Variation in Migratory Behavior Influences Regional Genetic Diversity and Structure among American Kestrel Populations (*Falco sparverius*) in North America

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**Abstract**

Birds employ numerous strategies to cope with seasonal fluctuations in high-quality habitat availability. Long distance migration is a common tactic; however, partial migration is especially common among broadly distributed species. Under partial migration systems, a portion of a species migrates, whereas the remainder inhabits breeding grounds year round. In this study, we identified effects of migratory behavior variation on genetic structure and diversity of American Kestrels (*Falco sparverius*), a widespread partial migrant in North America. American Kestrels generally migrate; however, a resident group inhabits the southeastern United States year round. The southeastern group is designated as a separate subspecies (*F. s. paulus*) from the migratory group (*F. s. sparverius*). Using mitochondrial DNA and microsatellites from 183 and 211 individuals, respectively, we illustrate that genetic structure is stronger among nonmigratory populations, with differentiation measures ranging from 0.060 to 0.189 depending on genetic marker and analysis approach. In contrast, measures from western North American populations ranged from 0 to 0.032. These findings suggest that seasonal migratory behavior is also associated with natal and breeding dispersal tendencies. We likewise detected significantly lower genetic diversity within nonmigratory populations, reflecting the greater influence of genetic drift in small populations. We identified the signal of population expansion among nonmigratory populations, consistent with the recent establishment of higher latitude breeding locations following Pleistocene glacial retreat. Differentiation of *F. s. paulus* and *F. s. sparverius* reflected subtle differences in allele frequencies. Because migratory behavior can evolve quickly, our analyses suggest recent origins of migratory American Kestrel populations in North America.

**Key words:** Falco sparverius, North America, partial migration, subspecies

Numerous vertebrate and invertebrate taxonomic groups include species that engage in seasonal migrations (Dingle 1996). Among these species, birds are perhaps most frequently associated with migratory behavior (Rappole 1995). The variety of migratory strategies displayed by different bird species is a response to geographic variation in the seasonal availability of high-quality habitats (Cox 1985; Berthold 1996). By engaging in migrations, birds may be better poised to exploit resources that are locally abundant for only short periods of time during the annual cycle (Pulido 2007). In general, avian migratory behavior follows a latitudinal gradient: nonmigratory behavior is most apparent among equatorial bird species, whereas seasonal migrations are more common among birds that breed at latitudinal extremes (Newton 2003). Migration to latitudinal extremes may have become prevalent during interglacial periods (following glacial retreat) and served as a mechanism to reduce intra- and interspecific competition for resources at more equatorial locales (Cox 1968). Although it is tempting to conceptually group bird species into 2 categories (migratory vs. nonmigratory), most birds display a range of patterns that include partial migration (Berthold 2001). In partial migration systems, a portion of a species’ populations or individuals (either
majority or minority) inhabits the breeding grounds year round, whereas the remainder engages in seasonal migration (Lack 1943; Lack 1944; Berthold 2001; Jahn et al. 2004; Sekercioglu 2010). Partial migration is expected to be most common at intermediate latitudes where there is the greatest interannual variation in winter climate (Cohen 1967; Lundberg 1988). Up to a point, partial migration becomes more prevalent as distance away from the equator increases. However, at latitudinal extremes, conditions become sufficiently unfavorable that all individuals must leave the breeding grounds to ensure survival.

Many bird species have extremely broad breeding distributions. Given extensive spatial variation in breeding population locations, variation in migratory behavior within species is frequently noted. An excellent example is the American Kestrel (*Falco sparverius*), a species that breeds throughout North and South America (Smallwood and Bird 2002) and has upward of 17 recognized subspecies (Ferguson-Lees and Christie 2001). American Kestrels are particularly widespread in North America, where the species is known to be a partial migrant (Bildstein 2006). In general, the tendency toward winter migration is clinal and decreases in more southern breeding populations (Smallwood 1990; Smallwood and Bird 2002; Smallwood et al. 2009). American Kestrels are year-round residents in the southeastern United States (Hoffman and Collopy 1987; Smallwood 1990), whereas individuals breeding throughout the remainder of the United States and Canada generally engage in seasonal migration (Layne 1982; Henny and Brady 1994; Hoffman et al. 2002). Migrants from the north are common in the southeastern United States during the winter (Layne 1982; Hinnenbusch et al. 2010), but because breeding occurs during summers, limited opportunities for reproduction between migratory and nonmigratory individuals likely exist.

Although variation in migratory behavior is established in American Kestrels, the population genetic repercussion of behavioral differences is unknown. Migratory movement, in the context of avian seasonal migrations, must be distinguished from the population genetics concept of migration. From a population genetics perspective, migration is typically defined to involve movements that result in gene flow between populations. Gene flow occurs when there is a difference between an individual's birth site and breeding site (Slatkin 1985). The 2 types of migration do not necessarily need to be coincident. A species can plausibly exhibit natal and breeding site fidelity such that little gene flow occurs among populations.

The nonmigratory southeastern American Kestrel has been designated as a separate subspecies (*F. s. paulus*) from the nominate subspecies found throughout the remainder of North America north of Mexico (*F. s. sparverius*). The southeastern subspecies, considered to inhabit Florida, Georgia, Alabama, South Carolina, Mississippi, and Louisiana (Smallwood 1990; Breen and Parrish 1997; Smallwood and Bird 2002; Maney and Parrish 2007; Beasley and Parrish 2009) was originally described based on subtle size and plumage differences from the main North American kestrel subspecies (Howe and King 1902). However, Layne and Smith (1992) indicated that size differences in North American kestrels could be consistent with Bergmann’s rule. Clinal variation in plumage is also recognized (Smallwood et al. 1999). Overall, these studies suggest that the distinction between *F. s. paulus* and *F. s. sparverius* may not be valid given the lack of diagnosability (see Patten and Unitt 2002) of the 2 groups based on these characters. Despite these findings, use of the subspecies designation continues in most literature, although it is recognized that the validity of American Kestrel subspecies requires reevaluation (Smallwood and Bird 2002).

In this investigation, we used mitochondrial DNA (mtDNA) sequences and microsatellite loci to address numerous questions about genetic structure, genetic diversity, population status and history, and subspecific designations of American Kestrels in North America. We asked the following questions: 1) Does regional migratory variation coincide with regional genetic diversity patterns? Do nonmigratory southeastern populations display lower genetic diversity levels due to smaller local population sizes and greater genetic drift? 2) Does regional variation in migratory behavior coincide with regional variation in genetic structure levels? Given that southeastern United States populations are considered to be nonmigratory, we may expect that genetic structure among southeastern populations will be greater than that observed among migratory populations if migratory behavior coincides with individual dispersal and movement tendencies that promote gene flow. 3) Does the migratory behavior of regional population groups provide insights about population status and history? For example, migratory populations may display the signal of population expansions if their origin coincided with glacial retreat following recent Pleistocene climate change. 4) Do our genetic data support or refute the validity of the *F. s. paulus* subspecific designation? No previous genetic data have been generated in American Kestrels to address this topic; therefore, our analyses may be used to help update or corroborate the existing taxonomic designations that are used within this species.

**Materials and Methods**

**Molecular Genetic Analyses**

Blood samples were obtained from American Kestrels during the breeding season at 13 sampling areas across North America between 1995 and 2007 (Table 1, Figure 1). Locations were selected primarily based on the availability of samples for analysis, but encompassed both representative locations from the 2 North American subspecies as well as geographically distal locations across the continent. Although long-term studies are needed to better understand typical life spans of American Kestrels in the wild, the species typically lives >5 years in captivity with some
individuals reported as living as long as 14 years (Smallwood and Bird 2002). Thus, the 12-year period over which specimens were collected in this study is not likely to be problematic. DNA extractions were performed as described in Haig et al. (1994). mtDNA sequence data were obtained from 183 specimens. An ~2.3-kb fragment containing the

Table 1  Sample sizes and measures of genetic diversity within American Kestrel populations in North America

<table>
<thead>
<tr>
<th>Sampling location</th>
<th>Putative subspecies</th>
<th>mtDNA sequences</th>
<th>Microsatellites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>A (A&lt;sub&gt;r&lt;/sub&gt;)</td>
<td>H</td>
</tr>
<tr>
<td>CA</td>
<td>11</td>
<td>7 (5.79)</td>
<td>0.909</td>
</tr>
<tr>
<td>OR</td>
<td>16</td>
<td>7 (4.85)</td>
<td>0.842</td>
</tr>
<tr>
<td>UT</td>
<td>15</td>
<td>7 (5.12)</td>
<td>0.857</td>
</tr>
<tr>
<td>ID</td>
<td>16</td>
<td>7 (4.74)</td>
<td>0.825</td>
</tr>
<tr>
<td>NV</td>
<td>9</td>
<td>6 (5.44)</td>
<td>0.833</td>
</tr>
<tr>
<td>CO</td>
<td>17</td>
<td>6 (4.12)</td>
<td>0.765</td>
</tr>
<tr>
<td>SK</td>
<td>14</td>
<td>5 (3.66)</td>
<td>0.659</td>
</tr>
<tr>
<td>NC</td>
<td>21</td>
<td>7 (4.33)</td>
<td>0.771</td>
</tr>
<tr>
<td>SC</td>
<td>9</td>
<td>2 (2.00)</td>
<td>0.500</td>
</tr>
<tr>
<td>GA-E</td>
<td>23</td>
<td>5 (2.63)</td>
<td>0.391</td>
</tr>
<tr>
<td>GA-SE</td>
<td>15</td>
<td>2 (1.80)</td>
<td>0.248</td>
</tr>
<tr>
<td>FL-N</td>
<td>8</td>
<td>3 (3.00)</td>
<td>0.750</td>
</tr>
<tr>
<td>FL-C</td>
<td>9</td>
<td>4 (3.78)</td>
<td>0.750</td>
</tr>
</tbody>
</table>

Reported quantities include sample size (n), average number of alleles per locus (A), rarefied values of A (A<sub>r</sub>) that account for sample size differences among sampling locations, haplotype diversity (H<sub>H</sub>), nucleotide diversity (π), expected heterozygosity (H<sub>E</sub>), and observed heterozygosity (H<sub>O</sub>).

* At each sampling location, specimens were obtained from the following counties: CA (California): Alameda, Contra Costa; OR (Oregon): Benton, Lane; UT (Utah): Box Elder, Davis; ID (Idaho): Ada; NV (Nevada): Churchill; CO (Colorado): Larimer, Weld; SK (Saskatchewan): Northern Saskatchewan; NC (North Carolina): Hoke; SC (South Carolina): Richland; GA-E (Eastern Georgia): Richmond; GA-SE (Southeastern Georgia): Evans, Toombs, Tattnall; FL-N (Northern Florida): Okaloosa; FL-C (Central Florida): Levy, Marion.

Figure 1. Map of North America highlighting the location of 13 sampling areas where American Kestrel samples were obtained for analysis. Squares reflect locations where specimens are considered to be the southeastern American Kestrel subspecies (*Falco sparverius paulus*). Circles indicate *F. s. sparverius* sampling locations.
mitochondrial ND6 gene and control region was obtained initially by Long polymerase chain reaction (PCR) (GeneAmp XL, Roche) using universal degenerate primers L15725 and H1251 (Sorenson et al. 1999). From this sequence, new primers were designed for the amplification of a ~950-bp section of the control region: AMKE-CR-1 (5’-CCATTACATTACACTCAAGTCA-3’) and AMKE-CR-2 (5’-GGTGTATTTTGTTTAATGACTTC-3’). PCs (25 µL) were performed using 1× PCR buffer (Perkin Elmer, Waltham, Massachusetts), 3.5 mM MgCl₂, 10 µM of each dNTP, 0.15 mM of each PCR primer listed above, and 1U AmpliTaq Gold DNA polymerase (Perkin Elmer, Waltham, Massachusetts). Thermal cycling parameters included an initial denaturation at 94 °C for 2 min followed by 32 cycles of the following: 94 °C for 30 s (denature), 50 °C for 30 s (annealing), and 72 °C for 1 min (extension). A final extension step (5 min at 72 °C) completed each reaction. Bidirectional sequencing of PCR products were performed using primers AMKE-CR-1 and AMKE-CR-2 with ABI Prism BigDye Terminator sequencing chemistry on an ABI 3730 automated DNA sequencer (Applied Biosystems, Foster Bay, California). Resulting sequences were aligned and trimmed to the final 798 bp length using SeqMan ver. 8.0.2 (DNAStar, Inc., Madison, Wisconsin). Sample sizes from each population are provided in Table 1.

PCR-based analyses of 5 microsatellite loci (NVHfp13, NVHfp31, NVHfp82-2, NVHfp86-2, and NVHfp107) were used to obtain multilocus nuclear genotypic data as described in Nesje et al. (2000). A total of 211 specimens from our 13 sampling regions were included in analyses (Table 1). PCR amplification products were analyzed on an ABI 3730 capillary DNA automated sequencer (Applied Biosystems, Inc.) housed at the Oregon State University Central Services Laboratory. ABI Genotyper software was used to score microsatellite allele sizes.

Genetic Diversity Patterns

We quantified genetic diversity within each population and geographic region in several different ways. For the mtDNA data set, we calculated haplotype diversity ($H$) and nucleotide diversity ($\pi$) using Arlequin ver. 3.1 (Excoffier et al. 2005). We also used HP-Rare (Kalinowski 2005) to calculate allelic richness ($\bar{A}$) within each sampling location, in addition to a rarefaction-based estimate of allelic richness ($A_\lambda$) that accounted for sample size differences among locations. For the microsatellite data, we used the computer program GDA version 1.1 (Lewis and Zaykin 2002) to calculate observed ($H$) and expected ($\bar{H}$) heterozygosity. HP-Rare was also used to calculate $A$ and $A_\lambda$ for the microsatellite data set. We used Mann–Whitney $U$ tests as implemented at http://faculty.vassar.edu/lowry/utest.html to determine if these diversity-associated statistics significantly differed between 1) putative subspecies as outlined in Table 1 and 2) southeastern and western North American breeding populations. Note that the sole difference between these 2 statistical tests was the placement of the North Carolina (NC) sampling location. In the former test, the NC sampling location was grouped with other populations from the southeastern United States. In the latter test, the NC sampling location was grouped with samples from western North America (Figure 1) because the subspecies boundary has been suggested to occur between the NC and SC sampling locations (Smallwood 1990; Smallwood and Bird 2002). We also used GDA to identify deviations from Hardy–Weinberg genotypic proportions and test for linkage disequilibrium between pairs of loci within each sampling region. Composite test results for Hardy–Weinberg disequilibrium within each sampling region were obtained by combining $P$ values from locus-specific analyses using the $Z$-transform test (Whitlock 2005).

Genetic Structure Patterns

We used the Analysis of Molecular Variance (AMOVA, Excoffier et al. 1992) as implemented in Arlequin to test for genetic structure at numerous hierarchical levels within each data set. Genetic structure in the mtDNA data set was quantified based on $\Phi_{ST}$ (Excoffier et al. 1992), whereas microsatellite genetic structure was based on $\theta$ (Weir and Cockerman 1984). Initially, we calculated global values of these statistics for each complete data set using the 13 separate sampling locations as independent units. Likewise, we obtained pairwise values $\theta$ and $\Phi_{ST}$ for all combinations of sampling locations. To complement these quantities, we also used a short computer program written by M.P.M. (see Draheim et al. 2010) to calculate global and pairwise estimates of genetic differentiation based on Jost's $D$ (see Equation 13 of Jost 2008). Jost's $D$ is unaffected by the underlying level of diversity at a locus, which can have a strong influence on the maximum value that traditional differentiation statistics such as $F_{ST}$ or $G_{ST}$ can achieve (Hedrick 2005). Separate analyses were performed using the mtDNA and microsatellite data sets, with the average value of $D$ over loci used as the summary statistic in the case of the latter. $P$ values associated with values of $\Phi_{ST}$, $\theta$, or $D$ were obtained using 10,000 randomization replicates. To better understand regional patterns of genetic structure, separate sets of analyses were performed that quantified the overall levels of differentiation among the 7 western sampling locations (CA, OR, UT, ID, NV, CO, and SK) and the 6 southeastern locations (NC, SC, GA-E, GA-SE, FL-N, and FL-C). We further quantified genetic structure patterns between geographic regions by combining all western samples and all southeastern samples into 2 distinct groups. A similar set of analyses was performed where sampling locations were grouped based on putative subspecies designations (F. s. sparverius: CA, OR, UT, ID, NV, CO, and SK; F. s. paulus: SC, GA-E, GA-SE, FL-N, and FL-C).

We also used STRUCTURE version 2.2.3 (Pritchard et al. 2000) to identify the number of genetic clusters suggested by the microsatellite loci and to perform individual cluster assignments. Analyses were performed using 10 replicates of each assumed number of clusters ($K$), where $K$ ranged from 1 to 6. Each analysis replicate was based on an initial $2 \times 10^5$ burn-in steps followed by $3 \times$
analyses from the value of $K$ that yielded the highest average likelihood score were summarized using the computer program CLUMPP version 1.1.1 (Jakobsson and Rosenberg 2007). CLUMPP summaries were produced using 100 replicates of the greedy algorithm search option.

Population Status and History
We used our mtDNA data to test for evidence of past population expansion events within each sampling location. Because our genetic structure analyses suggested little (or no) differentiation of the set of western sampling locations or the *F. s. sparverius* sampling locations (see Results), analyses were also performed on the pooled sets of samples from the locations associated with these groupings. Analyses were performed using DnaSP version 5.0 (Librado and Rozas 2009) and included calculation of the $F$ and the R2 statistic of Ramos-Onsins and Rozas (2002). These 2 statistics are considered to be among the most useful for detecting population expansion events (Ramos-Onsins and Rozas 2002). Coalescent-based simulations, as implemented in DnaSP, were used to determine the significance of these values based on 10 000 replicates. We also evaluated evidence for population expansions using mismatch distributions (Rogers and Harpending 1992). We used DnaSP to compare observed mismatch distributions with expected mismatch distributions from constant-sized and growing populations.

We used our microsatellite data set and the program BOTTLENECK (Piry et al. 1999) to evaluate evidence for recent bottlenecks within each sampling region. As with our tests for population expansion described above, bottleneck analyses were also performed on the pooled sets of specimens from the western group and all *F. s. sparverius* individuals. Analyses were initially run by assuming either a strict stepwise mutational model or the infinite alleles model (IAM). However, a recent survey of studies of avian microsatellite mutational dynamics (Miller et al. 2012) revealed that the two-phase model (TPM; Di Rienzo et al. 1994) appears to be more realistic, as only 60–80% of avian microsatellite mutations result in a strict stepwise change. We therefore also performed our bottleneck analyses under the TPM using values of either 60% or 80% pure stepwise mutations in an attempt to bracket those observed in empirical data sets. We likewise specified the variances of multistate mutational sizes to be 2, 4, 9, or 16, which corresponded to average multistate mutational jumps of ~1.4, 2, 3, or 4 steps (Di Rienzo et al. 1994). We used 10 000 analysis replicates for each bottleneck analysis, with results over loci derived from the Wilcoxon signed-rank test as suggested by Cornuet and Luikart (1996).

Support for the Validity of Subspecies Designations
We examined the distinction between *F. s. paulus* and *F. s. sparverius* by evaluating the extent of mtDNA differentiation and haplotype sharing between putative subspecies. We used the program NETWORK version 4.5 (www.fluxus-engineering.com) to generate a median-joining haplotype network (Bandelt et al. 1999) to estimate the genealogy of the mtDNA haplotypes and to determine if haplotype groups could be identified that corresponded to putative subspecific groupings. We also considered results of our STRUCTURE analyses (described above): if STRUCTURE identified more than one cluster, then patterns of individual assignments to different clusters may help inform assessments of the validity of subspecies (Miller et al. 2010).

Results

Genetic Diversity Patterns
We identified 25 unique haplotypes among the 183 American Kestrel specimens included in our analyses (Supplementary Appendix I: Genbank Accessions JQ348936–JQ348960). Likewise, we detected between 5 and 13 alleles across the 5 loci that were used to generate multilocus microsatellite genotypes from our samples (Supplementary Appendix II). No evidence for deviation from Hardy–Weinberg genotypic proportions was detected within any sampling location, and no evidence for linkage disequilibrium between pairs of loci was observed.

Considerable variation in genetic diversity levels was apparent among sampling locations (Table 1). Outcomes of analyses that compared average diversity levels between regional groups or putative subspecies groups produced similar results (Table 2). We detected significantly lower genetic diversity within southeastern sampling locations and the *F. s. paulus* group based on rarefied allelic richness ($A_r$ for both mtDNA and microsatellites), haplotype diversity ($H_r$), and expected microsatellite heterozygosity ($H_e$) (Table 2). No differences were detected based on nucleotide diversity ($π$) or observed heterozygosity ($H_o$).

Table 2  Average genetic diversity statistics for mtDNA sequences and microsatellite loci in 2 different groupings of American Kestrel sampling locations

<table>
<thead>
<tr>
<th>Regional grouping</th>
<th>mtDNA</th>
<th></th>
<th></th>
<th>Microsatellites</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$A_r$</td>
<td>$H_r$</td>
<td>$π$</td>
<td>$H_e$</td>
<td>$H_o$</td>
<td>$H_o$</td>
</tr>
<tr>
<td>Western</td>
<td>4.817</td>
<td>0.813</td>
<td>0.002</td>
<td>3.973</td>
<td>0.527</td>
<td>0.500</td>
</tr>
<tr>
<td>Eastern</td>
<td>2.923</td>
<td>0.568</td>
<td>0.001</td>
<td>3.370</td>
<td>0.481</td>
<td>0.488</td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.012</td>
<td>0.018</td>
<td>0.226</td>
<td>0.005</td>
<td>0.003</td>
<td>0.390</td>
</tr>
<tr>
<td>Subspecies groupings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>F. s. sparverius</em></td>
<td>4.756</td>
<td>0.808</td>
<td>0.002</td>
<td>3.933</td>
<td>0.525</td>
<td>0.498</td>
</tr>
<tr>
<td><em>F. s. paulus</em></td>
<td>2.642</td>
<td>0.528</td>
<td>0.001</td>
<td>3.314</td>
<td>0.475</td>
<td>0.488</td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.007</td>
<td>0.011</td>
<td>0.093</td>
<td>0.011</td>
<td>0.004</td>
<td>0.379</td>
</tr>
</tbody>
</table>

$P$ values reflect analysis results from nonparametric Mann-Whitney $U$-tests that tested the null hypothesis of no differences between groups. Raw data used in analyses and definitions of each diversity statistic are listed in Table 1.
Table 3  Genetic structure patterns among American Kestrel sampling locations, geographic regions, and putative subspecies as determined based on mtDNA sequences and microsatellite loci

<table>
<thead>
<tr>
<th>Sampling location sets</th>
<th>mtDNA</th>
<th>Microsatellites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ϕST</td>
<td>P value</td>
</tr>
<tr>
<td>13 Sampling locations</td>
<td>0.103</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Southeastern</td>
<td>0.155</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Western</td>
<td>0.007</td>
<td>0.3039</td>
</tr>
<tr>
<td>Southeastern versus Western</td>
<td>0.075</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>F. s. paulus</td>
<td>0.163</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>F. s. sparverius</td>
<td>0.011</td>
<td>0.2249</td>
</tr>
<tr>
<td>F. s. paulus vs. F. s. sparverius</td>
<td>0.105</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Genetic Structure Patterns

Results of STRUCTURE analyses provided the most support for the K = 1 solution, suggesting that no genetic structure existed among American Kestrel sampling locations (Supplementary Appendix III). Although the likelihood associated with the K = 2 solution was only slightly lower than that from K = 1, we note that individual cluster membership coefficients were generally on the order of ~0.5 in the K = 2 case (Supplementary Appendix III), which further highlighted the unsuitability of the K = 2 model and the overall lack of detectable genetic structure by this approach. These findings, however, were in contrast to our explicit tests for genetic structure using analogs of FST. In this case, both mtDNA and microsatellite data sets generally produced similar results and highlighted 2 main trends in the genetic structure patterns (Table 3). First, although significant overall genetic structure was evident when all 13 sampling locations were analyzed, not all locations contributed equally to the observed differentiation pattern. Specifically, little evidence for genetic structure was detected when we evaluated differentiation of the 7 western sampling locations, whereas appreciable structure was apparent among the 6 southeastern sampling locations regardless of the genetic data set (mtDNA vs. microsatellites) or approach for quantifying genetic structure patterns (Table 3). Second, similar patterns were noted when locations corresponding to each putative subspecies were analyzed: clear differentiation was noted among the 5 F. s. paulus populations, however, there was evidence for significant genetic structure among the 8 putative F. s. sparverius sampling locations only in the analysis of the microsatellite data that was based on Jost's D. In all cases, the magnitude of values of ϕST, θ, or D were lower for sets of western or F. s. sparverius sampling locations relative to the southeastern and F. s. paulus sampling locations. In general, these results were reiterated by the pairwise values of ϕST, θ, and D that were generated (Tables 4 and 5). In analyses based on ϕST and θ, significant pairwise values were observed only in contrasts involving pairs of putative F. s. paulus locations or contrasts involving one eastern and one western location (Table 4). Significant differentiation was generally not detected between pairs of western locations or between pairs of putative F. s. sparverius populations (Table 4). Similar trends were noted in analyses based on Jost's D (Table 5). From the mtDNA data, 5 of the 10 pairwise contrasts involving F. s. paulus were significant at the 0.05 level, whereas only two of the 28 F. s. sparverius contrasts were suggestive of any structure patterns. Based

Table 4  Pairwise estimates of genetic structure levels among 13 American Kestrel sampling locations

<table>
<thead>
<tr>
<th>CA</th>
<th>OR</th>
<th>UT</th>
<th>ID</th>
<th>NV</th>
<th>CO</th>
<th>SK</th>
<th>NC</th>
<th>SC</th>
<th>GA-E</th>
<th>GA-SE</th>
<th>FL-N</th>
<th>FL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>—</td>
<td>0.024</td>
<td>0.020</td>
<td>—</td>
<td>0.004</td>
<td>0.027</td>
<td>0.019</td>
<td>0.011</td>
<td>0.019</td>
<td>0.062</td>
<td>0.054</td>
<td>0.051</td>
</tr>
<tr>
<td>OR</td>
<td>—0.008</td>
<td>—</td>
<td>0.009</td>
<td>0.022</td>
<td>0.013</td>
<td>0.005</td>
<td>0.013</td>
<td>0.013</td>
<td>0.080</td>
<td>0.029</td>
<td>0.081</td>
<td>0.012</td>
</tr>
<tr>
<td>UT</td>
<td>—0.024</td>
<td>0.049</td>
<td>—</td>
<td>0.016</td>
<td>0.014</td>
<td>0.015</td>
<td>0.007</td>
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<td>0.052</td>
<td>0.025</td>
<td>0.013</td>
<td>0.036</td>
</tr>
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<td>—0.028</td>
<td>0.009</td>
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<td>0.001</td>
<td>0.002</td>
<td>0.009</td>
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<td>0.036</td>
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<tr>
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<td>0.089</td>
<td>0.002</td>
<td>0.010</td>
<td>0.004</td>
<td>0.047</td>
<td>0.047</td>
<td>0.020</td>
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<tr>
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<td>0.015</td>
<td>0.032</td>
<td>0.031</td>
<td>0.138</td>
<td>0.052</td>
<td>0.014</td>
<td>0.041</td>
<td>0.025</td>
<td>0.044</td>
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<tr>
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<td>0.077</td>
<td>0.006</td>
<td>0.002</td>
<td>—</td>
<td>0.056</td>
<td>0.036</td>
<td>0.034</td>
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<tr>
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<td>0.203</td>
<td>0.204</td>
<td>0.203</td>
<td>0.397</td>
<td>0.204</td>
<td>0.188</td>
<td>0.192</td>
<td>—</td>
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<td>0.068</td>
<td>0.164</td>
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<tr>
<td>GA-E</td>
<td>0.139</td>
<td>0.140</td>
<td>0.182</td>
<td>0.148</td>
<td>0.401</td>
<td>0.146</td>
<td>0.098</td>
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<td>0.030</td>
<td>—</td>
<td>0.105</td>
<td>0.075</td>
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<tr>
<td>GA-SE</td>
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<td>0.163</td>
<td>0.190</td>
<td>0.163</td>
<td>0.486</td>
<td>0.142</td>
<td>0.087</td>
<td>0.125</td>
<td>0.271</td>
<td>0.049</td>
<td>—</td>
<td>0.125</td>
</tr>
<tr>
<td>FL-N</td>
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<td>0.072</td>
<td>0.106</td>
<td>0.072</td>
<td>0.228</td>
<td>0.076</td>
<td>0.061</td>
<td>0.083</td>
<td>0.057</td>
<td>0.085</td>
<td>0.264</td>
<td>—</td>
</tr>
<tr>
<td>FL-C</td>
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<td>0.229</td>
<td>0.130</td>
<td>0.181</td>
<td>0.228</td>
<td>0.170</td>
<td>0.189</td>
<td>0.170</td>
<td>0.235</td>
<td>0.255</td>
<td>0.265</td>
<td>0.217</td>
</tr>
</tbody>
</table>

Site abbreviations are provided in Table 1 and Figure 1. Values below diagonal correspond to values of ϕST quantified from the mtDNA sequence data. Values above diagonal correspond to values of θ as estimated from microsatellite data. Numbers in bold represent values that were significant at the α = 0.05 level (highlighted only for the purposes of illustrating general trends).
on the microsatellites, 8 of 10 F. s. paulus contrasts reflected genetic structure whereas only 8 of the 28 F. s. spaverius contrasts showed comparable trends. Interestingly, although the NC population tended to display significant differentiation with other southeastern populations, it was generally not identified as being significantly different from any of the western populations despite the large geographic distances that separated them.

Population Status and History

Tests for population bottlenecks based on our microsatellite data provided little evidence for recent reductions in population size. Across the separate bottleneck analyses that involved different sampling locations (or groups of sampling locations), mutational models, and mutational model parameters, the signature of a bottleneck was observed just once at the α = 0.05 level (analysis of the OR population when assuming the IAM; \( P = 0.031 \)). This result is nonsignificant after Bonferroni corrections and suggests that it is likely spurious, as none of the other analyses with different mutational models or model parameters provided corroborating results.

The mtDNA data suggested that many of the western sampling locations have experienced population expansions (Table 6). The R2 statistic was significant in 4 of the 7 western sampling locations, whereas Fu's \( F_s \) was significant in 5 of those cases. Neither statistic revealed the signature of expansion in any southeastern population. In analyses of all pooled western samples or all F. s. spaverius samples, Fu's \( F_s \) was highly significant. Mismatch distributions (Supplementary Appendix IV) provided qualitative support for these patterns. In this case, all western populations (separately and combined), all pooled F. s. spaverius populations, and the NC population produced a mismatch distribution that was more consistent with an expanding population than with a constant population (Supplementary Appendix IV). In general, southeastern populations produced mismatch distributions that were more consistent with constant-sized populations (Supplementary Appendix IV).

Support for the Validity of Subspecies Designations

The median-joining network produced from the 25 haplotypes detected in this investigation suggested relatively shallow haplotype divergence (Figure 2). No groupings of haplotypes were present that suggested clear differences between eastern and western populations or between specimens from putative F. s. paulus and F. s. spaverius populations. Given that STRUCTURE produced results objectively distinguish (Supplementary Appendix IV).

Table 5  Pairwise estimates of genetic structure levels among 13 American Kestrel sampling locations based on Jost's D

<table>
<thead>
<tr>
<th></th>
<th>CA</th>
<th>OR</th>
<th>UT</th>
<th>ID</th>
<th>NV</th>
<th>CO</th>
<th>SK</th>
<th>NC</th>
<th>SC</th>
<th>GA-E</th>
<th>GA-SE</th>
<th>FL-N</th>
<th>FL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td></td>
<td>0.063</td>
<td>0.043</td>
<td>0.003</td>
<td>0.083</td>
<td>0.076</td>
<td>0.076</td>
<td>0.069</td>
<td>0.130</td>
<td>0.109</td>
<td>0.116</td>
<td>0.046</td>
<td>0.147</td>
</tr>
<tr>
<td>OR</td>
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<td></td>
<td>-0.056</td>
<td>-0.044</td>
<td>-0.022</td>
<td>0.011</td>
<td>0.017</td>
<td>0.205</td>
<td>0.175</td>
<td>0.087</td>
<td>0.109</td>
<td>0.052</td>
<td>0.026</td>
</tr>
<tr>
<td>UT</td>
<td>-0.344</td>
<td>0.004</td>
<td></td>
<td>-0.035</td>
<td>0.004</td>
<td>0.045</td>
<td>0.030</td>
<td>0.052</td>
<td>0.074</td>
<td>-0.003</td>
<td>0.158</td>
<td>0.035</td>
<td>0.150</td>
</tr>
<tr>
<td>ID</td>
<td>-0.233</td>
<td>-0.195</td>
<td>-0.101</td>
<td></td>
<td>0.025</td>
<td>0.016</td>
<td>0.035</td>
<td>0.020</td>
<td>0.126</td>
<td>0.089</td>
<td>0.076</td>
<td>-0.004</td>
<td>0.133</td>
</tr>
<tr>
<td>NV</td>
<td>-0.259</td>
<td>-0.112</td>
<td>-0.199</td>
<td>-0.137</td>
<td></td>
<td>0.013</td>
<td>-0.052</td>
<td>0.010</td>
<td>0.097</td>
<td>0.206</td>
<td>0.001</td>
<td>0.096</td>
<td></td>
</tr>
<tr>
<td>CO</td>
<td>-0.074</td>
<td>-0.008</td>
<td>0.130</td>
<td>-0.111</td>
<td>0.094</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SK</td>
<td>0.163</td>
<td>0.140</td>
<td>0.330</td>
<td>0.012</td>
<td>0.348</td>
<td>-0.066</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>0.222</td>
<td>0.186</td>
<td>0.420</td>
<td>0.131</td>
<td>0.361</td>
<td>0.082</td>
<td>0.042</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SC</td>
<td>0.587</td>
<td>0.487</td>
<td>0.720</td>
<td>0.375</td>
<td>0.778</td>
<td>0.246</td>
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<td>0.205</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>GA-E</td>
<td>0.601</td>
<td>0.472</td>
<td>0.724</td>
<td>0.372</td>
<td>0.780</td>
<td>0.239</td>
<td>0.062</td>
<td>0.200</td>
<td>0.121</td>
<td>0.064</td>
<td>0.043</td>
<td>0.019</td>
<td>0.084</td>
</tr>
<tr>
<td>GA-SE</td>
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<td>0.523</td>
<td>0.742</td>
<td>0.415</td>
<td>0.794</td>
<td>0.260</td>
<td>0.095</td>
<td>0.226</td>
<td>0.082</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FL-N</td>
<td>0.193</td>
<td>0.152</td>
<td>0.315</td>
<td>0.075</td>
<td>0.266</td>
<td>0.060</td>
<td>0.097</td>
<td>0.227</td>
<td>0.335</td>
<td>0.329</td>
<td>0.362</td>
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<td>0.704</td>
<td>0.218</td>
<td>0.116</td>
<td>0.158</td>
<td>0.210</td>
<td>0.202</td>
<td>0.241</td>
<td>0.167</td>
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</tr>
</tbody>
</table>

Site abbreviations are provided in Table 1 and Figure 1. Values below diagonal correspond to values of D quantified from the mtDNA sequence data, whereas values above diagonal were derived from microsatellite data. Numbers in bold represent values that were significant at the α = 0.05 level (highlighted only for the purposes of illustrating general trends).

Table 6  Results of tests that evaluated evidence for population expansions within American Kestrel sampling locations

<table>
<thead>
<tr>
<th>Sampling location</th>
<th>R2</th>
<th>( P ) value</th>
<th>( F_s )</th>
<th>( P ) value</th>
<th>Mismatch distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
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<td>0.008</td>
<td>-3.323</td>
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<td>Expanding</td>
</tr>
<tr>
<td>OR</td>
<td>0.100</td>
<td>0.009</td>
<td>-3.068</td>
<td>0.009</td>
<td>Expanding</td>
</tr>
<tr>
<td>UT</td>
<td>0.141</td>
<td>0.337</td>
<td>-2.046</td>
<td>0.027</td>
<td>Expanding</td>
</tr>
<tr>
<td>ID</td>
<td>0.100</td>
<td>0.018</td>
<td>-3.068</td>
<td>0.008</td>
<td>Expanding</td>
</tr>
<tr>
<td>NV</td>
<td>0.107</td>
<td>0.001</td>
<td>-3.459</td>
<td>0.003</td>
<td>Expanding</td>
</tr>
<tr>
<td>CO</td>
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<td>0.181</td>
<td>-1.884</td>
<td>0.073</td>
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</tr>
<tr>
<td>SK</td>
<td>0.126</td>
<td>0.078</td>
<td>-1.606</td>
<td>0.064</td>
<td>Expanding</td>
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<tr>
<td>NC</td>
<td>0.118</td>
<td>0.261</td>
<td>-2.064</td>
<td>0.081</td>
<td>Expanding</td>
</tr>
<tr>
<td>SC</td>
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<td>0.775</td>
<td>2.079</td>
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<td>0.057</td>
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<td>Constant</td>
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<tr>
<td>FL-C</td>
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<td>0.619</td>
<td>0.143</td>
<td>0.535</td>
<td></td>
</tr>
<tr>
<td>All western samples</td>
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<td>0.105</td>
<td>-12.005</td>
<td>&lt;0.001</td>
<td>Expanding</td>
</tr>
<tr>
<td>All F. s. spaverius samples</td>
<td>0.043</td>
<td>0.080</td>
<td>-12.946</td>
<td>&lt;0.001</td>
<td>Expanding</td>
</tr>
</tbody>
</table>

Significant results (\( P < 0.05 \)) consistent with an expansion are highlighted in bold. Results from mismatch distributions are based on qualitative comparisons of empirical mismatch distributions to expected distributions that would be produced under constant population sizes or expanding populations (Supplementary Appendix IV). Question marks ("?" reflect analyses where expectations under the constant population size and expanding population models were extremely similar and difficult to objectively distinguish (Supplementary Appendix IV).
that were most consistent with the $K = 1$ solution (Supplementary Appendix III), no genetic evidence for the distinction between subspecies was generated by that analysis approach.

**Discussion**

Extensive spatial variation in the breeding distribution of a species can arise when variation in dispersal ability permits some individuals to exploit geographically distant habitats and resources that are seasonally favorable from an ecological and energetic perspective (Pulido 2007). Such scenarios can lead to development of partial migration systems if other geographic regions are capable of supporting individuals throughout the annual cycle and if trade-offs between individual fitness and the migratory strategy employed exist (Lack 1968). In this investigation, we highlight the complex link between the geographic distribution of breeding populations, the seasonal need for long-distance movements (or lack thereof in southeastern populations), and the implications of these factors on population genetic processes in American Kestrels. We likewise interpret our data in the context of investigating the distinction between *F. s. sparverius* and *F. s. paulus*.

**Genetic Structure and Diversity**

Our analyses revealed clear regional differences in genetic structure and diversity that were consistent with regional variation in migratory behavior of American Kestrels in North America. Genetic structure was pronounced among the nonmigratory southeastern populations relative to migratory western populations (Table 3). Assuming drift/migration equilibrium conditions, our results suggest that dispersal and gene flow may be lower in the southeast than in other parts of North America. We likewise detected significantly reduced genetic diversity within southeastern populations (Tables 1 and 2). This pattern may be consistent with the effects of genetic drift and reflect reduced dispersal and movement of southeastern American Kestrels.

Our results closely resemble those from Buerkle’s (1999) analyses of Prairie Warblers (*Dendroica discolor*) in the eastern United States. Analyses of mtDNA revealed that populations of the nonmigratory subtropical Florida Prairie Warbler subspecies (*D. d. paludicola*) contained less genetic diversity and were more differentiated than geographically...
widespread populations of the migratory Prairie Warbler subspecies (D. d. discolor). Likewise, although no differences in genetic diversity levels were apparent, nonmigratory populations of the Florida Grasshopper Sparrow (Ammodramus savannarum floridanus) were differentiated from populations of the migratory Grasshopper Sparrow subspecies across North America. However, no differentiation was apparent among the migratory populations themselves (Bulgin et al. 2003).

In the case of American Kestrels, our results suggest that seasonal migratory behavior also reflects the general tendency for individuals to exhibit greater breeding and natal dispersal. Inferences made from our genetic data also appear to be consistent with empirical evidence on American Kestrel dispersal events obtained from banding studies. Miller and Smallwood (1997) documented relatively short (~5 km) median natal dispersal distances within a Florida breeding population and no differences in dispersal distances between males and females. These values may be typical of birds throughout the southeastern United States. In contrast, Jacobs (1995) reported median dispersal distances of 30 and 16 km for females and males, respectively, at a Wisconsin breeding location. Of only 10 resightings of banded birds, one female dispersed as far as 161 km, whereas one male dispersed 362 km. Jacobs’ finding may suggest that long-distance dispersal events are common outside of the southeastern United States because they were detected in spite of the small sample of birds used for analyses. Corroborating evidence of reduced site fidelity outside of the southeastern United States comes from Bowman et al. (1987), who estimated natal philopatry rates between 1.5% and 3.7% in Ontario and Quebec, Canada. In that study, philopatry was liberally defined based on the return of an individual to within 218 km² of its natal site.

Our analyses shed light on historical demographic trends within American Kestrel populations, and further provide insights about the origins of populations in different geographic regions. Reports exist that suggest widespread declining American Kestrel populations across North America (Layne 1980; Hoffman and Collopy 1988; Smith et al. 2008; Bird 2009; Farmer and Smith 2009; Smallwood et al. 2009). However, results of our analyses provided no evidence for recent genetic bottlenecks in any of the sampling locations examined. Although an apparent discrepancy, numerous nonexclusive factors could conceivably be affecting the outcomes of these statistical tests. The ability to detect a genetic bottleneck using genetic data will depend on 1) the severity and duration of the population bottleneck and 2) the size of the genetic data set (i.e., number of sampled individuals and loci) (Cornuet and Luikart 1996). American Kestrels are considered to be among the most common raptors, with a previously estimated population size in North America of over 1.2 million breeding pairs (Bird 2009). Thus, despite reported reductions in population sizes, it is likely that the recent population changes are of insufficient magnitude to have an impact on genetic diversity within this species. We are also unable to rule out the possibility that our data set was insufficient to detect a bottleneck. Given the level of diversity, number of loci, and sample sizes from each sampling location in our analyses (Table 1), a bottleneck that results in even a 10-fold reduction in effective population size may be difficult to identify (see Figure 2 in Cornuet and Luikart 1996).

Migratory behavior and exploitation of breeding habitat at high latitudes has been widely hypothesized to have developed during warmer interglacial periods of the recent Pleistocene (Cox 1985; Milá et al. 2000; Milá et al. 2006). Our results appear to be consistent with this notion, as our mtDNA analyses identified signals of population expansion, predominantly among migratory American Kestrel populations outside of the southeastern United States. By contrast, southeastern populations mainly displayed the signal of constant-sized populations (Table 6, Supplementary Appendix IV). Our data therefore appear to be consistent with a scenario whereby the migratory American Kestrel populations are recently diverged from the nonmigratory southeastern populations and have expanded northward and increased in size in concert with glacial retreat. The relatively low levels of differentiation between southeastern and western sampling locations (Tables 3–5) and slight differences in allele frequencies between groups (Figure 2, Supplementary Appendices I and II) also appear to be consistent with the recent origin of the migratory populations.

American Kestrel Subspecies in North America

Results of genetic analyses may help support (Miller et al. 2010) or refute (Draheim et al. 2010) preexisting subspecific designations that have been erected based on geographic variation in morphology, behavior, or other relevant characters (Haig et al. 2006, 2011). This investigation comprised the first analysis of genetic structure and diversity in American Kestrels and can therefore nominally shed light...
on the validity of American Kestrel subspecies. The taxonomic designation *F. s. paulus* is commonly used when referring to American Kestrels that are the nonmigratory year-round residents of the southeastern United States. Previous analyses of morphology and coloration (Layne and Smith 1992; Smallwood et al. 1999) suggest the existence of latitudinal clinal variation, which may make empirical subspecific diagnoses difficult and call into question the validity of the subspecies themselves (Patten and Unitt 2002). Migratory behavior may also be clinal (Smallwood 1990).

Our genetic data provide little clear evidence to either support or refute the distinction between *F. s. paulus* and *F. s. sparverius*. STRUCTURE analyses produced the highest likelihood scores for the $K = 1$ scenario, indicating to the absence of any detectable genetic structure. And while our $F$-statistics analysis detected significant genetic structure that could be accounted for by differentiation of putative subspecies (Table 3), we primarily identified subtle differences in allele or haplotype frequencies between putative subspecies (Figure 2, Supplementary Appendices I and II) rather than clear differences that reflect diagnosable units (Miller et al. 2010). Despite these findings, there are nonetheless attributes of our results that could be considered to reflect the existence of different subspecies, albeit in the very early stages of differentiation. Primarily, we highlight genetic structure patterns associated with the NC sampling location (Figure 1). This location is considered to reside outside of the distribution of *F. s. paulus* (Smallwood and Bird 2002), but is also geographically distant from the remainder of the *F. s. sparverius* sampling locations included in our analyses (Figure 1). Interestingly, the NC population demonstrated little differentiation with the western sampling locations but was clearly differentiated from the majority of the other southeastern sampling locations (Tables 4 and 5). This finding suggests that the NC population may migrate, in contrast to the largely nonmigratory behavior noted in other populations from the southeastern United States. Overall, our data can not allow us to rule out the possibility that the subspecies boundary suggested in Smallwood (1990) has a concrete biological basis. Additional sampling and analysis of individuals and populations from the vicinity of this putative boundary may be required to perform a definitive assessment.

Divergence at neutral genetic loci (such as those examined here) may not reflect patterns at rapidly evolving loci under selection (Greenberg et al. 1998; Crandall et al. 2000; Ballentine and Greenberg 2010). When describing American Kestrel subspecies in North America, the primary character mentioned by authors is the difference in migratory behavior. Although no formal analyses of fitness trade-offs have been performed to determine the adaptive significance of migration in American Kestrels, migration in general appears to be an evolutionary labile trait that can arise quickly within evolutionary lineages (Pulido 2007). The heritability of migratory behavior in partially migratory populations has also been demonstrated in other avian species (Biebach 1983). American Kestrels are routinely raised in captivity for research purposes, making them a preeminent model organism for a variety of wildlife research topics (Bardo and Bird 2009). This attribute may facilitate initiation of experiments to determine if variation in migratory behavior is under genetic control in this species. If true, documentation of such patterns may ultimately provide the most complete evidence to support or refute the taxonomic distinction between *F. s. paulus* and *F. s. sparverius*.

### Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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### References


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