
493 variance partitioning and redundancy analyses to further examine the relative
494 contributions of wing length, throat color, and geographic distance in explaining
495 variation in genome-wide divergence among the populations in our data set. The
496 variance partitioning approach enabled us to test hypotheses about the relative
497 significance of IBA and IBD, taking into account correlations among phenotypic,
498 environmental, and geographic distance variables.

499 The best model fit from the variance partitioning analyses (Table 7) included the
500 effects of all three matrices (geographic distance, throat color distance, and wing length
501 distance). These models, in which various combinations of matrices are conditioned
502 upon one another, demonstrated that collectively 73% of genome-wide divergence is
503 attributable to three variables: wing length, throat coloration, and geographic distance

between populations (Figure 8). Further analyses enabled us to analyze the association between each matrix (geographic distance, throat color, wing length) separately by conditioning each variable on the others in all possible combinations (Table 7). For example, when the matrix containing pairwise distance in wing length is conditioned on the matrices containing pairwise differences in geographic distance and throat color, the influence of wing length on its own accounted for 42% of pairwise genomic distance among the populations in our sample (Figure 8, Table 7). When the matrix containing pairwise distance in throat color is conditioned upon the matrices containing pairwise differences in geographic distance and wing length, the influence of throat color on its own explained 5% of pairwise genomic distance among the populations in our sample. Finally, when conditioned upon the wing and color matrices, the effect of geographic distance on its own did not explain additional variation in genomic distance among the populations in our sample (Figure 8, Table 7). Interestingly, the only place where the geographic distance matrix explained a significant amount of variation in genomic divergence is when both geographic distance and wing length were considered side by side and conditioned upon their correlation with the color matrix (Figure 8, Table 7).

Discussion

Geographic distance and phenotypic distance are strongly correlated among our study populations. Thus, we applied two complementary methods of variance partitioning—multiple matrix regression, and constrained redundancy analysis—which enabled us to analyze the relative contributions of correlated matrices (geographic distance and phenotypic distance) to genome-wide divergence. Overall, our results demonstrate clear

527 evidence that both IBA and IBD contribute to genome-wide divergence among these
528 closely related populations of barn swallows. When spatial autocorrelation between
529 phenotype and geographic distance are accounted for, our results suggest that
530 divergence in an ecological trait (wing length) and a sexual signaling trait (throat color)
531 play a larger role in population genetic divergence than does geographic distance.
532 Surprisingly, we found no evidence that elevation and temperature differences
533 were influential to genome-wide divergence. Although these features of the
534 environment, which are relevant for obligate aerial insectivores, did vary spatially, they
535 were neither associated with geographic distance nor genome-wide distance among the
536 populations in our sample. In a further investigation, we also found no evidence that
537 maximal temperatures or precipitation patterns at each location differed as a function of
538 geographic distance or were associated with genome-wide divergence (Safran,
539 unpublished data). Given the span of our sampling locations, ranging from Israel to
540 North America, these results either suggest that barn swallows occupy fairly similar
541 environments with respect to elevation and temperature or are not particularly sensitive
542 to these ecological variables. The latter explanation seems most likely as these
543 populations are very cosmopolitan in distribution both during the breeding and non-
544 breeding season and during long migratory trips where they likely inhabit a wide range
545 of environments.
546 ***Population Genomic Structure and Phenotypic Divergence.*** Our data demonstrate
547 genetic similarity of individuals within sampling localities, with greater differences
548 between populations that are separated by large geographic distances and genome-
549 wide clustering that generally corresponds to named subspecies. Principal component

analysis shows that the most genetically different populations along PC1 correspond to a highly supported east – west split in the current phylogenetic hypothesis for barn swallows (Zink et al. 2006, Dor et al. 2010). Populations in Asia (*H.r. gutturalis*) and North America (*H.r. erythrogaster*) are more closely related to one another than either are to populations in Europe (*H.r. rustica*) and the Middle East (*H.r transitiva*). PC2 further separates *H.r. gutturalis* (TW) from two samples from the North American *H.r. erythrogaster* populations (IA and CO), and the UK population from other mainland populations of *H.r. rustica* (TR, CR and RM). Samples from the Israeli subspecies (IL) are clustered closely with other mainland European populations of barn swallows, consistent with an unresolved relationship between this subspecies and *H.r. rustica* in the current phylogeny (Dor et al. 2010). Despite being relatively genetically similar, individuals from Israel and the continental European populations are fairly divergent in phenotype, particularly with respect to ventral color.

Despite shallow genomic divergence, phenotypic differentiation is apparent in all aspects of morphology we analyzed in this study, ranging from traits related to body size and flight (wing and tail length) to ventral color. Phenotypic variation despite shallow genomic divergence appears common among many taxa, including cichlid fishes (e.g., Wagner et al. 2012) and particularly in birds (e.g. Parchman et al. 2006, Rodrigues et al. 2014, Poelstra et al. 2014, Mason and Taylor 2015), where it is often the case that a few genes are implicated in morphological variation against a fairly homogenous genomic background (e.g., Poelstra et al. 2014, Kardos et al. 2015). In other words, although a larger number of genes may be involved in generating plumage coloration, studies to date have suggested that a large proportion of segregating trait

variation is due to variation in a small number of genetic loci. Collectively, these studies suggest an important role of divergent selection on signaling traits in population differentiation (Wagner et al. 2012, Poelstra et al. 2014), which might play a particularly important role during the earliest stages of speciation (Kraaijeveld et al. 2010). Finer-scale genomic analyses in a broader geographic context will enable us to test whether phenotypic differentiation is a barrier to gene flow when different subspecies of barn swallows are in secondary contact.

Isolation by Adaptation, Controlling for Isolation by Distance. Because trait divergence is correlated with geographic distance among populations, we applied two complementary statistical methods to tease apart the relative significance of geographic and phenotypic distance in explaining genome-wide divergence. Both sets of analyses reveal that wing length and, to a lesser extent, throat color, are most strongly associated with genome-wide divergence, when geographic distance among populations is accounted for statistically (Figure 8). The variance partitioning model enables us to directly quantify the influence of correlations among geographic distance, wing length, and throat color and to partition the contribution of each of these variables towards differences in genome-wide divergence (Figure 8, Table 6). Thus, our study reveals an important role of phenotypic divergence, and supports a model of IBA for explaining genomic differentiation of geographically isolated populations, bearing in mind that phenotypic divergence is also strongly correlated with geographic distance, and a model of IBD, among populations (Figure 8).

IBD is one of the most common patterns in population genetic data (e.g., Jenkins et al. 2010). Evidence is accumulating to suggest that IBA (which includes models of

Isolation by Environment, or IBE) is also well supported in a variety of empirical systems among closely related populations (e.g., Edelaar et al. 2012, Lasky et al 2012, Lee and Mitchell-Olds 2012, Shafer and Wolf 2013, Morgans et al. 2014, Wang and Bradburd 2014). A recent meta-analysis further revealed that the effect of geographic distance or spatial autocorrelation (between phenotypes, environmental variables, and geographic distance) is critical to control for in statistical tests of IBA but has rarely been done in previous studies (Shafer and Wolf 2013). Simulation studies reveal that a failure to account for spatial autocorrelation can lead to biased results (Shafer and Wolf 2013). In our own study, separate analyses of IBA and IBD each revealed significant effects on genome-wide divergence. Analyses that explicitly considered the correlations of phenotypic and geographic distance were critical to separating out the relative significance of each factor. Variance partitioning (Legendre and Fortin 2010) and multiple matrix regression (Wang 2013) are excellent methods for dealing with spatial autocorrelation, analyzing the relative significance of IBA and IBD, and are particularly important alternatives to partial Mantel tests (Bradburd et al 2013, Wang 2013, Wang et al. 2013), which are subject to false positive results (Diniz-Filho et al 2013).

Barn swallows and divergent selection. Our results support an important role of IBA in genome-wide divergence. In particular, differences in wing length, the most divergent phenotypic trait among populations, explained a significant amount of variation in among-population genomic divergence; throat color is also associated with genomic divergence, but to a lesser extent. From these results and our understanding of the function of these traits, we infer an influence of both natural selection and sexual selection in genomic divergence among these eight populations of barn swallows.

These results are intuitive for several reasons. First, wing length and shape are traits associated with migratory behavior (Marchetti et al 1995, Lockwood et al 1998). Barn swallows vary in migratory distance, and there is evidence of migratory divides throughout their range (e.g., Irwin and Irwin 2005, von Rönn et al. 2016). All four representatives of the *H.r. rustica* subspecies (UK, Czech Republic, Romania, and Turkey) have the longest wing lengths among populations sampled, whereas individuals from distantly-related Taiwan (*H.r. gutturalis*) have the shortest. Interestingly, the sole non-migratory population in our study, Israel, has intermediate wing lengths that overlap in variation with individuals from Turkey and Romania and is genomically indistinguishable from *H. r. rustica*, based on pairwise F_{ST}. In other migratory bird populations, variation in migratory behavior can influence both the evolution of wing shape and genomic differentiation (e.g., Ruegg 2007, 2008, Delmore et al. 2012, 2014, 2015, Rolshausen et al 2009, von Rönn et al. 2016). Thus, variation in migratory routes and behaviors (e.g., timing of arrival to breeding grounds, which may affect mate selection) is proposed as a potentially influential contributor to population divergence (Irwin and Irwin 2005, Rolshausen et al 2009). Further study is required to better understand the role of migratory behavior in divergence among barn swallow populations, as well as the extent to which these seasonal movement patterns influence trait evolution.

Our analyses of IBA also revealed that population-level differences in color contribute to genomic differentiation. Plumage color in various ventral regions (throat, breast, vent) varies both within and among closely related populations of barn swallows (Scordato and Safran 2014). Both throat and breast coloration are variable among the

642 populations in this study, but in different ways. For example, males in our North
643 American populations (Colorado, New York) have the darkest breast color, yet the
644 lightest throat color compared to other populations. Ventral coloration has been the
645 focus of several correlational and experimental studies in barn swallows. Together,
646 these studies indicate that melanin-based ventral color varies among populations
647 (Scordato and Safran 2014), is heritable (Hubbard et al. 2015), and relates to social
648 mate selection and paternity allocation (Safran and McGraw 2004, Vortman et al 2011).
649 Thus, patterns of ventral color differentiation are likely under divergent sexual selection,
650 although this hypothesis requires further testing.

651 Patterns of IBA may be underlain by divergent selection on traits that directly
652 impede gene flow or through the build up of an association between genome-wide
653 divergence and trait divergence in allopatric populations. Whereas models of IBA have
654 typically been applied to scenarios where gene flow is possible between populations,
655 most of the eight populations in our study are geographically isolated from one another,
656 with the exception of those within continental Europe (Czech Republic and Romania). It
657 is possible that gene flow occurs among populations sampled from western and central
658 Europe and Israel, though a formal analysis is needed to make direct inferences about
659 recent historical or ongoing gene flow in these regions. Nevertheless, divergence in the
660 absence of gene flow is an important aspect of avian diversification (e.g., Price 2008)
661 and thus it is highly relevant to ask questions about associations between phenotypic
662 and genomic divergence in order to better understand patterns and consequences of
663 trait evolution in isolated, yet closely related populations. Still, ongoing studies of IBA in
664 locations where divergent populations come into sympatry will be critical for inferring

665 whether trait divergence that may have evolved in isolation imposes a barrier to gene
666 flow.
667

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931 *Data accessibility*

932 Genome assembly: XXX

933 Phenotype and environmental data: Dryad XXX

934 Protocol and models: Dryad XXX

935

936 *Authors' contributions:*

937 R.J.S. designed the study, R.J.S., M.R.W, J.K.H, B.R.J., T.A., H.K., Y.V., P.P., S.S., and S.C.,
938 collected data in the field, N.C and E.S.C.S. completed the draft assembly of the genome, B.R.J.
939 conducted most of the lab work, R.J.S., E.S.C.S., S.M.F., T. P. analyzed the data with input from
940 P.N., R.J.S. wrote the manuscript with input from E.S.C.S., P.N, T.P, and N.K. All authors gave
941 final approval for publication.

942

943 **Table 1.** Sampling locations across four subspecies of *Hirundo rustica*. Acronyms are
944 given for each location that match with locations on Figures 1 and 2.
945
946

Sampling location [abbreviation for location on map]	**subspecies**	**Lat/Long**	**Final Sample size**	**Sampling dates: year (sample size)**
Boulder, Colorado, USA [CO]	*Hirundo rustica erythrogaster*	40.17, -105.10	144	2008 (50) 2009 (72) 2010 (22)
Czech Republic [CR]	*Hirundo rustica rustica*	49.06, 14.76	24	2010
Israel [IL]	*Hirundo rustica transitiva*	32.93, 35.54	45	2008 (3) 2009 (37) 2010 (5)
Ithaca, New York, USA [IA]	*Hirundo rustica erythrogaster*	42.44, -77.50	27	2002
Romania [RM]	*Hirundo rustica rustica*	46.75, 23.83	16	2010
Taiwan [TW]	*Hirundo rustica gutturalis*	25.09, 121.56	18	2010
Turkey [TR]	*Hirundo rustica rustica*	36.85, 31.16	50	2010 (50)
United Kingdom [UK]	*Hirundo rustica rustica*	50.50, -4.65	26	2009
Totals				
8 populations	*4 subspecies*		350	

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949

950 **Table 2.** Morphological and environmental traits measured in 350 individuals, across 8
951 sampling locations.

Types of Traits	Traits
Traits related to reproductive performance among sub-species of barn swallows	Tail streamer length, ventral color (throat breast and vent, % brightness)
Trait related to flight behavior and migratory distance	Wing length (mm)
Features of the environment that affect food availability	Mean, variability, and seasonal minima in breeding temperatures; elevation

952

953 Table 3. Average pairwise F_{ST} (upper diagonal) and geographic distance (km; lower
954 diagonal).
955

Sampling location	Colorado	Czech Republic	New York	Israel	Romania	Turkey	Taiwan	UK
Colorado	**NA**	*0.049*	*0.024*	*0.054*	*0.053*	*0.046*	*0.046*	*0.059*
Czech Republic	853.948	**NA**	*0.059*	*0.038*	*0.041*	*0.030*	*0.062*	*0.048*
New York	2397.257	6726.714	**NA**	*0.062*	*0.063*	*0.055*	*0.055*	*0.067*
Israel	10959.901	2454.926	9169.197	NA	*0.044*	*0.031*	*0.065*	*0.051*
Romania	9131.466	700.843	7399.893	1828.448	**NA**	*0.037*	*0.067*	*0.053*
Turkey	10381.422	1864.741	8578.322	591.36	1255.353	**NA**	*0.059*	*0.045*
Taiwan	11321.578	9083.006	12305.315	8199.932	8582.409	8425.255	**NA**	*0.073*
UK	7337.099	1443.954	5361.685	3818.056	2144.723	3230.591	10145.824	**NA**

956

Table 4. Percentiles for male morphological traits across all populations used for calculating ΔP differences. Lower wing and tail streamer percentiles indicate shorter wings and streamers, respectively. Higher color percentiles indicate *lighter* color. Greater elevation percentiles are indicative of higher elevations at breeding locations. Greater percentiles for variation, minima, and average temperatures are indicative of more variable, hotter climates measured over the period of data collection (1965-2015).

	Population Sampled								
Trait	Colorado	Czech Republic	Israel	New York	Romania	Taiwan	Turkey	UK	*Range in percentiles*)
Wing length	24.08	70.73	61.00	30.22	61.87	9.02	61.00	91.16	9.02-91.16
Streamer length	21.82	72.23	60.22	15.05	79.79	28.66	67.16	60.22	15.05-79.79
Throat Color	77.74	59.42	39.96	80.77	18.35	28.26	47.12	44.62	18.35-80.77
Breast color	21.03	58.63	27.61	21.99	58.72	80.77	55.41	70.39	21.03-80.77
Vent color	18.10	27.01	16.02	20.32	21.45	32.99	25.32	30.14	16.02-30.14
Elevation	100	62.5	75	37.5	50	12.5	87.5	25	12.5-100
Var temp	37.5	75	87.5	62.5	50	25	100	12.5	12.5-100
Min temp	62.5	25	12.5	87.5	50	100	87.5	37.5	12.5-100
Mean temp	62.5	25	12.5	75	50	100	87.5	37.5	12.5-100

Table 5. Results of ANOVAs to show geographic variation in morphological traits among eight closely related populations of barn swallows.

Trait	F	P	Adj R-squared
Wing length	$54.11_{7,353}$	< 0.001	0.52
Streamer length	$54.27_{7,352}$	< 0.001	0.52
Throat Color	$18.84_{7,346}$	< 0.001	0.25
Breast color	$28.16_{7,345}$	< 0.001	0.37
Vent color	$24.97_{7,345}$	< 0.001	0.34

969

970 **Table 6.** Results of Mantel tests. Tests of isolation by adaptation and phenotypic-
971 genetic distance correlations using traits related to mate selection (color, tail length) and
972 migratory behavior (wing length) and environmental traits including elevation and
973 various metrics of temperature during the breeding season. To control for multiple
974 testing, we have indicated the confidence intervals around the mantel test coefficient.
975

Variable	**Association with genome wide F_{ST}**			**Association with geographic distance**		
	Mantel r	*CI*	*P value*	*Mantel r*	*CI*	*P value*
Wing length	0.85	0.79-0.91	**0.002**	0.60	0.34-0.76	**0.010**
Tail length	0.67	0.60-0.81	**0.012**	0.59	0.38-0.85	**0.013**
Throat color	0.29	-0.08-0.66	0.091	0.48	0.27-0.78	**0.030**
Breast color	0.34	0.16-0.53	**0.023**	0.46	0.24-0.74	**0.031**
Vent Color	0.40	0.14-0.59	**0.037**	0.30	-0.11-0.54	0.094
Elevation	-0.04	-0.27-0.38	0.593	0.17	-0.07-0.35	0.202
Min breeding temp	0.07	-0.18-0.40	0.348	-0.01	-0.28-0.38	0.483
Mean breeding temp	0.20	-0.18-0.64	0.145	0.13	-0.14-0.54	0.274
Var breeding temp	0.07	-0.11-0.29	0.346	-0.05	-0.30-0.27	0.560

976 **Table 7.** Quantitative tests of variance partitioning (Figure 7): RDA analyses of the effects of
977 selection and geographic distance on genome-wide divergence. WING = pairwise differences in
978 wing length, COLOR = pairwise differences in throat color, DIST = pairwise differences in
979 geographic distance. Note that the matrix title before the symbol '|' indicates the variance due
980 to that matrix that contributed to genome-wide divergence, conditioned upon the matrices listed
981 after the '|' symbol.
982

Model	**F**	***P***	**Adjusted R-squared**
WING, COLOR, DIST	$WING_{1,24} = 78.36$	< 0.001	0.73
	$COLOR_{1,24} = 7.03$	0.010	
	$DIST_{1,24} = 0.03$	0.878	
WING|COLOR+DIST	$WING_{1,24} = 44.17$	< 0.001	0.42
COLOR|WING+DIST	$COLOR_{1,24} = 6.03$	0.018	0.05
DIST|WING+COLOR	$ECO_{1,24} = 0.03$	0.878	0.00
WING+COLOR|DIST	$WING_{1,24} = 38.66$	< 0.001	0.40
	$COLOR_{1,24} = 6.03$	0.021	
DIST+COLOR|WING	$DIST_{1,24} = 1.04$	0.321	0.05
	$COLOR_{1,24} = 6.03$	0.024	
DIST+WING|COLOR	$DIST_{1,24} = 32.15$	< 0.001	0.70
	$WING_{1,24} = 44.17$	< 0.001	

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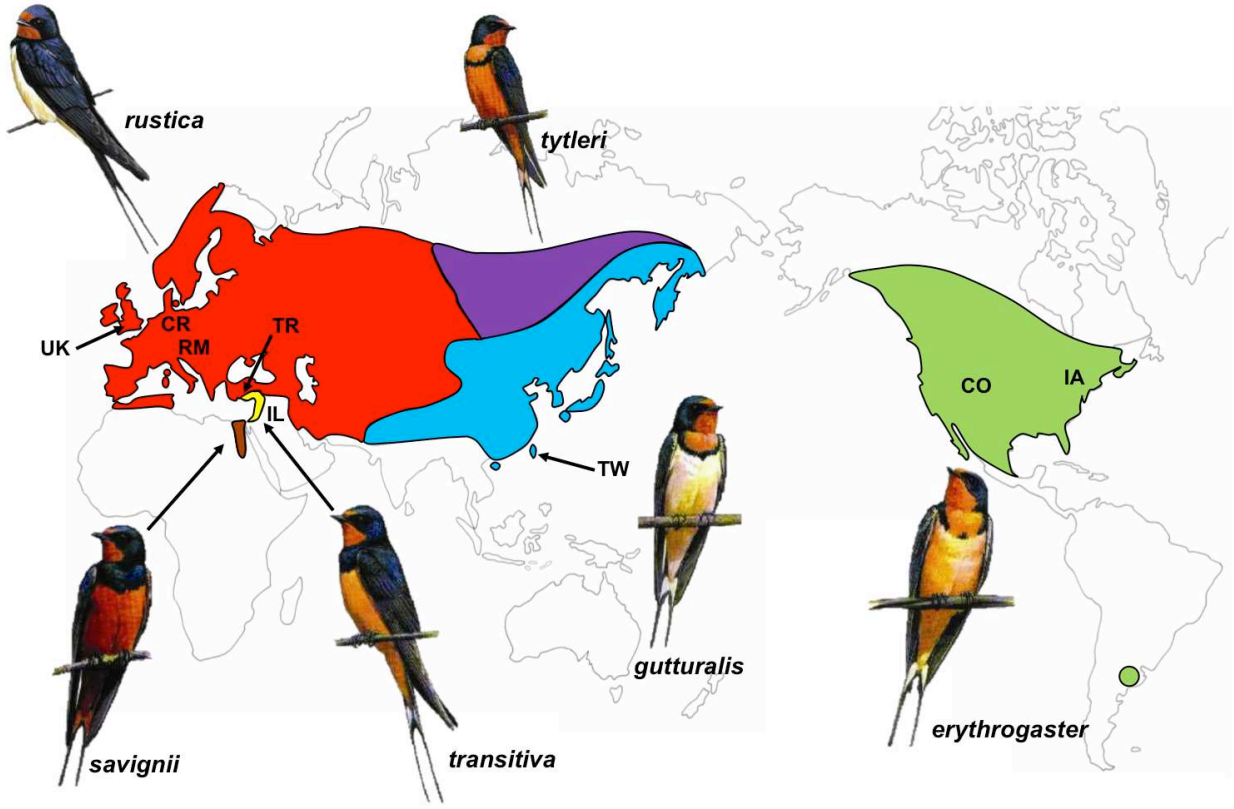
986

987 **Figure 1.** *Hirundo rustica* complex breeding distribution map indicating eight sampling
988 locations. Cartoons of male phenotypes are shown for each subspecies (with
989 permission by artist Hilary Burn). Acronyms for sampling sites are as follows: CO =
990 Colorado, USA; CR = Czech Republic; IA = New York, USA; IL = Israel; RM = Romania;
991 TR = Turkey; TW = Taiwan; UK = United Kingdom.
992



993

Figure 2. Statistical summary of population genetic structure based on principal component axes one (PC1), and two (PC2) derived from genotype probabilities. Acronyms for sampling sites are as follows: CO = Colorado, USA; CR = Czech Republic; IA = New York, USA; IL = Israel; RM = Romania; TR = Turkey; TW = Taiwan; UK = United Kingdom.

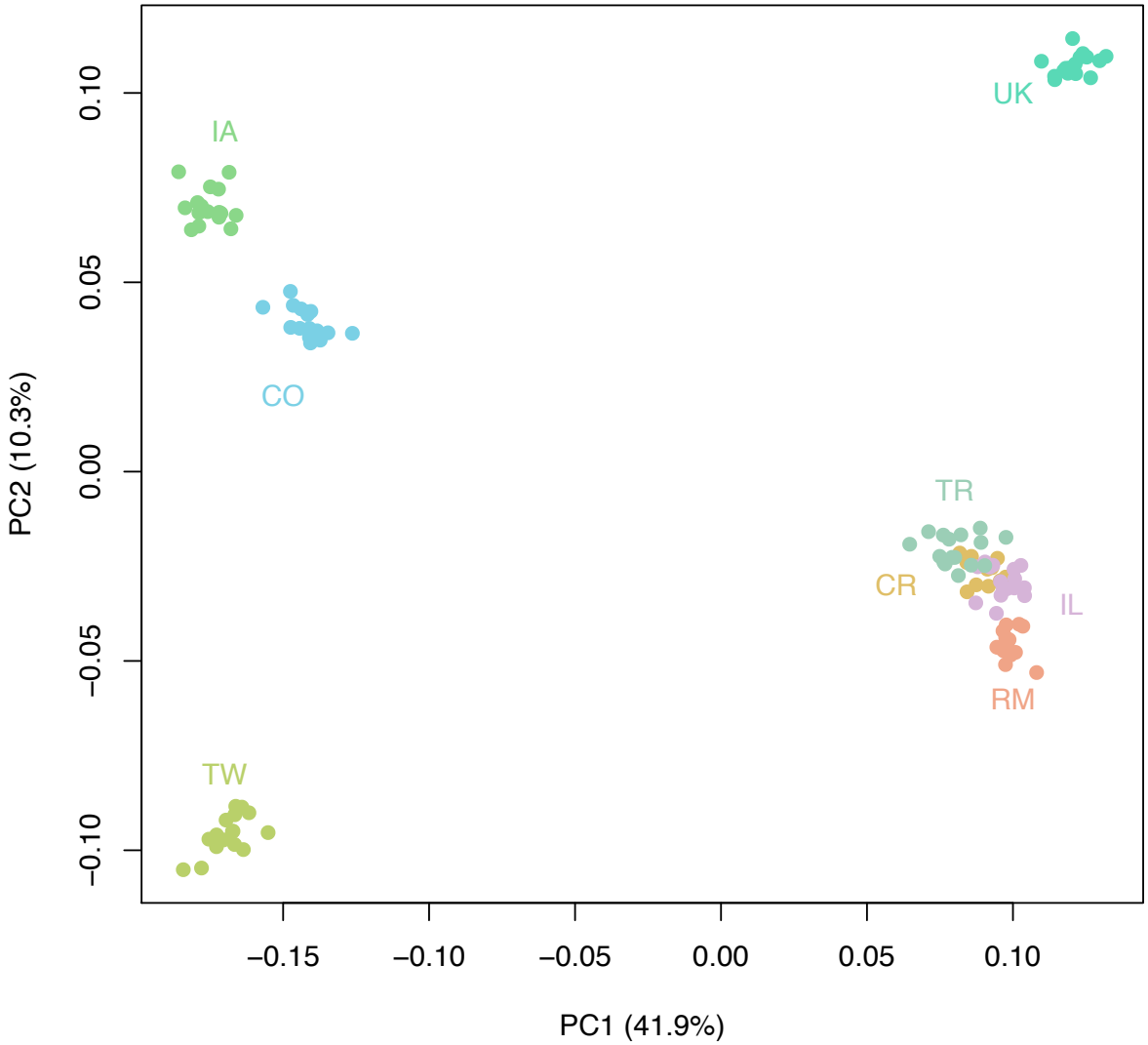


Figure 3. Average pairwise genetic distance (F_{ST}) as a function of geographic distance (km) among pairwise comparisons of eight barn swallow populations, consistent with a pattern of isolation by distance

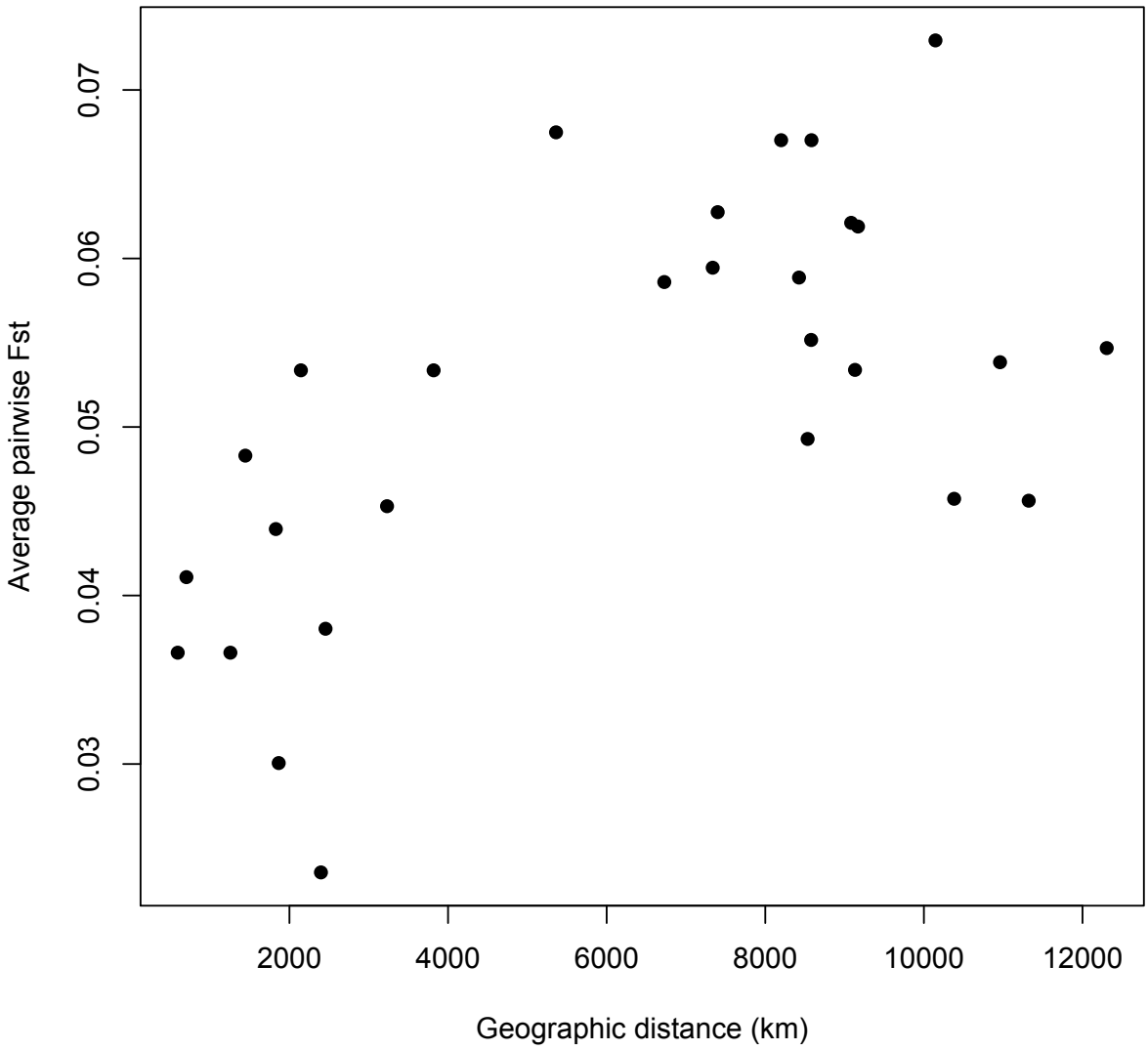


Figure 4. Average pairwise genetic distance (F_{ST}) is strongly correlated with phenotypic distance among pairwise comparisons of eight barn swallow populations, consistent with a pattern of IBA. Table 6 shows results of mantel tests between *F*st and these four phenotype measures; all are significantly associated with the exception of throat color.

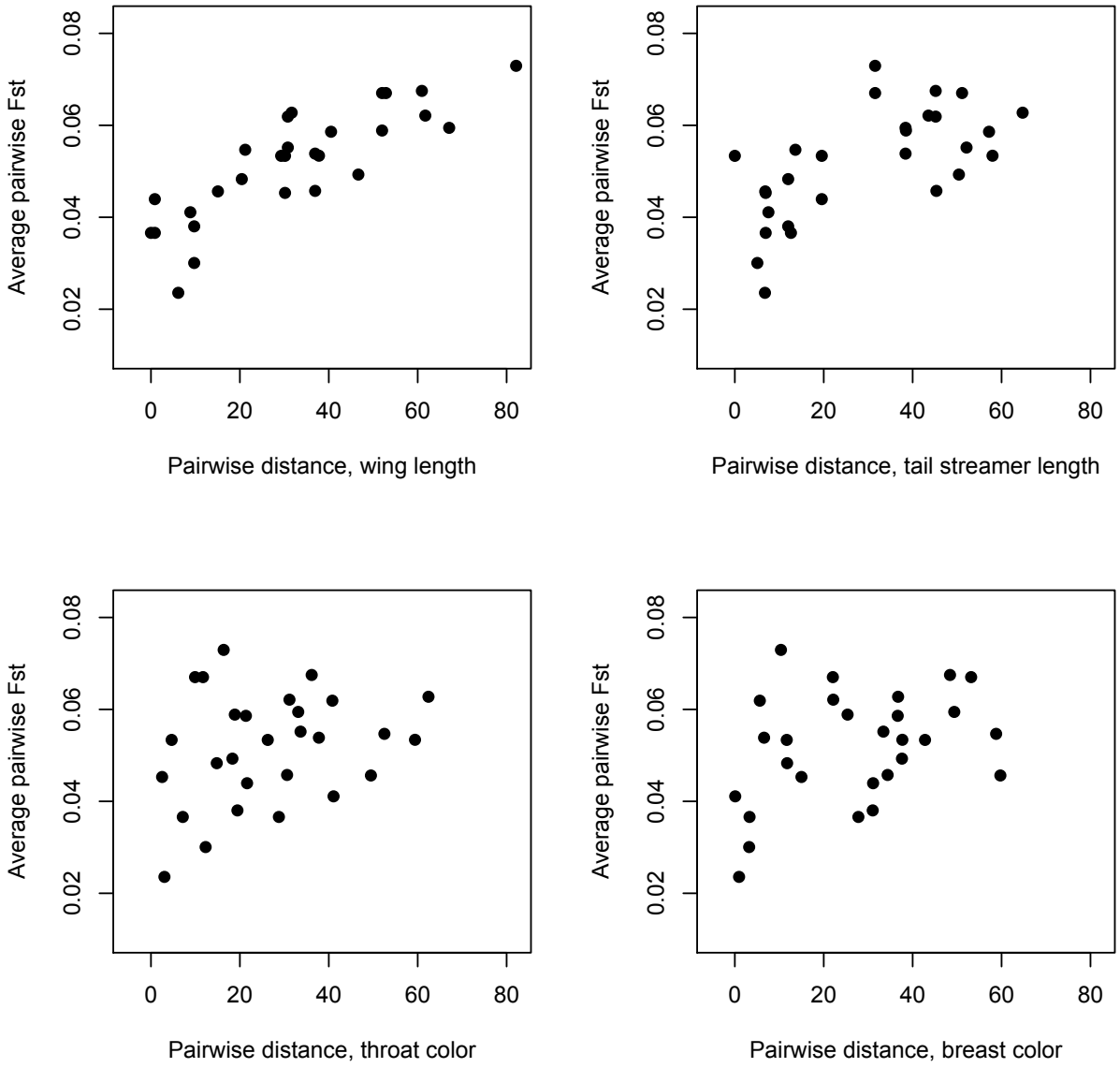


Figure 5. Average pairwise genetic distance (F_{ST}) as a function of environmental distance among pairwise comparisons of eight barn swallow populations. Table 6 shows results of mantel tests between F_{ST} and environmental distance; that there are no statistically significant associations between genetic distance and these environmental variables.

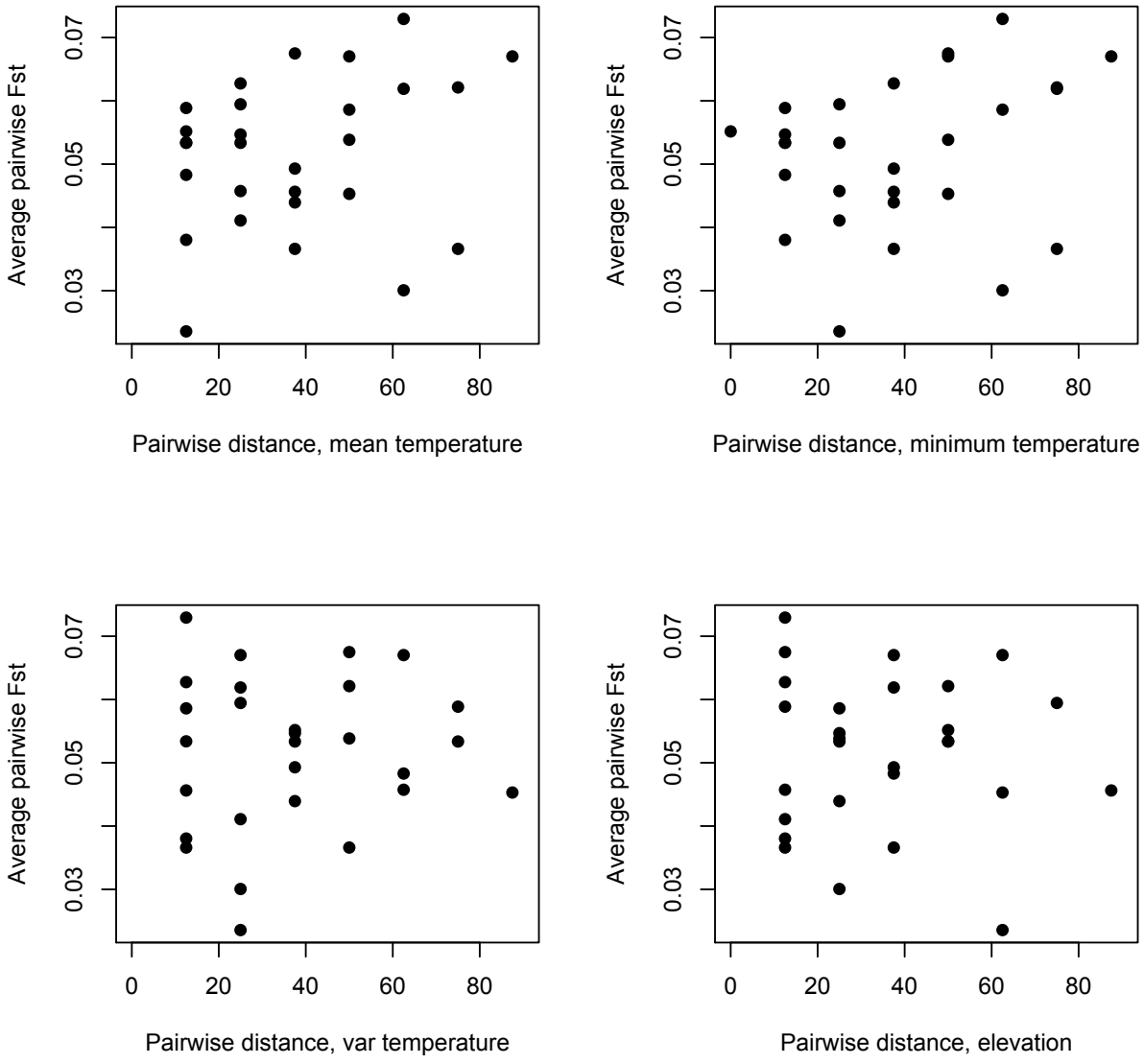


Figure 6. Average pairwise geographic distance as a function of phenotypic distance among pairwise comparisons of eight barn swallow populations indicate spatial autocorrelation between morphological and geographic distance. Table 6 shows results of mantel tests between geographic distance and phenotype distance; all of these associations are statistically significant.

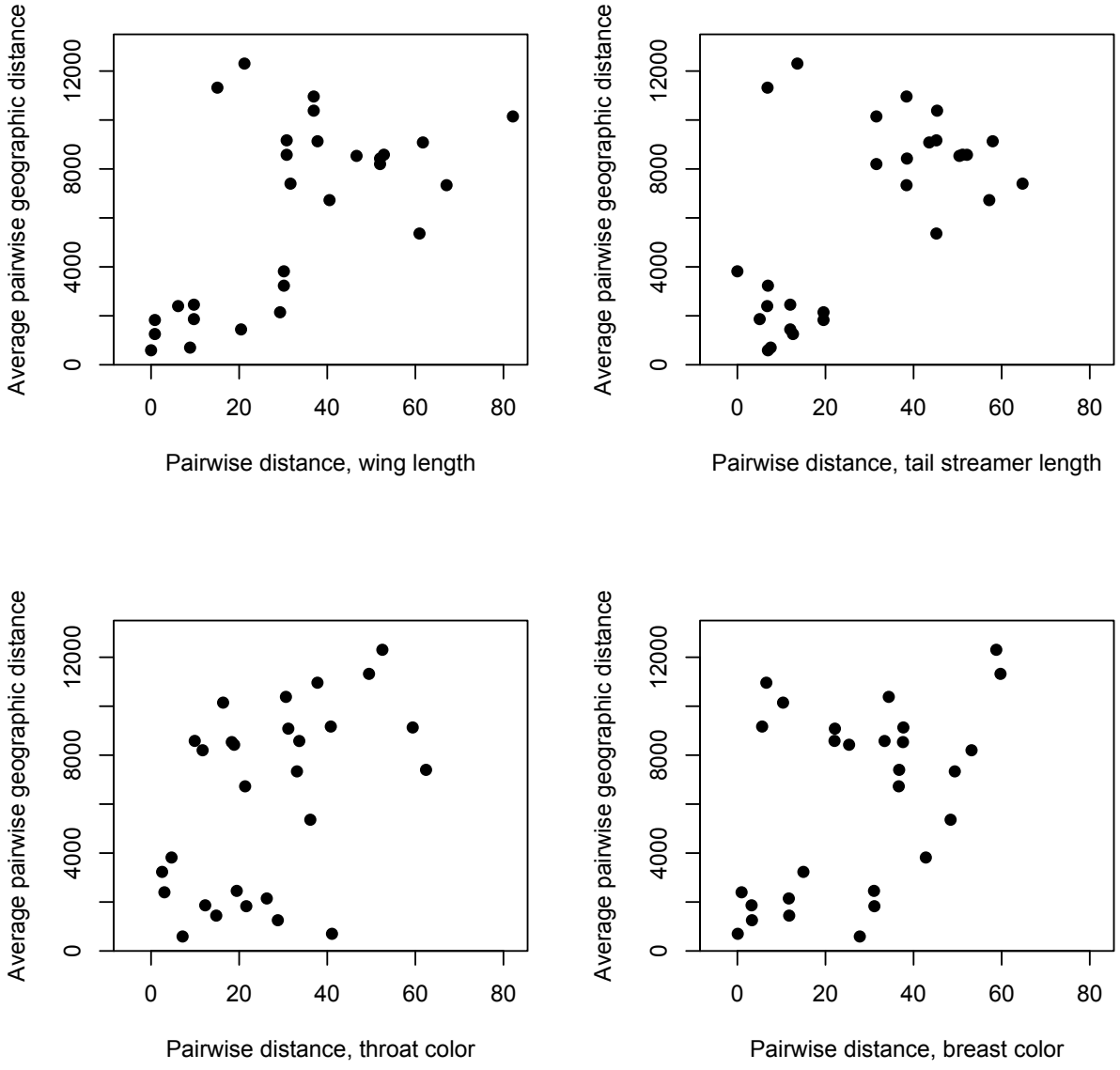


Figure 7. Average pairwise geographic distance as a function of environmental distance among pairwise comparisons of eight barn swallow populations. Table 6 shows results of Mantel tests between geographic distance and environmental distance; none of these associations are statistically significant.

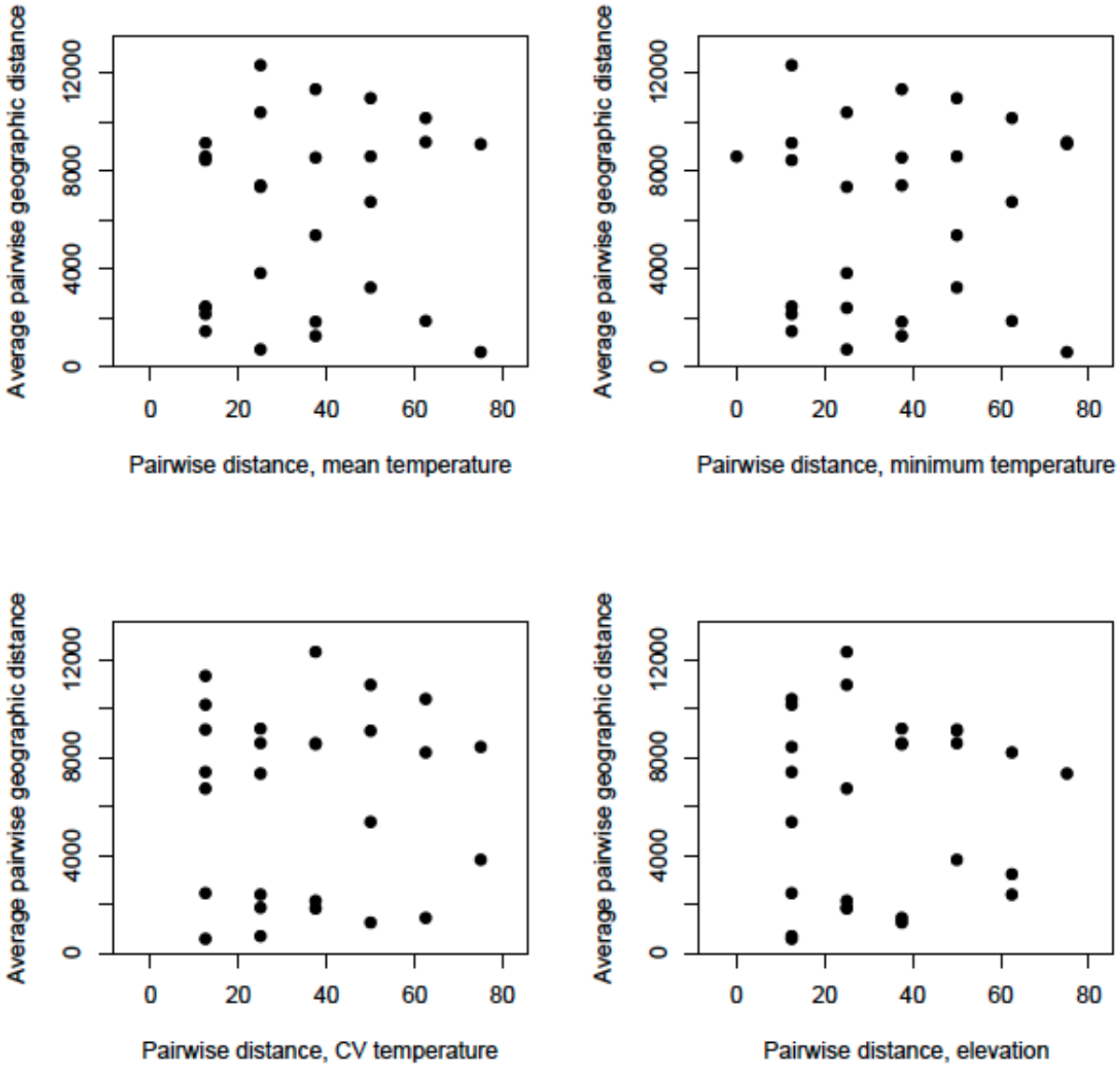
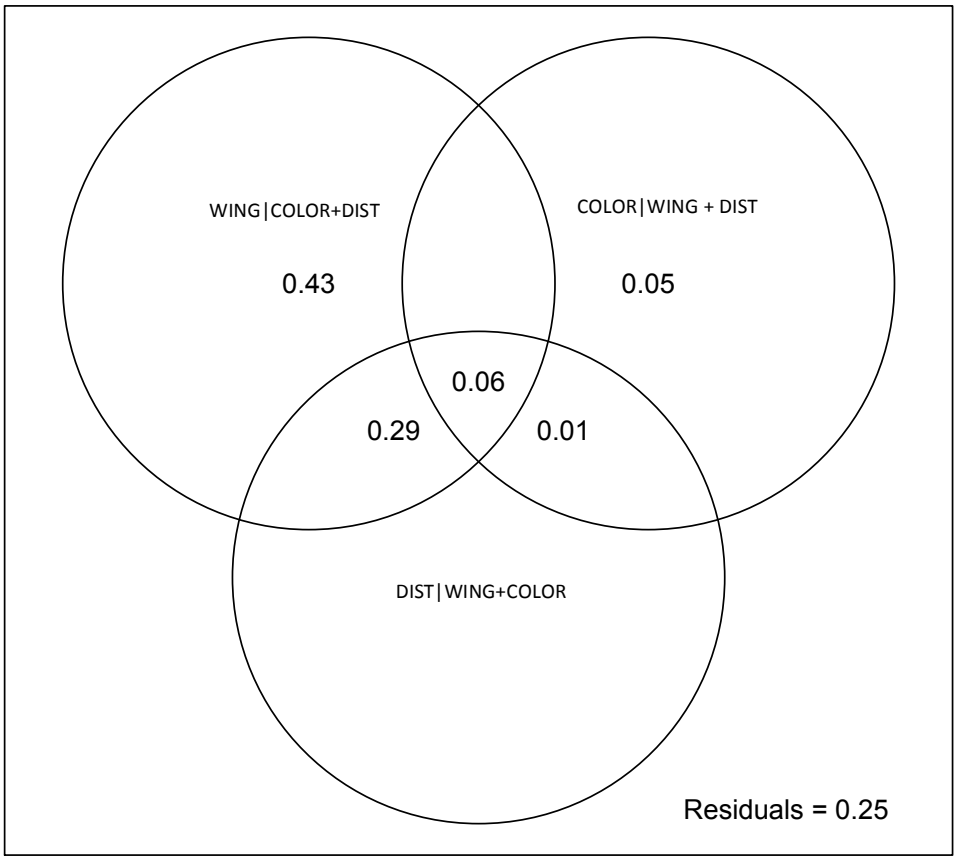


Figure 8. Variance partitioning plot to represent the extent to which natural selection (WING), sexual selection (COLOR), and geographic distance (DIST) explain variance in pairwise, genome-wide differences in F_{ST}. Note that the matrix title before the symbol '|' indicates the variance due to that matrix that contributed to genome-wide divergence, conditioned upon the matrices listed after the '|' symbol.



Values <0 not shown