

## **Genetic Differentiation of the South Florida Red-Shouldered Hawk (*Buteo lineatus extimus*) from the Nominate Subspecies (*Buteo lineatus lineatus*)<sup>1</sup>**

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### GENETIC DIFFERENTIATION OF THE SOUTH FLORIDA RED-SHOULDERED HAWK (*BUTEO LINEATUS EXTIMUS*) FROM THE NOMINATE SUBSPECIES (*BUTEO LINEATUS LINEATUS*)<sup>1</sup>

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ABSTRACT.—The south Florida subspecies of the Red-shouldered Hawk (*Buteo lineatus extimus*) is distinctly paler and smaller than other subspecies, reproduces at a lower rate, and can occupy very different habitats such as open marshes and grasslands with only scattered trees. We evaluated population differentiation between the south Florida population of Red-shouldered Hawks and two populations of eastern Red-shouldered Hawks (*B. l. lineatus*) in suburban (Cincinnati) and rural (Hocking Hills) environments in southern Ohio. Based on analysis of 11 microsatellite loci, we found significant genetic differentiation between the south Florida and eastern populations ( $F_{ST} = 0.077\text{--}0.097$ ), as well as significant differentiation between the two populations of the eastern subspecies ( $F_{ST} = 0.047$ ). Standardized genetic distance principal components analysis indicated two clusters, with individuals from the two Ohio populations forming a single cluster and those from Florida forming a second cluster. The substantial differences between the south Florida and other subspecies suggest this population warrants attention and possibly management as a distinct conservation unit, particularly in light of possible threats including habitat loss and rodenticide exposure.

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KEY WORDS: *Red-shouldered Hawk*; *Buteo lineatus extimus*; *Buteo lineatus lineatus*; *Florida*; *genetic differentiation*; *subspecies*; *suburban*; *urban*.

#### DIFERENCIACIÓN GENÉTICA DE *BUTEO LINEATUS EXTIMUS* EN EL SUR DE FLORIDA CON RESPECTO A LA SUBESPECIE NOMINAL *BUTEO LINEATUS LINEATUS*

RESUMEN.—La subespecie del sur de Florida *Buteo lineatus extimus* es claramente más pálida y más pequeña que otras subespecies, se reproduce a un ritmo menor y puede ocupar hábitats muy diferentes, como marismas abiertas y pastizales con solo árboles dispersos. Evaluamos la diferenciación poblacional entre la población de *B. l. extimus* del sur de Florida y dos poblaciones orientales de *B. l. lineatus* de ambientes suburbanos (Cincinnati) y rurales (Hocking Hills) en el sur de Ohio. Con base en el análisis de 11 loci de microsatélites, encontramos una diferenciación genética significativa entre las poblaciones del sur de Florida y las orientales ( $F_{ST} = 0.077\text{--}0.097$ ), así como una diferenciación significativa entre las dos poblaciones de la subespecie oriental ( $F_{ST} = 0.047$ ). El análisis estandarizado de componentes principales de la distancia genética mostró dos grupos, con individuos de las dos poblaciones de Ohio formando un solo grupo y aquellos de Florida formando un segundo grupo. Las diferencias sustanciales entre el sur de Florida y otras subespecies sugieren que esta población merece atención y posiblemente manejo como una unidad de conservación distinta, particularmente a la luz de las posibles amenazas que incluyen la pérdida de hábitat y la exposición a roentididas.

[Traducción del equipo editorial]

#### INTRODUCTION

The distributions of some raptor species can span entire continents or even the globe. Consequently, these raptor species can be made up of several subspecies that exhibit different plumages (Talbot et al. 2017), morphologies (Baladrón et al. 2015), and behaviors (Vrezec et al. 2018), resulting in separate subspecies occupying their own environmental niches. Many subspecies were described decades or centuries ago using plumage and morphological characteristics. Controversy has surrounded the use of avian subspecies designations in conservation because genetic investigations often fail to find genetic differentiation between described avian subspecies (Zink 2004, Phillimore and Owens 2006). However, United States law uses subspecies' designations to indicate which populations fall under conservation management (Haig et al. 2006).

The Red-shouldered Hawk (*Buteo lineatus*) comprises five subspecies: *B. l. elegans* in the western United States, *B. l. texanus* in eastern Texas, *B. l. alleni* in the southeastern United States, *B. l. lineatus* in the Northeast and Midwest, including southern Canada, and *B. l. extimus* in south Florida (Dykstra et al. 2020). Wheeler (2003) considers that *B. l. texanus* is not distinct from *B. l. alleni*.

The south Florida subspecies, *B. l. extimus*, inhabits the southern half of the Florida peninsula (Wheeler 2003) and is surprisingly little studied. Smaller and paler than the other subspecies (Wheeler 2003), *B. l. extimus* also has a generally lower reproductive rate (Nicholson 1930, Odgen 1974, Marain 2020). Habitat used can vary substantially from the relatively forested landscapes typically used elsewhere (Dykstra et al. 2000, Dykstra et al. 2020, and references therein). One study, conducted in south-central

Florida near the boundary between *B. l. extimus* and *B. l. alleni*, found Red-shouldered Hawks nesting in open grasslands interspersed with small ponds and wetlands, and small clusters of native and nonnative trees (Morrison et al. 2007). Similarly, in south Florida near Miami, tree cover in circular plots surrounding *B. l. extimus* nests averaged only 26% and varied widely (range = 0–71%; Marain 2020). Birds inhabiting the constructed wetlands of South Florida Water Management District's Stormwater Treatment Areas (STAs) west of Miami have nested on a lone palm tree, on utility poles, and on a water pumping building, all surrounded by sawgrass wetlands (Marain 2020).

Red-shouldered Hawk subspecies have been the subject of two genetic studies (Hull et al. 2008a, Barrowclough et al. 2019) that each used different sets of markers with regard to type (nuclear vs. mitochondrial DNA [mtDNA]) and number of loci and alleles, which influenced their ability to infer population differentiation (Kalinowski 2002, Ryman et al. 2006). Hull et al. (2008a) evaluated genetic differentiation of microsatellite loci and mitochondrial control region sequence data of Red-shouldered Hawks and detected significant differences between the western subspecies of the Red-shouldered Hawk (*B. l. elegans*) and the eastern subspecies groups (*B. l. lineatus/alleni/texanus*). However, Hull et al. (2008a) did not include any samples from the *B. l. extimus* population in south Florida and recommended future work with sampling from that population. More recently, Barrowclough et al. (2019) used sequencing data of a mitochondrial ND2 gene and two nuclear introns to evaluate the evolutionary relationships of 14 populations of Red-shouldered Hawks across North America, and also reported significant differentia-

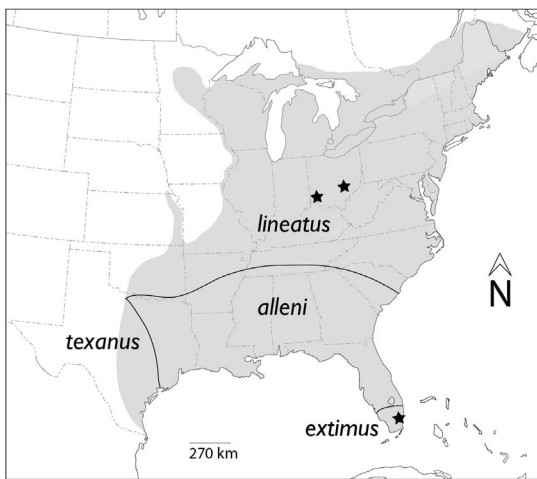


Figure 1. Study areas where Red-shouldered Hawks were sampled. Approximate extents of four subspecies in eastern North America are based on Dykstra et al. (2020).

tion between western and eastern subspecies. However, Barrowclough et al. (2019) did not identify significant population differentiation between the Florida peninsula and eastern populations. Their results suggested, based on phylogeographic analyses, that introgression in the Florida peninsula region occurs as a broad hybrid zone, but likely is the result of secondary contact following differentiation since at least the last glacial maximum.

Our aim in this study was to use microsatellite data to evaluate population differentiation between a south Florida population of Red-shouldered Hawks (*B. l. extimus*) and two populations of eastern Red-shouldered Hawks (*B. l. lineatus*) located in suburban and rural habitats in southern Ohio (Fig. 1). Although a previous study sampled widespread populations with large sample sizes, it compared mtDNA and nuclear introns and did not detect genetic differentiation between the south Florida birds and the eastern subspecies (Barrowclough et al. 2019). However, sampling microsatellite DNA may allow detection of finer-scale population structure that could be otherwise indiscernible using other techniques with only one or two loci, depending on mutation rate and allele frequencies (Ryman et al. 2006, Haas and Payseur 2010). Our approach will further contribute to a better understanding of subspecies differences. Red-shouldered Hawks in Ohio inhabit the central core of the eastern subspecies' range, yet Ohio populations were represented by only two samples in Barrowclough et al. (2019). The two populations in southern Ohio are similar in many ways, including nest-site selection (Dykstra et al. 2000) and reproductive rate (Dykstra et al. 2009, 2021), but exhibit noticeable behavioral differences (Dykstra et al. 2018), and little is known about genetic differentiation of Red-shouldered Hawks in this region.

## METHODS

**Study Areas.** *South Florida.* The study population included birds in Miami-Dade, Broward, and Palm Beach Counties (approximate center of study area: 26°12.051'N, 80°17.477'W) in the Southern Florida Coastal Plain ecoregion (Fig. 1; Omernik 1987). North-south boundary lines split each county nearly in half, with state- and federally protected Everglades wetlands to the west and human-dominated landscapes to the east (Nijman and Clery 2015). The climate is subtropical and most trees are evergreen.

*Suburban Cincinnati.* The study population included birds in Hamilton, Clermont, and Warren Counties in suburban southwestern Ohio (Cincinnati hereafter; approximate center of study area: 39°15.045'N, 84°21.517'W; Fig. 1) in the Interior Plateau ecoregion (Omernik 1987). Suburban development varied from densely to sparsely populated (Dykstra et al. 2000). Native forests are dominated by second-growth oak-hickory (*Quercus* spp. and *Carya* spp.) and beech-maple (*Fagus grandifolia* and *Acer saccharum*) associations, with lowland riparian forests characterized by sycamores (*Plantanus occidentalis*) and beech.

*Rural Hocking Hills.* The Hocking Hills study area (approximate center of study area: 39°32.032'N, 82°18.558'W; Fig. 1) in Hocking, Athens, and Perry Counties, Ohio, is approximately 180 km east of the Cincinnati study area and heavily forested with a sparse human population. Located in the Western Allegheny Plateau ecoregion (Omernik 1987), the study area's predominant forest type is oak-hickory, but plantations of white pine (*Pinus strobus*) and red pine (*P. resinosa*) are also common. Lowland forests are characterized by sycamores, silver maple (*Acer saccharinum*), beech, and river birch (*Betula nigra*). Most Hocking Hills nests we studied were located on federal- or state-owned land (Dykstra et al. 2000).

**Sample Collection.** At all three study sites, we used a bal chatri trap baited with a house mouse (*Mus musculus*) or European Starling (*Sturnus vulgaris*) to capture adult birds. Using standard climbing techniques, we accessed nest trees to hand-capture nestlings age 2–5 wk old.

In south Florida, we captured birds from 14 March–13 April 2018, 11 January–12 April 2019, and on 9 January 2020. All sampled birds were resident, paired adults, or nestlings. In Hocking Hills, we sampled nestlings on 15–16 May 2021. In the Cincinnati study area, we captured adult birds from 5 January–14 March 2018, and 20 February–7 March 2019. Most were associated with previously known breeding territories (Dykstra et al. 2009, 2021). Adult Red-shouldered Hawks in the Ohio study areas are year-round residents with small home ranges (Dykstra et al. 2001) and the breeding season generally begins in February (Dykstra et al. 2001, 2004, 2019). In addition, we included two Cincinnati birds sampled as nestlings in May 2011.

We used a sterile 28-gauge needle with syringe to collect approximately 50–100 microliters of blood from the brachial vein of each captured bird. The sample was applied onto a Whatman FTA™ bloodstain card (Whatman® BioScience, FTA™ Blood Collection Kit, Buckinghamshire, UK) and stored at room temperature for subsequent genetic analyses. We banded and weighed each bird, and made additional measurements as appropriate (Dykstra et al. 2012, Marain 2020, Wrona 2021).

**Sample Analyses.** DNA was extracted from blood using a Qiagen DNeasy Blood and Tissue Kit (Hilden, Germany) following manufacturer's protocol, or basic phenol-chloroform protocol followed by alcohol precipitation. We used eleven fluorescently labeled microsatellite loci (A317, D107, D127, D220, D310, D324, B220, D327, A312, A110, and B221), chosen based on prior work (Hull et al. 2007, 2008a). DNA was amplified by polymerase chain reaction (PCR) following the conditions described in Hull et al. (2007). PCR products were genotyped using an ABI 3500 Genetic Analyzer (Applied BioSystems) with allele sizes called using GeneMapper v.6 (Applied BioSystems).

**Data Analyses.** We analyzed data in two ways: using all birds sampled and using only one bird per family group (hereafter “unrelated birds”). When excluding relatives, one sample for each family group was selected based on maximizing total loci genotyped and one sample was randomly selected when samples from the same family group had an equal number of loci genotyped.

We used the program GDA v1.1 (Lewis and Zaykin 2001) to test for departures from Hardy-Weinberg Equilibrium (HWE) and Linkage Equilibrium among loci and each population. Because of multiple simultaneous comparisons, sequential Bonferroni corrections were applied (Rice 1989). Total number of alleles ( $A$ ), allelic richness ( $AR$ ), mean observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity, and inbreeding coefficient ( $f$ ) values were calculated using the R package DiveRsity v.1.9.89 (Keenan et al. 2013) in R v.3.3.2 (R Core Team 2017).  $AR$  was calculated to control for differences in sample size among populations (Leberg 2002) and we used 95% confidence intervals using 1000 bootstrap replicates to estimate statistical significance of  $AR$  estimates between populations. Values for  $f$  were significant if their 95% confidence intervals did not overlap with zero.

We used principal component analysis (PCoA) using GenAlEx v.6.51 (Peakall and Smouse 2012) to assess whether individual samples clustered by population. We produced a standardized genetic distance PCoA for both datasets using pairwise genetic distances among individuals based on the number of alleles shared and their respective heterozygosity levels (Smouse and Peakall 1999).

To assess genetic differentiation among study sites, we calculated pairwise estimates of population differentiation based on  $F_{ST}$  and Jost's  $D$  ( $D_{est}$ ; Jost 2008) using GenAlEx.

We used the Bayesian clustering program Structure v.2.3.4 (Pritchard et al. 2000) to identify the most likely number of clusters ( $K$ ) and assign individuals to the inferred population cluster(s). We used an admixture

model with correlated allele frequencies and a burn-in of  $1 \times 10^5$  followed by  $5 \times 10^5$  permutations for  $K = 1$  to 6 with four iterations for each  $K$ . Individual alpha values were used for each population to address concerns with unbalanced sampling (Wang 2017). We conducted analyses with and without prior information on the locality of samples (LOCPRIOR model), with the former model shown to provide the correct inference of population structure when data possess weak structure signals that cannot be detected by the default model (Hubisz et al. 2009). We used the web-based program Structure Harvester v. A.1 (Earl and vonHoldt 2012) and the Evanno  $\Delta K$  method (Evanno et al. 2005) to determine the mostly likely number of genetically distinct clusters ( $K$ ). We used the program Clumpak (Kopelman et al. 2015) to compile replicate run results from Structure and for visualization of results showing both distinct major and minor modes when applicable (i.e., groups of runs with highly similar results).

## RESULTS

In south Florida, we sampled 14 adults, 1 second-year (SY) bird that was a breeder, and 4 unrelated nestlings ( $n = 19$  total birds,  $n = 19$  unrelated birds). In Hocking Hills, we sampled 20 nestlings from 8 nests ( $n = 20$  total birds,  $n = 8$  unrelated birds). In the Cincinnati study area, we sampled 15 adults and 2 nestlings; when we included only one bird from each known family group in Cincinnati, the sample size was slightly smaller ( $n = 17$  total birds,  $n = 14$  unrelated birds). We conducted all analyses on both sets of data: (1) all birds and (2) unrelated birds. However, because all results were similar for both data sets, we here present only the data for the smaller dataset of unrelated birds, thus avoiding potential bias associated with the inclusion of close relatives for some of the tests conducted (results for the full dataset are shown in the Supplemental Material).

Significant departure in HWE in the form of heterozygote deficiencies was observed in only one locus (D324 in south Florida) in the unrelated birds dataset. No pairwise comparisons testing for linkage disequilibrium were significant after correcting for multiple comparisons. Given the lack of significant linkage disequilibrium across all sampling locations and after excluding related individuals, it is valid to assume that the loci can be treated as independent from each other. Genetic diversity measures were similar among the three sampled populations, and no indication of inbreeding ( $f$ ) was observed (Table 1).

The PCoA pattern showed the individuals from Cincinnati and Hocking Hills populations forming a single cluster and those from Florida forming a second cluster; however, some overlap among the two clusters was observed (Fig. 2). Axes 1, 2, and 3 explained 25.1%, 8.5%, and 7.9%, of the variation, respectively, or 41.5% of the total variation.

The estimated pairwise  $F_{ST}$  and  $D_{est}$  measures indicated significant differentiation between all pairwise comparisons after adjusting for multiple comparisons ( $P < 0.01$ ; Table

Table 1. Microsatellite DNA (11 loci) diversity estimates for three populations of Red-shouldered Hawks using the unrelated birds data set:  $n$ , sample size;  $A$ , number of alleles;  $AR$ , allelic richness (95% confidence interval);  $H_o$ , observed heterozygosity ( $\pm$  standard error);  $H_c$ , expected heterozygosity ( $\pm$  standard error);  $F_{is}$  ( $f$ ), inbreeding coefficient (95% confidence interval).

POPULATION	$n$	$A$	$AR$	$H_o$	$H_c$	$f$
Cincinnati	14	72	4.841 (3.818–5.727)	0.658 $\pm$ 0.096	0.653 $\pm$ 0.079	-0.009 (-0.092–0.053)
Hocking Hills	8	65	4.801 (3.727–5.636)	0.758 $\pm$ 0.099	0.700 $\pm$ 0.058	-0.082 (-0.243–0.035)
South Florida	19	80	4.854 (3.909–5.545)	0.628 $\pm$ 0.091	0.620 $\pm$ 0.092	-0.014 (-0.085–0.039)

2). Consistent with geographic distance, the highest genetic differentiation was observed between Cincinnati and Florida populations ( $F_{ST}=0.097$ ,  $D_{est}=0.336$ ), whereas the Cincinnati and Hocking Hills populations showed the lowest genetic differentiation ( $F_{ST}=0.047$ ,  $D_{est}=0.099$ ).

Clustering analyses indicated that both mean posterior probability  $\text{LnP}(K)$  and  $\Delta K$  values without (Supplemental Material Fig. S1b, S1d) and with (Fig. S2b, S2d) LOCPRIOR model supported  $K=4$  and  $K=2$ , respectively, as the most likely number of clusters. However, no obvious clusters were apparent at  $K=4$  with an equal proportion assigned to each cluster for each individual regardless of sample location for all runs without LOCPRIOR (Fig. 3a) and no increased resolution defining additional population clusters as observed with  $K=3$  when including the LOCPRIOR parameter (Fig. 3b). At the minor mode of  $K=2$ , the Cincinnati population was more differentiated from the Hocking Hills and south Florida populations, with the latter two populations forming the second cluster but with some admixture observed between Cincinnati and Hocking Hills. Although  $K=3$  was not supported as the most likely number of clusters based on  $\text{LnP}(K)$  and  $\Delta K$  (Fig. S1, S2), the Structure runs with the LOCPRIOR model all appear to support three population clusters consistent with the three

*a priori* identified sampled locations (Fig. 3b), which was also consistent with the significant  $F_{ST}$  and  $D_{est}$  pairwise population genetic differentiation results (Table 2). As a caveat, we note that low sample sizes, particularly with the Hocking Hills population, may have influenced our ability to identify the number of genetic clusters using Structure. The LOCPRIOR model has been shown to be informative in such cases when sample sizes are low (Hubisz et al. 2009, Porras-Hurtado et al. 2013).

## DISCUSSION

Based on analysis of 11 microsatellite loci, we found significant genetic differentiation between the south Florida (*B. l. extimus*) and eastern (*B. l. lineatus*) populations of Red-shouldered Hawks, as well as significant differentiation between the two populations of eastern hawks (Cincinnati and Hocking Hills) located in suburban and rural populations in southern Ohio. Consistent with geographic distance, the genetic differentiation between the Cincinnati and Florida populations exceeded that between the Cincinnati and Hocking Hills populations. Our analyses indicated at least two clusters, with the Florida birds well distinguished from all Ohio birds (PCoA analysis) or from Cincinnati birds (cluster analysis using Structure).

Our results align well with those of Barrowclough et al. (2019), who found that an mtDNA haplotype that was very common in the *B. l. extimus* population was present in a much lower proportion of birds in eastern North America (and absent in the California birds), though a population with a substantial presence of this haplotype in central Florida suggested a hybrid zone. The authors used

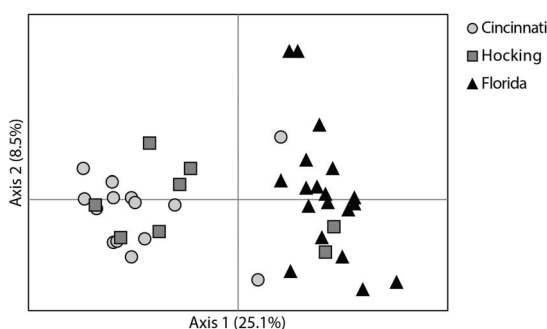
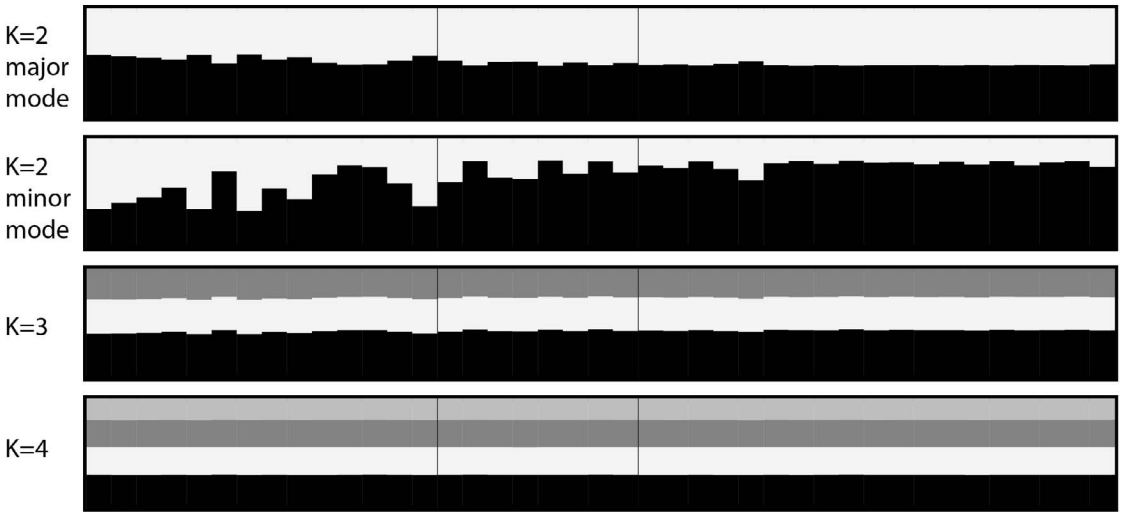


Figure 2. Standardized genetic distance principal coordinate analyses (PCoA) for three populations of Red-shouldered Hawks using pairwise genetic distances among individuals based on the number of alleles shared and their respective heterozygosity levels. Axis labels represent the first two coordinate axes with their respective explained variation.

Table 2. Pairwise estimates of  $F_{ST}$  and  $D_{est}$  among sampled populations of Red-shouldered Hawks for the unrelated birds data set.  $P$ -values based on 1000 permutations are indicated: \*  $0.01 > P > 0.005$ ; \*\*\*  $P \leq 0.001$ .

POPULATION 1	POPULATION 2	$F_{ST}$	$D_{est}$
Cincinnati	Hocking	0.047*	0.099*
Cincinnati	Florida	0.097***	0.336***
Hocking	Florida	0.077***	0.247***

## a) without LOCPRIOR



## b) with LOCPRIOR

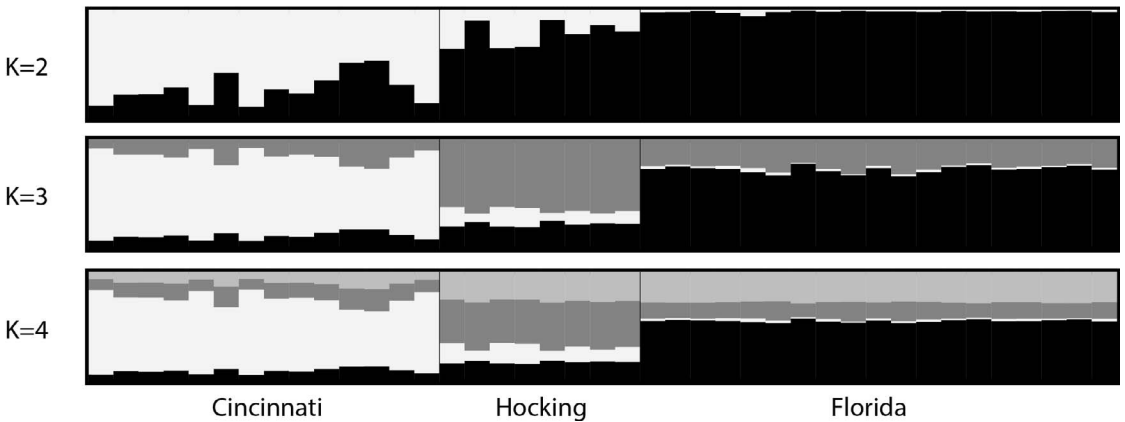


Figure 3. Results from Structure analyses (a) without and (b) with LOCPRIOR model for  $K = 2$  to 4 inferred clusters based on 11 microsatellite loci. Contrasting shading represents different inferred clusters and their magnitude indicates the posterior probability that the individual belongs to a particular cluster. Assignment of individuals to  $K = 2$  (without LOCPRIOR) included a major (2/4 runs; mean similarity score: 0.935) and minor (2/4 runs; mean similarity score: 0.920) mode.

ecological niche modeling to investigate the evolutionary history of the species and concluded that the south Florida population likely experienced a period of isolation during the last glacial maximum with more recent introgression among areas throughout Florida (Barrowclough et al. 2019). Barrowclough et al. (2019) suggest that this separate evolutionary lineage warrants elevation of the south Florida subspecies to full species status. This genetic differentiation and history agree well with the conspicuous differences in plumage, morphology, habitat use, and reproductive rate between the south Florida population and other popula-

tions (Nicholson 1930, Ogden 1974, Wheeler 2003, Morrison et al. 2007, Dykstra et al. 2020, Marain 2020).

As a caveat, we cannot discount that the substantial population genetic differences observed between the south Florida and other subspecies as shown here may reflect isolation by distance (Perez et al. 2018), which is commonly detected in genetic studies of raptors (Gousy-Leblanc et al. 2021). Intermediate samples from sites between southern Ohio and Florida would have helped to evaluate this possibility but were not available for this study. Barrowclough et al. (2019) studied multiple populations of Red-

shouldered Hawks, including in southern, central, and northern Florida, and did not detect genetic differentiation based on only a few loci (i.e., mtDNA ND2 and two nuclear introns) between all of the Florida birds combined as a single population and their remaining sampled eastern birds also grouped as a single population (Minnesota, Illinois, Ohio, New Jersey, Rhode Island, and North Carolina). However, by combining the three areas within Florida as a single population prior to conducting their pairwise  $F_{ST}$  analysis (Barrowclough et al. 2019), their ability to detect significant population differentiation or a pattern of isolation by distance may have been compromised due to unaccounted hierarchical substructure. Additional study is warranted to assess the importance of contemporary isolation by distance among geographic areas throughout the species' eastern distribution using an adequate number of polymorphic loci and sample sizes per subpopulation.

Nonetheless, our analysis, combined with that of Barrowclough et al. (2019) and the dearth of ecological data for the south Florida birds, suggests this population warrants attention and possibly management as a distinct conservation unit, whether subspecies or full species. Recent work suggests potential threats to this population include habitat loss and rodenticide exposure (Marain 2020). Although this population is able to inhabit urban/suburban environments, the human population of the Miami area is expanding rapidly, with associated habitat loss and conversion (Marain 2020). In addition, a substantial proportion of the population in the Miami area (49%) has been exposed to second generation anticoagulant rodenticides, based on liver samples of birds that were admitted to wildlife rehabilitation centers and then died or were euthanized there (Marain 2020). Additional research is needed to determine whether the south Florida population is increasing, stable, or declining in the rapidly urbanizing portions of its range.

We also found genetic differentiation between two populations of Red-shouldered Hawks located only approximately 180 km apart in southern Ohio. This finding was somewhat unexpected because the population of Red-shouldered Hawks is mostly continuous between the two study areas (Rodewald et al. 2016), and wide-ranging species with high mobility such as raptors are generally thought to exhibit low differentiation (Hull et al. 2008b), though habitat preferences can be associated with population structure even within raptors with wide distribution (Hull et al. 2008c). We cannot exclude the possibility of stochastic results due to low sample size and sampling error among the Hocking Hills birds after known siblings were excluded from the dataset. It is also possible that unknown relationships between sampled individuals might have affected our models; however, the fact that the inclusion of birds with known relationships in the dataset did not change the results (Supplemental Material 1) seems to refute that interpretation.

Available ecological data for Red-shouldered Hawks provide minimal evidence that might explain any genetic differentiation between these two local populations. Although the average natal dispersal for the species in this region is only 16–18 km (Dykstra et al. 2004, 2019), long-distance dispersal (>100 km) can occur; immature individuals have been recovered as far as 569 km away from the natal nest (Dykstra et al. 2019). Vagrancy occurs occasionally in the western subspecies (Bloom et al. 2011) and researchers consider that this species' potential for long-distance dispersal may be an important trait that allows it to succeed in urban/suburban areas (Dykstra et al. 2018).

In our previous studies of these same two populations, we found no differences in reproductive output (Dykstra et al. 2000, 2009, 2021) and no substantial differences in nest site selection, except that urban/suburban nests (Cincinnati study area) were located closer to houses and roads and more likely to be surrounded by lawn (Dykstra et al. 2000). However, behavioral differences are noticeable. Urban/suburban birds are more likely to defend their nests vigorously, sometimes striking researchers or pedestrians (Dykstra et al. 2018). Urban/suburban birds sometimes nest on human-made structures including rooftops and fire escapes (nine territories, 1997–2022, C. Dykstra and J. Hays unpubl. data), whereas rural ones do not (Dykstra et al. 2018). In addition, Red-shouldered Hawks are flexible generalists, and urban/suburban birds sometimes take advantage of novel food sources (e.g., pizza and other discarded human food; Dykstra et al. 2018) and may consume more birds than their rural counterparts (Miller 2013). Although genetic differentiation may be associated with urban adaptation (Teplitsky and Charmantier 2019 and references therein), we cannot speculate about that possibility in this case because we evaluated only one urban/suburban and one rural population, and because our sampled Hocking Hills population was small. Further studies from multiple paired populations in urban and rural areas will be required to evaluate whether the adaptation of Red-shouldered Hawks to human-dominated landscapes may include microevolutionary processes.

SUPPLEMENTAL MATERIAL (available online). 1. Results for the full dataset. Figure S1: Mean posterior probability ( $\text{LnP}(K)$ ) and  $\Delta K$  for Structure runs without LOCprior model. Figure S2: Mean posterior probability ( $\text{LnP}(K)$ ) and  $\Delta K$  for Structure runs with LOCprior model. Figure S3: Principal coordinate analyses (PCoA) using all samples. Figure S4: Results from Structure analyses (a) without and (b) with LOCprior model for all samples for  $K = 2$  to 4 inferred clusters based on 11 microsatellite loci. Table S1: Microsatellite DNA (11 loci) diversity estimates for each population using all samples. Table S2: Pairwise estimates of  $F_{ST}$  and  $D_{est}$  among sampled populations for the full dataset.

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