Assessing Genetic Diversity of Migratory and Non-migratory Birds in a Rapidly Developing Region of the Georgia Piedmont

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Abstract: Species richness, abundance, and genetic variability often decrease in bird populations when their habitats are subjected to anthropogenic activity. Regular and early monitoring of genetic diversity can give researchers and wildlife managers insight into the genetic health of populations so that action can be taken before inbreeding, loss of disease resistance, and population declines occur. We measured genetic diversity in populations of avian species that are increasingly exposed to anthropogenic changes. We analyzed samples from 89 individual birds from three locations in Gwinnett County, Georgia. Samples were collected from a total of seven species, four migratory [myrtle warbler (*Setophaga coronata*), American robin (*Turdus migratorius*), American goldfinch (*Spinus tristis*), and field sparrow (*Spizella pusilla*)] and three non-migratory [northern cardinal (*Cardinalis cardinalis*), brown-headed nuthatch (*Sitta pusilla*), and white-breasted nuthatch (*S. carolinensis*)]. DNA sequences of the mitochondrial gene *cytochrome c oxidase subunit 1* (*COI*) were compared to determine intraspecific genetic diversity. We found that genetic diversity varied among the seven species studied. Overall, nucleotide and haplotype diversity in *COI* was higher for non-migrants compared to migratory species. Comparisons of genetic diversity among study sites found that the least urbanized of the three locations had greater genetic diversity than the other two locations. As human development continues to eliminate natural areas, additional genetic monitoring is recommended for Gwinnett County and other rapidly developing urban areas.

Key words: cytochrome c oxidase, COI, avian, conservation

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Habitat loss is the greatest threat to wildlife in the United States and is a major driver of biodiversity declines (Wilcove et al. 1998). Anthropogenic activity that alters ecosystems results in a loss of biodiversity across taxa and has resulted in declines in bird species richness and abundance (Burleigh 1958, Smith and Smith 1994, Klaus and Keyes 2007, Murgui and Hedblom 2017). As urban areas are predicted to increase threefold world-wide by 2050, habitat availability for bird species will decrease and many more populations are expected to become confined to fragmented parcels (Westemeier et al. 1998, Amos and Balmford 2001, Angel et al. 2012). Fragmentation impedes conservation efforts in a variety of ways. Small, isolated populations are at greater risk of extinction due to demographic and environmental stochasticity; they are also prone to a loss of genetic diversity due to random genetic drift and inbreeding (Allendorf and Luikart 2009). Decreases in genetic variability can reduce disease resistance and make it difficult for populations to adapt to changing environments, possibly leading to local extinction (Amos and Balmford 2001, Bounas et al. 2018).

Surveys of intraspecific genetic diversity could be of use to conservation programs and land managers as this information can assist in determining appropriate strategies for maintaining healthy, diverse populations, including detecting initial losses of genetic diversity that may serve as an early warning of demographic declines (Haas et al. 2010, Bounas et al. 2018). While use of the mitochondrial gene *cytochrome c oxidase subunit 1* (*COI*) began as a method to identify species genetically (Hebert et al. 2004), *COI* is an advantageous gene to use when comparing the genetic diversity of multiple bird species. The development of universal primers to amplify *COI* allows for the same polymerase chain reaction (PCR) protocol to be used for a wide range of avian species, families, and orders and an extensive number of *COI* sequences has been collected over the past 16 years. (Hebert et al. 2004, Kerr et al. 2007, Stoeckle and Thaler 2014). *COI* variation can be measured in a large number of species with only small quantities of biological samples using a reliable and relatively inexpensive procedure (Hebert et al. 2004, Kerr et al. 2007).

Intraspecific genetic diversity can vary across species with different life histories. For example, seasonal migration is a strategy used by some bird species. Obligate migrants depend on multiple territories throughout the year for breeding, overwintering, and transient use during spring and fall migration periods (Rodewald 2015). At the other extreme, resident species do not migrate and require a single habitable territory year-round. Facultative migrants incorporate both strategies as they will remain on their breeding grounds when environmental conditions are good or migrate when conditions are too harsh. Therefore, it is possible for resident species to have satisfactory levels of genetic diversity while migratory species from the same geographical area have critically low levels, or vice versa.

Numerous studies have assessed avian genetic diversity and the results with respect to migratory status seem to be depend on the species and geographical location of the study. For example, universally low intraspecific variation in mitochondrial DNA (mtDNA) has been documented in migratory species such as red-winged blackbird (*Agelaius phoeniceus*), thick-billed murre (*Uria lomvia*), sandpipers, fox sparrow (*Passerella iliaca*), and New World warblers (Ball et al. 1988, Zink 1991, Birt-Friesen et al. 1992, Stoeckle and Thaler 2014). However, in studies of species for which there are distinct migratory and non-migratory populations, the migratory populations have higher levels of mtDNA diversity than the non-migratory populations (Buerkle 1999, Miller et al. 2012). Furthermore, large scale studies on the genetic diversity of microsatellite markers found migratory mammals, reptiles, amphibians, and fishes had less genetic diversity compared to non-migratory species, but migratory birds had more genetic diversity than non-migrants (Willoughby et al. 2017). Studies of genetic diversity, specifically in resident species, also came to opposite conclusions. In one example, mtDNA was found to be monomorphic in a non-migratory population of greylag geese (*Anser anser*) (Bounas et al. 2018). By contrast, high levels of nucleotide and haplotype diversity in *COI* have been detected in populations of non-migratory Eurasian collared-doves (*Streptopelia decaocto*) (Bagi et al. 2018). It is possible that differences in species, geographical locations, and the genes used to measure genetic diversity could account for the contrasting conclusions drawn from studies mentioned above.

In our study, intraspecific *COI* variability was measured for seven avian species (one obligate migrant, three facultative migrants, and three non-migrants) from three locations in Gwinnett County, Georgia. Results from this study indicate the extent of genetic diversity that exists in birds living in a habitat that is currently undergoing anthropogenic change. Furthermore, genetic diversity values from this study allow diversity to be compared to populations in other geographical locations and can serve as a baseline to compare values from the same locations in the future.

Study Area

Our study area was Gwinnett County, a suburban county of the Atlanta Metropolitan area in the Southern Appalachian Piedmont region of Georgia (Rummer and Hafer 2014). Gwinnett County is urbanizing faster than the surrounding region. The Piedmont region in the eastern United States holds overall approximately 62% of its total land area as forest land. In contrast, Gwinnett County has experienced rapid population growth, resulting in an increase in residential land use from 16.7% in 1984 to 44% in 2009, with an associated increase in land used for commercial, industrial, transportation and utilities (U.S. Bureau of Census 1995, Gwinnett County Board of Commissioners 2009, 2019). As of 2019, 10% of the land was categorized as forest land (including parks, recreation, and conservation), 12% was listed as undeveloped, and 5% was listed as non-agricultural estates (Gwinnett County Board of Commissioners 2019). Population growth is expected to continue in the future and limited amounts of land will be available for wildlife (Gwinnett County Board of Commissioners 2019). These recent and projected changes in land use make Gwinnett County a highly relevant area to study the effects of habitat loss and fragmentation on wildlife populations, in real time.

We sampled birds at three locations with varying levels of urbanization in Gwinnett County: 1) Georgia Gwinnett College, 1000 University Center Lane, Lawrenceville 2) Collins Hill Park, 2225 Collins Hill Road, Lawrenceville and (3) Harbins Park, 2550 Indian Shoals Road, Dacula. We used Google Earth Pro images (Google LLC, Mountain View, California) ArcGIS 10.5.1 (Environmental Systems Research Institute Inc., Redlands, California) to classify the levels of urbanization for each site. We classified George Gwinnett College with 57.3% urban (impervious surfaces [i.e., buildings, parking lots, or streets], gravel, and landscaped man-made yards) and 42.7% natural surface area (tree canopy, riparian areas, and grass meadows that are not landscaped), Collins Hill Park with 45.9% urban/54.1% natural, and Harbins Park with 7.3% urban/92.7% natural.

Methods

Field and Laboratory Methods

We captured live birds by mist netting between December 2016 and December 2018 and marked them with a unique U.S. Fish and Wildlife Service metallic band. Blood was drawn and/or body feathers were pulled after assessing age, sex, mass, and other measurable characteristics, and then birds were released. We reported all data collected on individual birds to the U.S. Geological Survey (USGS) Bird Banding Laboratory for their potential use in other studies. Banding activities and sampling were performed with a sub-permit from federal bird banding and marking permit num-

ber 23450 from USGS, and our research activities were approved by Georgia Gwinnett College (IACUC-2017-04).

We focused on seven species of migratory and non-migratory birds. Myrtle warblers (*Setophaga coronata*) are obligate migrants that winter in Georgia while American robins (*Turdus migratorius*), American goldfinches (*Spinus tristus*), and field sparrows (*Spizella pusilla*) are facultative migrants (Rodewald 2015). The remaining three species, brown-headed nuthatches (*Sitta pusilla*), white-breasted nuthatches (*Sitta carolinesis*), and northern cardinals (*Cardinalis cardinalis*) do not migrate (Rodewald 2015). We conducted monthly mist netting at each of the three study sites throughout the course of the study. Samples from the migratory species were obtained between the months of September and May in both years.

DNA was isolated either from the tips of approximately 20–30 body feathers or from approximately 20–50 µL of drawn blood. Feathers were stored at room temperature in paper envelopes, and blood samples were stored in 200 µL of PBS at –20° C. A Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc., Germantown, Maryland) was used to isolate DNA from feather and blood samples following the manufacturer's instructions. The mitochondrial gene *cytochrome c oxidase subunit 1* (*COI*) was amplified by PCR using the procedure described by Hebert et al. (2004). Primers used were Bird F1: TTCTCCAACCACAAAGACATTGGCAC and Bird R1: ACGTGGGAGATAATTCCAAATCCTG. 25 µL PCR reactions consisted of 12.5 µL of Go Taq Green Master Mix (Promega Corporation, Madison, Wisconsin), 0.5 µL Bird F1 primer, 0.5 µL Bird R1 primer, 6.5 µL nuclease free water, and 5 µL DNA template. The amplification protocol used was 5 min at 94° C followed by five cycles of 1 min at 94° C, 1.5 min at 45° C, 1.5 min at 72° C, followed by 30 cycles of 1 min at 94° C, 1.5 min at 51° C, 1.5 min at 72° C, and then a final extension of 10 min at 72° C, followed by a 4° C hold. PCR products were visualized on a 1% agarose gel. Samples that produced a visible band of approximately 700 bp were treated with ExoSAP-IT (Thermo Fisher Scientific, Waltham, Massachusetts) according to the manufacturer's instructions and sent to Eurofins Genomics (Louisville, Kentucky) for sanger sequencing. DNA sequences were aligned and analyzed using Mega 7 software (Kumar et al. 2016). GenBank accession numbers for the DNA sequences from this study are MN312092–MN312147.

Statistical Analysis

We determined single nucleotide polymorphisms (SNPs), nucleotide diversity, haplotypes, haplotype diversity, Tajima's D, and Fu and Li's D using DnaSP version 6 software (Rozas et al. 2017). The number of SNPs, number of haplotypes, nucleotide diversity, and haplotype diversity each measure genetic diversity slightly differently, and in each case larger values indicate more diversity. An SNP occurs when sequences from at least two individuals differ at any given nucleotide and a haplotype is the combination of SNPs found in an individual. Nucleotide diversity is the average number of nucleotide differences per site, whereas haplotype diversity is the probability that two randomly sampled alleles are different. Tajima's D and Fu and Li's D are neutrality tests and values significantly different from zero suggest selection or a change in population size. Haplotype maps were inferred using the median joining network method from PopART software version 1.7 (Leigh and Bryant 2015). We used two sample *t*-tests in GraphPad Prism 8.2.0 (GraphPad Software, La Jolla, California) to determine if nucleotide and haplotype diversity were significantly different between migrants and non-migrants, with $\alpha = 0.05$ to assess statistical significance for two-tailed tests.

Results

A total of 89 birds representing seven species were captured and analyzed in this study (Table 1). Of the seven species tested, the American robin, a short-distance migrant, had the fewest genetic variants with zero SNPs and only one haplotype. The non-migratory brown-headed nuthatch had the greatest number of genetic variants with ten SNPs and nine haplotypes (Table 1, Figure 1). Overall, migratory species had less genetic diversity in *COI* than non-migratory species. A *t*-test comparing nucleotide diversity of migratory and non-migratory birds indicated that non-migrants are more diverse than migrants (non-migrants: $\bar{x} = 0.00397$, SE = 0.00118; migrants: $\bar{x} = 0.00102$, SE = 0.00042; d = 1.43, df = 5, $P = 0.044$) (Figure 2A). The same was true for a comparison of haplotype diversity (non-migrants: $\bar{x} = 0.842$, SE = 0.045; migrants: $\bar{x} = 0.267$, SE = 0.125; d = 1.61, df = 5, $P = 0.013$) (Figure 2B). There was no indication of recent changes in population size and/or selection as values for Tajima's D and Fu and Li's D were not statistically significant (compared to zero) for any of the species tested (Table 1).

Differences in intraspecific genetic diversity were found among the three study sites. For field sparrows and American goldfinches, more genetic variants were found at Harbins Park compared to Collins Hill and Georgia Gwinnett College, respectively (Table 2). For field sparrows, both nucleotide and haplotype diversity were higher at Harbins Park than at Collins Hill (Figure 3A, D). Similarly, American goldfinches from Harbins Park had higher nucleotide and haplotype diversity compared to their counterparts from Georgia Gwinnett College (Figure 3B, E). For northern cardinals, nucleotide and haplotype diversity were higher at Georgia Gwinnett College than Collins Hill (Figure 3 C, F). While genetic diversity was lower at Georgia Gwinnett College and Collins Hill compared to the less urbanized Harbins Park study site, we found no indication of population declines. Values for Tajima's D and Fu and Li's D tests of neutrality were not significantly different from zero.

Table 1. Measures of genetic diversity and tests of neutrality for 89 birds sampled at three sites in Gwinnett County, Georgia, 2016–2018.

Species	*n*[a]	SNPs[b]	Pi[c]	H[d]	Hd[e]	Tajima's D	Fu and Li's D
Myrtle warbler (obligate migrant)	14	3	0.00095	2	0.143	−1.67053	−2.09051
American robin (facultative migrant)	10	0	0	1	0	n.d.[f]	0
Field sparrow (Facultative migrant)	20	3	0.00108	3	0.353	−0.97524	−1.25499
American goldfinch (facultative migrant)	14	3	0.00205	4	0.571	−0.70770	−1.03687
Northern cardinal (non-migrant)	13	4	0.00164	5	0.769	−0.76149	0.33450
Brown-headed nuthatch (non-migrant)	14	10	0.00481	9	0.923	−0.31502	−0.25762
White-breasted nuthatch (non-migrant)	4	5	0.00545	3	0.833	0.95621	0.95621

a. Sample size
b. Number of single nucleotide polymorphisms
c. Nucleotide diversity
d. Number of haplotypes
e. Haplotype diversity
f. n.d. = not determined

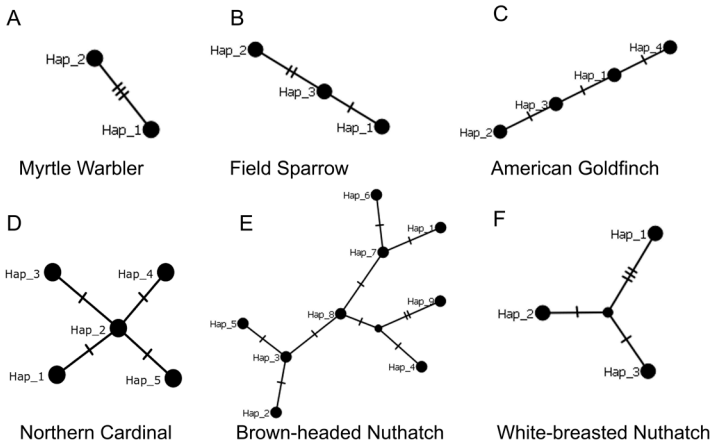

Figure 1. Haplotype maps for (A) obligate migrants, (B, C) facultative migrants, and (D-F) non-migrants for birds sampled at three sites in Gwinnett County, Georgia, 2016–2018. Perpendicular lines indicate the number of nucleotide differences between haplotypes.

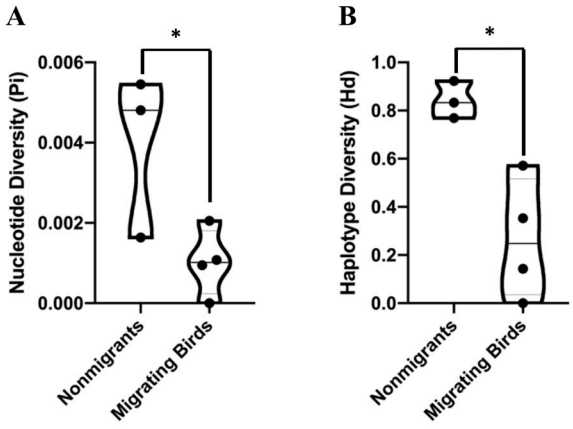

Figure 2. Violin plots show unpaired *t*-tests comparing A) nucleotide diversity (Pi) ($P = 0.0443$) and B) haplotype diversity (Hd) ($P = 0.0129$) between non-migrants and migratory species sampled at three sites in Gwinnett County, Georgia, 2016–2018. Solid line indicates median value.

Table 2. Number of *COI* haplotypes identified at three sites in Gwinnett County, Georgia, 2016–2018.

	Haplotypes (*n*[a])		
Species	GGC[b]	Collins Hill	Harbins
Myrtle warbler (obligate migrant)	2 (13)	1 (1)	n.d.[c]
American robin (facultative migrant)	1 (6)	1 (4)	n.d.[c]
Field sparrow (facultative migrant)	n.d.[c]	1 (7)	3 (13)
American goldfinch (facultative migrant)	2 (6)	n.d.[c]	4 (8)
Northern cardinal (non-migrant)	3 (4)	3 (8)	1 (1)
Brown-headed nuthatch (non-migrant)	9 (14)	n.d.[c]	n.d.[c]
White-breasted nuthatch (non-migrant)	3 (3)	1 (1)	n.d.[c]

a. Sample size
b. Georgia Gwinnett College
c. Not determined (no samples analyzed from location)

Discussion

Here we have shown evidence that there is less genetic variability of *COI* in migratory species compared to non-migrants captured in an area undergoing rapid anthropogenic change in the Piedmont region of northern Georgia. Other studies comparing migratory and non-migratory species have come to the opposite conclusion, however. American kestrel (*Falco sparverius*), populations in the western United States are migratory while populations in the southeastern United States do not migrate; comparisons of genetic diversity among American kestrels using both mtDNA and microsatellite markers indicated that the migratory populations had more genetic diversity than the non-migrants (Miller et al. 2012). Similarly, a study of prairie warblers (*Setophaga discolor*)

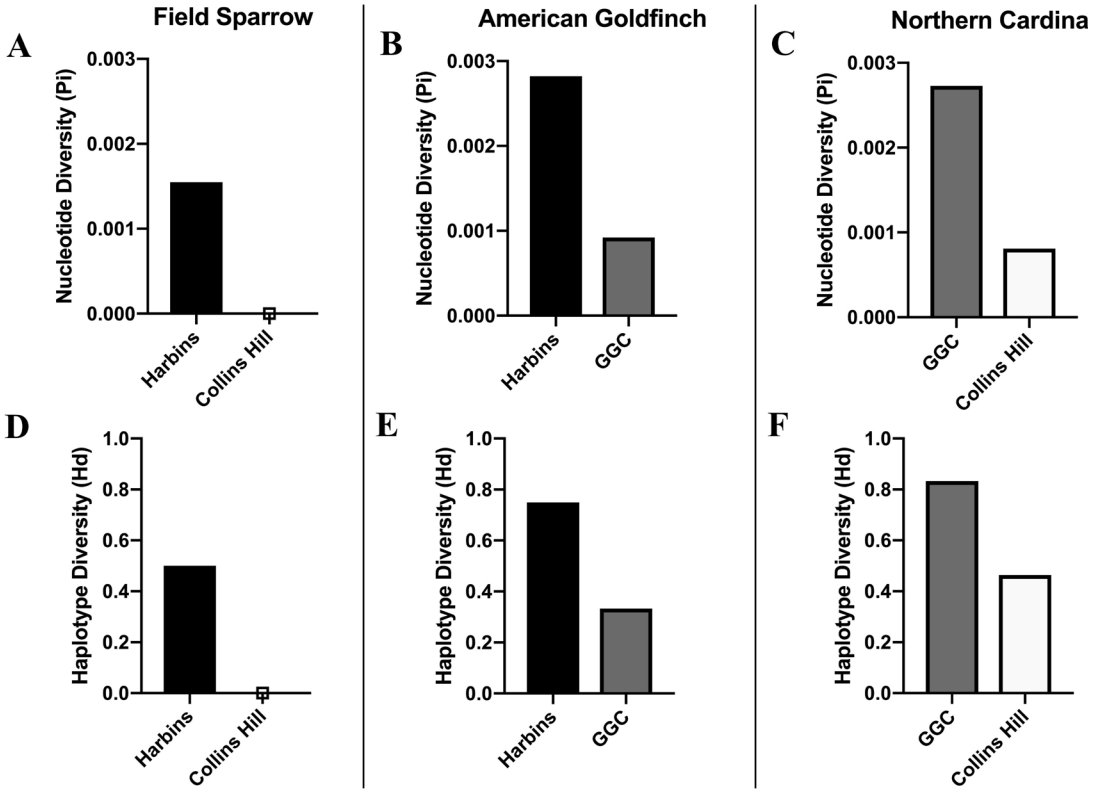

Figure 3. Comparison of nucleotide diversity (A-C) and haplotype diversity (D-F) for birds sampled at three sites in Gwinnett County, Georgia, 2016–2018: Georgia Gwinnett College (GGC), Collins Hill Park, and Harbins Park for field sparrow (A, D), American goldfinch (B, E), and northern cardinal (C, F).

compared migratory populations breeding in the eastern United States to non-migratory populations breeding in Florida and found that the migratory prairie warblers had more genetic diversity in mtDNA than their non-migratory counterparts (Buerkle 1999). Instead of comparing migrating and non-migrating populations of the same species in different geographical locations, we compared different species of migrating and non-migrating birds in the same geographical location. This could, in part, explain why our study came to a different conclusion.

Another difference in our study is that we used *COI* to analyze genetic diversity, whereas the studies mentioned previously, of the American kestrel and prairie warblers, used mtDNA Control Region, *ND6*, and nuclear microsatellite markers (Buerkle 1999, Miller et al. 2012) for the same purpose. It has previously been shown that measures of genetic diversity can differ depending on the individual gene(s) used when comparing groups. For example, migratory populations of graylag geese have multiple haplotypes for the mtDNA Control Region (Heikkinen et al. 2015), while a non-migratory population from Greece has only one haplotype which is unique to their population (Bounas et al. 2018). However, when nuclear genes were analyzed, a difference in genetic diversity between migratory and non-migratory populations was not detected. Levels of genetic diversity were only similar for the two populations when 11 nuclear microsatellite markers were compared (Bounas et al. 2018).

Consistent with our results showing high levels of genetic diversity in *COI* for the brown-headed nuthatch, previous studies for this species, which used up to twelve nuclear microsatellite markers, detected a large number of alleles and high levels of observed and expected heterozygosity (Haas et al. 2009, Haas et al. 2010). Brown-headed nuthatches have short natal dispersal distances, especially in males (Cox and Slater 2007). Limited dispersal behavior can cause the frequency of rare alleles to increase in small, isolated populations due to genetic drift and this behavior is thought to be one of the driving forces of fine-scale spatial genetic structure in brown-headed nuthatches (Haas et al. 2010, Aguillon et al. 2017).

Ultimately, there is a need for more research on genetic diversity in both migratory and non-migratory avian species. These studies may aid wildlife managers in assessing the genetic health of populations over time, especially as an area undergoes rapid anthropogenic change. Early and frequent surveys might allow for practical conservation measures to be implemented before more drastic interventions, such as translocating individuals between populations for the purpose of increasing genetic diversity (Tallmon et al. 2004, Haas 2010), are required. As our study detected lower genetic diversity in migratory species (especially myrtle war-

blers and American robins), additional studies are recommended to determine whether the same is true for nuclear DNA, or rather the low number of genetic variants seen here are unique to *COI* and/or mtDNA.

We believe this study highlights the importance of having conservation areas with a high percentage of natural surfaces in rapidly urbanizing regions as this may help species maintain populations with higher genetic diversity. Even though we did not detect evidence of population declines at the two most urbanized study sites, declines may be detected in the future if levels of genetic diversity continue to fall. In particular, we recommend frequent monitoring for resident species brown-headed nuthatches, white-breasted nuthatches, and northern cardinals in Gwinnett County as habitat loss and fragmentation continue. In general, additional studies should be considered to determine the amount of natural habitat required to maintain genetic health of wildlife populations and to prevent population declines.

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