Spring 2-17-2020

Assessing population genetic structure of Eastern Phoebes at a migratory stopover site

Daniel Jones
daniel.jones2@bobcats.gcsu.edu

ASSESSING POPULATION GENETIC STRUCTURE OF EASTERN PHOEBES AT A MIGRATORY STOPOVER SITE

By

Daniel P. Jones

A master's thesis submitted in partial fulfillment of the requirements for the degree of

Master of Biology

At

GEORGIA COLLEGE AND STATE UNIVERSITY

2020

Date of final oral examination 2/17/2020

Georgia College & State University

College of Arts and Sciences

Department of Biological and Environmental Sciences

We hereby approve the thesis of

ASSESSING POPULATION GENETIC STRUCTURE OF EASTERN PHOEBES AT A MIGRATORY STOPOVER SITE

Daniel P. Jones

Candidate for the degree of Master of Science

Dr. Katie Stumpf Major Professor	Date
Dr. David Weese Committee Member	Date
Dr. Melanie DeVore Committee Member	Date
Dr. Eric Tenbus Dean of College of Arts and Sciences	Date

ACKNOWLEDGEMENTS

This project would not have been successful without the guidance from my advisors, as well as the assistance of numerous individuals. I am very grateful for their mentorship provided during my graduate research. I want to thank my advisors Dr. Katie Stumpf and Dr. David Weese for their endless help and teaching during this time as I was implementing molecular techniques and analyses for the first time. I would also like to thank Dr. Melanie DeVore for her unending support and encouragement to me throughout this entire process. Their guidance and feedback were immensely helpful, from courses I took to reviewing the thesis, that all helped me and this project successfully progress and conclude. I truly, could not have asked for a better committee. Additionally, I would like to thank Dr. Bruce Snyder for his constant support during this process.

Thank you, Dr. Katie Stumpf, Charlie, Tracey and Allan Muise, for constantly supporting and helping me progress and hone my ornithological skills. Additionally, this project would not have been possible had it not been for Charlie Muise and all the volunteers that tirelessly help out at Panola Mountain Banding Station teaching me safe and efficient field protocols and giving an opportunity for this kind of research to be utilized. I became a birder because it was my first, and only, field-based class during my undergraduate career and I gave my heart to birds because of the love for wildlife that you all shared with me. I would not have the success today were it not for the PANO team.

I want to thank the Stumpf Lab of Avian Ecology and honorary members for helping me in a variety of ways from assisting in field collection to lab work: Harley Hunt, Kayla Allen, Kori Ogletree, Josi Giovinazzo, Kaleb Clifford, Christina Cortez, and Cole Brogden. My entire ornithological, ecological, and even molecular career started with this lab and I do not know who

or what I would be were it not for the variety of opportunities given to me within this lab and during this time. This was an opportunity of lifetime to help start this lab, and I thank Dr. Katie Stumpf for trusting in me to help her and her goals for this lab.

Lastly, I would like to thank all of my friends and family for their unending support and encouragement. I want to thank my mom and dad for helping me develop an appreciation for education at an early age and setting me up for the success I have today, and teaching me that hard work will always pay off as long as you keep at it. I want to thank my friends (too many to name), for supporting me and listening to me ramble about my problems when things went south (pun intended). Finally, I want to thank Blackbird Coffee for fueling me throughout this process, this family encouraged and supported me during my overlapping time while I was pursuing this degree. Milledgeville and Georgia College are a special place for a variety of reasons, but number one is because of the people. I am so thankful for the people I have met and the opportunities this town, college, and department have given me and I will cherish the memories forever.

TABLE OF CONTENTS

Acknowledgements……iii

Table of Contents……v

Chapter 1: Assessing population structure of migratory passerines……1

Abstract……...2

Migration……..3

Methods to Detect Population Structuring……5

Eastern Phoebes (*Sayornis phoebe*)……7

Literature Cited……9

Chapter 2: Genetic differentiation of migrating Eastern Phoebes (*Sayornis phoebe*) at a migratory stopover site……...14

Abstract……..15

Introduction……16

Methods……..18

Results……19

Discussion……..…21

Literature Cited……...25

Appendix……30

PREFACE

This thesis has been written in journal format and conforms to the style appropriate to my discipline. This manuscript will be submitted for publication in The Condor, a peer reviewed interdisciplinary scientific journal, and therefore reflects the required formatting for this publication. This thesis does not contain a list of tables or a list of figures since these are not included in the submission directions for contributors to this journal. Figures and tables follow the text of the manuscript as required by The Condor and this thesis committee.

Chapter 1

ASSESSING POPULATION GENETIC STRUCTURE OF MIGRATORY PASSERINES

ABSTRACT – Migration is an energetically costly and stressful event and migratory stopover sites are important areas along the migratory route that birds stop at to rest and refuel. Migration timing and routes vary across and even within species, including differences between sexes, ages and populations. Most genetic studies of migratory birds occur on breeding grounds, but when breeding locations are not known, it is likely that genetic patterns can be assessed accurately at migratory stopover sites if certain conditions exist. First, if a species is philopatric – where individuals return to the same sites every year, gene flow across geographically separated populations would be low, resulting in genetically distinct populations. Second, if geographically separated breeding populations converge on the same stopover sites, genetically distinct populations should be observed throughout the migratory season. Few studies, however, attempt to assess population structure at stopover sites, yet these are important areas to study in order to understand migration ecology and migratory origin. Traditionally, these types of studies use genetic markers like microsatellites or are coupled with field-based methods to determine population structure and migratory origin. However, using microsatellites alone should be sufficient if the previous conditions are met. Eastern Phoebes (*Sayornis phoebe*) are a philopatric migratory bird with a large breeding range throughout the US. If geographically isolated populations are converging on the same stopover sites along their migratory route, then genetic patterns may be revealed even when migratory origin is unknown.

Migration

Birds occupy different ranges during the three seasons (breeding, wintering and migrating) of their annual cycle based on how seasonal changes impact hormonal cues and foraging opportunities (Gill 2007). The breeding season, late spring and throughout summer months, is a time when food sources and nesting locations are plentiful (Gill 2007). The wintering season is when birds return to their nonbreeding territories for food resources (Gill 2007), and in the western hemisphere wintering sites are typically in southern portions of the United States, in Mexico and Central America, or northern portions of South America (Gill 2007). Birds travel between their breeding and wintering grounds during migration twice a year, once during the spring and once during the fall (Gill 2007). Depending on species, an individual may spend 13-17 weeks of the year migrating (Bonter et al. 2008) and the routes they take are referred to as flyways (Gill 2007, Buhnerkempe et al. 2016). In North America, there are four primary migration flyways, the Atlantic, Mississippi, Central, and Pacific flyways, that connect wintering and breeding regions. Birds that breed in northeastern parts of North America tend to follow the Atlantic flyway and travel southward to overwinter in the southeast or travel across the Gulf of Mexico into northern parts of South America (Lincoln 1935, Buhnerkempe et al. 2016). Birds that breed in middle northern parts of North America generally follow either the Mississippi or Central flyway and migrate southward overwintering in Central America and into northern parts of South America (Lincoln 1935, Buhnerkempe et al. 2016). Lastly, birds that breed in northwestern parts of North America follow the Pacific flyway west of the Rocky Mountains and migrate southward to overwinter in Mexico (Lincoln 1935, Buhnerkempe et al. 2016).

Migration timing may differ by sex, age, and population, even within a species (Woodrey and Chandler 1997). Males tend to migrate before female conspecifics to arrive at breeding grounds to establish foraging and nesting territories (Kokko 1999, Morbey and Ydenberg 2001, Kokko et al. 2006). After leaving the nest and becoming independent from parental care, hatch-year birds spend time exploring the area around their natal site, often to the north (Baker 1993, Brown and Taylor 2015). Because of this post-fledging exploration period, adults often migrate before hatch-years (Brown and Taylor 2015). Lastly, breeding populations found in geographically different areas may migrate at different times depending on resource availability in their specific breeding locations (Sutherland 1998, Ruegg and Smith 2002, Baker 2003, Pulido 2007). Like timing, the migration route of adults and hatch year birds of the same species may also differ (Woodrey and Chandler 1997). Adults usually take the same learned routes from previous migrations, but since hatch-year birds migrate after adults for their they often take different routes than adults (Ellegren 1991, Arguedas and Parker 2000, Brown and Taylor 2015). Some species of migratory birds are philopatric, where individuals return to the same breeding sites in successive years (Esler 2000). Since philopatry directly influences the location where birds breed, it may also influence their migration paths (Esler 2000). Additionally, natal dispersals are a major influence to gene flow and strongly influence population structure and occur regularly across migrant species and philopatric migratns (Sutherland et al. 2000). These dispersals inherently cause different migration paths when compared to adults, as hatch-year birds explore during this pre-migratory phase investigating future breeding sites (Greenwood and Harvey 1982, Baker 1993, Sutherland et al. 2000).

Birds often use sites along their migration route to refuel (Paxton and Moore 2017) or avoid unfavorable weather (Gill 2007), and birds stop at these sites for one to several days at a

time (Gill 2007). These migratory stopover sites are often near water, forest edge, and/or grasslands (Rodewald and Brittingham 2004, Bonter et al 2008). Riparian zones are crucial stopover sites that support many migrating species because they have a high abundance of insects for insectivorous birds (Bonter et al. 2008, Ruth et al. 2012). Forest edge provides protection from predation and have abundant food resources of fruits and insects (Rodewald and Brittingham 2004, Bonter et al. 2008). Grasslands, known migratory stopover sites for many species (Ruth et al. 2012), are crucial for migrating birds that need to forage heavily on seeds and other fat sources to meet the high caloric demand for long-distance flight (McWilliams et al 2002)

By definition, philopatric species should have few to no immigration events across breeding populations. However, philopatric species that breed in two geographically separated locations that migrate using different pathways may use the same migratory stopover site (Figure 1). Even if each population follows a different established migratory pathway (Figure 1; denoted by different colored arrows), they may still converge on the same migratory stopover site, because all of the flyways tend to funnel into narrower flyways near the southern part of the US.

Methods to Detect Population Differentiation

Population genetic studies tend to use either, or a combination of both, field techniques (i.e. recapture data or radio telemetry) that infer movement patterns or lab techniques (i.e. stable isotope analysis or genetic markers) that directly measure gene flow (Lopes et al. 2013, Thorup et al. 2014). Field techniques often predict different genetic patterns than lab techniques (Stumpf et al. 2014). Even though field techniques can track movements between known breeding populations, those movements do not inherently result in increased gene flow between those

populations, because not all dispersal events actually result in successful mating (Prugnolle and De Meeus 2002). Lab techniques are beneficial in that they require handling individuals once for a short amount of time, whereas field techniques require recapturing sampled individuals. However, genetic markers used on their own have been sufficient in understanding patterns of population structure (Gadek et al. 2017, Bounas et al. 2018).

Most population genetic studies of migratory birds have used samples collected from breeding grounds, or samples from individuals of known breeding origin. However, this is difficult if not impossible, when breeding locations are unknown. Recent studies have investigated whether population genetic differences can be detected during the non-breeding seasons (Lopes et al. 2013, Bounas et al. 2018), but no studies have investigated whether population differences can be detected when breeding origin is uknown. It should theoretically be possible to detect genetic differences by sampling individuals captured throughout the year at a migratory stopover site, if those populations use the same stopover site.

Genetic markers are regions of a gene or sequence of DNA that can be used to identify variation within individuals, populations, and species (Sunnucks 2000). Microsatellites are hypervariable, short, tandem di- or trinucleotide repeats of DNA found within non-coding regions (Tautz 1989) where variation across individuals arises from mutations impacting the length of the di- or trinucleotide repeats (Vieira et al. 2016). Natural selection does not act on changing allelic or genotypic frequencies in non-coding regions (Tautz 1989), so microsatellites can isolate the effects of gene flow thereby revealing evolutionary patterns (Vieira et al. 2016).

We previously assumed that little genetic differentiation exists between breeding populations of birds due to their high mobility (Rockwell and Barrowclough 1987), but this assumption is rarely tested. Migratory species exhibit greater gene flow than non-migratory

species and those with small breeding ranges (Winker et al. 2000), but natal dispersals may be more common in migratory birds increasing gene flow and leading to low genetic differentiation (Sutherland et al. 2000, Förschler et al. 2010, Ramos et al. 2016). However, high philopatry can counteract those effects, mitigating gene flow and resulting in genetically structured breeding populations.

Eastern Phoebes

Phoebes are a dull looking species of Tyrant flycatcher. They breed extensively throughout much of North America and winter throughout central America. Phoebes are often the first species to arrive at breeding grounds (Bent 1942, Graber et al. 1974). They winter along the east coast of the United States from North Carolina to the Florida Keys, and along the Gulf of Mexico to central Mexico (Ware and Duncan 1989, Weeks Jr. 1994). Non-migratory resident populations can be found across parts of the United States from Virginia/ Maryland to Texas (Weeks Jr. 2011). Their range has expanded in conjunction with human development and they readily nest on man-made structures (Bent 1942, Weeks Jr. 1994). In Georgia, migrant populations are common throughout the state during the winter and non-migrant resident populations are common in north and central Georgia year-round (Figure 2; Weeks Jr. 2011).

Phoebes are habitat generalists though their distribution patterns are driven by proximity to water with available nesting sites rather than resource availability, even during migration (Hill and Gates 1988, Weeks Jr. 1994, Weeks Jr. 2011). Phoebes are most common around woodland edges and streams and they often nest in banks, caves, cliffs and ravines but will just as often nest in or on car ports, bridges, barns, culverts, window canopies, door canopies, and decks (Cuthbert 1962, Weeks Jr. 1979). Phoebes are philopatric and during the breeding season have

been observed to reuse nests for multiple broods (Weeks Jr. 1979, 1994). Phoebes were placed on the National Audubon Society's Blue List for species of conservation concern following a harsh winter in 1980 (Weeks Jr. 1994), but their population sizes have since rebounded due to expantion of their breeding range (Smith et al. 2015).

Little is known about phoebe migration and the patterns that link their breeding and wintering grounds (Weeks Jr. 2011). Males are thought to arrive at breeding grounds slightly sooner than females, but this is unconfirmed (Johnsgard 1979). They are among the first to arrive from spring migration, following the emerging abundance of insect (Bent 1942) and their fall migration habitat preferences mirror that of the spring (Weeks Jr. 2011). Breeding begins in the spring, but may be as late as May in the northern breeding range (Bent 1942). Fall migration begins in September, peaking in October and extending into November (Bent 1942). However, some phoebes remain in breeding sites after fall migration and migrate in the winter months (Jenness 1994). Males are thought to migrate before females but that may not be true across the range, and the reasons for this are unknown (Johnsgard 1979).

LITERATURE CITED

Arguedas, N., and P.G. Parker (2000). Seasonal migration and genetic population structure in House Wrens. The Condor 102: 517-528.

Baker, R. (1993). The function of post-fledging exploration: a pilot study of three species of passerines ringed in Britain. Ornis Scandinavica 24:71-79.

Baker, A.J. (2003). The deep roots of bird migration: inferences from the historical record preserved in DNA. Ardea Wageningen 90:503-513.

Bent, A.C. ed. (1942). Life histories and North American flycatchers, larks, swallows, and their allies. U.S. National Museum Bulletin 179.

Bonter, David N., S.A. Gauthreaux Jr., and T.M. Donovan (2008). Characteristics of Important Stopover Locations for Migrating Birds: Remote Sensing with Radar in the Great Lakes Basin. Conservation Biology 23:440-448.

Bounas, A., D. Tsaparis, M. Gustin, K. Mikulic, M. Sarà, G. Kotoulas, and K. Sotiropoulos (2018). Using genetic markers to unravel the origin of birds converging towards pre-migratory sites. Scientific Reports 8:8326.

Brown, J.M and P.D. Taylor (2015). Adult and hatch-year blackpoll warblers exhibit radically different regional-scale movements during post-fledging dispersal. Biology Letters 11:20150593.

Buhnerkempe, M.G., C.T. Webb, A.A. Merton, J.E. Buhnerkempe, G.H. Givens, R.S. Miller, and J.A. Hoeting (2016). Identification of migratory bird flyways in North America using community detection on biological networks. Ecological Applications 26:740-751.

Cuthbert, N.L. (1962). The Michigan Audubon Society phoebe study, part 2. Jack-Pine Warbler 40:68-83.

Ellegren, H. (1991). Stopover ecology of autumn migrating Bluethroats *Luscinia s. svecica* in relation to age and sex. Ornis Scandinavica 22:340-348.

Esler, D. (2000). Applying metapopulation theory to conservation of migratory birds. Conservation Biology 14:366-372.

Föschler, M.I., E. del Val, and F. Bairlein (2010). Extraordinary high natal philopatry in a migratory passerine. Journal of Ornithology 151:745-748

Gadek, C.R., S.D. Newsome, E.J. Beckman, A.N. Chavez, S.C. Galen, E. Bautista, and C.C. Witt (2017). Why are tropical mountain passes "low" for some species? Genetic and stable-isotope tests for differentiation, migration and expansion in elevational generalist songbirds. Journal of Animal Ecology 00:1-13.

Gill, F.B. (2007). Migration and navigation. Pp. 273-306, *In* J. Ciprioni, A. Reid, and J. Correa (Eds.). Ornithology. 3rd ed., W. H. Freeman and Company, New York, NY.

Graber, R.R., J.W. Graber, and E.L. Kirk (1974). Illinois birds: *Tyrannidae*. Illinois Natural History Survey Biological Notes 86.

Greenwood, P.J., and P.H. Harvey (1982). The natal and breeding dispersal of birds. Annual Review of Ecology and Systematics 13:1-21.

Hill, S.R., and J.E. Gates (1988). Nesting ecology and microhabitat of the Eastern Phoebe in the central Appalachians. American Midland Naturalist 120:313-324.

Jenness, D. (1994). A late Eastern Phoebe. Loon 66:48-49.

Johnsgard, P.A. (1979). Birds of the great plains: Breeding species and their distribution. University of Nebraska Press, Lincoln, NE, USA.

Kokko, H. (1999). Competition for early arrival in migratory birds. Journal of Animal Ecology 68:940-950.

Kokko, H., T.G. Gunnarsson, L.J. Morrell and J.A. Gill (2006). Why do female migratory birds arrive later than males? Journal of Animal Ecology 75:1293-1303.

Lincoln, F. (1935). The migratory flyways of North America. USDA Circular 342. Pages 12.

Lopes, R.J., J.A. Alves, J.A. Gill, T.G. Gunnarsson, J.C.E.W. Hooijmeijer, P.M. Lourenço, J.A. Masero, T. Piersma, P.M. Potts, B. Rabaçal, S. Reis, J.M. Sánches-Guzman, F. Santiago-Quesada, and A. Villegas (2013). Do different subspecies of Black-tailed Godwit *Limosa limosa* overlap in Iberian wintering and staging areas? Validation with genetic markers. Journal of Ornithology 154:35-40.

McWilliams, S.R., S.B. Kearney and W.H. Karasov (2002). Diet preferences of warblers for specific fatty acids in relation to nutritional requirements and digestive capabilities. Journal of Avian Biology 33:167-174.

Morbey, Y.E. and R.C. Ydenberg (2001). Protandrous arrival timing to breeding areas: a review. Ecology Letters 4:663-673.

Paxton, K.L., and F.R. Moore (2017). Connecting the dots: Stopover strategies of an intercontinental migratory songbird in the context of the annual cycle. Ecology and Evolution 7:6716-6728.

Pulido, F. 2007. The genetics and evolution of avian migration. BioScience 57:165-174

Prugnolle, F., and T. de Meeus (2002). Inferring sex-biased dispersal from population genetic tools: a review. Heredity 88:161-165.

Ramos, R., G. Song, J. Navarro, R. Zhang, C.T. Symes, M.G. Forero, and F. Lei (2016). Population genetic structure and long-distance dispersal of a recently expanding migratory bird. Molecular Phlogenetics and Evolution 99:194-203.

Rockwell, R.F., and G.F. Barrowclough (1987). Gene flow and the genetic structure of populations. Pp. 223-255, *In* F. Cooke and P.A. Buckley (Eds.). Avian Genetics: a population and ecological approach. Academic Press, London, UK

Rodewald, P.G. and M.C. Brittingham (2004). Stopover Habitats of Landbirds During Fall: Use of Edge-dominated and Early-successional Forests. The Auk 121:1040-1055.

Ruegg, K.C., and T.B. Smith (2002). Not as the crow flies: a historical explanation for circuitous migration in Swainson's thrush (*Catharus ustulatus*). The Royal Society 269:1375-1381.

Ruth, J.M, R.H. Diehl and R.K. Felix Jr (2012). Migrating Birds' Use of Stopover Habitat in the Southwestern United States. The Condor 114:698-710.

Smith, A.C., M.-A.R. Hudson, C.M. Downes, and C.M. Francis (2015). Change points in the population trends of aerial-insectivorous birds in North American: Synchronized in time across species and regions. PLoS ONE 10:1-23.

Stumpf, K.J., T. Theimer, M.A. McLeod, and T.J. Koronkiewicz (2014). Disparity in population structuring of Southwestern Willow Flycatchers based on geographic distance, movement patterns, and genetic analyses. Journal of Experimental Zoology 321A:577-585.

Sunnucks, P. (2000). Efficient genetic markers for population biology. Trends in Ecology & Evolution 15:199-203.

Sutherland, W.J. (1998). Evidence for flexibility and constraint in migration systems. Journal of Avian Biology 29:441-446.

Sutherland, G.D., A.S. Harestad, K. Price, and K.P. Lertzman (2000). Scaling of natal dispersal distances in terrestrial birds and mammals. Conservation Ecology 4:16.

Tautz, D. (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research 17:6463-6471.

Thorup, K., F. Korner-Nievergelt, E. B. Cohen, and S. R. Bailli (2014). Large-scale spatial analysis of ringing and re-encounter data to infer movement patterns: A review including methodological perspectives. Methods in Ecology and Evolution 5:1337-1350.

Vieira, M.L.C., L. Santini, A.L. Diniz, and C. de Freitas Munhoz (2016). Microsatellite markers: what they mean and why they are useful. Genetics and Molecular Biology 39:312-328.

Ware, D.M., and R.A. Duncan (1989). First record of the Eastern Phoebe nesting in Florida. Florida Field Naturalist 17:22.

Weeks Jr., H.P. (1979). Nesting ecology of the Eastern Phoebe in southern Indiana. Wilson Bulletin 91:441-454.

Weeks Jr., H.P. (1994). Eastern Phoebe (*Sayornis phoebe*). *In* A. Poole and F. Gill (Eds.). The Birds of North American, no. 94. Philadelphia, Pa.: Academy of Natural Sciences; Washington, D.C.: American Ornithologists' Union.

Weeks Jr., H.P. (2011). Eastern Phoebe (*Sayornis phoebe*), version 2.0. In The Birds of North America (A.F. Poole, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA.

Winker, K., G.R. Graves, and M.J. Braun (2000). Genetic differentiation among populations of a migratory songbird: *Limnothylpis swainsonii*. Journal of Avian Biology 31:319-328.

Woodrey, M.S. and C.R. Chandler (1997). Age-related timing of migration: Geographic and interspecific patterns. The Wilson Bulletin 109:52-67.

Chapter 2

GENETIC DIFFERENTIATION OF MIGRATING EASTERN PHOEBES (*SAYORNIS PHOEBE*) AT A MIGRATORY STOPOVER SITE

ABSTRACT – While most population genetic studies focus on breeding populations, patterns of genetic structuring of migrating birds can provide similar insights of species distribution, yet population structuring is rarely studied at migratory stopover sites. Philopatric species such as the Eastern Phoebe (*Sayornis phoebe*) return to the same breeding sites, which reduces gene flow between breeding populations and leads to population structuring. We should be able to detect population differentiation at migratory stopover sites if genetically distinct breeding populations converge during migration. Our objective was to determine if whether we could detect population structuring among migrating Eastern Phoebes of unknown origin. Panola Mountain State Park (PANO) is a restored grassland in central Georgia used by both migrant and local phoebes. We captured 73 individuals at PANO between June 2018-January 2019 and genotyped thirteen highly variable microsatellite loci specific to phoebes. Since the phoebes were of unknown origin, we assigned a priori populations based on 1) capture location, 2) capture season, and 3) capture date. We assessed population structuring using the program STRUCTURE and by pairwise F_{ST} analysis. Our STRUCTURE results didn't reveal any genetic differences in any of the a priori populations, but F_{ST} results revealed that phoebes captured during the breeding season are genetically different than those captured during fall migration and the winter. Although most phoebe captures have been hatch-year birds, PANO most likely does not host residents based on banding data. The most likely scenario is that captured phoebes represent two genetically differentiated populations and use PANO as a pre-migratory site at different times throughout the year. Overall, this study is the first to show population structuring of a migratory species by solely using genetic markers at a migratory stopover site when distinct breeding grounds and populations are otherwise unknown.

INTRODUCTION

Gene flow is a result of movements with successful reproduction across bird breeding ranges which increases genetic similarity among breeding populations (Ramos et al. 2016). Conversely, populations become genetically differentiated when gene flow is absent or if movements are not followed by successful reproduction (Prugnolle and De Meeus 2002). Migratory behaviors directly affect movements and, therefore, patterns of gene flow across breeding populations. For example, breeding site philopatry, when individuals return to breed at the same sites annually, reduces gene flow and therefore increases genetic differentiation between populations (Brawn and Robinson 1996). Gene flow may be reduced further if philopatric species from geographically separated breeding sites use different flyways, as individuals will most likely not breed across those sites (Ruegg et al. 2014, Hayes 2015, Jiguet et al. 2019). Additionally, non-migratory resident birds that occupy the same area throughout the annual cycle are likely genetically different when compared to migrants because they do not breed together (Winker et al. 2000).

Migration is a metabolically stressful behavior so migrating birds often stop to rest and forage at stopover sites along their migratory pathways. Many migrant species stop at sites that provide similar resources like water, abundant food, and forest cover that offers protection from predators and bad weather (Bonter et al. 2008, Rodewald and Brittingham 2004, Mehlman et al. 2005). However, the timing that birds stop at these locations can vary according to age, philopatry, and/or breeding location. Adults and first season migrants (hatch-year birds) of the same species rarely migrate together from breeding grounds because adults often migrate before hatch year birds (Woodrey and Chandler 1997, Brown and Taylor 2015). Philopatric adults tend to have more efficient and faster migration routes due to previous migrations (Ellegren 1991,

Brown and Taylor 2015) and may therefore arrive at stopover sites earlier than hatch year birds. Additionally, early versus late migration may be an indication that individuals are coming from different geographic locations and are potentially genetically differentiated (Liechti et al. 2014).

Eastern Phoebes (*Sayornis phoebe*; hereafter phoebes) are philopatric passerines that breed throughout much of North America and then winter throughout Central and North America, though resident populations exist in the Southeastern United States (Weeks Jr. 1994). Depending on their migratory origin, phoebes likely use different migratory flyways following seasonal changes like most terrestrial species (La Sorte et al. 2014); however, migration ecology is understudied within this species (Weeks Jr. 2011). Based on their distribution within North America phoebes likely use either the Atlantic and Mississippi flyways for migration, but specific use may depend on migration origin and destination (Gill 2007). The Atlantic and Mississippi flyways both pass through Georgia, so if genetically distinct breeding populations use different flyways, they may use the same stopover sites in Georgia. Similarly, residential and migratory phoebes overlap in parts of Georgia so migratory stopover sites in Georgia could host individuals from nearby resident populations and/or migratory individuals

A 110-acre grassland at Panola Mountain State Park (Stockbridge, Georgia; hereafter, PANO; Figure 3) lies along phoebe migratory pathways in central Georgia. Restoration of this grassland from agricultural land began in 2005 with a goal of improving habitat for grassland species of birds by revegetating with native grasses such as Yellow Indiangass (*Sorghastrum nutans*), Gammagrass (*Tripsacum*), Little Bluestem (*Schizachyrium scoparium*) and Big Bluestem (*Andropogon gerardi*). Surrounding the grassland to the east, north, and west is the South River and hardwood forest, making it an ideal migratory stopover site

Most studies that examine genetic patterns within a species occur on known breeding grounds (Boulet and Norris 2006), however if breeding location is unknown, migratory stopover sites may be a viable alternative (Clegg et al. 2003, Lopes et al. 2013, Bounas et al. 2018) if those sites are used by individuals from genetically distinct populations. Our objective for this study was to determine if we could detect population genetic structuring using individuals of unknown breeding location at this potential migratory stopover site.

METHODS

We ran 11 passive mist nets from 30 min before sunrise until noon one to three times per month between November 2017–January 2019 at Panola. We collected approximately 20 µL of blood from phoebes by brachial venipuncture (Owen 2011) and stored samples in TES buffer (0.2M pH 8.0) on ice in the field and transferred to long-term storage in a -80°C freezer. Our sampling methods were approved by the Georgia College & State University Institutional Animal Care and Use Committee (#05-17).

We extracted DNA using the Qiagen™ DNeasy Blood and Tissue extraction kit. We evaluated the presence of DNA using gel electrophoresis and quantified DNA concentrations using NanoDrop (NanoDrop, Thermo Fisher Scientific Inc.). We amplified 13 microsatellite primers specific for phoebes (Watson et al 2002, Beheler et al. 2007) and performed PCR reactions at 20uL final volume of 11 µL 2X Eco-*Taq*$^{+}$ Master Mix (Midwest Scientific; 1.5 mM $MgCl_2$, 200 µM dNTPs, 2.5units/25µL of *Taq* DNA Polymerase), 0.4 µL each of forward and reverse primers, and approximately 15 ng of template DNA. PCR cycling conditions were one cycle at 95° C for 4 min, 35 cycles of 94° C for 1 min, 55°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 10 min. We ran PCR products on 4% agarose gels to ensure successful amplification before sending them to UC Davis for sequencing on a 3730xl DNA

Analyzer (Applied Biosystems, Inc.). We scored and edited alleles using Geneious Prime (version 2019.1.3).

We used the program STRUCTURE 2.3.4 (Pritchard et al. 2000) to describe genetic structure. We conducted ten runs of 100,000 iterations after 10,000 step burn-in periods assuming one to ten population clusters (K = 1–10) under the admixture model using correlated allele frequencies (Falush et al., 2007). Since we didn't know the population of origin, we tested three potential a priori population assignments; using 1) capture location (Panola Mountain Banding Station), 2) season capture, and 3) capture date (waves of phoebes caught in abundance on different dates throughout the year; C. Muise, unpublished data). We defined season based on capture dates of our samples and compared to published averages as: breeding, May–early August; fall migration, middle-August–middle November; wintering, late November–January (Weeks Jr. 2011). We used STRUCTURE Harvester (Earl and vonHoldt 2012) to determine modal ΔK, which is considered the best predictor of the true K (Evanno et al. 2005).

For each of the three a priori population scenarios, we calculated the number of alleles per locus (N_A), allelic richness (A_R), the expected (H_E) and observed (H_O) heterozygosity, and the inbreeding coefficient (F_{IS}), and tested for deviations from Hardy-Weinberg equilibrium. We calculated pairwise F_{ST} and performed AMOVA tests when there were multiple a priori populations (scenarios 2 and 3, described above). We used GenAlEx 6.5 (Peakall and Smouse 2006) for all analyses.

RESULTS

We caught 73 phoebes on 11 days between October 2017–January 2019 (Table 1). However, we excluded November 18, 2017 from statistical analyses within scenario 3, as one phoebe was captured and due to sample size this a priori population was unable to yield results.

We amplified DNA at 13 loci but excluded one because 99% of individuals at that locus were homozygous, so our final analyses are based on 12 loci. STRUCTURE analyses showed no conclusive results of a most likely K under any of the three scenarios, and the results for admixture and no admixture were the same (STRUCTURE and STRUCTURE Harvester, Figure 4).

Capture location

Under scenario 1 (a priori populations assigned based on capture location) all loci were polymorphic, average number of alleles per locus ranged from three to fifteen, and allelic richness ranged from 1.252–8.227 (Table 2). Observed heterozygosity did not differ from expected at any loci (Table 2). The inbreeding coefficient (F_{IS}) ranged from –0.132–0.378 (Table 2). Five loci deviated significantly from Hardy–Weinberg Equilibrium (SAP32 (X^2=81.605; df=55; P=0.011), SAP39 (X^2=27.128; df=6; P=0.004), SAP50 (X^2=128.088; df=91; P=0.006), SAP53 (X^2=101.821; df=36; P=0.000) and SAP94 (X^2=10.473, df=3, P=0.015); Table 2)).

Season capture

Under scenario 2 (a priori populations assigned based on season capture), polymorphism ranged from 7-12 loci (Table 3). The average number of alleles per locus across populations ranged from 0 to fifteen, and allelic richness ranged from 0.000–2.199 (Table 3). Allelic frequency analyses revealed that observed heterozygosity differed significantly from expected across all populations, however there were few situations where observed and expected heterozygosity were zero (Table 3). The inbreeding coefficient (F_{IS}) ranged from –0.455–0.395 (Table 3). Nine loci were monomorphic and deviated significantly from Hardy–Weinberg equilibrium across two populations (Table 3). Pairwise F_{ST}'s ranged from 0.000 to 0.185 with an overall F_{ST} of 0.175, with the strongest genetic differentiation observed between seasons 1

compared to seasons 2 and 3 (F_{ST}=0.161-0.185; Table 4). Our AMOVA test revealed that 71% of the molecular variance was within individuals (F_{IT}=0.288; *P*=0.001; Figure 5), 11% was among individuals (F_{IS}= 0.137; *P*=0.001; Figure 5) and 18% among populations (F_{ST}=0.175; *P*=0.001; Figure 5).

Capture date

Under scenario 3 (a priori populations assigned based on capture date), polymorphism ranged from 1-10 loci (Table 5). The average number of alleles per locus across populations ranged from zero to eleven and allelic richness ranged from 0.000–8.000 (Table 5). Observed heterozygosity differed significantly from expected across all 11 dates (Table 5) and observed and expected heterozygosity differed for capture date populations 1, 10 and 11 (Table 5). The inbreeding coefficient (F_{IS}) ranged from –0.847–1.000 (Table 3). Six loci deviated significantly from Hardy–Weinberg equilibrium, with 37 total instances of monomorphic loci across the 11 populations (Table 5). Pairwise F_{ST} values ranged from 0 to 0.323 with an overall F_{ST} of 0.174 (Table 6). There was little to no differentiation present between dates 1-4 (F_{ST} = 0-0.045; Table 6). There was little to no differentiation between dates 5-10 (F_{ST}=0.000-0.062; Table 6). We observed the strongest genetic differentiation between date 1 and 5-10 (F_{ST}=0.207-0.275; Table 6), date 2 and 5-10 (F_{ST}=0.189-0.271; Table 6), date 3 and 5-10 (F_{ST}=0.232-0.323; Table 6), and date 4 and 5-10 (F_{ST}=0.200-0.280; Table 6). Our AMOVA test revealed that 76% of the molecular variance was within individuals (F_{IT}=0.244; *P*=0.001; Figure 6), 7% among individuals (F_{IS}=0.085; *P*=0.001; Figure 6) and 17% among populations (F_{ST} =0.174; *P*=0.001; Figure 6).

DISCUSSION

Our pairwise F_{ST} results for both season and date of capture indicate that individuals captured early (in the breeding season) are genetically different than those captured later (fall migration and winter) in the year (Figure 3). This difference may be due to three reasons. First, it is possible that early and late phoebes are coming from genetically distinct breeding populations that migrate at different times, an idea which is consistent with previous research (Ruegg et al. 2014). Genetic differentiation increases with increasing geographic distance of breeding location in philopatric migrants (Hayes 2015, van Oosten et al. 2016, Bounas et al. 2018), so the early and late season captures may be from regions that are geographically isolated from one another. Philopatry is sufficiently strong to cause populations to diverge (Johnsen et al. 2007, Alda et al. 2012), which would only amplify the differences based on geographic distance. Given that phoebes are philopatric with an expansive breeding range (Conrad and Robertson 1993, Weeks Jr. 1994, Beheler et al. 2003, Weeks Jr. 2011), this explanation seems likely.

Second, the genetic differences between early and late season captures we detected may be due to genetic differences between migratory and resident breeding populations. PANO is situated at the northern edge of phoebe resident distribution so it is possible that it supports non-migratory resident populations in addition to migrating individuals. However, it is unlikely that we are capturing resident breeding populations because we rarely capture adult phoebes – only 12% (57/466) of all phoebe captures since 2007 were adults and only seven of those were in breeding condition (C. Muise, unpublished data). In our samples, only 11% (8/73) were adults and none showed morphological characteristics of breeding (C. Muise, unpublished data). Given all of this, it is unlikely that a resident breeding population is present.

A third explanation is that the genetic structuring is a result of differences between migratory individuals that are using PANO as a stopover site and hatch-year individuals from a

non-migratory resident population nearby that are using PANO as a staging area. Hatch year passerines do not have established migratory routes and may seek out potential future breeding grounds during an exploratory pre-migratory phase (Arguedas and Parker 2000, Bounas et al. 2018). Songbird fledges have been observed as far as 93 km from their natal sites during post-fledging exploration periods before fall migration (Baker 1993). Given the rarity of adults, and the prevalence of hatch years caught in large numbers on single days (i.e. waves), this seems a likely explanation as well. Distinguishing between causes of genetic distinction – breeding population differences and differences between migrating and fledging at staging areas – will require additional study.

While F_{ST} results demonstrate genetic differences, STRUCTURE results show inconclusive results. However, STRUCTURE is known to perform poorly with small sample sizes (Gilbert et al. 2012) while F_{ST} can accurately estimate population structuring with as few as 30 individuals (Chen et al. 2015). The presence of monomorphic alleles can effectively mask population differences using STRUCTURE, however with enough polymorphic data even weak differentiation can be detected using F_{ST} (Weir and Cockerham 1984, Bossart and Prowell 1998, Bhatia et al. 2013). F_{ST} is often and reliably used to determine population structure based on genetic differences between populations (Whitlock and McCauley 1999), thus we are confident in using the results of the F_{ST} tests.

Most population genetic studies use samples from known breeding locations to detect population structure, however this study has shown that it is possible to detect genetic differentiation in a philopatric species at a migratory stopover site, even when breeding grounds are unknown. Panola Mountain State Park hosts genetically distinct populations of Eastern Phoebes, possibly from different breeding locations, highlighting the need for continued

restoration to serve a wide geographic range of breeding populations. Given the possibility that hatch years are using it as a staging area to seek out future breeding locations, continued habitat improvements will also increase potential breeding habitat for resident birds, allowing for population expansion. While our study focused on Eastern Phoebes, these methods may also be applicable to other migratory species, especially those that overlap with resident populations along their migratory routes.

LITERATURE CITED

Alda, F., J. García, J.T. García, and S.Suárez-Seoane (2012). Local genetic structure on breeding grounds of a long-distance migrant passerine: The Bluethroat (*Luscinia svecica*) in Spain. Journal of Heredity 104:36-46.

Arguedas, N., and P.G. Parker (2000). Seasonal migration and genetic population structure in House Wrens. The Condor 102: 517-528.

Baker, R. (1993). The function of post-fledging exploration: a pilot study of three species of passerines ringed in Britain. Ornis Scandinavica 24:71-79

Beheler, A.S., and O.E. Rhodes Jr (2003). Within-season prevelance of extrapair young in broods of double-brooded and mate-faithful Eastern Phoebes (*Sayornis phoebe*) in Indiana. The Auk 120:1054-1061.

Beheler, A.S., J.A. Fike, and O.E. Rhodes Jr (2007). Eight new polymorphic microsatellite loci from the eastern phoebe (*Sayornis phoebe*). Conservation Genetics 8:1259-1261.

Bhatia, G., N. Patterson, S. Sankararaman and A.L. Price (2013). Estimating and interpreting F_{ST}: the impact of rare variants. Genome Research 1-8.

Bonter, David N., S.A. Gauthreaux Jr., and T.M. Donovan (2008). Characteristics of Important Stopover Locations for Migrating Birds: Remote Sensing with Radar in the Great Lakes Basin. Conservation Biology 23:440-448.

Bounas, A., D. Tsaparis, M. Gustin, K. Mikulic, M. Sarà, G. Kotoulas, and K. Sotiropoulos (2018). Using genetic markers to unravel the origin of birds converging towards pre-migratory sites. Scientific Reports 8:8326.

Bossart, J.L., and D.P. Prowell (1998). Genetic estimates of population structure and gene flow: limitations, lessons and new directions. Trends in Ecology and Evolution 13: 202-208.

Brawn, J.D. and S.K. Robinson (1996). Source-sink population dynamics may complicate the interpretation of long-term census data. Ecology 77:3-12

Brown, J.M and P.D. Taylor (2015). Adult and hatch-year blackpoll warblers exhibit radically different regional-scale movements during post-fledging dispersal. Biology Letters 11:20150593.

Chen, G., A. Yuan, D. Shriner, F. Tekola-Ayele, J. Zhou, A.R. Bentley, Y. Zhour, C. Wang, M.J. Newport, A. Adeyemo and C.N. Rotimi (2015). An improved F_{ST} estimator. PLoS ONE 10:1-10.

Clegg, S.M., J.F. Kelly, M. Kimura, and T.B. Smith (2003). Combining genetic markers and stable isotopes to reveal population connectivity and migration patterns in a Neotropical migrant, Wilson's warbler (*Wilsonia pusilla*). Molecular Ecology 12:819-830.

Conrad, K.F. and R.J. Robertson (1993). Clutch size in eastern phoebes (*Sayornis phoebe*) II: age effects and seasonal variation. Canadian Journal of Zoology 71:1738-1742.

Earl, D.A., and B.M. vonHoldt (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4:359-361.

Ellegren, H. (1991). Stopover ecology of autumn migrating Bluethroats *Luscinia s. svecica* in relation to age and sex. Ornis Scandinavica 22:340-348.

Evanno, G., S. Regnaut and J. Goudet (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611-2620.

Falush, D., M. Stephens and J.K. Pritchard (2003). Inference of population structure using multilocus genotype data: dominant markers and null alleles. Genetics 164:1567-1587.

Gilbert, J.K., R.L. Andrew, D.G. Bock, M.T. Franklin, N.C. Kane, J.S. Moore, B.T. Moyers, S. Renaut, D.J. Rennison, T. Veen and T.H. Vines (2012). Recommendations for utilizing and reporting population genetic analyses: the reproducibility of genetic clustering using the program STRUCTURE. Molecular Ecology 21: 4925-4930.

Gill, F.B. (2007). Migration and navigation. Pp. 273-306, *In* J. Ciprioni, A. Reid, and J. Correa (Eds.). Ornithology. 3rd ed., W. H. Freeman and Company, New York, NY. 758 pp.

Hayes, J.B. (2015). Dispersal and population genetic structure in two flyways of Sandhill cranes (*Grus canadensis*). Ph.D. Dissertation. University of Wisoncsin-Madison, Madison, WI. 299 pp.

Jiguet, F., A. Robert, R. Lorrillière, K.A. Hobson, K.J. Kardynal, R. Arlettax, F. Bairlein, V. Belik, P. Bernardy, J.L. Copete, M.A. Czajkowski, S. Dale, V. Dombrovski, D. Ducros, R. Efrat, J. Elts, Y. Ferrand, R. Marja, S. Minkeviscius, P. Olsson, M. Pérex, M. Piha, M. Raković, H. Schmaljohann, R. Seimola, G. Selstam, J. Siblet, M. Skiercyński, A. Sokolov, J. Sondell, and C. Moussy (2019). Unravelling migration connectivity reveals unsustainable hunting of the declining ortolan bunding. Science Advances 5:1-10.

Johnsen, A., A.E. Fidler, S. Kuhn, K.L. Carter, A. Hoffmann, I.R. Barr, C. Biard, A. Charmantier, M. Eens, P. Korsten, H. Siitari and J. Tomiuk (2007). Avian clock gene polymorphism: Evidence for a latitudinal cline in allele frequencies. Molecular Ecology 16: 4867-4880.

La Sorte, F.A., D. Fink, W.M. Hochachka, A. Farsnworth, A.D. Rodewald, K.V. Rosenberg, B.L. Sullivan, D.W. Winkler, C. Wood and S. Kelling (2014). The role of atmospheric conditions in the seasonal dynamics of North American migration flyways. Journal of Biogeography 41:1685-1696.

Liechti, F. C. Scandolara, D. Rubolini, R. Ambrosini, F. Korner-Nievergelt, S. Hahn, R. Lardelli, M. Romano, M. Caprioli, A. Romano, B. Sicurella, and N. Saino (2014). Timing of migration and residence areas during the non-breeding period of barn swallows *Hirundo rustica* in relation to sex and population. Journal of Avian Biology 46:254-265.

Lopes, R.J., J.A. Alves, J.A. Gill, T.G. Gunnarsson, J.C.E.W. Hooijmeijer, P.M. Lourenço, J.A. Masero, T. Piersma, P.M. Potts, B. Rabaçal, S. Reis, J.M. Sánches-Guzman, F. Santiago-Quesada, and A. Villegas (2013). Do different subspecies of Black-tailed Godwit *Limosa limosa* overlap in Iberian wintering and staging areas? Validation with genetic markers. Journal of Ornithology 154:35-40.

Mehlman, D.W., S.E. Mabey, D.N. Ewert, C. Duncan, B. Abel, D. Cimprich, R.D. Sutter, and M. Woodrey (2005). Conserving stopover sites for forest-dwelling migratory landbirds. The Auk 122:1281-1290.

Owen, J.C. 2011 Collecting, processing, and storing avian blood: a review. Journal of Field Ornithology 82:339-354.

Peakall, R., and P.E. Smouse (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research–an update. Bioinformatics 28:2537-2539.

Prugnolle, F., and T. de Meeus (2002). Inferring sex-biased dispersal from population genetic tools: a review. Heredity 88:161-165.

Pritchard, J.K, M. Stephens and P. Donnelly (2000). Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Ramos, R., G. Song, J. Navarro, R. Zhang, C.T. Symes, M.G. Forero, and F. Lei (2016). Population genetic structure and long-distance dispersal of a recently expanding migratory bird. Molecular Phlogenetics and Evolution 99:194-203.

Rodewald, P.G. and M.C. Brittingham (2004). Stopover Habitats of Landbirds During Fall: Use of Edge-dominated and Early-successional Forests. The Auk 121:1040-1055.

Ruegg, K.C., E.C. Anderson, K.L. Paxton, V. Apkenas, S. Lao, R.B. Siegel, D.F. Desante, F. Moore and T.B. Smith (2014). Mapping migration in a songbird using high-resolution genetic markers. Molecular Ecology 23:5726-5739.

Van Oosten, H.H., J.C. Mueller, J. Ottenburghs, C. Both and B. Kempernaers (2016). Genetic structure among remnant populations of a migratory passerine, the Northern Weatear *Oenanthe oenanthe*. Ibis 158:857-867.

Watson, C. J. W., A. A. Beheler, and O. E. Rhodes Jr (2002). Development of hypervariable microsatellite loci for use in Eastern Phoebes (*Sayornis phoebe*) and related Tyrannids. Molecular Ecology Notes 2:117-118.

Weeks, H.P., Jr. (1994). Eastern Phoebe (*Sayornis phoebe*). *In* A. Poole and F. Gill (Eds.). The Birds of North American, no. 94. Philadelphia, Pa.: Academy of Natural Sciences; Washington, D.C.: American Ornithologists' Union.

Weeks, H.P., Jr. (2011). Eastern Phoebe (*Sayornis phoebe*), version 2.0. In The Birds of North America (A.F. Poole, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA.

Weir, B.S., and C.C. Cockerham (1984). Estimating *F*-statistics for the analysis of population structure. 1983. Evolution 38:1358-1370.

Whitlock, M.C. and D.E. McCauley (1999). Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. Heredity 82:117-125.

Winker, K., G.R. Graves, and M.J. Braun (2000). Genetic differentiation among populations of a migratory songbird: *Limnothylpis swainsonii*. Journal of Avian Biology 31:319-328.

APPENDIX

Table 1: Sample size (N) of each a priori population assignment under three scenarios, 1) capture location, 2) season capture (breeding, May–early August; migration, middle August–middle November; wintering, late November–January; Week Jr. 2011), and 3) capture date of phoebes captured between November 2017 – January 2019 at Panola Mountain Banding Station (Stockbridge, Georgia, USA).

Capture Location (N)	Season Capture (N)	Capture Date (N)
Location 1 (73)	Breeding (46)	06.13.2018 (19)
	Migration (23)	06.22.2018 (12)
	Wintering (4)	07.4.2018 (4)
		07.13.2018 (3)
		08.3.2018 (8)
		08.18.2018 (12)
		09.29.2018 (6)
		10.14.2018 (4)
		11.25.2018 (2)
		01.12.2019 (2)

Table 2. Sample size (N), number of alleles (N_A), allelic richness (A_R), expected (H_E) and observed (H_O) heterozygosity, inbreeding coefficient (F_{IS}), and X^2 tests for Hardy-Weinberg Equilibrium with associated P-value, with statistically significant values in bold, for a priori population assignment based on capture location at 12 microsatellite loci of phoebes captured between November 2017 – January 2019 at Panola Mountain Banding Station (Stockbridge, Georgia, USA).

	N	N_A	A_R	H_E	H_O	F_{IS}	X^2 (P-value)
SAP22	35	15	6.825	0.853	0.829	0.029	82.545 (0.948)
SAP32	66	11	8.227	0.878	0.879	0.000	81.605 (**0.011**)
SAP39	73	4	1.348	0.258	0.178	0.310	27.128 (**0.000**)
SAP47	71	9	3.747	0.733	0.775	-0.057	46.984 (0.104)
SAP50	72	14	6.612	0.849	0.931	-0.096	128.088 (**0.006**)
SAP53	70	9	2.227	0.551	0.343	0.378	101.821 (**0.000**)
SAP66	39	13	6.882	0.855	0.872	-0.020	51.276 (0.992)
SAP73	73	3	1.570	0.363	0.411	-0.132	3.330 (0.343)
SAP94	72	3	1.504	0.335	0.264	0.212	10.473 (**0.015**)
SAP96	73	7	3.069	0.674	0.603	0.106	21.233 (0.445)
SAP104	39	12	3.267	0.694	0.538	0.224	68.969 (0.377)
SAP108	72	3	1.252	0.201	0.167	0.172	4.334 (0.228)

Table 3. Sample size (N), number of alleles (N_A), allelic richness (A_R), expected (H_E) and observed (H_O) heterozygosity, inbreeding coefficient (F_{IS}) and X^2 tests for Hardy-Weinberg Equilibrium with associated P-value for a priori population based on season capture at 12 microsatellite loci of phoebes captured between November 2017 – January 2019 at Panola Mountain Banding Station (Stockbridge, Georgia, USA).

Population	Locus	N	N_A	A_R	H_E	H_O	F_{IS}	X^2 (P-value)
Breeding	SAP22	35	15.000	2.199	0.829	0.853	0.029	82.545 (0.948)
	SAP32	39	11.000	2.185	0.872	0.869	-0.003	58.816 (0.338)
	SAP39	46	3.000	0.495	0.174	0.251	0.308	13.486 (**0.004**)
	SAP47	45	8.000	1.588	0.822	0.741	-0.110	25.960 (0.575)
	SAP50	45	13.000	2.191	0.956	0.858	-0.114	125.200 (**0.001**)
	SAP53	45	7.000	0.572	0.156	0.226	0.312	167.960 (**0.000**)
	SAP66	39	13.000	2.118	0.872	0.855	-0.020	51.276 (0.992)
	SAP73	46	3.000	0.797	0.587	0.468	-0.254	6.174 (0.103)
	SAP94	45	3.000	0.665	0.311	0.366	0.151	5.086 (0.166)
	SAP96	46	7.000	1.406	0.609	0.673	0.096	18.844 (0.595)
	SAP104	39	12.000	1.651	0.538	0.694	0.224	68.969 (0.377)
	SAP108	46	3.000	0.546	0.261	0.297	0.120	2.150 (0.542)
Migration	SAP22	0	0.000	0.000	0.000	0.000	-	Monomorphic
	SAP32	23	11.000	2.083	0.870	0.840	-0.035	71.790 (0.064)
	SAP39	23	4.000	0.447	0.130	0.200	0.349	9.597 (0.143)
	SAP47	22	9.000	1.709	0.727	0.741	0.018	35.200 (0.506)
	SAP50	23	12.000	2.052	0.913	0.825	-0.107	65.416 (0.497)
	SAP53	21	9.000	1.870	0.714	0.817	0.126	37.863 (0.384)
	SAP66	0	0.000	0.000	0.000	0.000	-	Monomorphic
	SAP73	23	2.000	0.241	0.130	0.122	-0.070	0.112 (0.738)
	SAP94	23	2.000	0.462	0.174	0.287	0.395	3.584 (0.058)
	SAP96	23	6.000	1.251	0.522	0.654	0.202	11.917 (0.685)
	SAP104	0	0.000	0.000	0.000	0.000	-	Monomorphic
	SAP108	22	1.000	0.000	0.000	0.000	-	Monomorphic
Wintering	SAP22	0	0.000	0.000	0.000	0.000	-	Monomorphic
	SAP32	4	6.000	1.733	1.000	0.813	-0.231	12.000 (0.679)
	SAP39	4	4.000	1.074	0.500	0.563	0.111	8.16 (00.227)
	SAP47	4	3.000	0.736	0.500	0.406	-0.231	0.444 (0.931)
	SAP50	4	5.000	1.494	0.750	0.750	0.000	8.444 (0.586)
	SAP53	4	4.000	1.255	0.500	0.688	0.273	8.444 (0.207)
	SAP66	0	0.000	0.000	0.000	0.000	-	Monomorphic
	SAP73	4	1.000	0.000	0.000	0.000	-	Monomorphic
	SAP94	4	2.000	0.377	0.250	0.219	-0.143	0.082 (0.775)
	SAP96	4	4.000	1.255	1.000	0.688	-0.455	4.889 (0.558)
	SAP104	0	0.000	0.000	0.000	0.000	-	Monomorphic
	SAP108	4	1.000	0.000	0.000	0.000	-	Monomorphic

Table 4. Pairwise F_{ST} values (below diagonal) and associated P-values (above diagonal) for three a priori populations based on season capture of phoebes captured between November 2017 – January 2019 at Panola Mountain Banding Station (Stockbridge, Georgia, USA), with significant values in bold print.

	Breeding	Migration	Wintering
Breeding		**0.001**	**0.001**
Migration	0.185**		0.312
Wintering	0.161**	0.000	

0 to 0.05 indicates little genetic differentiation, *0.05 to 0.15 indicates moderate genetic differentiation, **0.15 to 0.25 indicates great genetic differentiation, ***0.25 indicates very great genetic differentiation (Wright 1978).

Table 5. Sample size (N), number of alleles (N_A), allelic richness (A_R), expected (H_E) and observed (H_O) heterozygosity, inbreeding coefficient (F_{IS}) and X^2 tests for Hardy-Weinberg Equilibrium with associated P-value for a priori population assignment based on capture date at 12 microsatellite loci of phoebes captured between June 2018 – January 2019 at Panola Mountain Banding Station (Stockbridge, Georgia, USA).

Population	Locus	N	N_A	A_R	H_E	H_O	F_{IS}	X^2 (P-value)	Population	Locus	N	N_A	A_R	H_E	H_O	F_{IS}	X^2 (P-value)
06.13.2018	SAP22	18	10	5.538	0.819	0.833	-0.017	35.406 (0.847)	08.3.2018	SAP22	0	0	0.000	0.000	0.000	-	Monomorphic
	SAP32	20	10	7.273	0.863	0.950	-0.101	34.188 (0.880)		SAP32	2	3	2.667	0.625	0.500	0.200	4.000 (0.261)
	SAP39	20	3	1.225	0.184	0.200	-0.088	0.140 (0.987)		SAP39	8	2	1.438	0.305	0.125	0.590	2.782 (0.095)
	SAP47	19	7	4.298	0.767	0.789	-0.029	21.920 (0.404)		SAP47	8	5	2.000	0.500	0.625	-0.250	1.653 (0.998)
	SAP50	19	9	5.967	0.832	1.000	-0.201	29.045 (0.410)		SAP50	8	6	4.571	0.781	0.750	0.040	10.747 (0.77)
	SAP53	20	1	1.000	0.000	0.000	-	Monomorphic		SAP53	8	7	5.120	0.805	1.000	-0.243	28.267 (0.133)
	SAP66	20	11	5.839	0.829	0.900	-0.086	25.933 (0.990)		SAP66	0	0	0.000	0.000	0.000	-	Monomorphic
	SAP73	20	3	2.100	0.524	0.65	-0.241	2.977 (0.395)		SAP73	8	2	1.438	0.305	0.375	-0.231	0.426 (0.514)
	SAP94	20	3	1.504	0.335	0.400	-0.194	0.969 (0.809)		SAP94	7	2	1.690	0.408	0.286	0.300	0.630 (0.427)
	SAP96	20	7	2.963	0.663	0.550	0.170	9.295 (0.987)		SAP96	8	5	3.200	0.688	0.750	-0.091	5.819 (0.83)
	SAP104	20	9	2.827	0.646	0.500	0.226	19.454 (0.989)		SAP104	0	0	0.000	0.000	0.000	-	Monomorphic
	SAP108	20	3	1.559	0.359	0.350	0.024	0.329 (0.954)		SAP108	8	2	1.280	0.219	0.000	1.000	8.000 (**0.005**)
06.22.2018	SAP22	9	8	6.000	0.833	0.889	-0.067	18.750 (0.906)	08.18.2018	SAP22	0	0	0.000	0.000	0.000	-	Monomorphic
	SAP32	11	9	7.563	0.868	0.818	0.057	34.933 (0.519)		SAP32	12	8	5.143	0.806	0.917	-0.138	26.500 (0.546)
	SAP39	11	3	1.204	0.169	0.091	0.463	11.456 (**0.010**)		SAP39	12	4	1.548	0.354	0.250	0.294	4.790 (0.571)
	SAP47	11	5	3.903	0.744	0.909	-0.222	11.510 (0.716)		SAP47	11	7	3.723	0.731	0.727	0.006	16.840 (0.721)
	SAP50	11	7	5.261	0.810	1.000	-0.235	13.583 (0.887)		SAP50	12	9	4.431	0.774	0.917	-0.184	54.370 (**0.025**)
	SAP53	11	1	1.000	0.000	0.000	-	Monomorphic		SAP53	11	9	5.628	0.822	0.909	-0.106	26.434 (0.878)
	SAP66	11	7	5.628	0.822	0.727	0.116	25.173 (0.240)		SAP66	0	0	0.000	0.000	0.000	-	Monomorphic
	SAP73	11	3	2.180	0.541	1.000	-0.847	12.000 (**0.007**)		SAP73	12	2	1.087	0.080	0.083	-0.043	0.023 (0.88)
	SAP94	11	3	1.449	0.310	0.182	0.413	4.790 (0.188)		SAP94	12	2	1.492	0.330	0.250	0.242	0.703(0.402)
	SAP96	11	6	3.315	0.698	0.636	0.089	10.920 (0.758)		SAP96	12	4	2.796	0.642	0.417	0.351	8.147 (0.228)
	SAP104	11	6	3.507	0.715	0.545	0.237	27.960 (**0.022**)		SAP104	0	0	0.000	0.000	0.000	-	Monomorphic
	SAP108	11	3	1.458	0.314	0.364	-0.158	0.480 (0.923)		SAP108	11	1	1.000	0.000	0.000	-	Monomorphic
07.4.2018	SAP22	4	5	4.571	0.781	0.500	0.360	12.000 (0.285)	09.29.2018	SAP22	0	0	0.000	0.000	0.000		Monomorphic
	SAP32	4	6	5.333	0.813	0.750	0.077	16.00 (0.382)		SAP32	6	9	8.000	0.875	1.000	-0.143	33.000 (0.612)
	SAP39	4	3	2.133	0.531	0.250	0.529	4.160 (0.245)		SAP39	6	1	1.000	0.000	0.000	-	Monomorphic
	SAP47	4	4	3.556	0.719	1.000	-0.391	6.667 (0.353)		SAP47	6	6	4.000	0.750	0.833	-0.111	14.760 (0.469)
	SAP50	4	6	5.333	0.813	1.000	-0.231	14.00 (0.526)		SAP50	6	7	4.235	0.764	0.833	-0.091	14.160 (0.863)
	SAP53	4	1	1.000	0.000	0.000	-	Monomorphic		SAP53	5	4	2.941	0.660	0.400	0.394	9.200 (0.163)
	SAP66	4	5	4.571	0.781	1.000	-0.280	8.000 (0.629)		SAP66	0	0	0.000	0.000	0.000	-	Monomorphic
	SAP73	4	1	1.000	0.000	0.000	-	Monomorphic		SAP73	6	1	1.000	0.000	0.000	-	Monomorphic
	SAP94	4	1	1.000	0.000	0.000	-	Monomorphic		SAP94	6	2	1.180	0.153	0.167	-0.091	0.050 (0.824)
	SAP96	4	3	1.684	0.406	0.500	-0.231	0.444 (0.931)		SAP96	6	4	3.130	0.681	0.833	-0.224	3.060 (0.801)
	SAP104	4	4	2.909	0.656	0.750	-0.143	4.000 (0.677)		SAP104	0	0	0.000	0.000	0.000	-	Monomorphic
	SAP108	4	2	1.280	0.219	0.250	-0.143	0.082 (0.775)		SAP108	6	1	1.000	0.000	0.000	-	Monomorphic
07.13.2018	SAP22	3	4	3.600	0.722	1.000	-0.385	4.500 (0.609)	10.14.2018	SAP22	0	0	0.000	0.000	0.000	-	Monomorphic

SAP32	2	3	2.667	0.625	0.500	0.200	4.000 (0.261)
SAP39	3	2	1.385	0.278	0.333	-0.200	0.120 (0.729)
SAP47	3	3	2.571	0.611	1.000	-0.636	3.000 (0.392)
SAP50	3	6	6.000	0.833	1.000	-0.200	15.000 (0.451)
SAP53	2	1	1.000	0.000	0.000	-	Monomorphic
SAP66	3	5	4.500	0.778	1.000	-0.286	9.000 (0.532)
SAP73	3	1	1.000	0.000	0.000	-	Monomorphic
SAP94	3	2	2.000	0.500	0.333	0.333	0.333 (0.564)
SAP96	3	3	2.000	0.500	0.667	-0.333	0.750 (0.861)
SAP104	3	3	2.571	0.611	0.333	0.455	3.333 (0.343)
SAP108	3	1	1.000	0.000	0.000	-	Monomorphic

SAP32	4	6	5.333	0.813	0.750	0.077	16.000 (0.382)
SAP39	4	1	1.000	0.000	0.000	-	Monomorphic
SAP47	4	3	2.133	0.531	0.500	0.059	3.360 (0.339)
SAP50	4	6	5.333	0.813	1.000	-0.231	20.000 (0.172)
SAP53	4	4	3.556	0.719	0.500	0.304	7.111 (0.311)
SAP66	0	0	0.000	0.000	0.000	-	Monomorphic
SAP73	4	2	1.280	0.219	0.250	-0.143	0.082 (0.775)
SAP94	4	2	1.600	0.375	0.000	1.000	4.000 (**0.046**)
SAP96	4	2	0.662	0.469	0.250	0.467	0.871 (0.351)
SAP104	0	0	0.000	0.000	0.000	-	Monomorphic
SAP108	4	1	0.000	0.000	0.000	-	Monomorphic

Table 5 *Continued*. Sample size (N), number of alleles (N_A), allelic richness (A_R), expected (H_E) and observed (H_O) heterozygosity, inbreeding coefficient (F_{IS}) and X^2 tests for Hardy-Weinberg Equilibrium with associated P-value for a priori population assignment based on capture date at 12 microsatellite loci of phoebes captured between June 2018 – January 2019 at Panola Mountain Banding Station (Stockbridge, Georgia, USA).

Population	Locus	N	N_A	A_R	H_E	H_O	F_{IS}	X^2 (P-value)
11.25.2018	SAP22	0	0	0.000	0.000	0.000	-	Monomorphic
	SAP32	4	6	5.333	0.813	0.750	0.077	16.000 (0.382)
	SAP39	4	1	1.000	0.000	0.000	-	Monomorphic
	SAP47	4	3	2.133	0.531	0.500	0.059	3.360 (0.339)
	SAP50	4	6	5.333	0.813	1.000	-0.231	20.000 (0.172)
	SAP53	4	4	3.556	0.719	0.500	0.304	7.111 (0.311)
	SAP66	0	0	0.000	0.000	0.000	-	Monomorphic
	SAP73	4	2	1.280	0.219	0.250	-0.143	0.082 (0.775)
	SAP94	4	2	1.600	0.375	0.000	1.000	4.000 (**0.046**)
	SAP96	4	2	1.040	0.469	0.250	0.467	0.871 (0.351)
	SAP104	0	0	0.000	0.000	0.000	-	Monomorphic
	SAP108	4	1	0.000	0.000	0.000	-	Monomorphic
01.12.2019	SAP22	0	0	0.000	0.000	0.000	-	6.000 (0.423)
	SAP32	2	4	4.000	0.750	1.000	-0.333	0.222 (0.637)
	SAP39	2	2	1.600	0.375	0.500	-0.333	0.222 (0.637)
	SAP47	2	2	1.600	0.375	0.500	-0.333	4.000 (0.261)
	SAP50	2	3	2.667	0.625	0.500	0.200	4.000 (0.261)
	SAP53	2	3	2.667	0.625	0.500	0.200	Monomorphic
	SAP66	0	0	0.000	0.000	0.000	-	Monomorphic
	SAP73	2	1	1.000	0.000	0.000	-	Monomorphic
	SAP94	2	1	1.000	0.000	0.000	-	Monomorphic
	SAP96	2	3	2.667	0.625	1.000	-0.600	2.000 (0.572)
	SAP104	0	0	0.000	0.000	0.000	-	Monomorphic
	SAP108	2	1	1.000	0.000	0.000	-	Monomorphic

Table 6. Pairwise F_{ST} values (below diagonal) and associated P-values (above diagonal) for ten a priori populations based on capture date of phoebes captured between June 2018 – January 2019 at Panola Mountain Banding Station (Stockbridge, Georgia, USA), with significant values in bold print.

	06.13.2018	06.22.2018	07.4.2018	07.13.2018	08.3.2018	08.18.2018	09.29.2018	10.14.2018	11.25.2018	01.12.2019
06.13.2018		0.457	0.179	**0.044**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
06.22.2018	0.000		0.051	**0.036**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
07.4.2018	0.016	0.032		0.223	**0.001**	**0.001**	**0.001**	**0.001**	**0.005**	**0.001**
07.13.2018	0.044	0.045	0.026		**0.001**	**0.001**	**0.001**	**0.001**	**0.015**	**0.007**
08.3.2018	0.275***	0.271***	0.323***	0.280***		**0.002**	**0.003**	0.436	0.470	0.251
08.18.2018	0.262***	0.260***	0.293***	0.274***	0.056*		0.442	0.442	0.459	0.451
09.29.2018	0.232**	0.222**	0.278***	0.277***	0.062*	0.000		0.448	0.457	0.291
10.14.2018	0.226**	0.217**	0.264***	0.237**	0.003	0.000	0.000		0.448	0.413
11.25.2018	0.207**	0.189**	0.232**	0.205**	0.000	0.000	0.000	0.000		0.485
01.12.2019	0.239**	0.232**	0.245**	0.200**	0.024	0.000	0.019	0.000	0.000	

0 to 0.05 indicates little genetic differentiation, *0.05 to 0.15 indicates moderate genetic differentiation, **0.15 to 0.25 indicates great genetic differentiation, ***0.25 indicates very great genetic differentiation (Wright 1978).

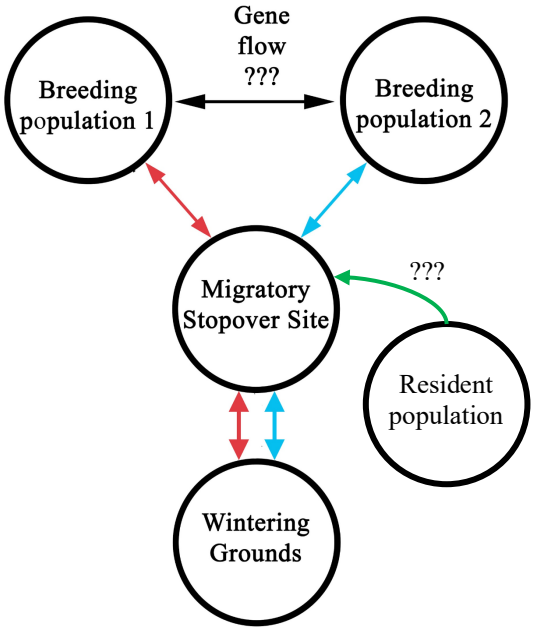

Figure 1: Conceptual model representing potential movements of two breeding populations and a residential population; breeding population 1 follows the red arrows, breeding population 2 follows the blue arrows, the resident population follows the green arrow and all are potentially converging on a migratory stopover site. Gene flow in this scenario is unknown, but is likely low for philopatric species.

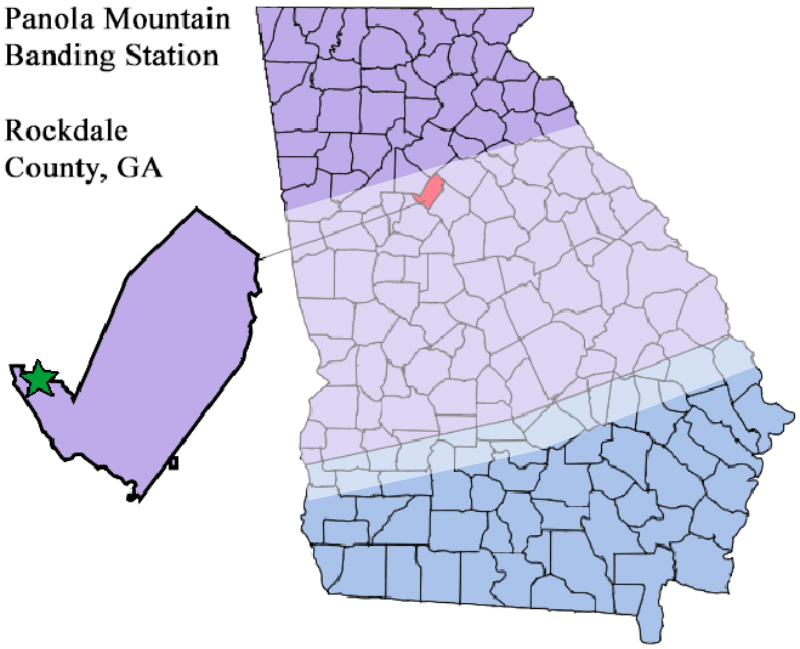

Figure 2: The wintering (blue) and resident (purple) range of Eastern Phoebes (Weeks Jr. 2011), as well as a greyed region indicating where residents and migrants potentially overlap in Georgia, USA. The study site is located in Rockdale County (red) with study site Panola Mountain Banding Station indicated by the green star.

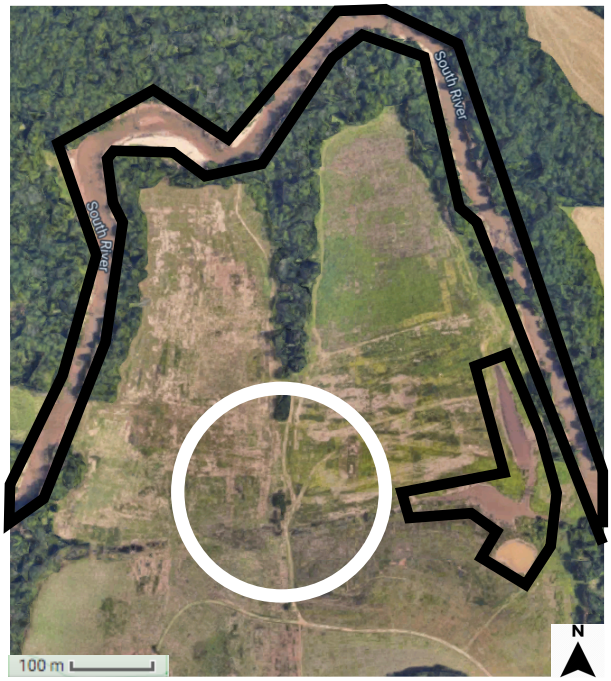

Figure 3: A satellite image of the Panola Mountain Banding Station (Stockbridge, Georgia, USA) showing 110 acres of grassland, with the surrounding scattered forest edge; riparian areas are outlined in black. The white circle represents where birds are captured during sampling days.

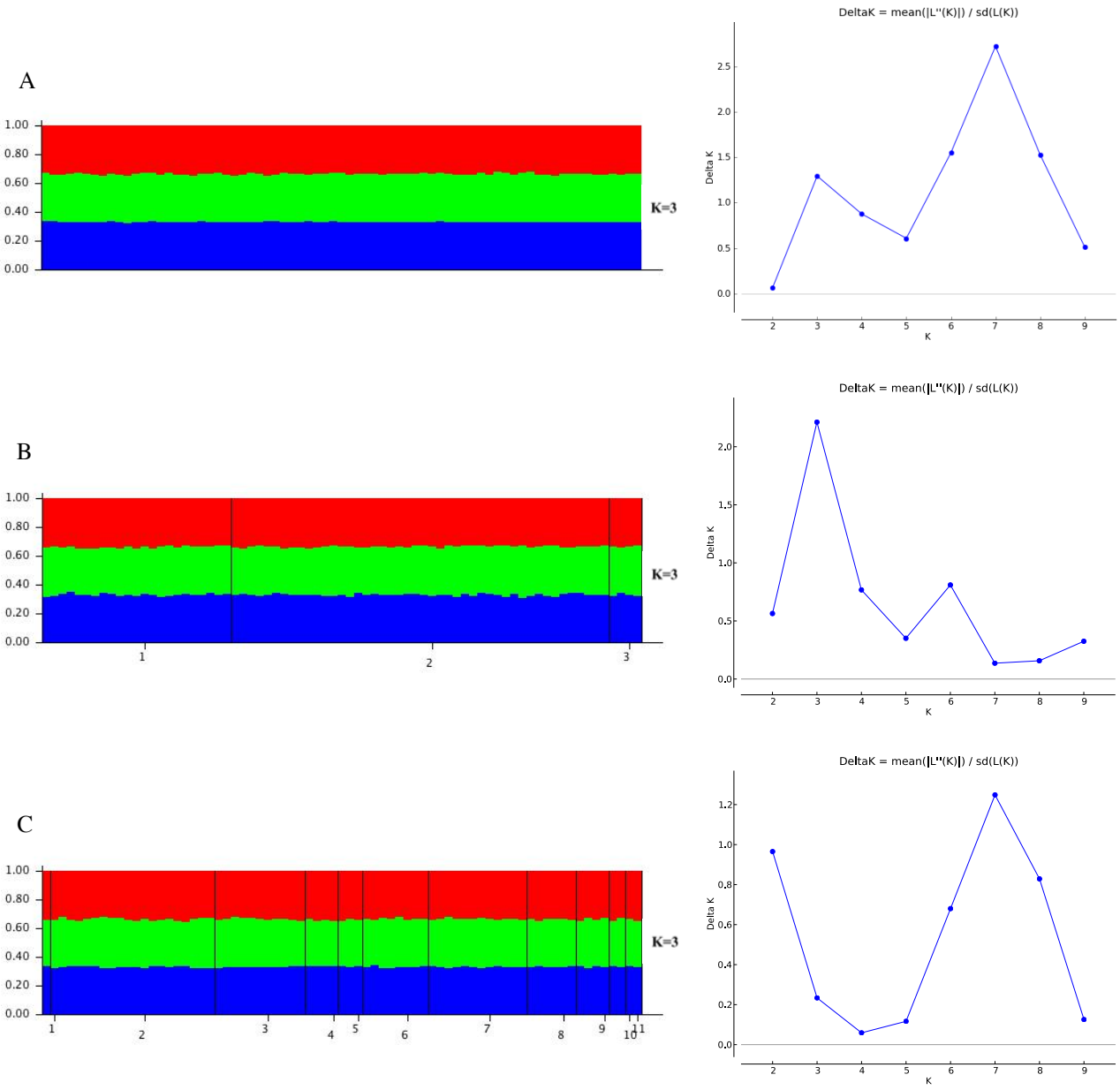

Figure 4: STRUCTURE bar graph results for K=3 and graph of ΔK for each STRUCTURE scenario of phoebes captured between November 2017 – January 2019 at Panola Mountain Banding Station (Stockbridge, Georgia, USA): capture location (A), season capture (B), and capture date (C). All graphs were made using STRUCTURE Harvester (Earl and vonHoldt 2012).

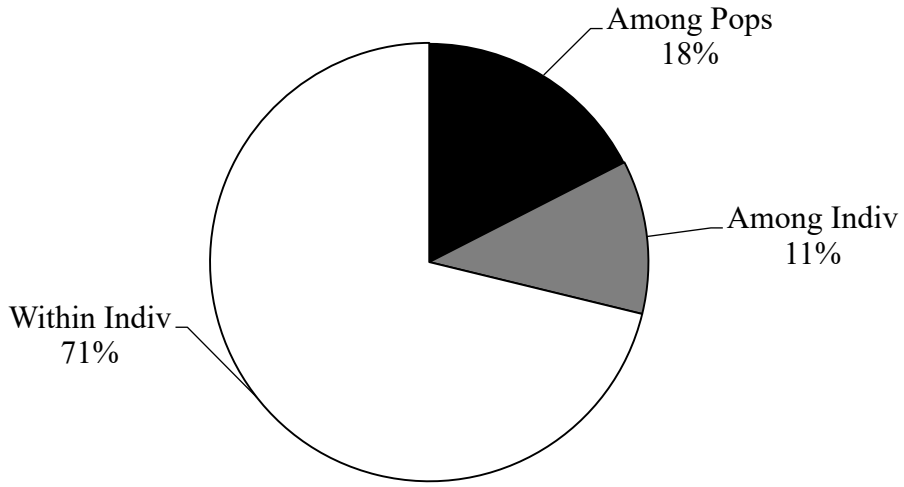

Figure 5: AMOVA (analysis of molecular variance) results showing distribution of variation among populations (black), among individuals (grey), and within individuals (white) for a priori population assignment based on season capture at 12 microsatellite loci of phoebes captured between November 2017 – January 2019 at Panola Mountain Banding Station (Stockbridge, Georgia, USA).

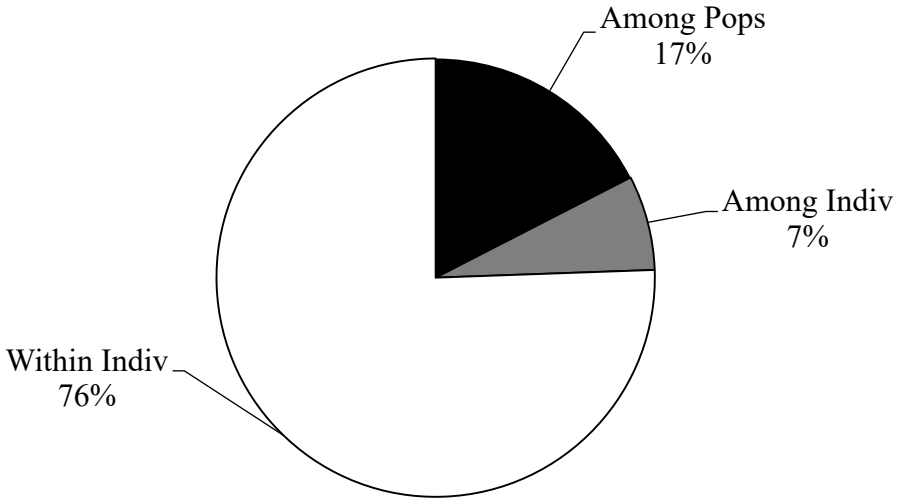

Figure 6: AMOVA (analysis of molecular variance) results showing distribution of variation among populations (black), among individuals (grey), and within individuals (white) for a priori population assignment based on capture date at 12 microsatellite loci of phoebes captured between June 2018 – January 2019 at Panola Mountain Banding Station (Stockbridge, Georgia, USA).