

THESIS

POPULATION-LEVEL ANALYSIS OF MIGRATORY PHENOLOGY USING GENOMIC DATA IN
A MIGRATORY SONGBIRD

Submitted by

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Graduate Degree Program in Ecology

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2022

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ABSTRACT

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The timing of spring migration for Nearctic-Neotropical birds is key for maximizing access to seasonal resources, such as food or territory on the breeding grounds, while minimizing risk of exposure to winter conditions. Many factors, including sex, weather, energetic condition, and food availability influence migration timing. However, the methods for disentangling within and between population drivers of migratory timing have historically been limited by challenges associated with identifying the breeding location of migrants passing through stop-over sites. For example, spring phenology hypotheses predict that the timing of migration will be driven by the date of spring onset at the breeding grounds, but spring onset often co-varies with distance to breeding site, making it difficult to disentangle the relative roles of each. Here, we utilize genomic data to identify the breeding ground of origin for over one thousand Common Yellowthroats (*Geothlypas trichas*) collected at key migratory stopover points to test the relative roles of sex, migration distance, and date of spring onset in driving migratory timing. We found different relationships for analyses at the species and population level. We conclude that while sex, estimated migration distance, genetic population, and breeding ground phenology are all highly significant predictors of migratory timing across the species, the relative importance each factor differs among genetically distinct populations and from the species-level pattern. Our results highlight the importance of including population-level differences when attempting to understand the multitude of factors that regulate migratory timing.

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INTRODUCTION

Each spring, millions of nearctic-neotropical migratory birds travel from their wintering grounds in Central and South America to breeding grounds in North America. Migration can be costly, both in terms of mortality during the migratory journey and the energetics associated with dramatic changes in physical condition (Silllett and Holmes 2002). These costs may be offset by the benefits of avoiding harsh winter conditions in northern latitudes and increased access to seasonally abundant resources across the annual cycle (Hedenström 2008). In order to maximize such benefits, migrants must time their journeys to be in sync with resource availability at their destinations (Pulido 2007). The timing of spring migration is known to have fitness consequences for some species, with migrants which arrive earlier on breeding grounds often having improved access to territory or mates, multiple broods, and higher chick survival rate (Smith and Moore 2005; Møller et al. 2009; Gienapp and Bregnballe 2012). Conversely, arriving on the breeding grounds too late may help avoid negative consequences associated with exposure to cold snaps, but will limit a migrant's access to resources and mates or restrict the length of the breeding season, which may lower overall fitness (Moller 1994; Brown and Brown 2000; Newton 2007; Ely et al. 2007). Despite the importance of migratory timing to avian life history evolution, many descriptions of timing have focused primarily on species-level patterns, with intraspecific drivers receiving comparatively little consideration (Pulido 2007).

Work on the genetics of migration in nocturnally migrating songbirds supports the idea that migratory timing, including both the timing of migratory movement and physiological changes undergone in preparation for this movement, is at least partially genetically controlled (Moller 2001; Berthold 2003; Pulido 2007; Hedenström 2008; Robson and Barriocanal 2011;

Studds and Marra 2011; Newton 2012; Ramenofsky, Cornelius, and Helm 2012). Classic experiments in blackcaps have shown that captive individuals experience migratory restlessness during the same period when they would typically be migrating in the wild (P. Berthold et al. 1990; Gwinner 1996). Further, hybrid offspring between individuals with different migratory timing exhibit intermediate migratory timing. One of the few studies that has assessed population genetic differences in a wild caught migratory songbird, the Wilson's warbler (*Setophaga petechia*), has shown that genetically distinct populations migrate through stopover sites at distinctly different times (Ruegg et al. 2014). However, it is not yet known whether such differences were due to underlying genetic differences between populations, environmental differences on their respective breeding/wintering grounds, or some combination of the two (Ruegg et al. 2014). Individuals belonging to the same genetic population share a common evolutionary history; therefore, population specific patterns in migratory phenology may have evolved over evolutionary timescales in response to differential selective pressures. As a result, a complete understanding of migratory phenology should consider population genetic structure, environmental factors, and demographic factors in concert when attempting to understand the factors that regulate the migratory timing of individuals.

Researchers have proposed several hypotheses to explain intraspecific variation in migratory timing, with investigations into the role of spring phenology on the breeding grounds and migration distance comprising the majority of the research. The spring phenology hypothesis predicts that the emergence of seasonal plants and insects (i.e. "spring onset") drives selection on the timing of migration, as migratory fitness often hinges on taking advantage of waves in resource availability. At the species scale, large, range-wide datasets have been used to describe the importance of synchronizing migration phenology with the emergence of spring foliage

(Jonzén et al. 2006; Both et al. 2006; Tøttrup et al. 2008; Horton et al. 2020). However, much of the research that ties spring onset to migration phenology focuses on the timing of arrival on the breeding grounds, which is likely highly dependent on conditions experienced *en route*. At earlier stages of migration, migrants have not yet experienced the conditions which would allow them to adjust their timing. For example, migrants in the early stages of their journey do not synchronize northward arrival timing across the Gulf of Mexico with annual spring phenology (Cohen et al. 2015) nor have they adjusted this timing to match the trend in earlier spring onset within temperate North America (Horton et al. 2019). Migratory timing at these early migration stopover points may therefore be under control of endogenous (i.e. genetic) cues, which in turn are suited to match migration to historical breeding ground conditions. Thus, if spring onset on the breeding grounds is a primary driver of migratory timing, we predict that individuals migrating to regions where spring has historically arrived earlier will also migrate earlier, while later migrants will be traveling to breeding locations with later spring onset.

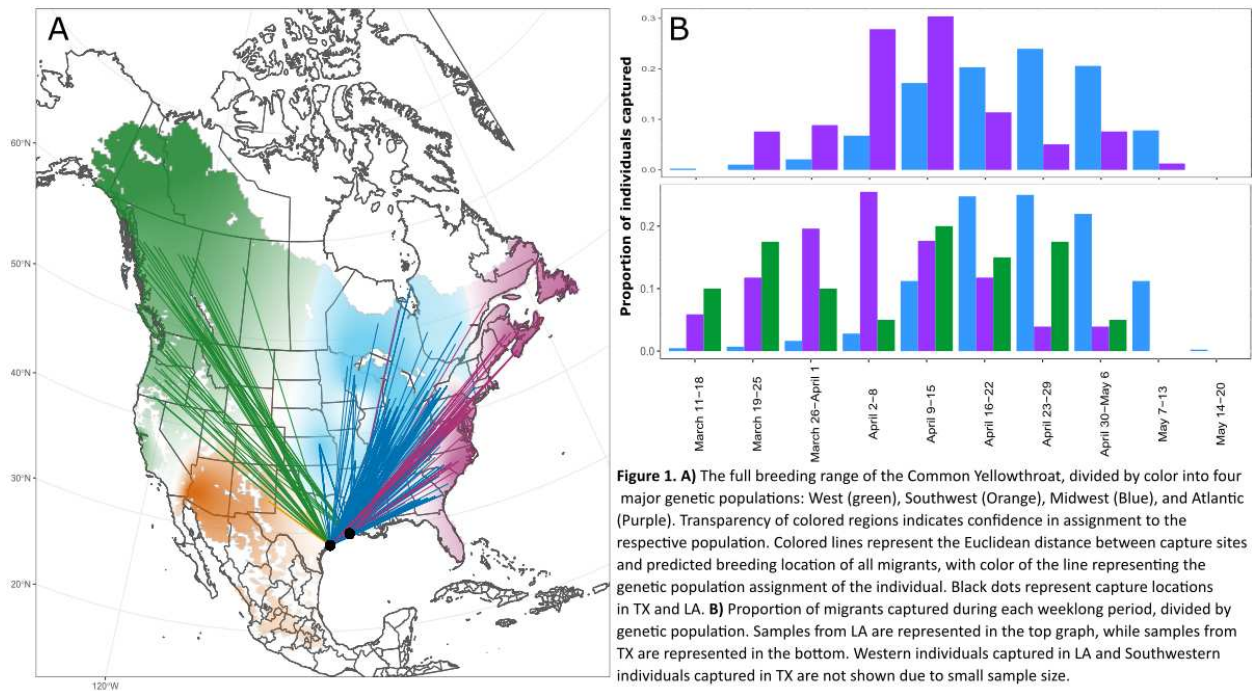
Migration distance has also frequently been hypothesized to play a role in the relative timing of migration, particularly in regards to the need for birds to balance the competing demands of flight distance, refueling rates, and flight speed in a way that minimizes energetic output (Alerstam and Lindström 1990; Alerstam 2011). In particular, optimality theory generally predicts that long-distance migrants will fly faster and spend less time on stop-over sites (i.e. “time-minimizers”) relative to short-distance migrants as a result of the energetic costs associated with longer flight distances and increased exposure risks along the migratory route (Alerstam and Lindström 1990; Alerstam 2011). Overall, predictions about which individuals will migrate first based on migration distance alone are often difficult to disentangle from predictions based on spring phenology, as migrants that travel farther are often traveling to

places where spring onset is later and, therefore, we would predict that they would also migrate later. In cases where both distance remaining to the breeding site and the date of spring onset at the breeding site can be approximated, it may be possible to tease apart the relative contributions of these two potential selective forces on migratory timing. For populations which migrate to breeding grounds of roughly equivalent spring onset, but have different migration distances, the population which migrates further has two options for maintaining arrival time: increasing migration speed, or migrating earlier. Empirical evidence largely suggests that long-distance migrant populations do indeed migrate faster (La Sorte et al. 2013), though some exceptions exist (Anderson et al. 2020). If spring onset is the same for two different breeding populations, but migratory distance is longer for one than the other, we may predict that, in keeping with time minimization strategies, individuals from populations that have further to migrate will migrate earlier to compensate for the additional distance. Here we use genetic tools to identify the breeding location and date of spring onset of individual migrants in order to begin disentangling the role of migratory distance and spring phenology in migratory timing.

While it has rarely been possible to account for population genetic structure during migration due to limitations in methodologies available for assessing population structure in migrating birds, this information is essential to understand intraspecific migration timing. The historical method of recapturing banded birds, though widely applied and useful for many applications, suffers from a low rate of recapture outside of the original capture site (Thorup et al. 2014). More recently, GPS trackers have offered extremely high precision records of individual movements, but can be cost-prohibitive for large scale research on population delineation (Arlt, Low, and Pärt 2013; Bridge et al. 2013). Alternatively, isotopic and genetic methods offer cost-effective methods for assessing the delineation of populations during

migration (Langin et al. 2009; Ruegg et al. 2014; Cohen et al. 2021) without the need for recapture, though the former can only assess population in a broad geographic sense, and cannot provide insight into the potentially important role of population structure. A particular advantage of genetic methods is that they allow for assessment of migratory patterns at both the population (Ruegg et al. 2014) and individual level (Bay et al. 2021), while simultaneously allowing one to consider the potential influence of shared evolutionary history on the migratory timing.

Here, we utilize genetic data collected from individual migrating birds to assess the relative importance of spring onset and migration distance on migratory timing, while accounting for range-wide population genetic structure. Our work focuses on the Common Yellowthroat (*Geothlypis trichas*), a widespread and abundant migrant species, with a breeding distribution spanning from Mexico to Alaska and from Atlantic to Pacific. The Common Yellowthroat makes a good model system for this work for a number of reasons. First, previous genomic analysis using low coverage whole genome sequencing supports genetic delineation between five subpopulations – two in the east (Atlantic and Midwest; Figure 1A) and three in the west (Western, California, and Southwestern; Figure 1A) (Ruegg et al. 2020) – making it possible to genetically assign individuals captured at migration stations to genetically distinct populations and predict the most likely breeding location of those individuals. Second, this species, like many neotropical migrants, exhibits sex specific migratory timing, with males typically migrating earlier than females (Morbey and Ydenberg 2001; Stewart, Francis, and Massey 2002) and sex specific migration patterns can easily be accounted for by sexing birds based on morphological features in the hand or using genetic markers to sex birds in the lab.



Third, Common Yellowthroats are highly abundant across their range, and therefore are frequently captured at many migrant banding stations, making it possible to capture large numbers of individuals and test hypotheses about the drivers of migratory timing.

To investigate the relative importance of migration distance and spring phenology in driving migration timing, we collected genetic samples from Common Yellowthroats captured in a time series throughout spring migration as they arrived at stopover sites in Texas and Louisiana. We used the previously designed panel of genetic markers to identify individuals to populations and predict the most likely breeding location of those individuals. We then used the resulting genetic assignments of individuals to breeding population and approximate breeding destination to calculate the remaining migratory distance and date of spring onset at the predicted breeding region. We integrated each of these datasets by combining the information on migration distance, the date of spring onset at predicted breeding location, and genetic assignments of sex into a multifactorial model designed to assess which factors are most important to driving

differences in migratory timing. Overall, we predicted that if population genetic structure is an important factor in determining migratory timing, then genetically distinct populations of Common Yellowthroats would migrate at distinct times. We further predicted that distinct populations would show differential responses to and differential interactions with spring onset and migration distance relative to each other and to species-scale patterns.

METHODS

Migrant Sampling, SNP Genotyping, and Genetic Sexing

Birds were captured with mist-nets throughout spring migration (March-May) at two long-term migration monitoring stations on the U.S. coast of the Gulf of Mexico in Johnson's Bayou, Louisiana (29°45'N, 93°37'W) during 2004-2012 and Clive Runnells Family Mad Island Marsh Preserve, Texas (28° 37'N, 96°6'W) during 2013-2019 (Figure 1A) (Cohen et al. 2015; 2019). Upon capture, one tail feather from each individual was collected for sexing and genotyping. Individuals captured at the Louisiana site were also sexed in the field when possible. In total, 1,105 samples were collected at the Louisiana site and 1,070 individuals were captured at the Texas site over the course of spring migration, late March to early May. Up to ten samples per week per year were selected at random for genetic identification of sex (if sex was not determined in the field) and of breeding population assignment.

DNA was extracted from migrant feathers using Qiagen Dneasy Blood and Tissue Kits. Genetic sexing was performed using methods outlined in Fridolfsson and Ellegren (1999) and elaborated on in Sehgal et al (2005). The CDH1 gene on the avian Z chromosome was amplified using the primers termed 2550F and MSZ1R. Fifteen microliter reaction mixtures were created containing 2.74 μ L of sample DNA, 1.36 μ L of each primer at 10 μ M, 0.68 μ L of 10 mg/mL BSA, 1.36 μ L of Q solution (Qiagen), and 7.5 μ L of Qiagen Master Mix. The thermocycling profile consisted of 15 minutes of initial denaturation at 94° C, followed by 43 cycles of 30s denaturation at 94°, 30s of annealing at 50°, and 45s of extension at 72°. The final extension period was for 10 minutes at 72°, after which the PCR product was stored at 4°. Sex-specific

PCR product was visualized using Invitrogen 2% 48-well Agarose E-gels, with two DNA bands representing females and a single band representing males.

Samples were genotyped using targeted SNPtype assays designed to diagnose population subdivision (Ruegg et al. 2020). DNA was extracted from migrant feathers using Qiagen Dneasy Blood and Tissue Kits. Each migrant was then genotyped using Fluidigm SNPtype assays at 96 loci (Ruegg et al. 2020- CEC report). Individuals with fewer than 90% of SNPs successfully genotyped were discarded. The R package *rubias* (Moran and Anderson 2019) was then used to assign samples to pre-defined breeding populations. Individuals with posterior probability of assignment less than 80% were considered as uncertain assignments, and were not used for remaining analyses.

Individual Breeding Location assignment

We linked successfully assigned individuals to breeding location by generating probability surfaces using the R package *OriGen* (Rañola, Novembre, and Lange 2014). The package created continuous allele frequency surfaces across the breeding range of the species using the 96 SNPs genotyped from breeding birds. From these probability surfaces, the probability that a migrant individual belonged to a given grid cell on a map was estimated. The probability surface of likely breeding locations for each individual was generated using 245 individuals of known breeding location and the program-specific parameters used in Bay et al. (MaxGridLength=70, RhoParameter=10; Rañola et al. 2014, Bay et al 2021). This created a probability grid for each individual, with grids cells spanning roughly 1.5° longitude and 0.5° latitude. The grid was then trimmed to the breeding range of the Common Yellowthroat and probabilities were rescaled to sum to 1.

Estimating Spring Phenology and Migration Distance

To incorporate environmental drivers of migration timing, we collected spring phenology data using the First Leaf Historical Spring Indices generated by the National Phenology Network (USA National Phenology Network, 1994-2013). This dataset uses historical leaf-out data to model the “start of spring” for all points in the US and Canada between 1880-2013 (Schwartz, Ault, and Betancourt 2013). A 20-year average of spring arrival for each point in North America was created by averaging across rasters from between 1994 and 2013. For each migrant, the probability of breeding in each cell as determined by *OriGen* was multiplied by the average spring onset at that location, and these values were summed (i.e., a weighted average). Similarly, migration distance was estimated for each individual by taking a weighted average of great circle distance between the individual’s capture location and each grid cell. Distances were calculated using the R package *geosphere* (Hijmans et al 2019).

Statistical Analysis

To assess the relationship between individual predictors (i.e. spring onset and migration distance) and migratory timing, we performed linear regressions at the species and population scale. Significance of these regressions was determined using the *Anova* function in the *car* package in R (Fox and Weisberg, 2019). Additionally, t-tests were performed to assess whether there were significant differences in timing between populations and sexes. For a complete model of migratory timing, linear mixed models were created using the *lmer* function from the R package *lme4* (Bates et al, 2015). Predictor variables included spring onset (logged Julian date), migration distance (km), genetic population, and sex, as well as an interaction term between

distance and spring onset and capture year as a random variable. The best performing models were selected using AICc, calculated via the `aictab` function from the R package *AICcmodavg* (Mazerolle 2020).

RESULTS

A total of 1051 samples were successfully genotyped from both locations, and roughly 80% of individuals belonged to the Midwestern population. Of the 510 individuals from Louisiana assigned with high confidence, 413 belonged to the Midwest, 81 belonged to Atlantic, and 16 to the Western population. Of the 541 Texas samples, 428 were assigned to the Midwest, 51 to Atlantic, 56 to the West, and 5 to the Southwestern population. All individuals had probable breeding locations determined by OriGen; the vast majority of these predictions fell within (coincided with) the broader population-specific geographic region determined by Rubias (Figure 1A).

A significant difference in timing was seen between the Midwest and Atlantic populations, with the Atlantic population migrating approximately ten days earlier than the Midwest (Figure 1B; $p < < 0.001$). This pattern was consistent between both sample locations and across years. The Western population also migrated significantly earlier than the Midwest population, though this pattern was not consistent between sample sites (Figure 1B). The Western population also showed a much greater variation in migration dates relative to the number of samples.

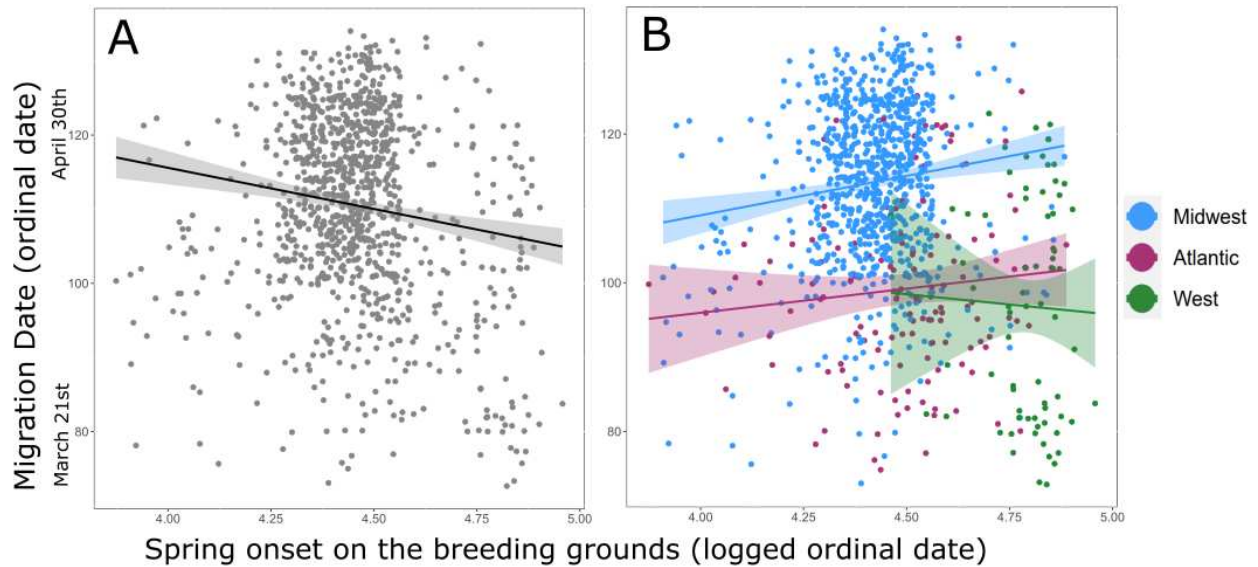


Figure 2. The differences in the relationship between spring onset on the breeding grounds and migration date at the species (A) and population (B) scale. Trendlines represent linear regressions with 95% confidence intervals. At the species level, spring onset was a significant predictor of timing ($p < 0.001$), with earlier migrants traveling to locations with later spring onset. At the population scale, however, spring onset was only a significant correlate in the Midwest population ($p = 0.0002$). Notably, the direction of the trend in the Midwest and Atlantic populations is opposite to the species level pattern.

At the species scale, the weighted mean spring onset on the breeding grounds was a significant predictor of migration timing ($p < 0.001$), with individuals captured earlier in the season predicted to be traveling to breeding grounds with a later spring arrival (Figure 2A). However, at the population level, spring phenology was only a significant predictor of timing for the Midwestern population ($p < 0.001$). Within this population, the pattern of timing was the reverse of the species scale, with early migrants traveling to locations with an earlier spring onset. Phenology was not significant for either group of Atlantic migrants or the Western samples (Figure 2B). No significant difference in pattern was seen between sexes (Figure 5B).

Migratory distance, calculated as the weighted average of the great circle distance from stopover site to the predicted breeding locations, was a highly significant predictor of migratory timing at the species scale ($p < 0.001$), with migrants which were predicted to travel longer distances migrating earlier in the season (Figure 3A). At the population scale, this pattern only

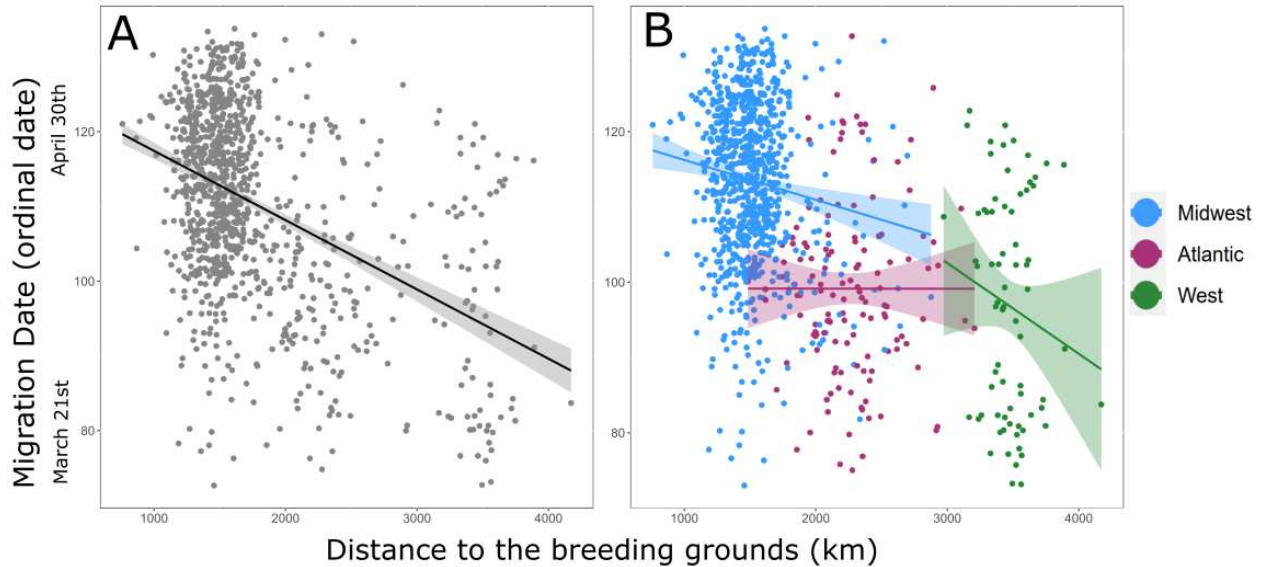


Figure 3. The differences in the relationship between migratory distance and migration date at the species and population scale. Trendlines represent linear regressions with 95% confidence intervals. At the species scale (A), distance is a highly significant predictor of migration date ($p < < 0.001$). At the population scale (B), distance is only a significant predictor within the Midwest population ($p < < 0.001$).

held within the Midwest population ($p < < 0.001$), while no significant relationship between migration distance and timing was found within the Atlantic or West populations (Figure 3B). No significant difference in trend was seen between sexes.

A total of 972 individuals across both sample sites were either successfully genetically sexed or sexed in the field. A significant difference in timing was seen between sexes with males migrating significantly earlier ($p < < 0.001$) in the season than females across all individuals and within the Midwest and Atlantic populations. In the Western population, males migrated slightly earlier than females, but the relationship was not significant.

To test the relative importance of each driver (shared evolutionary history, sex, distance, and spring onset) on migratory timing, linear mixed models were created at both the species and population level with capture year included as a random variable, and the highest performing model was selected by AICc (Table 1). At the species level, the top supported model included sex, population, migratory distance, and breeding ground spring phenology (AICc weight=0.44;

R squared = 0.32). At the population level, the top model for the Atlantic population was identical to the species scale. For the Atlantic population, an interaction term between distance and spring onset was included in the top performing model. The best performing model for the Western population was the only one to differ significantly from the species scale in terms of variables included, as no variables were included as important predictor within the population.

DISCUSSION

The timing of migration in birds is a highly complex behavior which is not fully understood, particularly at the intraspecific level. While species-level patterns of migratory timing have been shown to correlate with key fitness determinants, such as food availability on the breeding grounds or overall migration distance (Paxton et al. 2007; Langin et al. 2009; La Sorte et al. 2014; Cohen et al. 2019; Lehikoinen et al. 2019), migrants have no immediate knowledge of these factors upon departure, and must therefore rely on endogenous controls to properly time migration (Berthold 2003; Pulido 2007). The genetic underpinnings of migratory timing suggest that the evolutionary history of populations within a species may contribute to many patterns in said timing. We utilize modern advancements in genetic analysis to directly assess the role of evolutionary history in migratory timing and demonstrate that genetically distinct populations of common yellowthroat migrate at distinct times during the spring (Figure 1B). We also estimate spring onset on the breeding grounds and migration distance for each individual, showing that strong species-level patterns in timing are not consistent at the population scale. Our results demonstrate the importance of accounting for population genetic structure in attempting to assess the potential drivers of migratory phenology, such as the influence of spring onset or migration distance.

Population specific differences in migratory timing

Many studies have either indirectly observed or acknowledged the importance of population-level patterns in migratory timing (Paxton et al. 2007; Langin et al. 2009; Paxton and Moore 2017; Cohen et al. 2019), but without information on population genetic structure it is

often difficult to identify biologically meaningful populations boundaries (Funk et al. 2012). To overcome this limitation, we used population-specific genetic markers to identify migrants to populations to show differentiation in the relative timing of spring migration between three genetically distinct populations (Figure 1B). This differentiation was most pronounced between the Midwest and Atlantic populations, two closely related populations with significant geographic overlap, while the Western population showed wide variability in migration date. The Atlantic population migrated, on average, 7-10 days earlier in the season than the Midwest population, a trend which was consistent both across sample locations (Figure 1B) and across years. These results are similar to Ruegg et al (2014), who found that genetic populations of Wilson's Warblers migrated at distinct times (though with a smaller sample size and shorter timeframe). Alternatively, the Western population, whose breeding range spans a much larger and more northerly extent than either of its counterparts, migrated significantly earlier than the Midwest, but not the Atlantic. These results are similar to patterns noted in Langin et al (2009) who hypothesized that there would be higher variation in migratory timing in species which breed farther north because they would have more time to adjust their migration timing en route. Similarly, it is possible that the high variance in migration date within the more northerly breeding Western population relative to the Atlantic and Midwest populations is due to their broad latitudinal distribution. Overall, these results support the idea that when genetic markers are used it becomes clear that two of the three genetically distinct populations of the common yellowthroats exhibit distinct migratory timing which likely results from distinct selective pressures in different environments.

Spring phenology as a driver of migratory timing

While many migrants are known to shift the timing of arrival on the breeding grounds to sync with climate-induced shifts in spring phenology (Jonzén et al. 2006; Charmantier and Gienapp 2014; Lehikoinen et al. 2019; Horton et al. 2020), research suggests the timing of migrant passage through stopover sites early in the migratory journey are often less responsive to interannual variability in climate (Cohen et al. 2015; Horton et al. 2019). Instead, it has been suggested that migrant passage early in the migratory journey may be under control of endogenous (i.e. genetic) cues which have evolved in response to historical breeding ground conditions. Here we predicted that passage dates at stopover sites just north of the Gulf of Mexico would be earlier in individuals migrating to regions where spring has historically arrived earlier and later in migrants traveling to breeding locations where spring has historically arrived later. Interestingly, our results were dependent upon whether genetic population was considered. Our species-wide estimates of migration timing versus spring onset on the breeding grounds showed the opposite trend to our predictions, with earlier migrants traveling to destinations with *later* spring onset (Figure 2A). However, when genetic population was considered, the trend reversed in the Atlantic and Midwestern populations to come in line with predictions, though the trend only remained significant within the Midwest (Figure 2B). One possible explanation for this discrepancy is that the relationship between breeding phenology and migration timing is distinct for distinct populations, a pattern which is obscured at the species scale. More specifically, it is possible that individuals in the Midwestern population experience stronger selection on endogenous controls of timing relative to conspecifics from other populations, perhaps due to a more historically variable climate. Alternatively, the Midwest may experience stronger selection due to its proximity to the capture site, as migrants would have less distance in

which to gauge local climate conditions and therefore have a heavier reliance on endogenous controls. Given that two of the three populations showed a similar pattern, a third alternative is that it is possible that the failure to find significance in the Atlantic and Western populations is a result of the more limited sample sizes. Overall, our results support the idea that spring phenology is an important driver of migratory timing at stopover sites, but that the strength of its importance may vary by population. Further, these population-scale patterns tell a different story from patterns seen across the species, which may be obscured if population cannot be taken into account.

Distance as a driver of migratory timing

Migration distance has long been hypothesized to be a driver of migratory behavior, and, in turn, the timing of migration (Alerstam and Lindström 1990). Optimal migration theory predicts that to minimize energy costs, long-distance migrants will increase migration speed relative to short-distance counterparts (Alerstam and Lindström 1990; Alerstam and Hedenstrom 1998; Alerstam 2011). Evidence suggests that, among North American passerine migrants, long-distance migrants do tend to migrate faster (La Sorte et al 2013), and from this one can predict that long-distance migrants will reach migratory stopover points earlier in the season. However, for species migrating north across the Gulf of Mexico, previous work strongly indicates that southern-breeding migrants migrate through the Gulf earlier in the season (Langin et al. 2009; Cohen et al. 2019). Our results run counter to the observations of existing literature on spring migration in the region, but correspond with an energy cost minimizing strategy per optimal migration theory. Both species and population-level trends indicate that migrants which were captured earlier migrated to more distant breeding grounds (Figure 3). This dramatic difference

from previous observations is likely related to the extremely large breadth of migration distances this species is predicted to travel, as the minimum estimated migration distance is 831 km and the maximum is 4,153 km. This relationship between timing and distance may explain inter-population patterns of timing. The Midwest population consistently migrated later than either other population, and migrants from this population traveled significantly less distance (mean migration distance for Midwest: 1519.4 km; for Atlantic: 2275.8 km; for West: 3469.6 km). Of extreme note is that the Midwest and Atlantic populations, which showed highly consistent differences in migratory timing, have a broad overlap in predicted spring phenology, but not in migration distance (Figure 4). This suggests that if breeding ground phenology is similar between two populations, the population which migrates a longer distance will migrate earlier to compensate for the additional distance. It is also possible that extremely long-distance migrants,

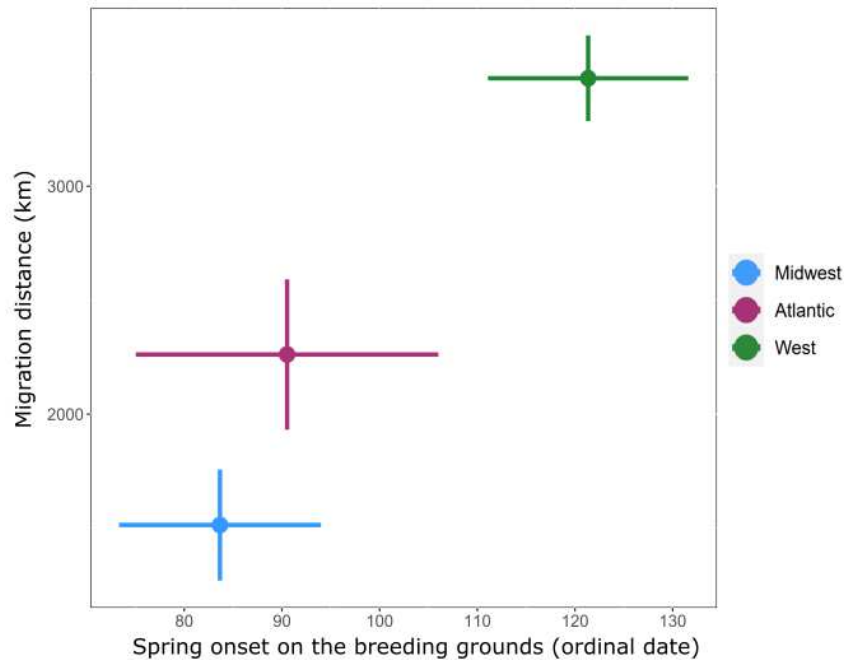


Figure 4. Overview of each genetic population's distribution of data for two predictors of migratory timing: spring onset and migration distance. Central dots represent the mean values of the population, while bars extend to one standard deviation. The Midwest and Atlantic populations largely overlap in the distribution of spring onset across their breeding range, but are distinct in migration distance. The West population shows no overlap with either other population, being both a much greater distance and later spring onset than either counterpart.

such as those represented in the Western population, cannot account for the extra distance simply by increasing speed, but must also migrate earlier in the season. Coupled with the above observation that intra-population relationships between timing and spring onset follow predictions more closely than the species-level pattern, our results indicate that, at very large geographic scales, migration distance will drive inter-population patterns in migration timing, while spring onset may drive intra-population patterns.

Interactions between distance, sex, and phenology

While spring phenology and migration distance were found to be associated with the timing of migration, it is almost certain that additional factors contribute to the total variation in timing (Alerstam and Hedenstrom 1998; Pulido 2007). Considered separately, it appears that spring onset drives intra-population patterns of timing, at least within the Midwestern population,

Table 1. Top performing linear mixed models of migratory timing at the species scale and within each genetic population, as well as the marginal R squared value for these models. A checkmark indicated inclusion of the variable in the top performing model, while an x indicated exclusion. Migration distance refers to the predicted distance of migrants to their breeding site, while spring onset at breeding site refers to the predicted timing of spring onset at the predicted breeding site. Overall, the results indicate that all predictors are valuable to prediction of migratory timing at the species scale. This pattern remains true in two of the three populations, though in the geographically

	Predictors					R ² (marginal)
	Population	Sex	Migration Distance	Spring Onset at Breeding Site	Distance x Spring Onset (Interaction term)	
Species scale	✓	✓	✓	✓	✗	0.323
West Population		✗	✗	✗	✗	0
Midwest Population		✓	✓	✓	✗	0.179
Atlantic Population		✓	✓	✓	✓	0.109

while migration distance drives inter-population differences. In our creation of linear mixed

models of migratory timing, the model which explained the most variation in timing included both of these factors, as well as genetic population, sex, and migration year (included as a random effect; Table 1). Even with the ability to include a larger number of samples and predict variables such as spring onset with more precision than many previous studies, the explanatory power of the top performing models was still limited ($R^2=0.32$ for the top performing model). This indicates that these variables operate in concert with many unmeasured factors, which may include wintering ground conditions, individual body condition, or weather. We also only considered linear relationships between these variables, while non-linear patterns may be present. Additional genetic data in unsampled regions of the breeding grounds would likely also improve predictions of spring onset and distance, and therefore model performance.

Models of timing within populations showed similar patterns to the full-species model, as the highest performing model for both the Midwest and Atlantic populations included all of the variables (Table 1). The notable exception was the Western population, for which no predictors were identified in the best performing model. This modeling appears to be in line with observations made above: populations breeding farther north have more distance and time in which to encounter environmental conditions, and therefore may be able to adjust their speed accordingly, leading to an overall weakening of relationships between breeding-ground variables and timing. Indeed, nearly a third of Western migrants were predicted to breed farther north than the totality of Midwestern and Atlantic individuals. The inclusion of sex in the highest performing models of two of the three populations fits one of the most well-documented trends in many neotropical migrants, wherein males migrate earlier in the season than females in order to stake out territory and attract mates (Kokko 1999; Morbey and Ydenberg 2001; Kokko et al.

2006). This pattern of protandry was seen in all three populations, though was not significant in the West.

CONCLUSIONS

In this study, we genetically assign migrating individuals to breeding populations to show that evolutionary history is an important determinant of migration timing in the Common Yellowthroat. In so doing, we demonstrate that genetics can offer a powerful tool for studying migratory phenology, both in assessing population structure and in making predictions of breeding ground metrics without the need for recapturing individuals. Our estimates of spring onset on the breeding grounds show that population-level patterns of migratory timing differ dramatically from species-level patterns, a discrepancy that both brings the results in line with the literature, and would otherwise be obscured without genetic data. Further, our results suggest that populations with southern breeding ranges may rely more heavily on endogenous cues to match migratory timing to spring phenology, while northern-breeding populations have a much weaker relationship with timing. Our predictions of migration distance may help explain the consistent differences in migratory timing between populations, as the long-distance Atlantic migrants migrated earlier than short-distance Midwestern, despite large overlap in spring phenology between the two populations. Overall, our results show that in a behavior as complex as the timing of migration, species-wide patterns likely result from a highly complex interaction of endogenous and exogenous drivers, which in turn may impact distinct genetic populations in distinct ways. Therefore, future attempts to describe this behavior should incorporate population genetic structure to most accurately reflect reality.

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