



Comparative phylogeography and population genetics within *Buteo lineatus* reveals evidence of distinct evolutionary lineages

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ABSTRACT

Traditional subspecies classifications may suggest phylogenetic relationships that are discordant with evolutionary history and mislead evolutionary inference. To more accurately describe evolutionary relationships and inform conservation efforts, we investigated the genetic relationships and demographic histories of *Buteo lineatus* subspecies in eastern and western North America using 21 nuclear microsatellite loci and 375-base pairs of mitochondrial control region sequence. Frequency based analyses of mitochondrial sequence data support significant population distinction between eastern (*B. l. lineatus/alleni/texanus*) and western (*B. l. elegans*) subspecies of *B. lineatus*. This distinction was further supported by frequency and Bayesian analyses of the microsatellite data. We found evidence of differing demographic histories between regions; among eastern sites, mitochondrial data suggested that rapid population expansion occurred following the end of the last glacial maximum, with *B. l. texanus* population expansion preceding that of *B. l. lineatus/alleni*. No evidence of post-glacial population expansion was detected among western samples (*B. l. elegans*). Rather, microsatellite data suggest that the western population has experienced a recent bottleneck, presumably associated with extensive anthropogenic habitat loss during the 19th and 20th centuries. Our data indicate that eastern and western populations of *B. lineatus* are genetically distinct lineages, have experienced very different demographic histories, and suggest management as separate conservation units may be warranted.

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1. Introduction

The taxonomic rank of subspecies has a controversial history in evolutionary biology (Wilson and Brown 1953; Mayr 1982; Phillimore and Owens 2006). Ideally, subspecies should reflect evolutionary potential and relationships within species, and provide a framework for prioritizing conservation efforts (Zink 2004; Haig et al. 2006); however, inconsistent application and definition have often limited the utility of the subspecies rank (Zink 2004). While more recent species concepts have been developed to describe evolutionary differentiation (Ryder 1986; Mortiz, 1994; de Queiroz, 2005), the rank of subspecies is still widely applied.

Use of the subspecies rank is common in ornithology (Zink 2004), where subspecies are often described as geographic segments of a species that, although not reproductively isolated from each other, differ in morphology or coloration, and may be associated with differences in habitat and behavior (AOU, 1983). Use of

these particular distinctions may mislead evolutionary inference (Zink 2004; Phillimore and Owens 2006); during initial periods of divergence, relative differences in the rates of morphological and neutral genetic evolution may result in conflicting patterns of differentiation (AOU, 1983; Bromham et al., 2002; Hull et al., in press). Therefore, some named subspecies may be phenotypically distinct yet genetically similar (e.g., Avise and Nelson 1989; Ball and Avise 1992; Zink, 2004) while other evolutionarily distinct lineages remain unrecognized due to retained similarities in morphology or habitat preferences (Isler et al. 2002).

Molecular genetic investigations of wide-ranging species with several recognized subspecies provide an opportunity to understand the ecological, behavioral, and evolutionary patterns responsible for differentiation, and evaluate the support for subspecific status. Such studies may be particularly useful in avian taxa where a high degree of mobility may obfuscate detection and description of within-species divergence. Additionally, molecular genetic analyses of subspecies complexes may aid conservation efforts by more clearly defining within-species differentiation and identifying the factors responsible for maintaining observed patterns of diversity.

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The Red-shouldered Hawk (*Buteo lineatus*) is a North American forest raptor composed of five recognized subspecies. *B. l. elegans* occurs along the Pacific coast from Baja California northward to Oregon. Four eastern subspecies, *B. l. alleni*, *extimus*, *lineatus*, and *texanus*, are separated by hundreds to thousands of miles from *B. l. elegans* and occur contiguously throughout riparian and deciduous forest regions of eastern North America (Fig. 1, Dykstra et al. 2008). No exchange of individuals between east and west has been reported through long-term ringing studies. Subspecies classification within Red-shouldered Hawks has been based on morphology and coloration (Clark and Wheeler 1987; Palmer 1988). However, there is some disagreement in the literature regarding the subspecific delineations within eastern North America. For example, Wheeler (2003) considered *B. l. texanus* as part of *B. l. alleni* based on variability of adult plumage and an inability to distinguish between juveniles of these two subspecies throughout their reputed breeding ranges (Clark and Wheeler 2001). No information is available on genetic relationships or validity of subspecies classification within Red-shouldered Hawks; indeed, debate as to the genus-level classification of Red-shouldered Hawks as *Buteo* or *Asturina* (Millsap 1986; Amadon and Bull 1988) was only recently resolved through molecular phylogenetic studies which place the Red-

shouldered Hawk within *Buteo* (Riesing et al. 2003; Lerner et al. 2008).

While Red-shouldered Hawks are currently a widespread species, important region-specific threats may be associated with particular subspecies and require focused conservation strategies (state conservation status is summarized in Table 1). A clearer understanding of the genetic relationships and demographic histories among putative Red-shouldered Hawk subspecies across their distribution is warranted to identify particular regions/taxa of concern. For example, if *B. l. elegans* is genetically and demographically isolated from eastern subspecies, then the two regional groups may be best managed as separate distinct lineages. Similar conservation issues may be addressed within the eastern range where the withdrawal and potential decrease of Red-shouldered Hawks from their previously published distribution in southern Texas and eastern Mexico has been of recent conservation concern (Howell and Webb 1995; Benson and Arnold 2001). If *B. l. texanus* is distinct from *B. l. alleni* (in contrast to Wheeler, 2003) suggestion), a population decline may warrant closer inspection and initiation of focused conservation planning for *B. l. texanus*.

In order to more accurately describe evolutionary relationships and inform conservation efforts, we investigated the current

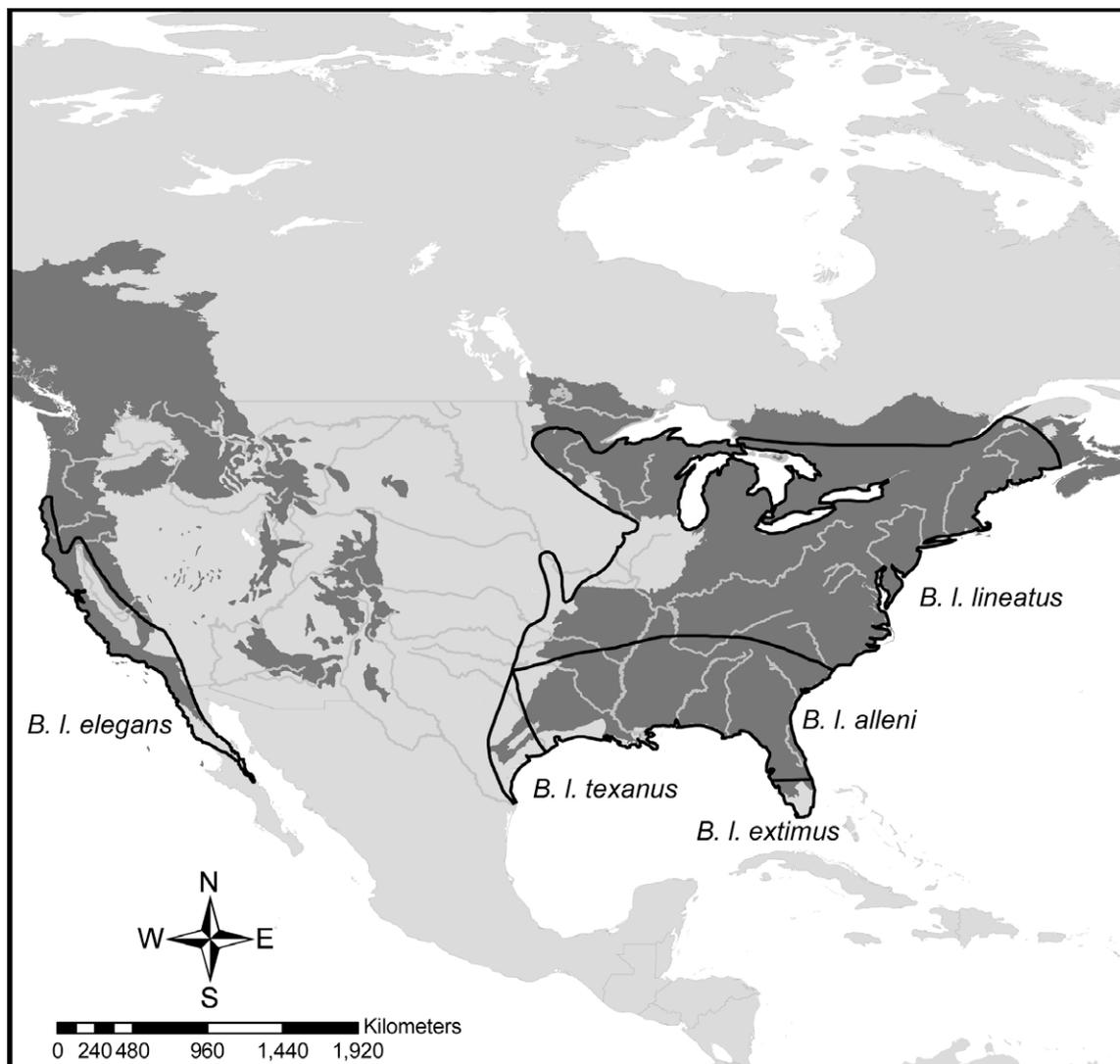


Fig. 1. Breeding range of Red-shouldered Hawk subspecies (black lines, following Wheeler, 2003), sampling locations (black triangles), and Mediterranean forest, and temperate broadleaf, mixed, and coniferous forests (dark gray shaded areas) in North America.

Table 1
Conservation status of Red-shouldered Hawks in the United States

State	Conservation status
Iowa	Endangered
New Jersey	Threatened
Michigan	Threatened
Wisconsin	Threatened
Minnesota	Special concern
New York	Special concern
Indiana	Special concern

genetic diversity, population structure, and historical demography of Red-shouldered Hawks sampled from across North America. We used molecular genetic data to (1) assess the validity of current subspecific designations, (2) examine the contemporary and historical evolutionary processes responsible for observed differentiation, and (3) evaluate the conservation status among continental populations.

2. Materials and methods

2.1. Sample collection

We collected samples from 112 Red-shouldered Hawks: 53 from *B. l. elegans* (all from California) and 59 from eastern North America (11 from within the range of *B. l. alleni* sampled in South Carolina and Florida, 28 from the range of *B. l. lineatus* sampled in Kentucky, Ohio, and North Carolina, and 20 within the range of *B. l. texanus* sampled in Texas). In the geographically isolated and non-migratory western population, samples were collected throughout the year and included juvenile dispersers from a broad region of California, while sample collection from the contiguously distributed and migratory eastern subspecies was limited to adults and nestlings during the breeding season to ensure that samples were accurately associated with the range of purported subspecies. Approximately 0.2 ml blood was drawn from the medial metatarsal or cutaneous ulnar vein and 2 feathers were plucked from the breast. Blood samples were stored in 1.5 ml Longmire's lysis buffer (100 mM Tris pH 8.0, 100 mM EDTA, 10 mM NaCl, 0.5% SDS) at ambient temperature in the field and at -80°C when delivered to the laboratory. Feathers were stored in paper envelopes and kept cool and dry. Genomic DNA was extracted from 25 μl blood/buffer solution or a single feather calamus using QIAGEN DNeasy kits (QIAGEN Inc.). DNA was stored at -80°C following extraction.

2.2. Microsatellite data collection

Using DNA extracted from whole blood and/or feathers, each individual was genotyped at 21 microsatellite loci (A110, A302, A303, A312, A317, B111a, B111a2, B220, B221, D107, D122, D123, D127, D207, D220, D223, D234, D235, D310, D327, D330) in six multiplex PCRs following the conditions described in Hull et al. (2007a). PCR products were separated with a 3730 DNA Analyzer (Applied Biosystems Inc.), and then scored using STRAND version 2.3.89 (Toonen and Hughes 2001).

2.3. Mitochondrial data collection

A 375-base pair segment of domain I of the mitochondrial control region was amplified for each individual using primers 16065F (Kimball et al. 1999) and H15414 (Bollmer et al. 2006). PCR products were prepared for sequencing using 0.5 μl exonuclease I and 1 μl shrimp alkaline phosphatase per 25- μl PCR. Clean PCR products were submitted to the UC Davis Sequencing Facility for sequencing using primers 14965 F and H15414 (Hull et al.

2008a). Sequences were aligned using sequencher version 4.7 (Gene Codes Corporation).

2.4. Microsatellite data analysis

All loci were tested for deviations from Hardy–Weinberg equilibrium using GENEPOP version 3.4 (Raymond and Rousset 1995). Each pair of loci in each region (east and west) was also tested for genotypic disequilibrium in GENEPOP. We assessed significance following sequential Bonferroni corrections for multiple tests (Rice 1989), with $\alpha = 0.002$. We tested for the presence of null alleles and scoring error using the program MICROCHECKER (van Oosterhout et al. 2004). Scoring error was further investigated through reamplification and scoring of 10% of the individuals at all loci and calculating the percent discrepancy between analyses. The program CONVERT 1.31 (Glaubitz 2004) was used to determine the number of private alleles (alleles occurring within only a single population) in each population, and MICROSATELLITE TOOLKIT (Park 2001) was used to calculate the observed and expected heterozygosity of each population and the mean number of alleles per locus. Allelic richness corrected for sample size was determined in FSTAT version 2.9.3.2 (Goudet 1995). Because occasional hybridization among North American *Buteo* species has been documented (Clark and Witt 2006; Hull et al. 2007b), we performed assignment tests in the program STRUCTURE version 2.1 (Pritchard et al. 2000) and inspected the relationship of samples to each other using a factorial correspondence analysis in GENETIX 4.05.2 (Belkhir et al. 2000).

Population pairwise F_{ST} , a measure of population differentiation, was calculated between all subspecies pairs in ARLEQUIN version 3.11 (Excoffier et al. 2005). Analysis of molecular variance was used to test for degree of differentiation between the *a priori* groupings of three eastern subspecies (*B. l. lineatus*, *alleni*, *texanus*) and the single western subspecies (*B. l. elegans*). A sequential Bonferroni correction was used to correct for multiple tests (Rice 1989).

A multilocus Bayesian clustering algorithm (STRUCTURE version 2.1; Pritchard et al. 2000) was used to determine the most likely number of population groups (K) and to probabilistically group individuals without using known geographic location or putative subspecies classification of the individual. Bayesian clustering analysis of population structure was performed for the total set of samples and separately for eastern samples. For both analyses we used the population admixture model with a flat prior and assumed that allele frequencies were correlated among populations. The simulation was run with a 500,000 iteration burn-in and a run length of 750,000 iterations. This parameter set was used to explore $K = 1$ through $K = 10$ across ten replicate runs. The $\log \text{Pr}(X|K)$ statistic for each value of K was averaged across runs. The most likely value for K was determined by first selecting the set of K values where the $\log \text{Pr}(X|K)$ value was maximized and subsequently selecting the minimum value for K that did not sacrifice explanatory ability (Pritchard and Wen, 2002; Waples and Gaggiotti 2006). We defined membership to a cluster based upon the highest proportion of ancestry to each inferred cluster.

We tested for evidence of recent population size reductions in eastern and western populations with one-tailed Wilcoxon sign-rank tests for heterozygote excess in the program BOTTLENECK version 1.2.02 (Piry et al., 1999). We tested for population bottlenecks using both the infinite alleles (IAM) and two-phase (TPM, default setting) models of microsatellite evolution.

2.5. Mitochondrial data analysis

The number of haplotypes, nucleotide diversity, and haplotype diversity were calculated in DnaSP version 4.10.9 (Rozas et al. 2003). Population differentiation was estimated through pairwise

Φ_{ST} comparisons in ARLEQUIN. Hypothesized difference between *B. l. elegans* and eastern subspecies and between *B. l. texanus* and *B. l. lineatus/alleni* were tested with AMOVAs in ARLEQUIN.

To evaluate the relationship among haplotypes and qualitatively assess demographic history, a reduced median-joining network was generated in NETWORK version 4.2.0.1 (Bandelt et al. 1999). Star-like networks are often indicative of a recent population expansion while extensively structured networks are suggestive of long-term stable populations. To statistically test for population expansion we calculated Fu's F_S statistic in ARLEQUIN (Fu 1997) for each genetically distinct population. Populations that have experienced recent expansion show significant deviations from the null hypothesis.

To estimate the age of distinct populations we used reduced median-joining networks to calculate $\rho \pm \sigma$ (Forster et al. 1996). Here ρ is the average distance from all descendant haplotypes to the ancestral node of the median-joining network and σ is a variance estimator. To translate the ρ statistic into years we used a mutation rate for the avian control region of 14.8% per million years (Wenink et al. 1996; Merilä et al. 1997). No fossil calibrated estimate of mutation rate is available for Red-shouldered Hawks or for any raptor species. Therefore, the absolute age estimations should be interpreted with caution.

3. Results

3.1. Microsatellite data

None of the 21 microsatellite loci significantly differed from Hardy–Weinberg equilibrium expectations and no evidence of linkage disequilibrium was detected. We found no evidence of null alleles or significant scoring error. The 10% of re-amplified individuals revealed a scoring error of 1.1% between runs. An absence of multi-cluster individuals in structure or intermediate individuals in the FCA indicated that no hybrid individuals were present in

our sample set (data not shown). Observed heterozygosity was 0.53 ± 0.01 for the eastern birds and 0.40 ± 0.02 for the western. The eastern population had 118 private alleles, an average of 10.6 ± 6.4 alleles per locus and an average allelic richness of 9.78, while the western population had six private alleles, a mean of 5.3 ± 3.4 alleles per locus, and an average allelic richness of 5.13.

The pairwise F_{ST} value between eastern and western samples was 0.17 ($P < 0.0001$), indicating substantial genetic differentiation. Comparisons among eastern subspecies resulted in lower F_{ST} values, summarized in Table 2. An AMOVA comparing the western subspecies (*B. l. elegans*) with three eastern subspecies (*B. l. lineatus*, *texanus*, and *alleni*) indicated significant differentiation ($F_{ST} = 0.18$, $P < 0.001$, $F_{CT} = 0.16$, $P < 0.001$). Factorial correspondence analysis resulted in a clear separation of eastern and western groups. The western group was much more tightly clustered than the eastern group (Fig. 2).

Our Bayesian clustering analysis of the total data set revealed very similar maximum $\log \Pr(X|K)$ for $K = 2$ and $K = 3$. Geographic correlation of clusters was evident for both $K = 2$ and $K = 3$, with eastern and western samples, respectively, comprising the two distinct clusters for $K = 2$. For $K = 3$, western birds formed one cluster and eastern birds were split into two clusters; one corresponded roughly with the range of the *B. l. texanus* subspecies and the second primarily occurring within the ranges of *B. l. lineatus* and *alleni*. Further analysis of only eastern samples confirmed this pattern, with a maximum $\log \Pr(X|K)$ at $K = 2$; the *B. l. texanus* sample consisting of primarily one cluster and the sample of *B. l. lineatus* and *alleni* consisting of a majority of the second cluster (Fig. 3).

A significant excess of heterozygotes was detected in the western population ($P < 0.001$ IAM, $P = 0.009$ TPM, Wilcoxon sign-rank test) indicating that a relatively recent population bottleneck has occurred within this population. The three eastern subspecies we sampled displayed no evidence of an excess of heterozygotes ($P = 0.019$ IAM, $P = 0.594$ TPM, Wilcoxon sign-rank test).

3.2. Mitochondrial data

Control region sequence data was collected for 83 individuals. Of these, 25 were from western Red-shouldered Hawks and 58 from the eastern range (10 from *B. l. alleni*, 29 from *B. l. lineatus*, and 19 from *B. l. texanus*). There were a total of 14 haplotypes, three of which were found in the western population and 12 in the eastern population; sequences have been deposited in GenBank under the accession numbers FJ376542–FJ376555. Total haplotype diversity was 0.65 ± 0.06 and total nucleotide diversity was 0.0024. Western hawks had a haplotype diversity of 0.36 ± 0.12

Table 2

Pairwise comparisons of genetic differentiation between presumed subspecies

	<i>B. l. elegans</i>	<i>B. l. lineatus</i>	<i>B. l. texanus</i>	<i>B. l. alleni</i>
<i>B. l. elegans</i>	—	0.09 [*]	0.23 [*]	0.14 [*]
<i>B. l. lineatus</i>	0.17 [*]	—	0.06	0.00
<i>B. l. texanus</i>	0.22 [*]	0.01	—	0.10
<i>B. l. alleni</i>	0.20 [*]	0.00	0.01	—

F_{ST} (microsatellites) below diagonal, Φ_{ST} (mitochondrial sequences) above diagonal. ^{*}Significant pairwise comparison following sequential Bonferroni correction.

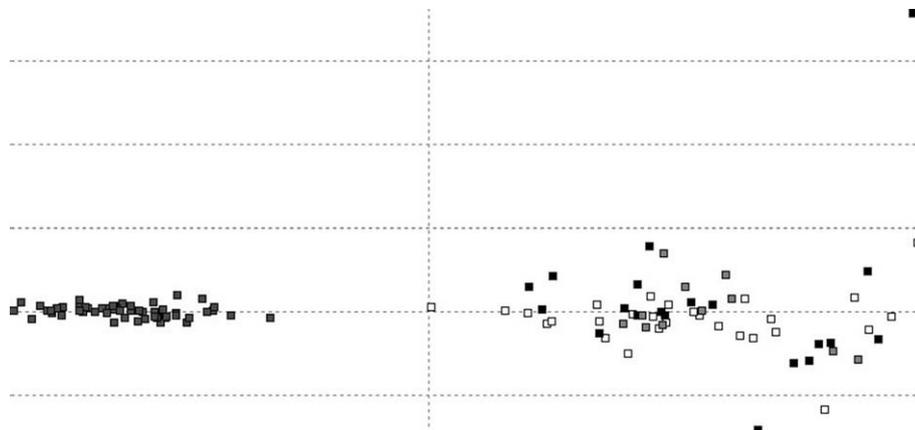


Fig. 2. Two dimensional factorial correspondence plot of multilocus genotypes for 112 Red-shouldered Hawks, 53 from the western subspecies (*B. l. elegans*; dark gray squares) and 59 from the eastern subspecies; *B. l. lineatus* ($n = 28$; white squares), *B. l. alleni* ($n = 11$; light gray squares), *B. l. texanus* ($n = 20$; black squares).

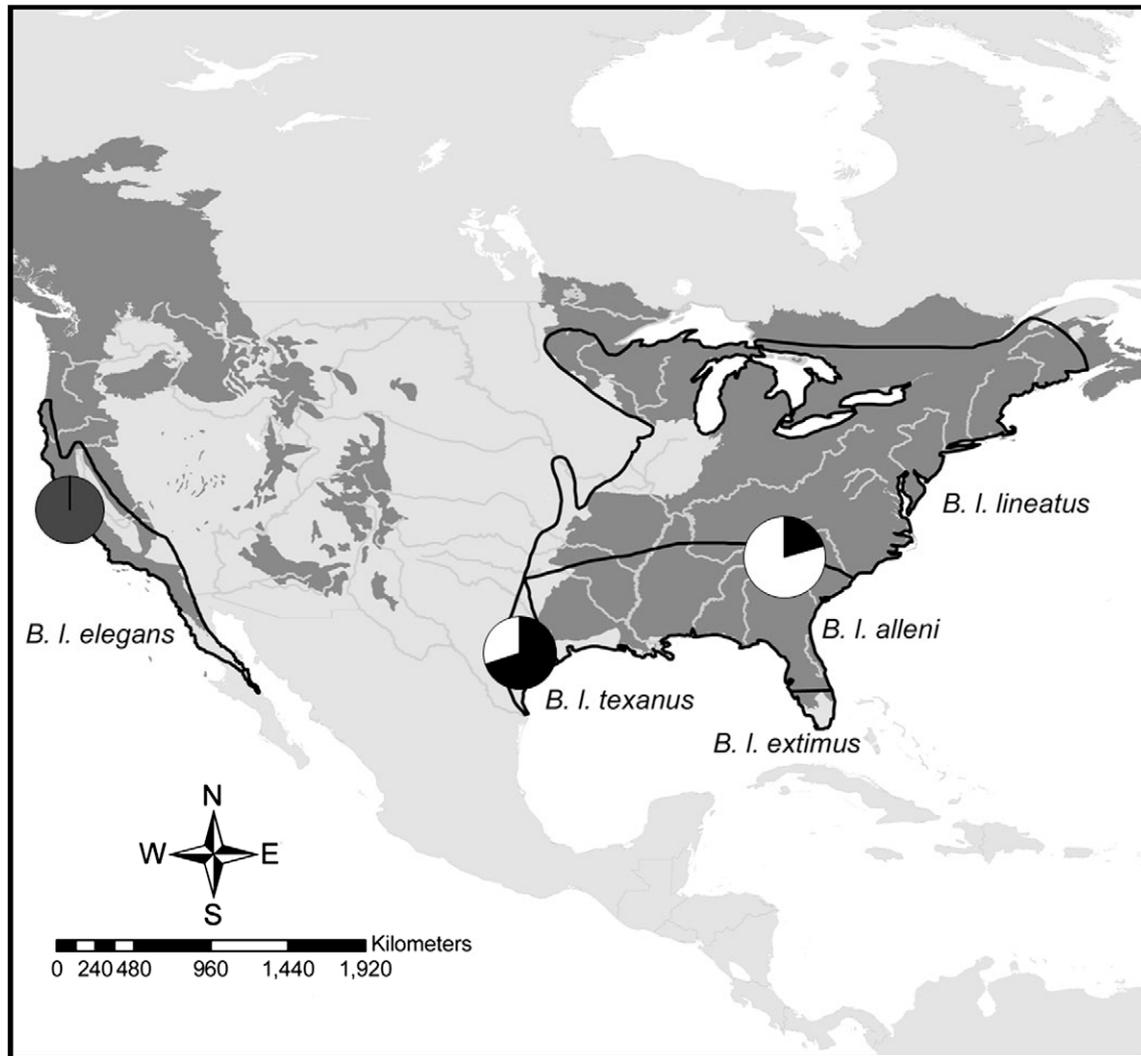


Fig. 3. Bayesian clustering analysis of Red-shouldered Hawks showing a strong distinction between western and all eastern sampling sites for $K = 3$; cluster A (dark gray) only occurred within the western range while clusters B (black) and C (white) only occurred in the eastern range.

and a nucleotide diversity of 0.0011, while the eastern population had haplotype and nucleotide diversities of 0.73 ± 0.05 and 0.0028, respectively. There were a total of 11 polymorphic sites (2.9%) in the 375-base pair region sequenced, with eight occurring solely in the eastern population and two solely in the western population. Pairwise comparison of all subspecies revealed significant differentiation between the western *B. l. elegans* and all eastern subspecies (Table 2). An AMOVA between *B. l. elegans* and eastern samples revealed a significant differentiation ($\Phi_{ST} = 0.11$, $P < 0.001$). No significant differences were found among eastern sampling sites. However, an AMOVA comparing *B. l. texanus* with *B. l. lineatus/alleni* revealed a significant differentiation ($\Phi_{ST} = 0.08$, $P < 0.001$).

Overall, the minimum spanning network displayed a star-like structure (Fig. 4), suggesting recent population expansion. However, the occurrence of only three haplotypes within the western population suggests that the expansion signature is primarily associated with the eastern populations. The majority of individuals were identified as haplotype A, which appears to be the progenitor haplotype for both eastern and western birds. All other haplotypes were represented by nine or fewer individuals and seven haplotypes were singletons. Fu's F_S was significant in both eastern populations (*B. l. texanus* and *B. l. lineatus/alleni*), supporting an interpretation of population expansion within the eastern populations, but not significant for the western population (Table 3). Be-

cause evidence of population expansion was documented only among eastern populations, ρ statistics and estimates of time since population expansion were not determined for the western population. Times since expansion and ρ statistics for eastern populations range from 9702 to 13,276 years before present and are summarized in Table 3.

4. Discussion

4.1. Differentiation and genetic diversity

Our results show substantial genetic differentiation suggestive of prolonged isolation between eastern and western Red-shouldered Hawks, as indicated by the significant and large (especially for avian species) microsatellite F_{ST} and mitochondrial Φ_{ST} estimates (0.18 and 0.11, respectively) and the exclusive eastern and western clusters recovered by Bayesian and FCA cluster analyses of microsatellite data. The observed mitochondrial differentiation is an order of magnitude greater than that observed for the same mitochondrial region between western North American populations of Swainson's Hawks ($\Phi_{ST} = 0.016$, *Buteo swainsoni*, Hull et al. 2008a) but is similar to that reported between eastern and western North American populations of Sharp-shinned Hawks ($\Phi_{ST} = 0.17$, *Accipiter striatus*; Hull and Girman 2005). As with

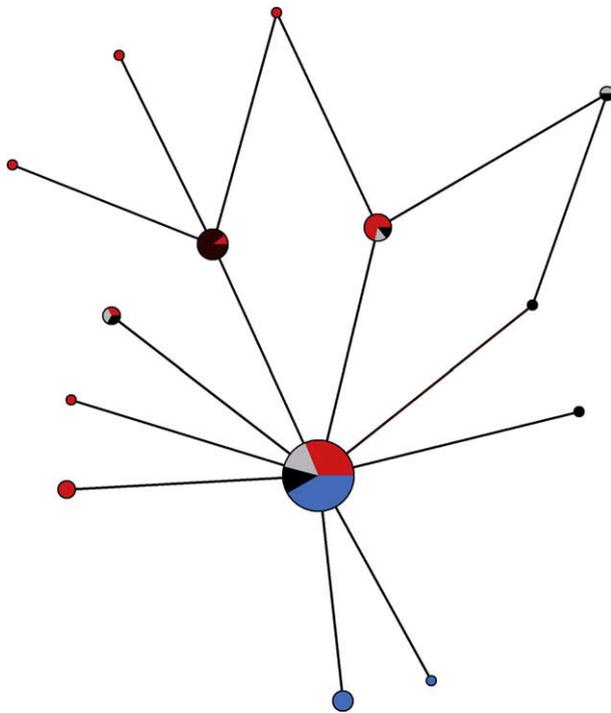


Fig. 4. Reduced median-joining network of Red-shouldered Hawk control region haplotypes (dark gray = *B. l. elegans*, black = *B. l. texanus*, white = *B. l. lineatus*, light gray = *B. l. alleni*). The relative abundance of each haplotype in the sample is represented by the size of the circle. Each branch represents a single nucleotide change. Haplotype A appears to be the progenitor of eastern and western birds.

Table 3

Expansion statistics and age of population expansion for genetically distinct populations of Red-shouldered Hawks

	<i>B. l. elegans</i>	<i>B. l. lineatus/alleni</i>	<i>B. l. texanus</i>
Fu's (1997) F_S	-0.67; $P = 0.21$	-6.24; $P = 0.001$	-3.23; $P = 0.003$
ρ statistic $\pm \sigma$	—	0.54 ± 0.24	0.74 ± 0.45
Years since expansion $\pm \sigma$	—	$9,702 \pm 4,407$	$13,276 \pm 8,046$

Sharp-shinned Hawks, eastern and western populations of Red-shouldered Hawks are separated by hundreds of miles of unsuitable habitat, which has likely acted as a barrier to dispersal between populations. In contrast, eastern and western North American populations of Red-tailed Hawks (*B. jamaicensis*) are not as restricted by habitat and come into contact and interbreed in a region concordant with the Rocky Mountain crest (Preston and Beane 1993). As a result, the differentiation between eastern and western Red-tailed Hawk populations ($F_{ST} = 0.031$; Hull et al. 2008b) is much less than that observed among Red-shouldered Hawks in this study.

The eastern and western populations display drastically different degrees of genetic diversity with the eastern population being much more diverse at both nuclear and mitochondrial markers than the western population. We observed a large number of private alleles overall, and a far higher number for the eastern set of subspecies than for the western subspecies (118 and six, respectively). Observed heterozygosity, allelic richness at each locus, and mean number of alleles per locus were all lower for the western population, indicating that western Red-shouldered Hawks are less genetically diverse than eastern birds. Similarly, eastern Red-shouldered Hawks had a much higher haplotype and nucleotide diversity than did western Red-shouldered Hawks.

Within the eastern range of Red-shouldered Hawks, data suggest that *B. l. texanus* may be genetically distinct from *B. l. lineatus/alleni*. While both pairwise F_{ST} and Φ_{ST} values are not significant, Bayesian clustering analysis and control region AMOVA suggest that *B. l. texanus* may be a distinct demographic unit from *B. l. lineatus/alleni*. The current sampling regimen (both sample number and geographic extent) prevents a definitive determination of the relationships among the eastern subspecies and additional investigation is warranted. To further examine relationships among eastern subspecies, future sampling should focus on the northern range of *B. l. lineatus*, several sites within the western and central range of *B. l. alleni*, and within the putative range of *B. l. extimus*.

4.2. Demographic history

In addition to stark differences in genetic diversity, the eastern and western populations of Red-shouldered Hawks have divergent demographic histories as indicated by analyses of microsatellite and mitochondrial data. Within eastern North America there is evidence of a recent population expansion in both the *B. l. texanus* and *B. l. lineatus/alleni* groups. In the *B. l. texanus* group, the estimated date of population expansion is roughly 13,200 years before present whereas the *B. l. lineatus/alleni* expansion appears to have occurred about 9700 years before present. While these estimates are extremely coarse, they are consistent with population expansion following the end of the last glacial maximum 20,000 years ago (Delcourt and Delcourt, 1991). Further, the older estimate for the *B. l. texanus* population suggests that population expansion in eastern North America may have initiated from the south-central portion of North America and subsequently proceeded through the northeast as appropriate habitat became available. Future analysis of samples from the southern Floridian range of *B. l. extimus* may help to identify whether a second (and potentially older) glacial refugium existed for eastern North American Red-shouldered Hawks.

No evidence of post-glacial population expansion was detected within the western population of Red-shouldered Hawks. Rather, the microsatellite data suggest that this population has experienced a population bottleneck within the relatively recent past as evident by a significant excess of heterozygotes (relative to the expected equilibrium heterozygosity). A bottleneck is consistent with both the loss of up to 90% of wetland and riparian habitat that occurred throughout California during the 19th and 20th centuries (Brown and Pasternak, 2005; Bloom 1980) and documented range contraction of western Red-shouldered Hawks (Friedmann, 1950; Dykstra et al. 2008). The recent population bottleneck effectively eliminates our ability to identify previous demographic signatures associated with Pleistocene refugia and post-Pleistocene climatic and habitat changes. Historical loss of riparian habitat has also occurred throughout eastern North America. However, the percent of total habitat loss was much less than in California, and the eastern Red-shouldered Hawk population was most likely not reduced to the same small numbers as in California. Consequently, the eastern population does not appear to have experienced a recent genetic bottleneck (no excess of heterozygotes detected) and retains a higher level of genetic diversity than the western population.

Similar evidence of a population bottleneck has been documented among Swainson's Hawks in California (Hull et al. 2008a). As with Red-shouldered Hawks, the historical range of Swainson's Hawks in California has been severely restricted by loss of riparian habitat (Bloom 1980). While Swainson's Hawk census numbers remain low and the species is listed by the state of California as threatened, Red-shouldered Hawks in California appear to be increasing in number and extent of range (Sauer et al.

2007). Anecdotal reports suggest that western Red-shouldered Hawks have begun to colonize regions of Oregon, Washington, and Arizona (B Anderson, Falcon Research Group, pers. comm.). This appears to be a relatively recent phenomenon, within the past 40 years, and may be associated with tolerance of human activity, the maturation of suburban tree cover, and the expansion of suburban development into areas of previously inhospitable southwestern deserts.

4.3. Taxonomy

In a taxonomic context these results suggest that at least two separate and distinct evolutionary lineages exist within Red-shouldered Hawks: (1) a western group comprised of *B. l. elegans*, and (2) an eastern group including *B. l. alleni*, *B. l. lineatus*, and *B. l. texanus*. Although the eastern and western populations share an ancestral mitochondrial haplotype, none of the derived haplotypes are shared between regions. Given the comparatively large census size of both eastern and western Red-shouldered Hawk populations, the shared ancestral haplotype may reflect incomplete lineage sorting following a relatively recent divergence (e.g., Hull et al. in press; Johnson 2008; Omland et al. 2006). Additional evidence of divergent evolutionary histories is observed in the microsatellite data (F_{ST} estimation, differing patterns of genetic diversity, and genetic clustering). These differences support the subspecies status of *B. l. elegans* and at least one, and possibly two or more, eastern subspecies (*B. l. lineatus*). The degree of genetic differentiation suggests that the eastern and western populations of Red-shouldered Hawks have been isolated without gene flow for an extended period and may be in the process of diverging into separate species. While species concepts and definitions are controversial, the observed differentiation between eastern and western populations would already warrant independent species status under some definitions. For example, the unified species concept, which attempts to combine the common elements of all species concepts, calls for “a separately evolving lineage segment as the only necessary property of species” (de Queiroz, 2005). In the context of ornithology, the biological species concept (Mayr 1957) has been the standard; therefore the taxonomic distinctions between eastern and western Red-shouldered Hawks minimally warrant subspecific separation, but deserve additional investigation and discussion.

4.4. Conservation

Although limited field studies have not identified obvious effects of inbreeding depression within *B. l. elegans*, continued monitoring of population health and genetic diversity may be warranted. While the range of *B. l. elegans* is currently expanding northward (Tweit, 2005; Scheuring and McAtee, 2006) and eastward (Wheeler 2003), the lower levels of extant genetic diversity suggest that the western subspecies may be more vulnerable to adverse effects, should the population experience another bottleneck or environmental perturbation in the near future. Although our knowledge of the factors driving recent population trends is anecdotal, availability of appropriate habitat appears critical to maintenance of western Red-shouldered Hawk populations (Bloom and McCrary, 1993, Rottenborn 2000). Focused research to better define the relationship between census numbers and habitat may help to direct conservation efforts.

In eastern North America, both the possible distinction of *B. l. texanus* from other eastern subspecies and the apparent similarity of *B. l. lineatus* and *B. l. alleni* should be interpreted with caution in a conservation framework. In general, Red-shouldered Hawk declines have been attributed to loss of wetland associated forests (Bednarz and Dinsmore 1981; Gehring 2003). Within the range

of *B. l. lineatus* decline has been attributed to loss of wetland associated forests (Bednarz and Dinsmore 1981; Gehring 2003) and fragmentation of contiguous forest tracts, which has created habitat more suited to the larger Red-tailed Hawk, a species that may outcompete Red-shouldered Hawks in the region (Bednarz and Dinsmore, 1981, 1982; Bryant 1986). Similarly, agriculture and urban development have already altered or eliminated 95% of the historic native vegetation and riparian woodlands in south Texas (Jahrsdoerfer and Leslie 1988). The loss has been accompanied by a 52% increase in the human population along the coastal region of Texas (Crossett et al. 2004) and continued human population increase is likely to result in greater habitat degradation. Given the potentially severe impact of Gulf Coast development on the population of Red-shouldered Hawks in the range of *B. l. texanus*, a precautionary approach to conservation planning for the subspecies may be appropriate. However, additional genetic and ecological data (e.g., Strobel 2007), are required before the subspecific relationships within the eastern group can be completely delineated. As discussed earlier, additional sampling throughout in the core ranges of putative subspecies *B. l. lineatus*, *alleni*, and *extimus* is required to describe the population genetic relationships of these subspecies.

5. Conclusion

Our findings provide an example of the conservation importance of testing the described boundaries of populations and subspecies. Although genetic evidence has frequently called into question the evolutionary significance of avian subspecies (Zink 2004), we found that the distinction of *B. l. elegans* from *B. l. texanus/lineatus/alleni* does, in fact, reflect important evolutionary lineages. In addition to identifying the genetic differentiation of *B. l. elegans* from eastern subspecies, our results suggest their recent passage through a genetic bottleneck. The genetic distinction of *B. l. elegans*, combined with its limited genetic diversity, indicate the importance of well planned conservation and monitoring efforts for this disjunct population of Red-shouldered Hawks. Within eastern North America, further data are required to definitively describe the evolutionary relationships of putative subspecies. However, our data suggest that a distinction may exist between *B. l. texanus* and *B. l. lineatus/alleni*. Prior to further interpretation and application of the results for eastern populations, we suggest current data be augmented through continued sample collection and interpreted in the context of ecological data. In conclusion, the increasing rate of suburban development and the dramatic loss of native vegetation communities throughout much of North America along with the genetic distinctness of *B. l. elegans* from its eastern counterparts suggest that sound conservation strategies for maintenance of genetic diversity among Red-shouldered Hawks may be prudent.

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