DISSERTATION

GENETIC STUDIES OF NORTHERN GOSHAWKS (*ACCIPITER GENTILIS*): GENETIC TAGGING AND INDIVIDUAL IDENTIFICATION FROM FEATHERS, AND DETERMINING PHYLOGEOGRAPHY, GENE FLOW AND POPULATION HISTORY FOR GOSHAWKS IN NORTH AMERICA

Submitted by

Shelley Bayard de Volo

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY SHELLEY BAYARD DE VOLO ENTITLED "GENETIC STUDIES OF NORTHERN GOSHAWKS (*ACCIPITER GENTILIS*): GENETIC TAGGING AND INDIVIDUAL IDENTIFICATION FROM FEATHERS, AND DETERMINING PHYLOGEOGRAPHY, GENE FLOW AND POPULATION HISTORY FOR GOSHAWKS IN NORTH AMERICA" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

[redacted]

Richard T. Reynolds

[redacted]

Marlis R. Douglas

[redacted]

Kenneth P. Burnham

[redacted]

Advisor: Michael F. Antolin

[redacted]

Department Head/Director: William K. Lauenroth

ABSTRACT OF DISSERTATION

GENETIC STUDIES OF NORTHERN GOSHAWKS (*ACCIPITER GENTILIS*): GENETIC TAGGING AND INDIVIDUAL IDENTIFICATION FROM FEATHERS, AND DETERMINING PHYLOGEOGRAPHY, GENE FLOW AND POPULATION HISTORY FOR GOSHAWKS IN NORTH AMERICA

The Northern Goshawk (*Accipiter gentilis*) is a large, highly mobile, mostly non-migratory and widespread forest raptor. It ranges across the Boreal forests from Alaska to Newfoundland, and south into forests of the Great Lakes, and montane regions of the Appalachian, Cascade, Sierra Nevada, Rocky Mountain, and Sierra Madre Occidental.

There has been much interest in the population and taxonomic status of Northern Goshawks, especially for populations occurring west of the 100th meridian. Because goshawks require large tracts of forest for foraging and nesting, their needs are often in conflict with forest resource management. These conflicts prompted concerns that changes in forest structure that fragment and alter landscapes (e.g., timber harvest, catastrophic fire, fire suppression and arthropod infestations) negatively impact goshawk populations. Also of concern is whether goshawk populations in Western North America are genetically distinct from those in the East and North, and whether some populations or subspecies should be candidates for listing and protection under the Endangered Species Act (ESA).

Beginning in 1991, these concerns prompted several petitions to list populations in the West as endangered, but the U.S. Fish and Wildlife Service (USFWS) has repeatably

cited insufficient data to support claims of population declines and distinct population segments. In 1998, the USFWS, however, identified data needed to evaluate goshawk populations, and recommended genetic analyses of gene flow and subspecies, and of standardizing measures of demography across the species' range. Similar conclusions were drawn by the Goshawk Technical Committee, who concluded that while much is known of goshawk natural history (e.g., nest site characteristics, food habits) little is known of the species' demography and genetic variation.

In an attempt to identify effects of forest management on goshawk populations, the USDA Forest Service, Rocky Mountain Research Station (RMRS), initiated a long-term study of goshawks on the Kaibab Plateau, Arizona. Objectives of this research (1991-2008) were to use capture-recapture techniques to investigate the ecology of the Kaibab goshawk population, specifically to determine: (1) age and gender specific vital demographic rates; (2) mate and territory fidelity of breeding goshawks; (3) effects of variation in prey density on goshawk reproduction; (4) survival and dispersal of post-fledgling goshawks; and (5) habitat correlates of reproduction and survival.

The Kaibab Plateau study provided a wealth of information on factors influencing goshawk demography on the Kaibab Plateau, and perhaps the Southwest. However, because capture-recapture methods are labor intensive and costly, they have been implemented in only a few other studies of North American goshawk populations. Further, the question of whether goshawks in the West constitute unique population segments has not been addressed.

The objectives of my dissertation were to (1) determine whether alternative methods to capture-recapture methods were feasible; and (2) evaluate the genetic relationships among goshawk populations across their continental U.S. range.

To address the first objective, I conducted two studies. First, I examined whether individual goshawks could be genetically "marked". This approach involved development of individual genetic profiles based on highly variable genetic markers. To examine the potential for genetic "marking" in Northern Goshawks, I sampled blood from 113 goshawks, banded as part of the Kaibab Goshawk study, and screened a suite of 20 microsatellite genetic markers for allelic variability. I found five microsatellites to be variable, and used them to: (1) quantify genetic variation (allelic diversity) for the Kaibab population; (2) determine the degree of relatedness among individuals in the population; and (3) develop individual genetic profiles for all goshawks sampled. I also evaluated an intron genetic marker to test its usefulness for determining gender of goshawks and used a probability of identity analysis to determine the statistical confidence in the uniqueness of genetic profiles. Lastly, I used variation in goshawk reproduction over a 13-year period to estimate effective population size for the Kaibab population. I found that the Kaibab population was variable at five of the genetic markers I tested and that individuals were less related than expected by chance. Five-locus genetic profiles for all 113 goshawks were unique, demonstrating that Kaibab goshawks could be genetically "marked" for capture-recapture studies. Probability of identity analysis also supported the conclusion that genetic profiles were unique, and the probability of error was exceptionally small. I also found that I could determine the gender of goshawks using the intron genetic marker. Lastly, I determined that effective population size for Kaibab

goshawks was small; a result that contrasted with the high levels of genetic diversity in the population. This contrast indicated the Kaibab population was open to gene flow from surrounding regions.

Second, I examined the question of whether goshawks could be genetically "marked and recaptured" using non-invasively sampled tissues. Goshawks are territorial, exhibiting high nest-site fidelity. They also molt their flight and tail feathers at their nests throughout each breeding season. I was interested in whether DNA from molted feathers collected at nest sites could be used to construct genetic profiles and provide a means to genetically "mark" and "recapture" goshawks. To answer this question, I used molted feathers from the Kaibab goshawk population collected over a 10-year period. Objectives were to: (1) optimize a method for DNA extraction from feathers; (2) quantify DNA yield for different sized feathers; (3) measure accuracy of genetic "marking" by comparing genetic profiles among feathers molted by the same banded individuals, and among genetic profiles derived from blood and feathers sampled from the same banded individuals. I optimized a DNA extraction method useful for feathers, and found that feathers provided relatively high yields of DNA. Large tail feathers provided significantly more DNA than other feather types, and genetic profiles were consistent among both feathers, and among blood and feathers, from the same individuals.

Third, I examined the genetic relationships among 21 populations of goshawks from across a large portion of their geographic range. The majority of samples came from goshawks in California, the Southwest, the Rocky Mountains, and the Eastern U.S. I used a 450 bp portion of the mitochondrial DNA (mtDNA) control region to determine (1) genetic diversity and structure, and gene flow among populations; (2) geographic

distribution of mtDNA lineages and their agreement with a priori defined biogeographic hypotheses of historical isolation in forest refugia since the end of the Pleistocene; (3) potential population expansions from historical forest refugia; (4) whether genetic data support the presence of unique population segments, especially in the West. I found that goshawks are variable for mitochondrial DNA across their continental U.S. range, and gene flow is high among populations within major geographic regions, but restricted between populations in the East and West. Restricted contemporary gene flow, in combination with historical isolation in the forests of California, the Southwest, and the East, has resulted in genetic differentiation among these regions. Populations in the Rocky Mountains and on the Colorado Plateau show signals of high gene flow from surrounding areas, and thus represent two unique areas of genetic admixture. Populations in the East underwent demographic growth more recently than populations in the West. Lastly, results support genetic divisions among populations of California, Southeast Arizona, Colorado Plateau, New Mexico, and the Rocky Mountains and Eastern U.S.

The genetic "marking" studies (Chapters 1 and 2) provided feasible and cost-effective alternative methods for capture-recapture, and are logistically easier to implement. If used, these methods have the potential to standardize the collection of demographic data across the species' range. Assessment of the genetic status among goshawks (Chapter 3) indicated populations are subdivided at a regional scale, with some gene flow within and among regions.

Shelley Bayard de Volo
Graduate Degree Program in Ecology
Colorado State University
Fort Collins, CO 80523
Spring 2008

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CHAPTER I

POPULATION GENETICS AND GENOTYPING FOR MARK-RECAPTURE STUDIES OF NORTHERN GOSHAWKS (*ACCIPITER GENTILIS*) ON THE KAIBAB PLATEAU, ARIZONA.

ABSTRACT

Advances in molecular techniques have facilitated use of genetic data in demographic wildlife studies. An important first step in genetic mark-recapture is selecting markers that uniquely "mark" and reliably "recapture" individuals. Markers should be tested on reliable DNA from known individuals (blood) before being used on non-invasively sampled DNA (hair, scat, or molted feathers). To evaluate whether Northern Goshawks (*Accipiter gentilis*) can be uniquely identified by genotyping, 113 known (banded, sexed) goshawks from the Kaibab Plateau, Arizona, were genotyped using DNA from blood and five microsatellite markers and a sex-linked gene. I used average relatedness to test whether adults in the population were related, and probability of identity ($P_{(ID)}$; probability that two random individuals from the population have the same genotype) to test the ability of multi-locus genotyping for uniquely identifying goshawks. I used genetic data to assess inbreeding and demographic data to estimate the effective population size. Sixty-nine adult goshawks were correctly sexed and genotyped. Expected heterozygosity was high ($H_E = 0.81$) and relatedness amongst adults was low

(r = -0.017). All individuals sampled (69 adults, 44 nestlings) had unique five-locus genotypes, the overall probability of identity was low ($P_{(ID)\ unbiased} = 7.03 \times 10^{-7}$), and the observed $P_{(ID)}$ was 0.00. Thus, Kaibab goshawks were uniquely "marked" by genotyping. Despite a small effective population size (N_e = 37 individuals), goshawks on the Kaibab Plateau functioned as a large breeding population with no inbreeding (F_{IS} = -0.001). I hypothesized that genetic diversity is maintained by gene flow via immigration of individuals from distant forests.

INTRODUCTION

Northern Goshawks (*Accipiter gentilis*) are highly secretive and are most easily detected during the breeding season when they aggressively defend their nests and young. While their defensive behavior at nests enables capture-recapture studies of breeding individuals, population monitoring is difficult because individuals often forgo breeding or their nests fail early in a breeding season. Even in years of high productivity, mark-recapture studies can be prohibitively expensive, because population sampling requires large field crews and multiple nest visits to many breeding territories to capture and recapture breeding goshawks (Reynolds et al. 2005).

Because of recent improvements in molecular techniques (Haig 1998, Parker et al. 1998), genetic capture-recapture may be a viable alternative to traditional capture-recapture methods for goshawks. Collecting molted feathers requires fewer nest visits than traditional capture-recapture methods. Breeding goshawks begin an annual molt during spring (Squires and Reynolds 1997) and because they spend much of the breeding season near their nests, they drop many of their molts within their nest areas including

years when nesting attempts fail. Thus, goshawk feathers are readily collected from nest areas, and may provide an efficient means to non-invasively sample their populations.

Several factors influence the success of genetic capture-recapture studies. An appropriate number of highly variable genetic markers for identifying individuals are required, and potential biases must be identified. ”Shadow effects” (lack of discrimination of individuals because of low variability or sampling too few markers) can negatively bias estimates of population abundance and positively bias estimates of survival (Mills et al. 2000). On the other hand, when more markers than necessary are used, population abundance may be overestimated and survival underestimated if genotyping errors add unique “genotypes”, and thus individuals, to population samples (Lukacs and Burnham 2005). Both biases will inflate variance and lower precision of parameter estimates (Lukacs and Burnham 2005).

Microsatellites are currently a preferred molecular marker for identifying individuals because they are easily interpreted (i.e., heterozygous genotypes are easily distinguished from homozygous genotypes), highly variable, bi-parentally inherited, and generally appear to be selectively neutral. Further, a large body of literature exists on microsatellite evolution (Jarne and Lagoda 1996, Goldstein and Pollock 1997, Estoup et al. 2002), which has facilitated the development of much statistical theory and analytical software (Hedrick 2005). Microsatellites are, however, expensive and time consuming to develop for each newly studied species. Occasionally primers used to amplify microsatellite markers in one species can be used in related species (Ellegren 1992, Primmer et al. 1996, Galbusera et al. 2000, Martinez-Cruz et al. 2002).

Prior to starting a non-invasive genetic study, establishing intra-population genetic structure (i.e., levels of inbreeding and relatedness) and the frequency of null alleles (alleles that fail to amplify) is necessary for providing baselines against which feather samples can be compared (Mills et al. 2000). Likewise, it is important to establish statistical power of multi-locus genotyping for identifying individuals with an independent population sample. We present results from a pilot study where we assessed the feasibility of implementing a non-invasive genetic capture-recapture study on a population of Northern Goshawks on the Kaibab Plateau, Arizona. Before assessing the utility of molted feathers as a viable source of DNA, we established a dependable genotyping marker set using DNA derived from blood (Taberlet and Luikart 1999).

Objectives were to: 1) screen species-specific and cross-specific (among species) microsatellite markers; 2) test a sex-linked gene in goshawks known to distinguish males and females in other raptors (Kahn et al. 1998); 3) assess the power of multi-locus genotyping to uniquely identify individuals using probability of identity analysis ($P_{(ID)}$; probability that two individuals drawn at random from the same population share the same multi-locus genotype); and 4) estimate average relatedness, inbreeding, and effective population size for the goshawk population on the Kaibab Plateau.

METHODS

Field Methods - The goshawk study population is located on the Kaibab Plateau in northern Arizona, an area that includes the North Kaibab Ranger District of the Kaibab National Forest and the North Rim of the Grand Canyon National Park (for descriptions of the study area see Reich et al. 2004, Reynolds and Joy 2005). It is a forested plateau

surrounded by shrub-steppe habitat, the nearest forests being 97 km to the north, 250 km to the east, 80 km to the west and 89 km to the south, except for a small patch of forest 18 km south on the south rim of the Grand Canyon. Sampled nests were well distributed across the study area. Sixty-nine adult, breeding goshawks were captured (1991-1993, 2000-2002) along with 44 of their nestlings (Reynolds et al. 1994). Adult goshawks were sexed using morphometrics (weight, tarsus length) and behavior. Blood was sampled from the brachial vein with 22-gauge needles and non-heparinized capillary tubes (volume $\leq$ 0.10 ml). Blood was transferred into STE (Sodium Chloride-Tris-EDTA) buffer-filled storage tubes kept cool in insulated containers with frozen cold-packs until crews returned to the field station, where samples were subsequently frozen (-20º C). At the close of the field season, blood was transferred to, and stored at -80º C at Colorado State University, Fort Collins, CO.

Laboratory Methods - I extracted DNA using QIAamp mini blood kits (Qiagen, Inc., Valencia, CA) following the manufacture's protocol. To find microsatellites, I screened published and unpublished primer sets that included microsatellites originally isolated from Northern Goshawks (Topinka and May 2004), and European Goshawks (*Accipiter gentilis gentilis*), Golden Eagles (*Aquila chrysaetos*), and Red Kites (*Milvus milvus*) (Peck 2000). I also tested primers that amplify an intron within the avian CHD (chromo-helicase-DNA binding) gene and that successfully sexed Red-Tailed Hawks (*Buteo jamaicensis*) and Great-Horned Owls (*Bubo virginianus*) (Kahn et al. 1998). The CHD gene is located on the Z and W sex chromosomes. I expected males to be homozygous (ZZ genotype) and females to be heterozygous (ZW genotype).

I used PCR (Polymerase-Chain-Reaction) to amplify microsatellites in 25µl reactions using 0.5µl (AGE1a) or 1.0µl (all other markers) of template DNA, 2.5µl 10x buffer containing 15mM Mg_2Cl_3 (Promega Corp. Madison, WI; for markers AGE1a , AGE 2 and AGE 4 an additional 3mM Mg_2Cl_3 was added), 20mM dNTPs, 25pM each primer, 1U Taq Polymerase, and one drop mineral oil to prevent evaporation. Negative controls (reactions that include all reagents except template DNA) were included in every set of reactions, and I used "cold start" PCR where tubes (in racks) were kept on ice to prevent premature non-specific priming. I used MJR PTC-100 thermocyclers programmed for the following protocol: denature at 94º C for 4 min., then 31 cycles of denature at 94º C for 40 sec., annealing at 58º C for 40 sec., and chain extension at 72º C for 40 sec., with a final extension at 72º C for 5 min.

I used PCR to amplify the CHD sex-linked gene in 25µl reactions using 1.0µl template DNA and the same reaction buffer described above. The PCR protocol included an initial 5 min. 95º C denature, then 11 cycles of denature at 94º C for 30 sec., annealing at 52º C for 35 sec., and chain extension at 72º C for 2.0 min., then 31 cycles of denature at 92º C for 30 sec., annealing at 56º C for 35 sec., and chain extension at 72º C for 2.0 min, with a final extension at 72º C for 7 min.

I used gel electrophoresis to separate alleles. For microsatellites, I used 8% polyacrlyamide (Long Ranger, Cambrex Corp., Rockland, MA) denaturing gels (55 cm long) that were run at 45 watts for 4-5 hrs, depending on allele size. For the CHD sex-linked gene, I used single-strand-conformation-polymorphism (SSCP) methods (Hiss et al. 1994) and electrophoresed alleles on non-denaturing gels at 5 watts for 15 hrs.

For microsatellites, I established allele standards using representative samples from our first gel and then standardized all other gels using those same samples. Gels were scored visually, and allele standards were run on both sides of a gel to account for gel ambiguities that cause slight variations in migration distances. Further, a subset of individuals (N=23) was genotyped a second time to validate scores for microsatellite markers. For the CHD marker I ran all known females together (n = 40) and all known males (n = 29) together to familiarize ourselves with allele morphology. Although not sequenced, the fragments were approximately 240-260 base pairs, and Z and W alleles were similar in size, but were differentiated by the SSCP analysis (Hiss et al. 1994).

DATA ANALYSIS

Population Genetic Analysis - Population substructure, inbreeding, and genetic drift can reduce heterozygosity in populations. However, low yield and degraded DNA sampled from sources such as molted feathers can artificially reduce population heterozygosity if allelic dropout (ADO; one of two alleles in a heterozygous individual fails to amplify) at one or more markers occurs. It is, therefore, important to use high yield sources of DNA (typically blood) from a known reference population to determine frequency of ADO (or null alleles) and true levels of heterozygosity (Taberlet et al. 1999).

I used CERVUS 2.0 (Marshall et al. 1998) to estimate observed (H_O) and expected (H_E) population heterozygosity and null allele frequencies. CERVUS provides estimates of null allele frequencies with an iterative algorithm based on differences between observed and expected homozygote frequencies. I used GENEPOP 3.4 (Raymond and Rousette 1995) to test for departures from Hardy-Weinberg equilibrium (random mating)

and GDA 1.0 (Lewis and Zaykin 2001) to test for linkage-disequilibrium (genotypes at one marker are independent from genotypes at other markers) and to estimate F_{IS}, an indicator of population substructure and inbreeding. For a review of F-statistics and microsatellite genetic markers *see* Balloux and Lugon-Moulin (2002)

To test our assumption that our sample of adult goshawks was not comprised of closely related individuals, I used IDENTIX 1.1 (Belkhir et al. 2002) to estimate mean pairwise relatedness. I used Queller and Goodnight's (1989) estimator option, and tested the null hypothesis of no relatedness by comparing our estimate to a distribution of coefficients derived through conventional Monte Carlo resampling procedures (1000 permutations).

Probability of identity - The uniqueness of an individual's genotype depends on the number and polymorphism (heterozygous) of the markers. Multi-locus genotypes based on few highly variable markers can be as powerful as those based on many less variable markers (Waits et al. 2001). Mills et al. (2000) suggested for studies of genetic demography that profiles should be based on multi-locus genotypes capable of discriminating individuals with 99% certainty. Estimating probability of identity ($P_{(ID)}$) is one way to establish this certainty when it is expressed as 1- $P_{(ID)}$. $P_{(ID)}$ is similar to the match probability used in human forensics (see Evett and Weir 1998, Avise 2004, Hedrick 2005) but is less susceptible to violations of linkage-disequilibrium and Hardy-Weinberg equilibrium, both of which can be prevalent in small, isolated, or substructured populations (Waits et al. 2001).

$P_{(ID)}$ analysis includes two steps. First, two theoretical $P_{(ID)}$'s, one for unrelated individuals ($P_{(ID)unbiased)}$) and one for siblings ($P_{(ID)sibs}$), are estimated (for equations see Waits et al. 2001). Both estimators use population allele frequency data, and $P_{(ID)unbiased}$ is corrected for bias in small samples. The two estimators provide lower and upper confidence bounds on the number of markers needed to accurately discriminate individuals. If the study population is composed of many related individuals, then resolving those individuals requires more markers. Step two involves calculating an observed $P_{(ID)obs}$ based on actual multi-locus genotypes from a known population sample and is simply the proportion of all possible pairs of individuals with identical multi-locus genotypes (Waits et al. 2001).

To estimate both theoretical $P_{(ID)}$'s and to quantify $P_{(ID)obs}$ (the proportion of individuals that share genotypes), I used PROB-ID5 (provided by L. Waits; Waits et al. 2001). I used multi-locus genotypes derived from 69 adult goshawks, which I assume to be unrelated (see below), and 44 of their nestlings (sibling groups of 2-4 nestlings). I first analyzed the adults and then added the offspring/sibling groups. I used all five microsatellite markers and the CHD sex-linked gene, and added markers sequentially starting with those having the highest number of alleles.

Effective Population Size - To evaluate whether immigration and gene flow influenced genetic structure of the Kaibab population I estimated the effective size of the Kaibab population (N_e). Effective population size is the idealized number of individuals in a population measured either demographically, in terms how many individuals actually contribute to breeding (i.e., variance in productivity), or genetically, using F-statistics and

measures of inbreeding where the assumption of non-overlapping generations exists (Barton and Whitlock 1997, Hedrick 2005). Goshawks sampled on the Kaibab Plateau during the study period likely represent at least three overlapping generations, thus I relied on reproductive data to estimate N_e.

Effective population size is generally smaller than the censused population (N). Counts of breeding pairs of goshawks can be used to index N_e, but not all goshawk pairs on the Kaibab produced an equal number of offspring during the study (Wiens and Reynolds 2005). I therefore estimated annual N_e's (Eq. 6.8a in Hedrick 2005) as:

$$N_e = \frac{N\bar{k} - 1}{\bar{k} - 1 + \frac{V_k}{\bar{k}}}$$

where $\bar{k}$ is mean productivity measured as the number of young fledged per active nest (1991-2003; Reynolds et al. 2005), V_k is the variance in annual mean productivity and N is the annual count of breeding pairs for the year. I then calculated a 13-yr harmonic mean of annual N_e's (Eq. 6.12b in Hedrick 2005) for our final size estimate.

RESULTS

Genetic Markers - Of nine cross-specific and sub-specific markers tested, two did not successfully resolve alleles, six amplified successfully but lacked variability, and one both amplified and was polymorphic (AGE 1a, Table 1). All four microsatellites originally isolated from Northern Goshawks amplified and were polymorphic (Table 1). The CHD sex-linked gene amplified and SSCP genotypes were consistent within the sexes (females n=40; males n=29), making it useful for distinguishing between male and

female goshawks. I validated our amplification and scoring of microsatellite markers after all individuals were genotyped and scored the first time. I genotyped the 23 individuals used as standards a second time, using DNA that was archived and remained untouched in our freezers from the time of original DNA extraction. I found only a single scoring error out of 230 opportunities (23 samples genotyped twice for five markers). This was a recording error; the sample had actually been genotyped correctly.

In comparisons of expected (H_E) and observed (H_O) heterozygosity at each marker, four of the five microsatellites were similar. One marker (AGE 6), however, significantly departed from Hardy-Weinberg expectations ($P < 0.00$) due to a deficiency of heterozygote genotypes (Table 1). Based on our data, I suspected this marker was sex-linked, as I found strong linkage-disequilibrium between AGE 6 and the sex-linked CHD marker ($p < 0.001$, Fisher's method, 3200 runs) indicating that the two markers segregate together. It appeared that the marker is located on the Z sex-chromosome because all females (ZW) had only a single allele (homozygous), while most males (ZZ) were heterozygous. I hypothesize that alleles on the female's W-chromosome are non-amplifiable (null) because of mutations in the priming sequences flanking the marker (Scribner and Pearce 2000) or because the marker on the Z-chromosome simply has no homologous region on the W-chromosome. I found no evidence for linkage-disequilibrium between the other four microsatellite markers (Table 1) when AGE 6 and CHD were excluded from the analysis. I found no evidence of null alleles, a result important for future assessments of genotyping error when using feathers as a source of DNA.

Population Genetics - F_{IS} measures departures of observed and expected heterozygosity under assumptions of random mating and indicates either inbreeding (F_{IS} > zero) or inbreeding avoidance (F_{IS} < zero). Thus, highly structured or isolated populations that experience genetic drift, generally exhibit positive F_{IS} values. Alternatively, large populations or those experiencing high gene flow generally exhibit non-significant or negative F_{IS} values.

I found no evidence of inbreeding or inbreeding avoidance (F_{IS} = -0.001; 95% CI: -0.070, 0.063; AGE 6 excluded, Table 1), suggesting that Kaibab goshawks mate randomly. Lack of inbreeding could result from large population size, gene flow by immigrants, or both. Our demographically derived estimate of effective population size (N_e = 37 individuals; range = 10-86), however, indicates that the population is demographically small, thus making gene flow a more likely source of genetic variability. This is consistent with our estimate of relatedness among adults goshawks, where average relatedness (r = -0.017) was less than expected by random (Fig. 1).

Probability of Identity - Certainty of individual identification is equal to 1- $P_{(ID)}$, and therefore the goal in estimating probability of identity analysis is to obtain small values of $P_{(ID)}$. Waits et al. (2001) suggest a value ≤ 0.0001 for forensic investigations where estimates of demographic parameters are needed. This threshold is interpreted as a 1:10 000 chance two individuals sampled from the same population will have the same multi-locus genotype.

I found that all 69 unrelated adults had unique multi-locus genotypes with the inclusion of the first three markers ($P_{(ID)obs}$ = 0.00), and likewise the estimated $P_{(ID)}$ met

the 0.0001 threshold ($P_{(ID)\ unbiased} = 1.13 \times 10^{-4}$) (Fig. 2a). With five markers, the same sample had a $P_{(ID)\ unbiased} = 7.03 \times 10^{-7}$. Based on demographic data, however, I know that siblings and parent-offspring can nest simultaneously in the Kaibab population (R. Reynolds, unpubl. data). To model this effect I added 44 nestlings-siblings to the sample. While the two theoretical $P_{(ID)}$'s did not change, all five markers were required to differentiate individuals ($P_{(ID)obs} = 0.00$, Fig. 2b).

In both cases (adult only and adults with offspring-sibling groups) our sample of markers was insufficiently large to bring the $P_{(ID)sibs}$ to the 0.0001 threshold. Thus, I was not able to estimate an upper number of markers needed for this resolution. Nonetheless, with five markers $P_{(ID)sibs} = 6.17 \times 10^{-3}$, which translates into a six in a 1000 chance of drawing two identical genotypes. Because I sampled many parent-offspring pairs that I could nevertheless distinguish, I was confident the five combined markers provide unique genetic marks.

DISCUSSION

Our intent in this study was to develop a set of genetic markers that uniquely identified individual goshawks. I desired to establish this marker set using high-yield DNA (blood) sampled from a known reference population. While most microsatellites tested did not amplify or were monomorphic (most *A. g. gentilis* markers), I did find a set of highly variable markers that consistently amplified DNA from blood. Elsewhere (S. Bayard de Volo, *unpubl. data*), I found that the same genetic markers consistently and reliably amplified DNA from molted feathers. It should be noted that because AGE 6 is probably on the Z sex chromosome, its utility for estimating within population relatedness and

levels of inbreeding is limited to samples from males. However, differences in allele frequencies between populations will still be useful for larger scale studies comparing populations. In a study of goshawks in Utah, Sonsthragen et al. (2002) used a different set of microsatellite markers than ours. Of eight markers, only one of theirs exhibited the same number of alleles ($H_O = 0.73$, $H_E = 0.74$) as AGE 6 did in our study. This alternative marker (BV 20; Gautschi et al. 2000) would be useful if it exhibited similar levels of heterozygosity and allelic diversity in the Kaibab population. Replacing AGE 6 with a less variable marker would result in having to add more markers to the entire genetic profile, which would introduce more opportunities for genotyping error.

With the five microsatellite markers tested (Table 1), all 113 goshawks sampled had unique multi-locus genotypes resulting in a $P_{(ID)obs} = 0.00$ (Fig. 2b) and a $P_{(ID)unbiased} = 7.03 \times 10^{-7}$. This was a powerful result considering that our sample included many parent-offspring and sibling pairs from the same nest. Likewise, the five microsatellites showed a high level of expected heterozygosity ($H_E = 0.81$). Others have shown that marker sets composed of five markers that result in $H_E \geq 0.80$ will have a theoretical $P_{(ID)} \leq 0.0001$ (Waits et al. 2001). In Paetkau's (2003) retrospective analysis of 21 non-invasive genetic studies in bears, the number of markers used was determined by whether the first five most variable microsatellite markers together had $H_E > 0.80$. He found that for some black bear (*Ursus americanus*) populations H_E was > 0.80 for five markers, however, for others, and for all grizzly bear (*U. arctos*) populations, H_E was < 0.80, requiring the marker set to be increased to six or seven in order to confidently discriminate among individuals.

It is important to note that our estimates of $P_{(ID)}$ are specific to the Kaibab goshawk population; I can not predict with complete certainty that these same markers will uniquely mark goshawks from other populations. Power of discrimination depends on population-specific levels of genetic variability (heterozygosity); goshawk populations that are less variable because of geographic isolation or habitat fragmentation may require more markers to uniquely genotype individuals (Paetkau and Strobeck 1994). However, goshawks are highly vagile and I suspect gene flow is likely high among other populations in Arizona and Utah. These goshawk populations will probably exhibit similar heterozygosity and the marker set tested here should prove useful for other studies.

The Kaibab goshawk population exhibits high genetic variability (Table 1) despite its geographic isolation and small effective population size (based on demographic data; 13-yr mean equaling 37 individuals). Several explanations may account for this. First, it is possible that the markers used in this study are under selective sweeps with genes that are affected by balancing selection for heterozygous genotypes. Such selection has been found for the genes of the MHC (major histocompatability complex) in mammals (Avise 2004) where heterozygous individuals experience a fitness advantage. It is unlikely, however, that all four non sex-linked markers would be under the same selective pressures given that they exhibit independent segregation (no evidence of linkage disequilibrium; see Black et al. 2001).

A second and more likely explanation is that actual N_e for this goshawk population is much larger because geographically distant populations in the region are connected by migration and gene flow. While adult goshawks are mostly sedentary on

breeding territories (Detrich and Woodbridge 1994, Squires and Ruggiero 1995, Reynolds and Joy 2005), band recoveries of first year goshawks from the Kaibab Plateau indicate dispersal distances of up to 440 km (Wiens 2004). In addition, telemetry data show that juvenile goshawks disperse from the Kaibab Plateau in their first year, with the majority moving beyond the 80 km detection distance (Wiens 2004). Further, Wiens (2004) showed that only 11% of 70 banded nestlings returned to be recruited into the Kaibab breeding population, indicating high first year mortality and/or low natal site fidelity. Evidence for the latter is indicated by the lack of population structure for goshawks in Utah, just north of the Kaibab Plateau, which can be attributed to gene flow from beyond the study area. In order to better assess actual effective population size for goshawks in western North America, I are expanding our studies to include populations in the western portion of the species range. Data from these studies should allow a more comprehensive evaluation of the genetic structure and effective population size for goshawks in the West.

CONCLUSIONS

Genetic marking of Northern Goshawks on the Kaibab Plateau is both feasible and reliable. Likewise, non-invasive genetic sampling will provide an alternative method for demographic and genetic data collection, as I have found that molted feathers are as reliable a source of DNA as blood (S. Bayard de Volo unpubl. data). Because goshawks show high territory fidelity (Detrich and Woodbridge 1994, Reynolds and Joy 1995, R. Reynolds unpubl. data), they are particularly well suited for non-invasive genetic sampling. I recommend that monitoring programs implement rigorous field collection of

molted feathers. As with any demographic study, valid inferences to the population depend on appropriate spatial and temporal sampling from that population. Researchers and managers interested in implementing non-invasive genetic mark-recapture to study goshawks should contact the corresponding author or refer to http://lamar.colostate.edu/~sbdv for a feather sampling and storage protocol.

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Table 1. Statistics for microsatellites tested on blood-derived DNA sampled from female (N=40) and male (N=29) Goshawks, Kaibab Plateau, AZ (1991-1993, 2000-2002).

Marker	Species	Alleles	NA[d]	H_O	H_E	H-W[e] *p* (SE)	F_{IS} (95% CI)[f]
AGE 1[a]	*A. g. atricapillus*	15	0.0425	0.77	0.84	0.21 (0.020)	0.0825
AGE 2[a]	*A. g. atricapillus*	11	0.0006	0.83	0.83	0.08 (0.009)	0.005
AGE 6[a,c]	*A. g. atricapillus*	11	0.3805	0.38	0.85	0.00 (0.000)	0.556
AGE 4[a]	*A. g. atricapillus*	10	-0.0062	0.86	0.85	0.06 (0.005)	-0.007
AGE 1a[b]	*A. g. gentilis*	5	-0.0506	0.77	0.7	0.09 (0.005)	-0.1
All markers		52		0.72[g]	0.81	*See text*	0.115 (-0.039, 0.346) -0.001[h] (-0.070,0.063)

[a] Topinka and May 2004. [b] Peck 2000. [c] Suspected sex-linked marker

[d] Frequency of null alleles estimated with CERVUS 2.0.

[e] Departures from Hardy-Weinberg expectations. Exact p-values and SE derived using Markov-Chain methods (1000 Dememorizations, 200 batches and 1000 iterations) in GENEPOP 3.4.

[f] 95% CI calculated using bootstrapping over markers (10 000 replicates) in GDA 1.0

[g] Simple average. [h] AGE 6 omitted.

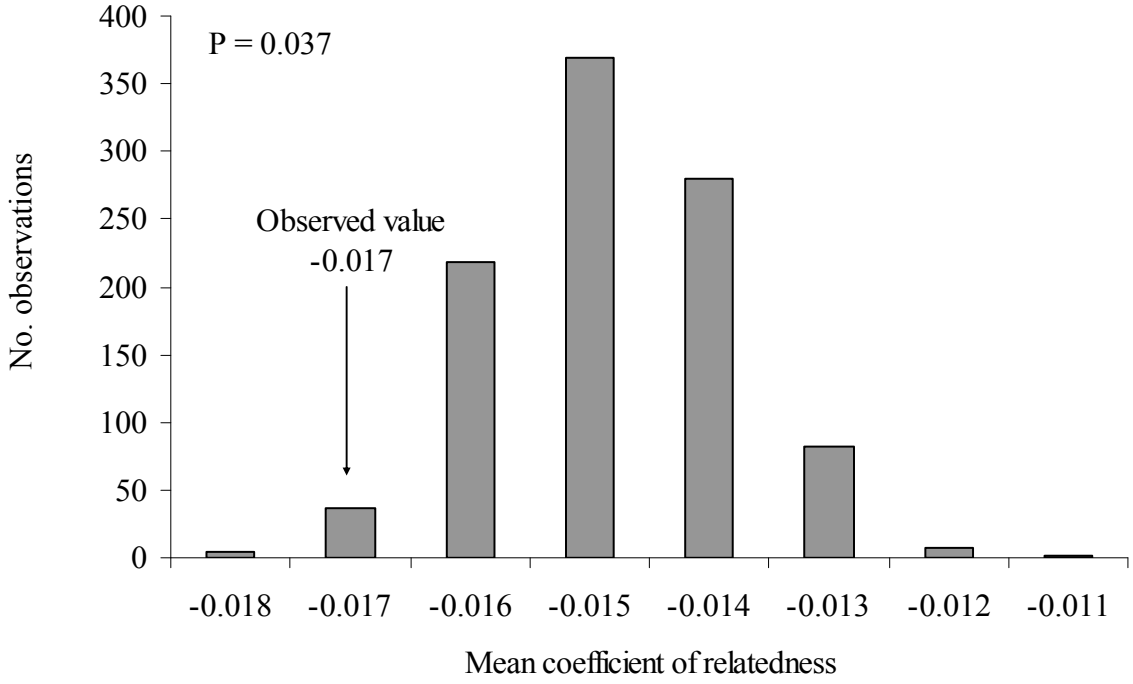

Figure 1. Observed average relatedness relative to 1000 randomized populations assumed to lack relatedness. The observed mean falls below that which is expected at random, occurring with a probability of 3.7%, indicating that Northern Goshawks (*Accipiter gentilis*) on the Kaibab Plateau, AZ, are less related than expected at random.

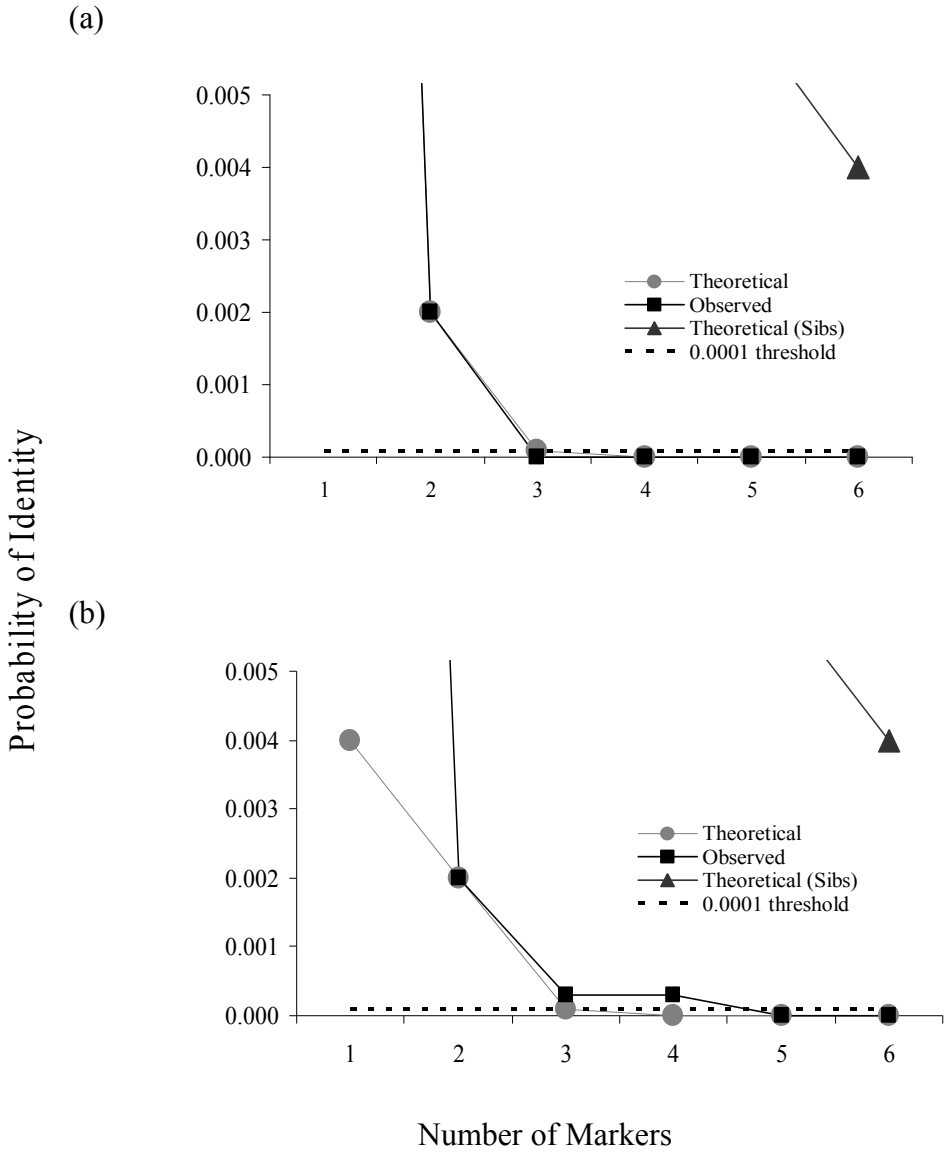

Figure 2. Relationship between theoretical, observed, and sib probability of identity ($P_{(ID)}$) for Northern Goshawks (*Accipiter gentilis*) on the Kaibab Plateau, AZ. The first five markers are microsatellites and the sixth is the CHD sex-linked gene. Observed data closely tracked that of the theoretical estimator; however, while (a) all 69 unrelated adult goshawks were resolved after the first three markers (observed $P_{(ID)} = 0$), (b) it took an additional two markers to resolve sibling and parent-offspring goshawks when 44 nestlings were added to the sample. In both cases, the theoretical $P_{(ID)}$ met our 0.0001 threshold (a 1:10 000 chance that two individuals sampled from the same population have identical multi-locus genotypes). I did not analyze enough markers, however to bring the sibling $P_{(ID)}$ to our threshold level

Appendix I. Source, repeat qualities and accession numbers or primer sequences for microsatellites found to be useful for genotyping Northern Goshawks (*Accipiter gentilis*) on the Kaibab Plateau, AZ.

Marker	Repeat Motif[a]	Allele size in base pairs	Accession #'s or Primer Sequences 5' to 3'	Author
AGE 1	$(gggaa)_8..(gaga)_9..(gagaa)_3$	216	AY312451	Topinka and May (2004)
AGE 2	$(gagaa)_{10}(ga)_4$	170	AY312452	
AGE 6	$(gagaa)_4..(gagaa)_2..(gagaa)_5$	259	AY312456	
AGE 4	$(gagaa)_{19}$	275	AY312454	
AGE 1a	$(ggat)_5$	208[b]	**f** acaactgggctgtgctttgc **r** cttcccggtggctgaggctt	Peck 2000

[a] Sequenced by authors.
[b] Average allele size in European goshawk (*A. g. gentilis*)

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CHAPTER II

MOLTED FEATHERS AS A NON-INVASIVE SOURCE OF DNA FOR GENETIC STUDIES OF NORTHERN GOSHAWKS

ABSRACT

To assess the value of molted feathers as a non-invasive source of DNA for genetic studies of Northern Goshawks (*Accipiter gentilis*) I isolated DNA from blood and molted feathers, then used five microsatellite markers and a sex-determining gene to test the effects of an optimized DNA extraction method and feather size on DNA yield (ng/μL) and genotyping errors (PCR success and allele dropout). I determined genotyping consistency between two replicates of the same feather sample, and among multiple feathers and blood from the same individuals. The optimized precipitation extraction method significantly increased DNA yield. Tail feathers yielded significantly more DNA than primary, secondary and smaller feathers, yet all feather sizes produced equally high rates of PCR success. Small (coverts, alular) feathers were, however, susceptible to allelic dropout for the largest of the five microsatellite markers tested. Genotypes were highly consistent among the two replicates of the same feather sample and rates of allelic dropout were low (~2%). Likewise, genotype consistency among blood and feathers, and multiple feathers, from the same individual was equally high (allelic dropout = 2.2% and 0.93% respectively). Feathers molted at breeding sites provided on average 24 ng/μL of

DNA, which is a relatively high DNA yield compared to other non-invasive tissue sources, and resulted in the low rates of genotyping errors.

INTRODUCTION

Most non-invasive genetic studies have focused on mammals like bears (Schwartz et al. 2006), wolves (Hausknecht et al. 2007), primates (Morin et al. 2001, McGrew et al. 2004), and elephants (Eggert et al. 2003), which are often times difficult to capture. DNA has been sampled from hair (Goossens et al. 1998), feces (Tikel et al. 1996), urine (Nota and Takenaka 1999, Hausknecht et al. 2007), fish scales (Lucentini et al. 2006), shed snake skin (Bricker et al. 1996, Eguchi and Eguchi 2000), egg shells (Schmaltz et al. 2006) and blood spots left on snow from injured or proestrus female wolves (Scandura 2005). Likewise, small mammal bones from owl pellets (Taberlet and Fumagalli 1996) have been used to detect species presence, identify trends in abundance and to determine phylogeography (Miller et al. 2006).

Raptors (hawks, eagles, owls, etc.) can also be difficult to capture, especially in the numbers required for demographic studies. Capture-recapture methods require capturing and marking individuals, and then resighting those marks (usually leg bands or patagial tags) in subsequent years. The ability to capture and resight individuals often depends on defensive behavior at breeding sites, limiting data collection to a few months over the breeding season and to years when individuals nest. Raptors typically have large territories making capture-recapture prohibitively expensive, which can be further exacerbated when species breed in remote, rugged landscapes and exhibit elusive behavior (McDonald 2004, Thompson 2004).

Because DNA can be sampled from feathers, it is possible to use non-invasive genetic sampling to study raptor genetics and ecology. Several studies have used DNA from molted feathers to identify species (Rudnick et al. 2007), determine sex (Griffiths and Tlwarl 1995), identify parentage and individual turnover at nests (Rudnick et al. 2005), assess population genetic structure (Segelbacher et al. 2003), phylogeography (Bayard de Volo, Chap. 3, this publication) and interspecific phylogeny (Seki 2006). I are aware of no studies that used molted feathers to identify individuals to estimate demographic parameters in a capture-recapture framework (White and Burnham 1999).

Raptors are well suited for non-invasive genetic sampling for demographic studies. Most raptors molt their feathers over several months during the spring and summer, when they are also tending their nests with eggs or nestlings. Likewise, many raptors are territorial, using the same nests or nest areas over many years. Thus, nests can be used over many decades as territory "ownership" passes from one individual to another, making sampling locations relatively fixed and reliable. Collecting molted feathers at nest areas is also less costly, as it requires fewer nest visits than capturing and resighting marked individuals.

Molted feathers are a good source of DNA, as the microenvironment within feather shafts protects DNA. As feathers grow, they are supplied with blood containing nucleated red-blood cells. When feather growth is complete, the blood supply is removed, but residual cells remain inside of the feather shaft and as part of the superior umbilicus (Horvath et al. 2005). Skin cells on the outside of the feather shaft can supply DNA, although these cells are not as protected from DNA-degrading conditions (e.g., solar radiation, hydrolysis, and repeated freezing-thawing).

Correctly identifying individuals from feather DNA requires experimental validation of rates and sources of genotyping errors. Genotyping errors for hair and fecal DNA have been well studied (Paetkau 2003, Broquet and Petit 2004, Waits and Paetkau 2005), but only three studies have formally tested for such errors in DNA from molted feathers (Segelbacher 2002, Horvath et al. 2005, Rudnick et al. 2005). Our larger research goals involve testing the efficacy of non-invasive genetic sampling to estimate adult survival rates in a population of Northern Goshawks (*Accipiter gentilis*) in northern Arizona, where I have used traditional capture-mark-resight methods (Reynolds et al. 2005) while collecting molted feathers from active nest sites (1991-2007). This combination of data will provide an opportunity to assess the feasibility and accuracy of genetic capture-recapture in studies of long-term demography in Northern Goshawks. Because genotyping error associated with feather DNA is poorly understood, our goals in this study were to address the following objectives:

1. Optimize a DNA extraction method for feathers.
2. Test the effects of feather type (size) on DNA yield, success of PCR amplification, and genotyping error.
3. Assess consistency of genotypes derived from replicates of the same feather sample (within feather comparison)
4. Assess consistency of genotypes derived from multiple feathers and blood samples from the same individual (across sample comparison).

METHODS

Sample Collection and Preservation - I sampled goshawks from across the North Kaibab Plateau in northern Arizona, USA. This forested plateau is surrounded by large expanses of pinyon-juniper woodland and desert sage-scrub habitats, spatially isolating it from other forested regions. Goshawk nesting density on the plateau was high (~ 150 territories), but not all nests were occupied or active in every year (Reynolds et al. 2005, Reynolds and Joy 2006, Wiens et al. 2006b).

I sampled blood from 69 breeding adult goshawks (Bayard de Volo et al. 2005). Molted feathers were collected from active nest sites weekly throughout the breeding season, by searching under the active nest and other large trees, downed logs and stumps. Most feathers were labeled by territory, nest, and date directly on the feather shaft with a fine "Sharpie" brand marker (1991-1999) or were placed in a labeled paper envelope (territory, nest, date, names of collectors, and distance and bearing from the active nest; 2000). Some feathers collected early in the study were labeled with year only. Feathers were stored according to year in temperate dry, dark storage.

All feathers at nest sites were collected during each visit; nest sites were thus "cleaned up" each week and year, and I was confident that feathers were molted and collected in the same year. When new nests were located, I used only obviously freshly molted feathers and avoided those that were dirty, dry and brittle, as these were likely molted in previous years.

Genetic Markers and Probability of Identity - Previously, I assessed genetic diversity and structure for Kaibab goshawks and tested twelve microsatellite markers and the CHD sex determining gene (Bayard de Volo et al. 2005). I also calculated probability of identity (Waits et al. 2001) to show I could confidently [$P(\text{ID})<0.0001$] identify 114 individuals using five microsatellite markers (Bayard de Volo et al. 2005). This sample included 44 nestlings, all the offspring of the 69 adults used in the study. Thus, the sample included related individuals, all of which were resolved using five-locus genetic profiles. In this study, I used the same five microsatellite and sex determining markers.

General Methods for Molted Feather DNA Extraction - I extracted DNA from 100 molted feathers randomly selected from our larger feather collection (10/yr, 1991-2000). Sixty-five of the 100 feathers were labeled with at least year and territory, whereas the other 35 were labeled with year only (territory ID was unknown). In many cases, several feathers were selected from the same territory (over the same or several years). Feather samples included primary, secondary, tail, alular, and wing and body covert feathers.

I reduced the risk of sample contamination from DNA extraction or PCR amplification by performing all feather extractions in a lab separate from where blood samples were processed. Additionally, amplified DNA never entered the room where feather DNA extractions took place, and I always used aerosol-resistant tips on pipettes that which were dedicated to low concentrate DNA extracts. Following T. Glenn (unpubl. protocol, "Getting DNA out of old stuff", 1996, Smithsonian Institute) I routinely wiped all surfaces with 10% bleach (sodium hypochlorite; Prince and Andrus

1992). Likewise, all scissors, blades and forceps were sterilized by soaking in a 50% bleach solution, and then rinsed in sterile ddH_2O.

The entire calumus tip was separated from the rest of the feather and soaked in 70% EtOH for 30 min., rinsed once in ddH_2O, and soaked again in ddH_2O for another 30 min. I produced replicates of each feather by splitting the calumus tip down its entire length (from tip to where feather vanes start), cutting the shaft horizontally into small (~ 0.3 cm) pieces (placed on a sterile Petri dish), and randomly placing each piece into one of two tubes (first piece random, and then alternating between tubes). I ensured that each tube had one of the two proximate calumus-tip halves, as this part of the feather likely contains much of the DNA. I then isolated DNA from all replicates-1, and 53 of corresponding replicates-2 (Fig. 1) using Proteinase-K digestion, followed with a ammonium acetate separation of proteins and a cold ethanol precipitation of nucleic acids (*hereafter* PPT). The other 47 replicates-2 were extracted using QIAamp (Qiagen Corp., Valencia, CA) extraction kits. In the case of the latter, subsequent PCR amplifications were unreliable, potentially a consequence of our altering the manufacturer's protocol. I therefore omitted these replicates from this study.

Digestion mix contained: 600 µL TNE (Tris-NaCl-EDTA; pH 7.5), 60 µL 1M Tris-HCL (pH 8.0), 25 µL Proteinase-K (25 mg/ml), 10 µL 25% SDS (sodium-dodecyl-sulfate), and 5 µL 1M DTT (dithiothreitol). I prepared the DTT just prior to use, because of the reagent's tendency to quickly lose activity upon going into solution (M. Sorenson pers. comm.). Feathers were digested for 24-hrs in a water bath at 55° C and pulse-vortexed twice during that period. DNA pellets were re-suspended in 20µL TE.

Following extractions, I quantified DNA yield in ng/μL for all replicates using a flourometer (DyNAQuant 200, Hoefer, Bio-Rad Laboratories, Inc., Hercules, CA) and a Fluorescent DNA Quantitation Kit (Hoechst 33258 Dye; Bio-Rad Laboratoires, Inc., Hercules, CA). Instrument accuracy was checked every 5th sample using a concentration standard (calf thymus DNA prepared at 100 ng/μL). Although, the flourometer reports yields <10 ng/μL, the quantitation kit's lower limit of accuracy is 10 ng/μL. Nevertheless, I recorded all quantities including those less <10 ng/μL.

Optimizing a DNA Extraction Method - To optimize the extraction protocol, I extended the digestion time from 24 hrs to 5-7 days, increased freezer time for the isopropanol precipitation from 1 hr to 12 hrs and increased cold centrifugation from 10min. to 30min. (S. Talbot, USGS, pers. comm.). I also increased Proteinase-K from 25 μL to 45 μL and DTT from 5μL to 30μL, quantities more consistent with protocols cited by T. Glenn (unpubl. protocol, "Getting DNA out of old stuff", 1996, Smithsonian Institute). These protocol changes were applied to only a subset of sample replicates (*n*=53, replicates-2 in Fig. 1). I then determined whether these changes increased DNA yield.

Effects of Feather Type - I determined the effects of feather type (size) on DNA yield, PCR success and allelic dropout using 100 feather replicates (replicates-1, Fig. 1), which all had the same PPT extraction method. Feather age (time in archive) ranged 2-11 years.

Genotyping Consistency Between Replicates of Same Feather - To determine the degree of consistency between genotypes derived from replicates of the same feather sample, I used only feathers for which both replicates had the same precipitation extraction method (n = 53 feathers, Fig. 1). Most of these were remiges and rectrices.

Genotyping Consistency Among Samples from the Same Individual - To evaluate consistency of genotypes among samples from the same goshawk, I compared: (1) blood collected from banded individuals (genotyped in Bayard de Volo et al. 2005) to feathers collected from their nest sites; and (2) multiple feathers collected from the same nest site, either in the same year or over several years. However, because goshawks rarely disperse to new territories following a "divorce" or disappearance of a mate, there are cases where blood and feathers were not sampled from the same nest site, and/or in the same year. In all cases however, band-resight data were used to confirm the same goshawk occupied the sampled nests in all years.

PCR, GENOTYPING AND SEX DETERMINATION

Preparation of all feather PCR reactions was performed in an enclosed box containing two germicidal ultraviolet (UV) light bulbs (240 nm spectrum, 15 watt, UV Process Supply #G15T8) to degrade contaminating DNA on PCR racks, pipettes and tubes. I used the UV lights for 20-30 min. prior to processing each set of PCR reactions.

Microsatellite markers were amplified as described in Bayard de Volo et al. (2005) with the following exceptions: (1) in some cases I added an additional 3mM

Mg_2Cl_3 to the PCR buffer conditions; (2) I used positive controls (blood derived DNA) to determine whether non-amplification was due to procedural errors or degraded feather DNA; (3) the number of PCR cycles was increased to 40 (56 for AGE-4). PCR conditions for the CHD marker followed Bayard de Volo et al. (2005).

Gel electrophoresis was used to separate and visualize alleles (Bayard de Volo et al. 2005). To assess the reliability of each genotype, I employed a confidence scoring system similar to that used by Paetkau (2003). Samples with intense, unambiguous bands received a "1", while those with less intensity, yet unambiguous bands were a "2"; those with questionable bands were given a "3", (i.e., a relatively faint band compared to the other in a heterozygote individual; bands resulting from possible leakage between lanes; bands that were difficult to score against standards because of voltage inconsistencies in the gel); and non-amplifying samples were scored with a "4". All score-4 samples were re-amplified a second time, using 2μL starting template. For the CHD sex-linked gene typing methods followed those outlined in Bayard de Volo et al. (2005).

All replicates were processed independently of one another, from DNA extraction to genotyping. For example DNA extraction for all replicates-1 was completed before performing DNA extraction on replicates-2, and all replicates-1 were PCR amplified together and then run on gels together, independent of their respective replicates-2. Thus, multi-locus genotypes were independently derived, which allowed us to assess the reliability of our overall genotyping strategy (e.g., bookkeeping, scoring gels).

DATA ANALYSIS

Typically, allele dropout is identified with a multi-tubes approach, where as many as six PCR's are conducted for each marker and sample. In this study, I defined allele dropout as occurring when a genotype for: (1) one feather replicate was heterozygous, and the corresponding replicate was homozygous; and (2) one feather or blood sample was heterozygous and the other feather from the same individual was homozygous. Under this definition, I assumed that the heterozygous genotype was the correct one (*see* Bayard de Volo et al. 2005 for details of marker specific levels of heterozygosity).

Optimizing the Extraction Method - I used ANOVA to test a simple linear model where variation in DNA yield was explained by year of feather collection, extraction method (optimized vs. non-optimized), and sample within year (treated as a random variable). I tested the model using PROC GLM and PROC MIXED in SAS (2001), SAS Institute Inc., Cary, North Carolina.

Effects of Feather Type - I used ANOVA (PROC GLM, SAS 2001.) to test two simple linear models looking at the effect of feather type on DNA yield, and feather type and DNA yield on number of loci amplified while controlling for year in both models. To assess rates of locus- and tissue-specific allelic dropout I used the Brookfield estimator (Brookfield 1996) in MICRO-CHECKER 2.2.1 (Van Oosterhout et al. 2004) to test three tissue categories: (1) small feathers; (2) large feathers; (3) blood derived DNA. MICRO-

CHECKER identifies allele dropout (or null alleles) through a determination of heterozygote deficiency under the assumption of Hardy-Weinberg equilibrium.

Genotyping Consistency between Replicates of the Same Feather - I used GIMLET 1.3.3 (Valière 2002) to derive "consensus" genotypes from replicates and used the program's default threshold for retaining alleles (retained if alleles occur more than once). Under this limit, replicate genotypes not matching because of allelic dropout defaulted to the heterozygous genotype (i.e., 10/10 vs. 10/12 defaults to 10/12), and single-locus, non-matching genotypes default to missing data (i.e., 07/10 vs. 06/09 defaults to 00). Rates of allelic dropout were then estimated through comparisons of consensus genotypes back to replicate genotypes.

Genotyping Consistency among Samples from the Same Individual - I had two goals for comparing multiple samples from the same individual. First, I directly compared blood samples and multiple feathers from 11 goshawks and looked for allelic dropout with the assumption that the blood sample's genotype was correct. To quantify allelic dropout I counted the instances where a feather typed homozygous for a locus and the blood sample typed heterozygous. I also identified 15 groups of feathers as having come from 15 different individuals based on band-resight data and feather collection histories. Multiple-feather groups included 2-5 feathers and were assumed to have come from the same individual. Allelic dropout was measured as above.

Second, I tested a software package that would accurately group together samples that came from the same individual, while allowing for some degree of genotyping error. Such groups are important to identify as they represent individual capture histories, data needed for capture-recapture analysis. To test a grouping method I used blood/feather and multiple-feather groups, as well as feathers without field sampling information (n=36) and implemented the "grouping function" in GIMLET 1.3.3 (Valière 2002).

All feather genotypes were based on: (1) consensus genotypes derived from both replicates of feather samples (*n*=53); and (2) single replicates from feather samples (*n*=43). Thus, multi-locus genotypes for 43 feathers were based on single PCR amplifications, while multi-locus genotypes for 53 feathers were based on two PCR amplifications. Single-locus genotypes were typed as "missing data" if results for those loci were considered "low quality" (score-3 fragments, which were of uncertain nature; *n*=9 samples). Four samples were excluded from analysis because genotypes for >60% (3/5) of their loci were of low quality (Paetkau 2003).

RESULTS

Sex Determination - Because PCR success was high for replicates-1 (Figure 1) I opted not to run the second set to save time and resources. Of 100 replicates, four did not amplify and two produced weak and unreliable amplifications. Of the 94 samples with reliable PCR amplifications, only two sexed male, both of which genotyped as different individuals.

Optimizing the Extraction Method - Optimizing the PPT extraction method resulted in significantly higher DNA yields (F=3.3, df=51, P<0.0001), where mean yields were $\overline{X}$ = 15.22 ng/µL (range 1-81) and $\overline{X}$ = 27.31 ng/µL (range 0-99) for PPT and Optimized PPT respectively (Figure 2). The number of loci amplified, however, was not affected by DNA yield or year of feather collection (F=0.91, df=51, P=0.63), where all but one replicate amplified all five microsatellite markers.

Effects of Feather Type - Feather type significantly influenced DNA yield (F = 6.74, P < 0.0001, df = 4), however, neither DNA yield nor feather type influenced success of PCR amplification (Figure 3). Interestingly, tail feathers yielded significantly more DNA ($\overline{X}$ = 24.61 ng/µL, range 1-81, n=13), than all other feather types, including primary flight feathers ($\overline{X}$ = 13.82 ng/µL, range 1-59, n=28; Figure 3). MICRO-CHECKER results for the three tissue categories indicated no allelic dropout, except in the case of small feathers for AGE-4 (Table 1) and all categories for AGE-6. In the case of the latter, I expected this result as AGE-6 was shown previously to have a null allele (Bayard de Volo et al. 2005).

Genotyping Consistency Between Replicates of Same Feather - Results from GIMLET indicated that allelic dropout among paired replicates was rare; 1.7% across loci, 2.2% across PCR's, and 2.1% across samples.

Genotyping Consistency Among Samples from the Same Individual - Allelic dropout between blood and feathers from the same individual was rare (2.2%, 4 events/180 PCR reactions). I also found it to be rare among multiple feathers from the same individual (0.93%, 3 events/323 PCR reactions).

Ten of the 11 blood/feather groups were confirmed by the GIMLET's grouping analysis (Figure 4 provides one example). The single group not confirmed included a blood sample and two feather samples, both of which grouped together with a different genetic profile (Figure 5). Twelve of the 15 feather groups were completely confirmed. The three groups not confirmed resulted from: (1) a possible genotyping error associated with a PCR or a gel loading error (a different genotype for AGE-4 in one feather, as compared with the three others in the group); (2) field mislabeling of a feather, which subsequently grouped with several other feathers and a blood sample from different territory; (3) a genotyping error associated with an allelic dropout event. This latter case involved a heterozygous genotype for AGE-4 in two feathers, and a homozygous genotype for the same locus in the group's third feather (based on a single PCR). Normally the third feather would not group alone, however the second feather had missing data for AGE-2. The two differences forced a separate grouping by GIMLET.

DISCUSSION

Our objectives were to establish optimal methods for recovering and amplifying DNA from non-invasively collected molted feathers. I was especially concerned with assessing rates and sources of genotyping errors (PCR failure and allelic dropout) and in assessing whether some markers or tissue sources were more prone to these errors. I also wanted to

test a computerized method for grouping together samples having the same genetic profile, and which would assign sample-specific match probabilities.

Sex Determination - I found that molted feathers can be used to determine sex in Northern Goshawks and that most feathers sampled were from female goshawks. Our GIMLET groupings indicated the 100 feathers represented 53 individuals, two of which were male. I suspect non-invasive sampling will be biased toward females because males spend a majority of time away from the nest hunting while females remain at their nests tending their young. As a result, males molt a majority of their feathers away from the nest site and our results confirm this.

Optimized DNA Extraction Method - Increasing digestion time and reagent concentrations facilitated the increased break down of feather keratin and increasing freezer time and cold centrifugation helped to increase precipitation of nucleic acid molecules. Both steps increased processing time however, and compared with other commonly used methods (silica-filter methods) ours takes at least three times longer to process samples. One thing to note is that results in this study are based on half-feather samples. Using the whole feather calumus tip, as well as the superior umbilicus (Horvath et al. 2005), will yield even more DNA allowing final template volumes to be higher (30-50µL instead of the 20µL used in this study) while maintaining similar concentrations. However, for smaller feathers, which yielded on average <10 ng/µL, using final volumes of 20-30µL would allow for higher DNA concentrations. For details of the extraction protocol see Appendix 1.

Feather Type - Our results compare with one other study (Horvath et al. 2005) that quantified DNA yield, but the authors did not report yields less than 10 ng/μL. I was not surprised that smaller covert and alular feathers yielded less DNA, but was surprised to find that they still yielded high quality PCR amplifications for most loci, the only exception being AGE-4 (Table 1). This finding stresses the need to understand the interactive effects among feather size and marker locus, and I recommend testing for such effects using both high and low copy DNA. Sefc (2003) also found larger fragment sizes more susceptible to allelic dropout, and they found this trend continued despite increased DNA concentration (two feathers vs. a single feather).

Genotyping Consistency Between Replicates of Same Feather - Our comparisons of replicates from the same feather allowed us to directly assess rates of allelic dropout under the assumption that the heterozygous genotype was correct. The fact that I found little allelic dropout (~ 2%) is not surprising when I consider that DNA yield for replicates averaged 24 ng/μL (SD=33, range 0-288) across all feather types. This concentration is much higher than the 56 pg threshold cited by Taberlet and Luikart (1999) for avoiding allelic dropout 99% of the time and higher than what Morin et al. (2001) recovered from chimpanzee hair and feces [fecal yields were 192 pg/μL (0-2550); single hair was 38 pg/μL (0-228)]. Rates of allelic dropout found in this study agree with others that used molted feathers; 1.1% in Black Grouse (Segelbacher 2002), 0.33% in Eastern Imperial Eagles (Rudnick et al. 2005), 8.33% (feather tip) and 0% (superior umbilicus) in Spanish Imperial Eagles (Horvath et al. 2005).

Genotyping Consistency Among Samples from the Same Individual - Comparisons between blood and feathers and among multiple feathers from the same individual also indicated low rates of allelic dropout. In the few cases where allelic dropout occurred, or where loci were typed as missing data, GIMLET 1.3.3 accurately grouped those samples with others from the same individual instead of separately as "new individuals". This is important for capture-recapture studies, because genotyping errors that erroneously introduce new individuals into a population sample will inflate estimates of abundance and bias estimates of survival low (Mills et al. 2000). These questionable samples, however, are flagged by GIMLET, which also sometimes includes them simultaneously into more than one group, and lists samples differing by only a single locus. With this information, questionable samples can be reexamined.

A question that might be asked in any non-invasive study is how many PCR's are required to reliably genotype samples. While some of our samples were based on two PCR's, others were based on only a single PCR. In the case of the latter, there was only one example in which a sample was grouped alone by GIMLET as a result of allele dropout (Case 3 above). Without further examination, this sample would erroneously be considered a new individual. All other samples with allelic dropout were correctly assigned to groups with several other samples. As well, all single-PCR samples with single-locus homozygous genotypes, matched the homozygous genotypes of all other samples in their GIMLET group. Thus, it appears our method of culling low quality samples and forcing low quality loci to be "missing data" was effective for correctly assigning single-PCR samples.

I agree with Mowat and Paetkau (2002) who suggest re-examining (repeating PCR's) all homozygous genotypes and samples differing at only a single locus. I also agree with Lukacs and Burnham (2005) that amplifying and processing more loci than are needed for development of unique genetic profiles (those with $P(\text{ID}) < 0.0001$; Waits et al. 2001) introduces unnecessary chances for genotyping errors (*but see* McKelvey and Schwartz 2004) and adds unnecessary costs and labor. However, as illustrated in this study, molted feathers from large raptors have the potential to provide significantly more DNA (ng/μL) than hair and scat (pg/μL), and are therefore not subject to the same rates of genotyping errors.

A major source of error I found was mislabeling of feathers in the field and/or errors associated with reading labels written on the feather shaft. One way to guard against such errors is to process several feathers a year from each nest, being careful to use feathers collected on different dates. While this would be an optimal strategy for studies processing small sample sizes, it would at the very least double expenses and labor. Our field sampling for feathers was part of our weekly nest monitoring and included the potential for ~ 500 sampling events per year (7 persons sampling 6 nests/day, one day/week * 12 weeks); over 10 years, a few sampling errors are expected. Most monitoring programs do not conduct weekly visits and if crews sampled twice a field season it is doubtful feathers would be mislabeled. Digital photos can be used to guard against misreading of labels on the feather shaft, and I recommend that feathers be stored in individual envelopes labeled with collection information.

I have since used molted feather DNA to successfully amplify ~500 bp of the mtDNA control region for ~300 feathers (unpublished data), but have not tested it on

other nuclear genes. I recommend that all research and monitoring programs collect and archive molted feathers, even if there are no immediate plans to use them. They store easily, and as shown here, feathers stored for 10 or more years still provided useful DNA. I believe that the collection of molted feathers for genetic based studies is not only important for studies of Northern Goshawks, but also for other raptor species and especially for species in regions where conservation efforts are logistically and financially limited. The use of feathers should not be limited to raptors, as all birds molt their feathers annually and if nests can be located, feathers are likely to be found.

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Table 1. Yes/no answers to whether null alleles/allelic dropout exists and estimates of their frequency for each locus based on tissue source. Secondary feathers were considered "small" or "large" depending on their contribution to initial starting volumes for DNA extraction. AGE-6 (a sex-linked locus) is known to have null alleles on the female's W-sex chromosome and this is strongly reflected in its estimated null allele frequency across all categories. AGE-4, however, does not show high frequencies of null alleles (or allelic dropout) in the higher-concentrate DNA samples (blood and larger feathers), yet in the lower concentrate samples it does. Estimates were derived in MICRO-CHECKER using the Brookfield estimator 1.

DNA Source	AGE-1 216 bp	AGE-2 170 bp	AGE 6 259 bp	AGE-4 275 bp	AGE-1a 208 bp
Small Feathers[a]	No	No	Yes	**Yes**	No
Coverts, alulae, secondary	0.031	0.035	0.376	**0.101**	-0.076
Large Feathers[b]	No	No	Yes	No	No
Secondary, primary, tail	0.009	0.038	0.452	0.021	-0.022
Blood[c]	No	No	Yes	No	No
	0.031	-0.002	0.259	-0.002	-0.042

[a] n= 38

[b] n= 54

[c] n=69

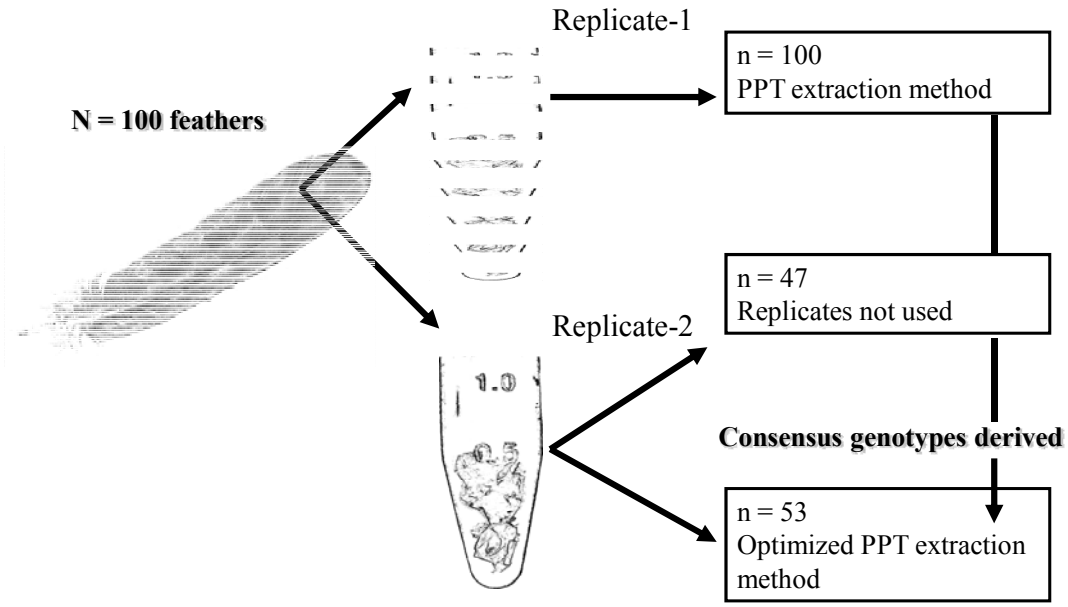

Figure 1. Schematic of sampling design showing how each molted feather was split into two replicates. Repicate's-1 all had the same precipitation DNA extraction method (PPT), and some replicate's-2 had an optimized PPT extraction method, while others a silica-filter extraction method. The latter replicates proved problematic, and were therefore not used in this study (see text for further explanation).

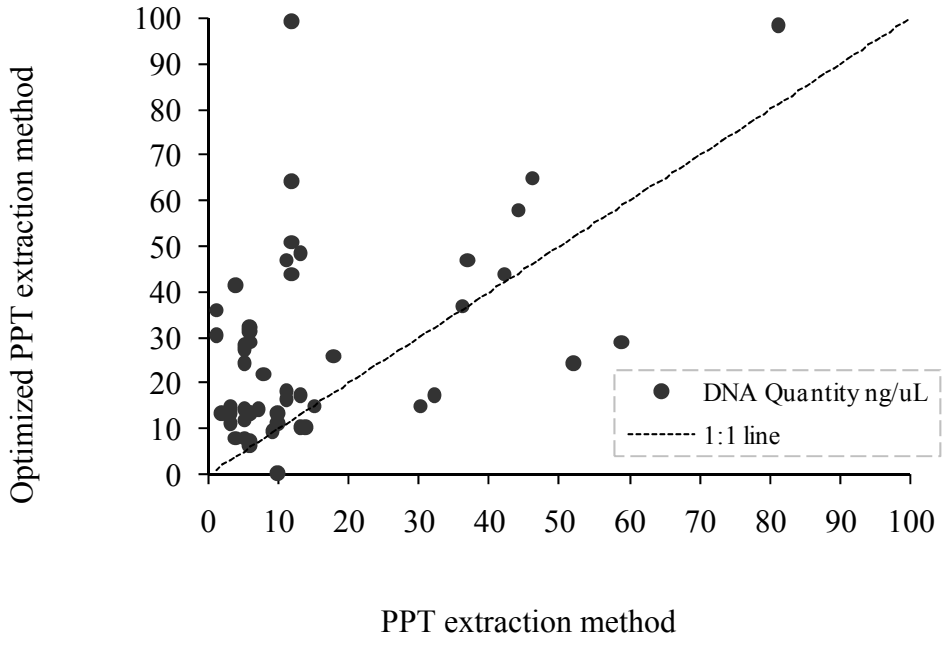

Figure 2. Pair-wise correlation's for replicates of feather samples having either the precipitation (PPT) or the optimized PPT DNA extraction method. Points represent paired-replicates and those falling on or near the line indicate equal performance. In some cases multiple paired-replicates had the same values and are therefore represented by a single point. Optimizing the PPT method increased DNA yield, which is indicated by the number of points that lie above the line. N=102 replicates (51 feathers).

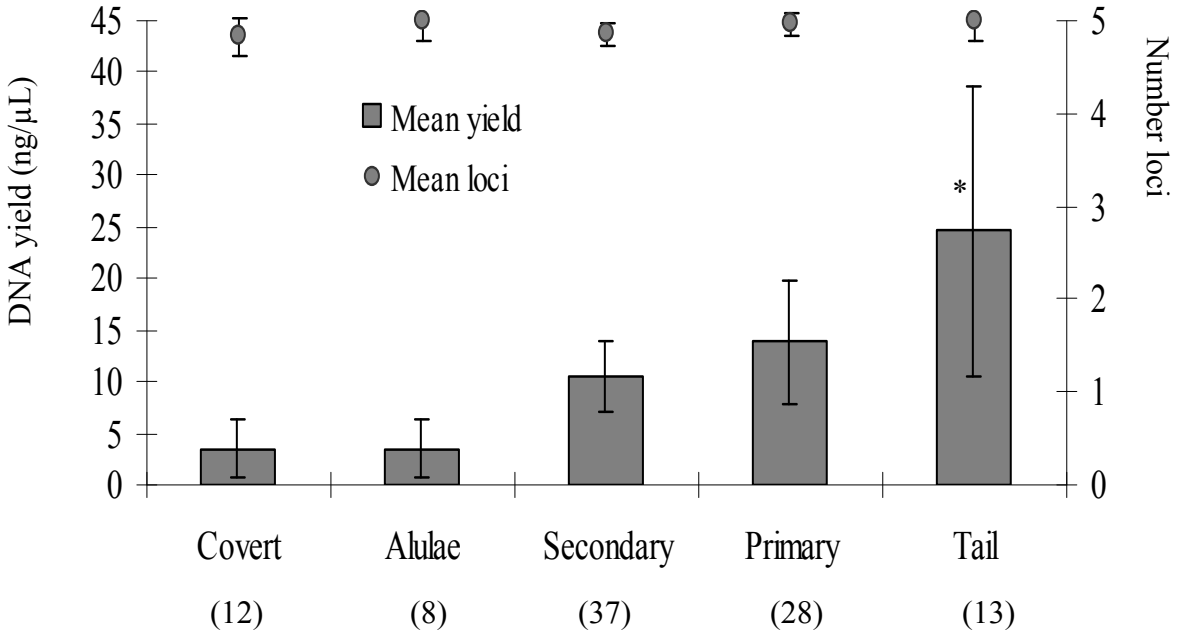

Figure 3. Means and 95% CI for DNA yield (bars) and success of PCR amplification (points) based of feather type/size. Samples were replicates-1 only (half-feather samples). While feather type affected DNA yield, only tail feathers yielded significantly more DNA ($P < 0.001$ for all comparisons). Feather type did not influence success of PCR amplification, where all but one sample amplified all five loci.

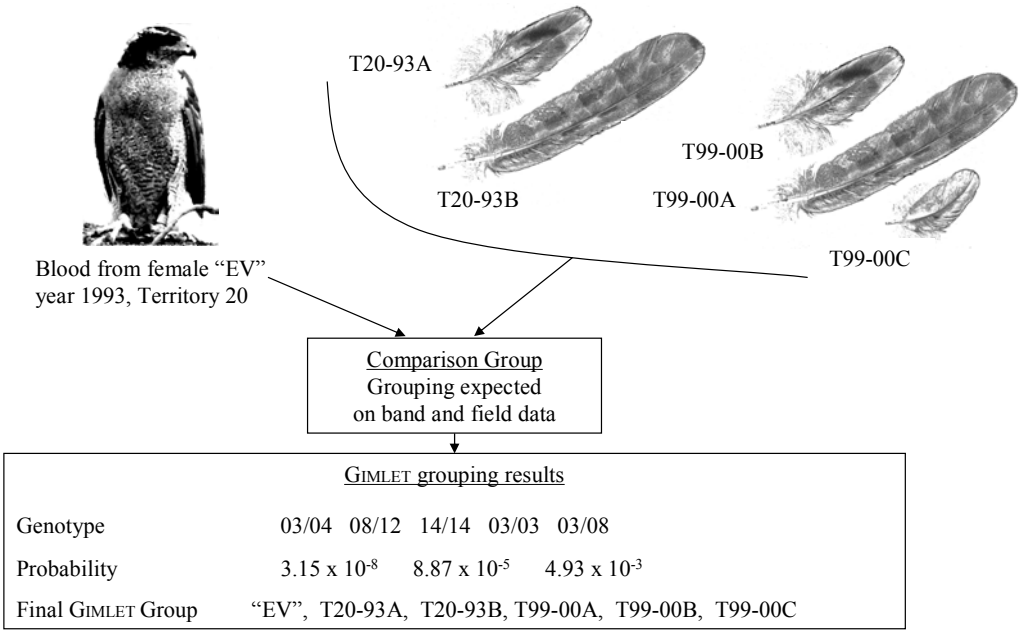

Figure 4. An example of comparison groups (blood from banded Northern Goshawks and molted feathers collected at their nest sites) that was tested for genetic-grouping accuracy using GIMLET 1.3.3. Feathers were labeled with territory ID (e.g., T20, T99), year collected (e.g., 93, 00) and feather letter (A, B, etc.). "Probability" is defined as the probability of a false match assuming either non-related, half-sib, or full-sib relationships. "Final GIMLET group" is the group of samples sharing the same genetic profile. The figure shows an example of where the group produced by GIMLET 1.3.3 matched the comparison group. The genetic profile for the female "EV" matched those of five feathers collected from nests at two different territories (years 1993 and 2000). All five feathers have the same match probabilities indicating exact matches across all five loci.

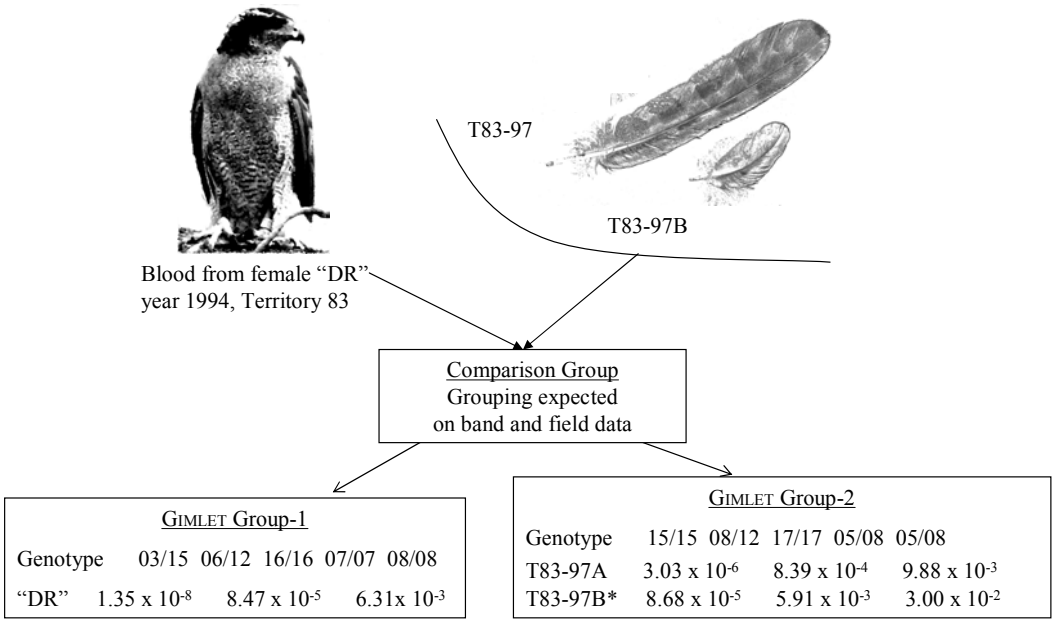

Figure 5. Example of comparison groups (blood from banded Northern Goshawks and molted feathers collected at their nest sites) that was tested for genetic-grouping accuracy using GIMLET 1.3.3. Feathers were labeled with territory ID (e.g., T83), year collected (e.g., 97) and feather letter (A, B, etc.). "Probability" is defined as the probability of a false match assuming either non-related, half-sib, or full-sib relationships. "Final GIMLET group" is the group of samples sharing the same genetic profile. The figure shows an example of a comparison group not confirmed by genetic data. Female "DR" grouped alone based on a genetic profile that was different from the two feathers. Although, female "DR" nested at Territory 83 in 1997, the feathers (determined to be female) were collected ~200m from the active nest. Each feather had a unique set of match probabilities because 83-1997B had missing data for locus AGE-4.

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Appendix 1.

FEATHER PREPARATION

- Wipe all surfaces with 10% bleach. Run UV lights (if available) for 20 min. Heat waterbath to 55˚C.
- Make sure there are plenty of sterile scissors, forceps and plastic Petri dishes before you start. We have about 25 each sterile scissors and forceps ready for use to process ~ 20 samples at time.
- Using Nitrile gloves helps to reduce static electricity that causes difficulty in handling feather material.

1. Set out 1.5 ml centrifuge tubes (label 1 – whatever).
   a. Separate calumus tip from rest of feather and place in tube. Make sure to cut above the superior umbilicus, so you can include it Later.
   b. Put feather back into envelope and write extraction date on envelope.
2. Wash tip - Fill tube with 70% EtOH and soak for **30 min**.
3. Set out another set of 1.5 ml centrifuge tubes and fill with ddH_20 and soak feathers for **30 min**.
4. Label 1.5 ml centrifuge tubes. These will be your digestion tubes, so label with 1-whatever **and date**.
   - Use sterile Petri dish as a catching surface, sterile scissors and sterile forceps **for each sample**
   - Cut feather calumus longitudinally along its length. Stop before superior umbilicus.
   - Then cut horizontally ~ 5cm of the feather tip into 2 -3 mm pieces and place into tube.
   - Cut out the superior umbilicus and place in tube. Cut it out as a small square around the "blood-dot".
   - Pull out the papery material from inside the calumus and place in tube.

DNA EXTRACTION

1. Mix up the digestion mix, using **newly mixed DTT** (we add 1ml sterile H_2O to a tube containing a pre-measured solid to make 1M solution).
2. Pipette **700 µL extraction mix** to each tube
3. Incubate at 55˚C until most of the material dissolves, overnight to one week, usually 3-4 days. In some cases we add more Proteinase-K (20 uL of 25mg/µL) if material is not completely digested in two days.

Following Digestion

4. Cool to room temperature.
5. Pipette **233µL protein precipitation mix** to each tube
6. Pulse Vortex to mix.
7. Place in freezer for 30 min.
8. Centrifuge (cold) on high for 30 min.
   a. Debris should be at the bottom of tube.
9. Label new 1.7 ml tube, fill w/ **600 µL 100% isopropanol (DNA grade)**
10. Pour supernatant into new tube (step 8) and leave debris behind.
11. Add **1 µL glycogen** to each tube (DNA carrier)
12. Mix samples by inverting 50 times
13. Place in freezer overnight

NEXT DAY

14. Centrifuge (cold) at 13,000-16,000 rpms for 30 min.

a. Look for pellet at bottom of tube and record size & appearance (white, clear, reddish, smear).

15. Pour off supernatant and drain tube on clean, absorbent paper.
16. Add **600 µL 70% EtOH (DNA grade)**
17. Centrifuge (cold) at 13,000-16,000 rpms for 2 min.
18. Carefully pour off EtOH
19. Air dry tubes to remove all excess EtOH. Sometimes we leave overnight to dry.

When Dried

20. Add **20 - 50 µL TE** to rehydrate pellet, flicking tube to mix. Can let rehydrate overnight before use, or warm (35 C˚) in a heating block to facilitate re-suspension. 1-2µl for PCR should be good.

Appendix 2. "Digestion Mix"; reagents used for feather digestion.

Reagents	Each tube µL
1X TNE	600
1M Tris-HCl, pH 8.0	60
Protease K (20mg/ml)	45
25% w/v SDS	10
1M DTT (newly mixed)	80
TOTAL	795

1X TNE	
100mM NaCl	5.844 g
50 mM Tris	6.055 g
25 mM EDTA	9.306 g
pH 7.5 w/ HCL	
Bring to 1 L volume w/ dd H2O	
Autoclave	

Appendix 3. "Protein precipitation" reagent.

Reagents	Each tube µL
7.5 M Ammonium Acetate	233

CHAPTER III

PHYLOGEOGRAPHY, GENE FLOW AND POPULATION HISTORY OF NORTHERN GOSHAWKS IN NORTH AMERICA

ABSTRACT

The Northern Goshawk is Holarctic in distribution. Its Nearctic breeding range encompasses much of North America's montane and forested regions from Newfoundland to Alaska, and south into Northern Mexico. Because of their wide distribution, there is much interest in understanding the genetic relationships among goshawk populations. Three subspecies have been suggested, two of which are well recognized (*Accipiter gentilis atricapilus* and *A. g. laingi)*. A third subspecies, the Apache goshawk (*A. g. apache*) of southeast Arizona and Mexico, was proposed based on its larger size and darker color, but too few data exist to support a subspecific status. The focus of this study was to elucidate the genetic relationships among 21 goshawk populations, from across much of the species' North American range. Domain-I of the mitochondrial control region (450bp) was sequenced and used to determine: (1) genetic diversity, population genetic structure, and gene flow among populations; (2) concordance of phylogeographic patterns with presumptions of allopatry in four hypothesized Late-Pleistocene forest refugia; (3) timing of population expansion from historical forest refugia; and (4) genetic relationships among *A. g. atricapilus* and putative *A. g. apache* populations.

I sampled DNA from 259 North American and six German goshawks using blood, plucked and molted feathers, and footpad tissue, and included an additional 56 mtDNA sequences from another study of goshawks from Utah. Among all North American populations, I identified 26 haplotypes, which had low nucleotide (0.003), but high haplotype, diversity (0.78). A minimum spanning tree of haplotypes resulted in a star-like topology, with shallow divergence among haplotypes. Forty-one percent of samples had a main, geographically unstructured haplotype and 42% of the remaining samples had one of four geographically structured haplotypes. Bayesian clustering of samples also supported four genetic clusters. Haplotype diversity was significantly structured among populations ($H_{ST} = 0.14$, $P < 0.001$), and analysis of molecular variance indicated 18% of the variation in haplotype diversity was explained by regional population structure. Significant genetic structure existed between (1) Arizona Sky Island populations and all others ($\Phi_{ST} = 0.283\text{-}0.472$), and (2) the Jemez Mountain population and all others ($\Phi_{ST} = 0.111\text{-}0.330$). Female mediated gene flow among most adjacent regional populations was high and asymmetrical, tending from periphery populations (California, Southeast Arizona and the Appalachian Mountains) to central-continental populations (Intermountain west and Great Lakes), but low amounts of reciprocal gene flow was supported in some cases. The geographic structure of haplotype diversity supported a priori hypotheses of population allopatry in three Late-Pleistocene forest refugia (Pacific Coastal, Southwestern, and Eastern forests), but did not support a hypothesized fourth, Rocky Mountain population. Lastly, while percent pairwise sequence divergence within North American populations was low (0-1.33 %), it ranged

7.83 % to 14.63 % among North American and German samples, which translated into an intercontinental divergence date of roughly 529,391 to 988,783 YBP.

I concluded that gene flow in goshawks is facilitated by directional, stepping-stone dispersal achieved by juvenile goshawks, and that high mate and breeding site fidelity of adults contributed to significant regional-scaled genetic differentiation. Historically, goshawk populations were isolated into three forest refugia, and populations in the East expanded rapidly with Early Holocene forest expansion into the north. Significant genetic differentiation existed between populations in the Arizona Sky Islands and all other populations, and these differences may reflect genetic drift in these small and isolated populations.

INTRODUCTION

At the height of the last glacial period (Wisconsin, Pleistocene), two large ice sheets covered most of the northern latitudes of North America (Pielou 1991). The climate was cool and dry, causing shifts of forested landscapes relative to their current distribution. The ranges of forest-dependent fauna would have also shifted with their preferred habitats, and these long term habitat changes are expected to have left their genetic marks on the today's populations. It is of much interest to understand the historical distributions of species and how such history shaped the diversity evident today. Historical faunal biogeography has been primarily accomplished by documenting the distribution of fossils in asphalt fields, caves and packrat middens (Bentancourt et al. 1990). While these methods provide a wealth of information, conclusions are limited by the paucity of available data for birds, which have hollow bones with a poor capacity for preservation.

Recently, the history of population dynamics and species biogeography has been inferred by determining geographic patterns of genetic lineages (Avise 2000). Of particular interest is differentiating demographic processes, such as dispersal and range expansion, from vicariant events that result in population divergence.

Comparative phylogeography of co-distributed species from defined ecosystems are especially informative (Arbogast and Kenagy 2001). For instance, Carstens *et al.* (2005) used genetic data from four animal and two plant species to test three hypotheses concerning historical connectivity among mesic forests of the Pacific Northwest and Rocky Mountains. They found deep divergence among three coastal and inland amphibians, a pattern that supported their "ancient vicariance" hypothesis. Patterns of genetic divergence in the water vole, willow and white-bark pine supported their hypothesis of a northern post-glacial dispersal route as opposed to a southern route. Such concordance among species suggests similar historical processes shaped the genetic structure of populations (Carstens et al. 2005). The study of phylogeography in highly vagile species, like birds, must be conducted at the scale at which populations function (Avise 2004). Only a few studies have evaluated the phylogeography of widely distributed species at their continental or inter-continental range (Mila et al. 2000, Waltari and Cook 2005, Omland et al. 2006). Such studies are critically important when considering wide-ranging and vagile species, as they provide an appropriate phylogeographic picture from which to draw inferences.

The Northern Goshawk

Goshawks (*Accipiter gentilis*) are Holarctic in distribution and the Northern Goshawk (*A. g. atricapilus)* is thought to be a "recent" colonizer from the European subspecies (*A. g. gentilis*) (Wattel 1973). Their distribution in North America includes most forested regions, from Alaska to Mexico, the Pacific Coast to the Northern Atlantic and Appalachian Mountains. Fossilized remains place goshawks in California during the Pleistocene ($\leq$ 38,000YA) in La Brea (Compton 1931, Miller and DeMay 1942) and Carpinteria (Miller 1931). Two northern goshawk subspecies are recognized by the AOU (American Ornithologists' Union 1983): the continental type, *A. g. atricapilus,* ranges the forested mountains of North America and Canada, while the Queen Charlotte goshawk, *A .g. laingi*, is restricted to the coastal islands of British Columbia and Southeast Alaska (Tavener 1940). In a study of museum specimens Whaley and White (1994) identified considerable variation in body size among the *A. g. atricapilus* types. They reported a size cline from the largest in Alaska, to smaller birds in both Eastern and Pacific Northwestern individuals. A third sub-species, the Apache goshawk (*A. g. apache*), has been suggested (Van Rossem 1938, Friedmann 1950, Whaley and White 1994) to inhabit the Sky Islands of Southeast Arizona into Northwest Mexico. It is described as darker in plumage, almost black dorsally, larger in size and long winged (Van Rossem 1938). Van Rossem's (1938) identification was based on only three birds and has been disregarded by most. Subsequently, Whaley and White (1994) described six male and 16 female Apache types as being larger than all other *A. g. atricapilus* (Alaskan, Eastern and Western) and much larger than *A. g. laingi* types. Thus, recognition of the Apache

subspecies is controversial and still debated. The USFWS considers its status of *A. g. apache* unresolved (USFWS 1998).

Goshawks are top level forest predators that prey on a variety of mammal and bird species, most of which are medium-sized forest and woodland inhabitants (grouse, woodpeckers, corvids, pigeons, lagomorphs and various sciuids). Juvenile goshawks exhibit low natal-site fidelity and are capable of long-distance natal dispersal (Wiens et al. 2006b) however, mate and breeding site fidelity is high in adult breeders (Detrich and Woodbridge 1997, Reynolds and Joy 2006). A genetic study of goshawks in Utah found no population subdivision, which was attributed to frequent immigration from outside the study area (Sonsthagen et al., 2004). Similarly, a population of goshawks on the Kaibab Plateau in northern Arizona showed no inbreeding despite having an effective population size of only N_e = 37 individuals (range = 10-86 over 13-years, Bayard de Volo et al., 2005). Such small populations could be inbred unless they are open to immigration and gene flow.

The degree to which North American goshawk populations were, and are, demographically and genetically connected is yet unknown, as no studies have assessed range-wide patterns of dispersal or gene flow. The goshawk's preference for forest habitats and its large range make it model species for testing biogeographical hypotheses about the distributions of forest refugia since the end of the last glacial period (Wisconsin Glaciation 70-11,000 before present (BP). Because expansion of goshawks would have been limited not only by forest habitat, but also by the co-distribution of their prey, studies of goshawks provide a framework for comparative phylogeography of other forest species such as corvids, woodpeckers, sciuids, lagomorphs and galliform species.

Based on the distribution of forests during the Late Pleistocene, I identified four possible forest refugia in which goshawk populations might have been isolated; (1) Eastern forest refugia; (2) Pacific Coast forest refugia; (3) Southwestern/Northern Mexico forest refugia; (4) Rocky Mountain forest refugia. My goals were to determine:

(1) Mitochondrial DNA diversity, population genetic structure and gene flow among contemporary North American populations;

(2) Geographic distribution of genetic lineages and their agreement with hypothesized Late Pleistocene forest refugia;

(3) Historical demography of goshawk populations, specifically testing the hypothesis of recent population expansion from forest refugia;

(4) Genetic divergence, if any, among *A. g. atricapilus* and putative *A. g. apache* populations;

Forest History and Refugia

At the height (18,000 BP) of the last glacial period, the Wisconsin (70-10,000 BP), two large ice sheets covered the northern latitudes of North America (Pielou 1991). The Laurentide Ice Sheet extended across Canada and met with the Cordilleran Ice Sheet, which covered British Columbia. The Pleistocene climate was cooler and drier than today, especially near the ice sheets, but a warming trend following the height of the Wisconsin period resulted in the melting of these large glaciers at the end of the Pleistocene era. The Holocene (10,000 BP to present) brought continued warming, with the period 7,000-5,000 BP, being warmer than today. The Mid-Holocene warm period

(sometimes referred to as Hypsithermal period) was then followed by a cooling trend, which has persisted into contemporary times (Pielou 1991).

These climatic shifts greatly influenced vegetation patterns across North America, and changes in distribution of forested landscapes are expected to influence the distribution of goshawks and their forest prey. Thus, an understanding of paleoclimate and paleovegetation is necessary for developing hypotheses of the effects of historical forest refugia on goshawk populations. My objective is not to evaluate which kinds of forests (deciduous or coniferous, open or closed understory) goshawks historically used. Goshawks are generalists with regard to tree species used for nesting and foraging (Squires and Reynolds 1997). My objective was to simply survey paleofloristic literature in order to map the distribution of historical conifer (arboreal and woodland) and arboreal hardwood forests. I made the assumption that these forests comprised suitable habitat for historical goshawk populations and their prey.

Eastern forests have changed significantly since the Late Pleistocene in tree species composition (Jackson et al. 1997), but remained more or less contiguous forested landscapes until well after European settlement (500 BP). As Late- Pleistocene (18-10,000) climates warmed, Eastern forests expanded in range northward and westward, following and skirting the melting Laurentide Ice Sheet , but did not make contact with Pacific Northwestern forests until relatively recently (<3,000 BP) (Hebda 1982). Forest expansion northward and westward was rapid (100-300m/yr) and substantially enlarged the forested landscape of Canada, and Mid-western and Eastern U.S regions.

Forests in the west, where mountain ranges and intermountain valleys are taller and deeper than in the East, have had a more mixed history, with forested regions

expanding and contracting with climatic fluctuations (Barnosky et al. 1987, Van Devender et al. 1987). Pleistocene climate was cooler and drier than today until the early Holocene (10,000 BP) when it began to warm rapidly. The height of climatic warming was about 7,000 BP, at which time a cooling trend brought increased moisture. These climatic fluctuations influenced western forests by shifting floristic elevational zones, altering tree and shrub species composition, and forest structure.

In the Pacific Northwest, Late Pleistocene mesic forests dominated the regions around the Coast and Cascade ranges from Washington to California (Barnosky et al. 1987, Daniels et al. 2005), but the inland Columbia Basin was a xeric landscape characterized by tundra like vegetation (20-10,000 BP) or sage-brush steppe (10-7000 BP) (Delcourt and Delcourt 1993). This large expanse of periglacial steppe spanned from eastern Washington to Wyoming, separating the mesic forests of the Coast and Cascade ranges from those in the Northern Rocky Mountains. Pacific Coast forests changed from closed-canopied mesic (20-10,000 BP) to open xeric parkland of arboreal tree species (10-7000 BP) (Delcourt and Delcourt 1993).

Where the Coast and Cascade ranges meet the Sierra Nevada, and south into the Sierra Nevada, Late Pleistocene climate (>13,000 BP) was cold and dry, which supported open subalpine parkland vegetation (Cole 1983, Daniels et al. 2005). Subsequent climate warming brought Early Holocene (10,000-7,000 BP) forests comprised of open conifer stands fragmented by montane chaparral (Davis et al. 1985, Anderson 1990, Daniels et al. 2005) . Following this period the climate cooled, and moisture increased allowing for the establishment of more mesic forests (pine, fir, hemlock, Douglas fir) characteristic of today (Anderson 1990, Davis 1999, Daniels et al. 2005) .

Pleistocene vegetation reconstructions for the Southern Great Basin and Mohave regions indicated a cooler and wetter environment than today, which allowed for pinyon-juniper-oak woodlands at lower elevations and spruce, fir and limber pine at higher elevations (Thompson 1990). As the climate warmed with the Early Holocene (10,000 BP), aspen increased and ponderosa pine swept through the region, advancing at a rate of 100-200m/yr. Pleistocene climate of the Chihuahuan and Sonoran regions was also cooler and wetter climate until the Early Holocene (9,000 BP), after which conditions warmed but remained wetter than today because of summer monsoon development (Delcourt and Delcourt 1993, Metcalfe et al. 2000). Pinyon-juniper woodlands covered much of what is today desert sage-scrub, and mesic conifer forests were present at elevations 500-1000 m lower than current distributions (Anderson 1993, Metcalfe et al. 1997, Weng and Jackson 1999, Jaramillo-Correa et al. 2006). Modern desert vegetation did not appear until ~4000 BP. Thus, forests of the Southwest and Great Basin were more widespread than today and likely provided important Pleistocene forest refugia, which extended well into Mexico. Today, ponderosa pine is restricted to above 2200m (7200 ft) and is thus fragmented across higher elevation peaks and plateaus.

Intermountain west (Great Basin and Colorado Plateau) vegetation was quite variable, as distributions depended on the variable topography characteristic of the region and the presence of many Pleistocene pluvial lakes, which were scattered across the landscape (e.g., Lake Bonneville and Pyramid Lake). Late Wisconsin vegetation in the northern latitudes of the Intermountain West was impoverished, consisting mainly of bristlecone pine (*P. longaeva*) woodlands down to 1600 m and spruce down to 1900 m (Wells 1983, Thompson 1990). In the lower latitudes (south of Lake Bonneville)

bristlecone pine woodlands were replaced by limber pine, Douglas-fir, and montane cedar (Wells 1983). Thus, significant differences with latitude and altitude existed in species composition of woodlands and subalpine forests. As temperatures warmed in the Early Holocene, woodlands and subalpine forests started to retreat to higher elevations, but moisture increased, facilitating the immigration of mesophylic species like quaking aspen, and Rocky Mountain maple (Thompson 1990). By 7000-6000 BP, modern woodlands (pinyon-juniper) appeared, and white fir and ponderosa pine immigrated from the south into the eastern Great Basin. Thus, during the Mid-Holocene the region became very fragmented with respect to forested regions, isolating them into an archipelago of island habitats.

Late Pleistocene climates in the Rocky Mountains were cold and dry, which supported many glaciers in higher elevations. The Pinedale Glaciers were scattered across the Rocky Mountain Range and were large in some areas, covering 5,000 km^2 of the San Juan Mountains (Carrara et al. 1984) and extended across the Yellowstone Plateau of Wyoming. Glacial retreat began as early as ~16,000 BP across Colorado and as the climate warmed in the early Holocene (~10,000), alpine tundra was replaced by such subalpine conifers as spruce and pine, including ponderosa pine (Anderson et al. 2008). By mid-Holocene Mixed Conifer forests were present at the higher elevations and they persisted there throughout the Holocene. Lower elevations in the Southern Rocky Mountains, experienced mid-Holocene drought, which caused a shift to steppe habitats in intermontane valleys (Anderson et al. 2008).

To summarize the paleovegetation reconstructions: (1) Long term and persistent forests were present along the Pacific Coast Ranges, as far south as Southern California;

(2) The southern Cascade and Sierra Nevada regions were xeric and without forests until a gradual Late Holocene establishment of forested landscapes (6000 BP); (3) Southern Sierra Nevada was also xeric, but Early Holocene forests were present by about 10,000 BP, including the Giant Sequoia at lower elevations; (4) Southwest Deserts, including Northern Mexico, were characterized by higher elevation mesic forests on peaks and plateaus, surrounded by large expanses of pinyon-juniper-oak woodlands, which persisted until ~3000 BP resulting from Mid-Holocene increased monsoonal moisture; (5) The Great Basin and Colorado Plateau was also xeric and cold during the Late Pleistocene, with subalpine vegetation at lower elevations and many large lakes across the landscape. Early Holocene increases in warmth and moisture allowed migration of mesophylic conifers and aspen, which were followed by mid-Holocene retreat of woodlands and subalpine forests to higher elevations; (6) Southern, and Northern, Rocky Mountain regions were cold and dry during the Pleistocene, where higher elevations were glaciated and covered by sparse tundra-like vegetation. Late Pleistocene-Early Holocene climate brought warming and glacial melt, followed by rapid development of mixed conifer forests that persisted throughout the Holocene.

METHODS

Sampling - Samples ($N = 470$) consisting of blood, and plucked and molted feathers, or footpad tissue were contributed from several research and monitoring projects representing much of the goshawk's range in western North America, and part of its range in the East (Table 1). Most sampling was from North America and represented mainly the *Accipiter gentilis atricapilus* subspecies; however the seven Southeast

Alaskan samples were from the *A. g. laingi* subspecies and the 25 Arizona Sky Island samples were from the range of the putative *A. g. apache* subspecies. The seven samples from Germany represented the *A. g. gentilis* subspecies.

Blood (stored in STE buffer or ethanol) and plucked feathers (stored in paper envelopes) were from banded individuals, thus the identity of hawks was known. All molted feathers were collected from active nest sites where adults were observed nesting and only feathers with collection data (year of collection and territory name/number) were used. The single footpad tissue sample was provided by the Museo de las Aves de Mexico, from a specimen labeled as "*Accipiter gentilis gentilis*".

DNA isolation - Genomic DNA was isolated from blood using silica-filter methods (QIAamp, Qiagen, Inc.) following the manufacturer's protocol. DNA was isolated from feathers using a long proteinase-K/DTT digestion, followed by an ammonium acetate separation of proteins and cold ethanol precipitation of nucleic acids (Bayard de Volo et al. In Review, 2008). To guard against possible contamination of feather samples by other high concentrate DNA samples in our laboratory (i.e., blood, PCR amplification products) I took the following precautions: (1) for each sample new gloves and newly sterilized instruments were used, which involved soaking in 50% bleach, rinsing in ddH_2O (Glenn 1996), and then exposure to UV light for 20min, (2) all procedures carried out in a preparation station (box) equipped with two germicidal UV light bulbs for sterilization between each set of DNA extractions; (3) dedicated pipettes and reagents for feather-DNA isolation, (4) all PCR amplification was conducted in a different room

physically separated from the DNA isolation and PCR-prep room; (5) all blood-DNA isolation was carried out in a separate part of the lab, with separate pipettes and supplies.

Genetic markers and PCR procedures - PCR was used to amplify a 514 base pair fragment of the mitochondrial (mtDNA) control region (domain-1). I used primers previously developed for North American goshawks (Sonsthagen et al. 2004); L16064 (5'-TTGGTCTTGTAAACCAAAGA-3'), H15426 (5'- ACCAAAGAGCAAGTTGTGC-3'). Reaction conditions included an initial denature at 94ºC for 3 min. followed by 40 cycles of denature at 94ºC for 40 sec., annealing at 54ºC for 40 sec and chain extension at 72ºC for 40 sec., followed by a final chain extension for 7 min at 72ºC. PCR reactions were performed on Bio-Rad Laboratories MyCycler thermocyclers, in 96-well plates.

Following PCR, samples were electrophoresed on 1.5% agarose gels to determine amplification success and concentration of PCR products. Samples were then purified for direct sequencing using either Qiagen's PCR min-prep purification kit (higher concentrate PCR products) or ExoSap-IT (USB Corp., Cleveland, OH) enzyme purification (lower concentrate PCR products). I found ExoSap-IT maintained PCR product concentrations, and therefore improved sequencing success of weak reactions. Sequencing reactions were conducted on an ABI 3130 Genetic Analyzer. Sequencing reactions for molted feathers involved a modified protocol that included 99 amplification cycles, which improved the strength of sequencing reactions for low concentrate PCR reactions.

Sequences were aligned by eye using Bio-Edit v. 7.07 (Hall 1999). To begin the alignment, I obtained sequences from GenBank (AY699828 - AY699835, Sonsthagen et

al 2004) and aligned all my sequences against these eight haplotypes. Therefore, haplotypes from this study are directly comparable with Sonsthagen et al (2004). All sequence chromatograms were printed and checked for ambiguous (weak or double peaks) and all polymorphisms were checked and accepted only if peaks were unambiguous. Samples having questionable sequences were re-amplified and re-sequenced. In some cases final sequence haplotypes were based on consensus of light and heavy strands, however in other cases they were based on consensus of two light strands. All new haplotypes for each population were verified with a second PCR and sequencing reaction. In all, 51% of samples were sequenced at least twice.

DATA ANALYSIS

I used several analyses to test our hypotheses of population history and phylogeography. For some analyses, samples were grouped by "sampled population" (sampling sites), while for other analyses, samples were grouped into larger "regional groups" (Table 1).

Genetic variation and diversity – Number of polymorphic sites, haplotypes, and rare haplotypes were determined and nucleotide diversity (π) and haplotype diversity (h) were calculated for each sampled population. I also determined nucleotide base frequencies, and transition and transversion rates for the entire North American dataset.

Population genetic structure – Corrected pairwise genetic distances between haplotypes were estimated using PAUP v 4.0(b10) (Swofford 1999), based on the best fit

model of molecular evolution as determined by hierarchical likelihood ratio tests in MODELTEST v 3.7 (Posada and Crandall 1998). The corrected distance matrix was used to assess population subdivision across North American sampling sites by estimating pairwise Φ_{st} in ARLEQUIN v 3.1.1. Global Φ_{st} for North America was estimated in DNAsp (Rozas and Rozas 1999), which uses equation (2) of Hudson et al. (1992) for non-recombining sequence data. Significance for global Φ_{st} was determined by permutation using 1000 replicates. I also determined the scale at which goshawk populations fell into broader regional groups using an analysis of molecular variance (AMOVA) (Excoffier et al. 1992), which partitions genetic variation into nested groups (within populations, among populations within regional groups and among regional groups). I grouped sampled populations based on; (1) geographical proximity, (2) possible barriers to gene flow (i.e., the Continental Divide, desert regions), (3) geographic distribution of haplotypes (Table 1). Populations normally considered single units (Northern Utah, Arizona Sky Islands) were partitioned into sub-population for the purposes of estimating among sub-population (V*b*) molecular variance. Vancouver Island, Alberta and Jemez Mts., NM could not be partitioned, because population samples from these areas comprised too few goshawks.

Genetic structure was also assessed using STRUCTURE v 2.2 to implement a Markov chain Monte Carlo (MCMC) algorithm to cluster individuals into genetic populations (Pritchard et al. 2000, Falush et al. 2003). This method assumes a model of *K* populations (clusters), and then estimates the likelihood the data fit the model. I modeled both North American and German samples together for *K*=1-23 clusters, under the assumption of population admixture. I used a 10,000 step burn-in and a MCMC of

100,000 steps for five iterations. Sequences were reduced to 32 polymorphic sites, each of which represented a locus with potentially four alleles. I assumed allele frequencies within populations were correlated. Because loci were linked (all polymorphic sites are matrilineally inherited as a single haplotype), the degree of uncertainty associated with ancestry estimates were possibly underestimated, and may be biased or imprecise; as might be the estimation of K (Pritchard et al. 2000). Therefore, I implemented this analysis with caution, and primarily looked for agreement with other analyses.

Historical demography – To determine whether goshawk populations were historically stable, or if they had undergone periods of decline followed by I used DNAsp v 4.20.2 (Rozas and Rozas 1999) and ARLEQUIN v 3.1.1 to conduct several analyses. First, nucleotide diversity (π) and haplotype diversity (h) was determined for each sampled population. Low nucleotide diversity coupled with high haplotype diversity suggests rapid and recent population growth, while high nucleotide and low haplotype diversity indicate long-term population stability. Second, I calculated the expansion coefficient (S/d), the ratio of the number of variable sequence positions (S) to the mean number of pairwise nucleotide differences (d) between haplotypes within a population (Peck and Congdon 2004). Large values indicate recent population expansion, while small values indicate long-term population stability (von Haeseler et al. 1996). I also calculated several neutrality indices that together provide information on population stability/expansion by testing whether the pattern of sequence polymorphism fits a neutral modal of evolution, as opposed to non-neutral forces like selection. Fu and Li's (1993) F* and D* statistics evaluate the genealogical pattern of mutations among

sequences, by comparing the frequency of internal to external alleles. An increased frequency, when compared to a neutral modal, of old (internal) mutations indicates background selection. Fu's F_S indicates population expansion when an excess of external low frequency mutations (alleles) is present (Fu 1997). Thus, non-significant F* and D* coupled with significant and large F_S indicate recent population expansion.

Mismatch distributions provide information on the frequency of pairwise nucleotide differences among individuals and can elucidate population demography (Rogers and Harpending 1992). Distributions of pairwise differences in populations that went through a bottleneck and subsequently expanded generally follow a unimodal (usually Poisson) distribution, while long-standing populations at equilibrium exhibit more pairwise differences, and thus show multi-modal distributions. I used DNAsp v 4.20.2 (Rozas and Rozas 1999) to compare the observed distribution of pairwise nucleotide differences to one based on a model of population stability, and then used the resulting estimate of τ to model the observed distribution under the assumption of population expansion (θ initial = 0; θ final =1000). I also used ARLEQUIN to estimate the raggedness index (*rg*), which indicates the shape of mismatch distributions, and assessed statistical significance from the distribution of the statistic determined by simulations.

Estimating divergence times – I used a generalized non-linear least-square approach to calculate mismatch distributions and estimate τ (a parameter of demographic expansion) under the assumption of population expansion in Arlequin v 3.1.1. Parametric bootstrapping was used to estimate corresponding 95% confidence intervals for $\hat{\tau}$ (Schneider and Excoffier 1999). Time since expansion (number of generations between

initial and current population) was determined using the relationship $\tau = 2u$, where $u = 2(\mu k)$, and μ = mutation rate per million years and k = sequence length (Rogers 1995). Time, in thousands of years, is determined by standardizing for a per-year mutation rate and accounting for generation time. I chose a mutation rate of 14.8% per million years for domain-I of the control region as this was previously determined for Dunlin (*Calidris alpina*) (Wenink et al. 1996), and used for Greenfinch (*Carduelis chloris*) (Merilä 1997) and sharp-shinned hawks (*A. striatus*) (Hull and Girman 2005). I used a generation time of three years based on observational data from goshawks on the Kaibab Plateau, Arizona (R. Reynolds, pers. comm), but goshawks are capable of breeding their first year, and age of first reproduction varies across individuals, and across space and time (Squires and Reynolds 1997).

Gene flow – The number of female migrants exchanged between any two populations per generation ($N_f m$) was calculated two ways. First, I used ARLEQUIN to estimate the number of female migrants ($N_f m$) among populations using Slatkin's (1995) method. This method estimates the number of migrants exchanged, but says nothing about directionality. I used MIGRATE v 2.4.2 (Beerli and Felsenstein 1999, Beerli 2008) to estimate the directional $N_f m$ among larger regional populations. To limit program running time I combined some regional groups (Cascade-Sierra and Vancouver Island into a "CA-VAIS" group; Northern Rockies, Northern Utah, Colorado Rockies and Rocky Islands into a "Rocky Mountain West" group) into larger groups based on similarity of haplotype frequencies and lack of divergence. Full models, θ ($N_f \mu$, a composite measure of effective population size and mutation rate), and all pairwise

migration parameters, were estimated individually from the data and were compared to a restricted island model for which θ was averaged and pairwise migration parameters were symmetrical between populations.

MIGRATE was run using maximum likelihood search parameters; ten short chains (2,000 trees sampled out of 400,000 recorded), five long chains (10,000 trees sampled out 2,000,000 recorded), and five adaptively heated chains (start temperatures: 1, 1.5, 3, 6, and 12; swapping interval = 1). Full models were run three times to ensure the convergence of parameter estimates. Restricted models were run once. The alternative model was evaluated for goodness-of-fit given the data using a log-likelihood ratio test. The resulting statistic from the log-likelihood ratio test is equivalent to a Chi-square distribution with the degrees of freedom equal to the difference in the number of parameters estimated in the two models (Beerli and Felsenstein 2001).

Phylogenetics - Because traditional phylogenetic methods (parsimony, neighbor-joining and maximum likelihood) make assumptions that are generally invalid for population level studies, I used TCS (Clement et al. 2000) to determine the evolutionary relationship among control-region haplotypes. The program estimates an absolute distance matrix from all pairwise comparisons of haplotypes, which were connected using the maximum number of mutations justified by parsimony (i.e., haplotypes are linked to each other by the minimum possible mutations). The result is displayed as a minimum spanning network or tree. All North American samples were included to insure that haplotype frequencies were properly calculated and haplotype age was determined.

I evaluated the relationship within and between North American and German sub-species using PAUP v 4.0. I calculated pairwise distances from a neighbor-joining tree of all North American and German samples, after correcting for the model of sequence evolution as determined by MODELTEST (Posada and Crandall 1998). Distance settings included an HKY+G+I correction for sequence evolution, proportion of invariable sites=0.80, and gamma=0.60. Pairwise differences were converted to percent divergence and plotted as a frequency histogram.

RESULTS

Genetic variation and diversity - Of 391 samples tested, 321 produced unambiguous sequences and were used for assessing genetic diversity and structure. North American goshawks were variable for Domain-1 of the mitochondrial control region, with 28 (3.78%) polymorphic sites over the 450 base pairs analyzed, 23 of which were parsimony informative. From these 28 polymorphic sites, 26 North American haplotypes and two German haplotypes were identified (Table 2). Most haplotypes differed by 1-3 mutational steps within continental clades (Figure 1) and 21-24 mutational steps among continental clades. I found no insertions/deletions, and of the 17 polymorphic sites within North America three were identified by a transversion and 14 by a transition. Nucleotide frequencies among all North American goshawks were: A=30.86%, C=28.45%, G=13.36%, T=27.33%. Haplotype diversity across all North American samples was high (78%), while nucleotide diversity was low (0.3%) (Table 3). Population specific diversity indices are presented in Table 3.

Haplotype-B was most abundant (41%) and found in all sampled populations (Table 4, Figure 1). Haplotype-A was primarily a Cascade-Sierra type, although it was also found in British Columbia and the Rocky Mountains. Haplotype-D was most abundant in Cascade-Sierra and the Rocky Mountains, although it was also found in the Eastern populations. Haplotype-E was dominant (68%) in the Arizona Sky Islands, but also occurred in the Colorado Plateau and singly (one hawk) in the Central Appalachian Mountains. Haplotype-G was dominant (58%) in the Jemez Mountains of New Mexico, but also occurred in the Colorado Rockies, Colorado Plateau and as the single hawk from Lake Tahoe, California (Sierra Nevada). Rocky Mountain and Eastern populations were dominated Haplotype-B, with the addition of many low frequency haplotypes. This was contrasted by the Colorado Plateau, in which seven nearly equally frequent haplotypes were found, and the Cascade-Sierra which had only three equally frequent haplotypes (plus the singleton "G" from Lake Tahoe). Vancouver Island was very similar to the Cascade-Sierra populations, although our sample size for this location was small, limiting inference. The Southeast Alaska sample was also small, but four of the seven haplotypes were unique to that location.

Population genetic structure - Hierarchical likelihood ratio tests performed in MODELTEST indicated that control region sequences fit the HKY+I+G model of evolution (Kimura 1980). Overall, regional goshawk populations exhibited subdivision (Φ_{ST} measured as $H_{ST} = 0.14$, $P = 0.0000$, $\chi^2 = 726$, df = 264, DNAsp). Pairwise estimates of Φ_{ST} showed differences in haplotype diversity, especially among the Arizona Sky Island population and all other regions ($\Phi_{ST} = 0.283$-0.472), and the Jemez Mountain population

and all other regions (Φ_{ST} = 0.111-0.330) (Table 5). The Cascade-Sierra populations showed large differences from populations in the Rocky Mountains (Φ_{ST} = 0.057-0.311) as well as the Eastern regions (Φ_{ST} = 0.184-0.345), but not to the same degree with the Colorado Plateau populations (Φ_{ST} = 0.005-0.105) and Northern Utah (Φ_{ST} = 0.052) (Table 5).

Analysis of molecular variance (AMOVA) indicated most the variation in haplotype diversity was found within populations, but about 16% was attributed to among regional groups and very little (~4%) variation was found among populations within regional groups (indicating our selection of groups was well supported; Table 6).

The Bayesian clustering method implemented in STRUCTURE indicated three to seven mtDNA genetic clusters, with five clusters (Germany included) the most likely number (Figure 5). For *K*=5, a proportion of individuals from each population fell into each of the various clusters, except for the Arizona Sky Islands and Germany populations. A majority (59%) of samples from the Sierra Nevada populations fell into cluster-1, 77% of Arizona Sky Islands fell into cluster-2, 99% of German samples fell into cluster-4, and a proportion of all North American populations fell into cluster-3 and cluster-5 (Figure 6, Table 7). The four North American clusters correspond well with the hypothesis of haplotype evolution as determined in TCS 1.21 (Figure 7).

Historical demography - Some regional populations were further grouped into larger populations (Southwest = Colorado Plateau and Arizona Sky Islands; Rocky Mountain west = N. Rockies, Colorado Rockies, Northern Utah and Rocky Islands; Eastern = Great Lakes and Central Appalachians) to improve estimation of neutrality indices and dates of

expansion (Table 3). Nucleotide diversity (π) was low (0.00199 – 0.00419) and haplotype diversity (h) was high (0.38 – 0.90) for regional groups. The expansion coefficient (S/d) was about 5.00 for most regional groups, but > 10.00 for the Great Lakes, and for the Rocky Mountain West, East, and for all North American populations combined (Table 3). Significantly large values of Fu's F_S indicate an excess of recent mutations and thus recent population expansion, and non-significant Fu and Li's F* and D* also indicate population expansion. Fu's F_S was negative and significant ($P < 0.05$) for most regional populations and larger population groups. It was largest for all North American populations combined (-20.80, $P < 0.05$) and for the Central Appalachian population (-16.49, $P = 0.001$). It was not large for Colorado Rockies, Cascade-Sierra, Arizona Sky Islands and other smaller populations. Fu and Li's F* and D* were not significant for regional populations nor for larger population groups (Table 3).

The raggedness index was not significant for most populations, with the exception of the Central Appalachians, Colorado Plateau and all North American Populations combined (Table 3). Mismatch distributions for most populations and larger population groupings were unimodal and Poisson distributed, indicating historical bottlenecks and subsequent expansion (Figures 8-10). Jemez Mountains, however exhibited the greatest number of pairwise differences among the three haplotypes found in the population.

Estimating divergence times - Goshawks appear to have expanded following the end of the last glacial period (Wisconsin), where expansion for all North American populations combined dated to 15,618 YA (95% CI = 12,275-19,595 YA) (Table 3). The youngest population was the Great Lakes and Arizona Sky Islands, although the 95% CI for the

latter was wide. The oldest population was the Jemez Mountains of New Mexico, although the 95% CI for this estimate is also wide, making the estimate unreliable. More realistically, the Southwest (*N*=105, Colorado Plateau and Arizona Sky Islands combined) dates to 17,883 YA (95%CI = 11,723-26,351YA).

Gene flow - Number of female migrants ($N_f m$) per generation among populations, as determined by Slatkin's (1995) method, was lowest for the Arizona Sky Islands compared to all other populations ($N_f m$ = 0.56 – 1.27), and the Jemez Mountains and all other populations ($N_f m$ = 1.01 – 4.02) (Table 5). There were many cases of an infinite number of female migrants among populations, resulting from a lack of pairwise differentiation in haplotype diversity. This method assumes symmetrical gene flow, an assumption not supported by the data (see below), and does not provide confidence intervals on the estimates.

Likelihood ratio tests in MIGRATE indicated better support for the full model (all parameters allowed to vary independently) than the restricted model (symmetrical gene flow) (LnL (Full) = 92.429; LnL (Restricted) = -369.015; P<0.0001, df = 64) supporting asymmetrical gene flow among regional populations. Several other hypotheses were tested involving forcing migration to zero for some population pairs, but in all cases the full model was better supported.

Estimate of θ ($N_f \mu$ - effective number of females and mutation rate) under the restricted model was 0.0099. Estimates of θ under the full model were smallest in Jemez Mountains (θ = 0.005, 95% CI = 0.003-0.008) and largest in SE Alaska-Coastal BC

(θ = 0.022, 95% CI = 0.006-0.205) (Table 9-10). Estimates of $N_f m$ (number of female migrants) varied across populations but ranged from 0 to 76 (Tables 8-10).

Directional migration occurred from: (1) California-Vancouver Island to SE Alaska-Coastal BC (2) Colorado Plateau to SE Alaska-Coastal BC; (3) Arizona Sky Islands to Colorado Plateau; (4) California-Vancouver Island to Rocky Mountain West; (5) Rocky Mountain West to Great Lakes; (6) Appalachians to Great Lakes; (7) Rocky Mountain West to Appalachians. Reciprocal migration among these populations was not supported (Figure 11).

Phylogenetics - Evolutionary relationships among control region haplotypes indicated little sequence divergence within North American samples (Figure 1). Most haplotypes were separated by 1-3 mutational steps (Figure 1). The star-like topology is typical of expanding populations and ~90% of all samples had one of seven primary haplotypes.

Pairwise comparisons (N = 51,630) of sequence divergence among all North American (*A. g. atricapilus*) and German (*A. g. gentilis*) samples revealed little divergence within continental sub-species (North America = 0-1.33 %; German = 0.00 %), but much divergence between them (7.83 % to 14.63 %; Figure 12). This indicates a divergence date of roughly 529,391 to 988,783 BP assuming a mutation rate 14.8% per million years (Wenink et al. 1996, Merilä 1997, Hull and Girman 2005). Percent sequence divergence among goshawks in the Arizona Sky Island populations and all other North American populations ranged 0-1.04 % (Figure 13).

DISCUSSION

The Northern Goshawk is a widespread forest predator, having a breeding range that encompasses much of North America, yet little is known of connectivity among populations, both historical, and contemporary. My objective was to describe genetic relationships among goshawk populations in the continental U.S. and to identify populations with unique genetic characteristics, especially the putative Apache subspecies. I did not comprehensively sample the entire breeding range of goshawks in North America (did not include Interior Alaska, Yukon Territories, Interior British Columbia, New England and Mexico), and thus limit most of my discussion to populations sampled.

Mitochondrial DNA Diversity, Population Genetic Structure, and Gene Flow - Mitochondrial DNA diversity in North American goshawks was high (78% among all sampled goshawks), but divergence among haplotypes was shallow (low nucleotide diversity) indicating relatively young lineages. These results compare well with haplotype and nucleotide diversity found for other co-distributed forest birds; Sharp-shinned hawks (Hull and Girman 2005), Spotted Owls (Barrowclough et al. 1999), Blue Grouse (Barrowclough et al. 2004).

Despite being a highly mobile species, goshawks exhibited significant genetic structure ($H_{ST} = 0.14$, $P < 0.0001$) among all populations. Some populations exhibited exceptionally high levels of pairwise differentiation (e.g., Arizona Sky Islands, Jemez Mountains), but differences also occurred at the regional scale (Table 5). Based on

pairwise Φ_{ST}, I identified several larger regions in which goshawk populations could be distinguished with respect to mtDNA gene flow: (1) South-East Alaska/Coastal British Columbia; (2) Cascade-Sierras; (3) Colorado Plateau; (4) Arizona Sky Islands; (5) Jemez Mountains; (6) Rocky Mountains, Great Lakes and Central Appalachian populations. Similarity for mtDNA structure among the last group of populations was driven by the dominance in all three regions of Haplotype-B; however, each region had its own set of region-specific low frequency haplotypes (Figure 2, Table 4). To test the strength of this Rocky Mountain-Eastern regional group, I performed an AMOVA, which considered the Rocky Mountains, Great Lakes and Appalachian populations combined. The degree of variation explained by the regional groups dropped to from 16% (13 regional populations; Table 6) to 9%. Thus, although pairwise Φ_{ST} among these populations did not always indicate differences in genetic distance, there was significant support to consider these regions as independent population segments (13 groups explained more variation in haplotype diversity than did nine groups).

The amount of genetic structure was a little surprising, given that goshawks are capable of long-distance movements, which has the potential to homogenize genetic structure (Hedrick 1999). Band returns of first year goshawks from the Arizona Kaibab Plateau indicated dispersal distances 52-440 Km (Wiens et al. 2006b). Likewise, only 8 of 70 banded nestlings returned to nest in their natal Kaibab population, indicating either high juvenile mortality and/or low natal site recruitment (Wiens et al. 2006b). Low (1.7%) natal-site recruitment was also found for goshawks in California (Detrich and Woodbridge 1997). Telemetry studies further showed that juvenile goshawks dispersed from the Kaibab Plateau their first year, with a majority of individuals permanently

leaving the study area, which is separated from the closest suitable habitat by a minimum of 80 km (Wiens et al. 2006b).

Adult goshawks are mostly sedentary on breeding territories, exhibiting high mate and territory fidelity (Squires and Ruggiero 1995, Detrich and Woodbridge 1997, Reynolds and Joy 2006). Some adults make winter movements to lower elevation foraging grounds (Squires and Ruggiero 1995, Sonsthagen 2006), or as irruptions (irregular mass movements of individuals from a large geographic location) to latitudes with more hospitable winter climates (Doyal and Smith 1997, Squires and Reynolds 1997). But, these adults generally returned to their breeding territories the following Spring (Squires and Ruggiero 1995, Squires and Reynolds 1997). The band and telemetry studies suggest that gene flow is achieved by dispersing juveniles, but the strong genetic structure found among the study populations suggests breeding site fidelity limits continent-scale gene flow and panmixia (Avise 2004).

Another factor likely influencing genetic structure was directionality and a stepping-stone nature of gene flow. MIGRATE revealed that female-mediated gene flow was asymmetrical among populations (Tables 8-10, Figure 11). For example, gene flow from California and Vancouver Island indicates immigration into populations of Coastal Alaska, British Columbia, the Colorado Plateau and the Rocky Mountain West, while reciprocal gene flow was not supported. Emigrants from the Arizona Sky Islands moved to the populations of the Colorado Plateau, while support for reciprocal gene flow was lacking. In general, female gene flow tended from the West Coast and Southwest to the interior Intermountain West and then to the Great Lakes region. As well, gene flow from the Appalachians tended toward the Great Lakes region.

In many cases, the MIGRATE analysis did not indicate reciprocal gene flow, but the geographic pattern of haplotypes suggests it occurred. For instance, the presence of an individual in Lake Tahoe (most southern Sierra sampling site) with the G-haplotype suggests gene flow from the Colorado Plateau into the Sierra Nevada Mountain region. Likewise, the presence of several individuals with the B-haplotype in both the Sierra and the Cascade populations suggests gene flow from the North and/or East. In the Arizona Sky Islands, the presence of the B-lineage in two adults, both nesting (1994) in separate territories in the Pinaleno Mountains (most northward Sky Island) indicates gene flow from the north.

The significant amount of gene flow into the Colorado Plateau, and the character of equally frequent haplotypes from surrounding regions, suggested this region is important for admixture of genetic diversity from surrounding populations. Likewise, substantial gene flow from California to Northern Colorado populations indicates it too is an important region for the spread of genetic diversity into Eastern populations.

Phylogeographic Patterns and Refugial Populations - The Bayesian clustering method implemented in STRUCTURE suggested four North American genetic clusters, all of which overlapped with the primary haplogroups A, D, E and G (Figure 7), with Haplotype-B represented as a mix of all other clusters. The four clusters were geographically structured, while the mixed clustering for Haplotype-B resulted from the lineage's lack of geographic structure. The wide geographic distribution of Haplotype-B suggests an ancestral history; however, its dominance in the East and Rocky Mountains also suggests an Eastern origin. Its presence elsewhere could be from rapid post-glacial gene flow

with population expansion from Eastern forest refugia, or it may have been historically present, but rare, in the West.

The star-like pattern of the minimum-spanning network (Figures 1 and 7) coupled with the a majority (83%) of goshawks represented by one of five geographically structured haplogroups (genetic clades A, B, D, E, G; Figure 2) supports the hypothesis of recent population expansion from three forest refugia (Avise 2000). Most remaining haplotypes were represented by less than 1% of individuals and most of these were from Rocky Mountain and Eastern populations. The increased number of low frequency and singleton haplotypes in the East and Rocky Mountains supports the hypothesis of relatively more recent and substantial population growth in these regions as compared to the West (Figure 2).

Pacific coast refugia - Support for a Pacific Coast Refugia is found in the dominance of Haplotypes A and D in California populations and their presence in Vancouver Island. Haplotype-D also shared phylogenetic history with most of the Southeast Alaskan lineages (Haplotypes K, L and T; Figure 1). The presence of lineages descendent from Cascade-Sierra haplotypes in Southeast Alaska, suggests population expansion from Pacific Coast refugium like that seen among flora (Soltis et al. 1997), mammals (Byun et al. 1997, Wooding and Ward 1997, Demboski and Cook 2001), and birds (Burg et al. 2005, Burg et al. 2006). There are two possible explanations for this pattern. First, the Cascade-Sierra population was part of larger Pacific Coast refugia population and as coastal forests advanced northward with the retreat of the Cordilleran ice sheet (13,000 BP), goshawks colonized the newly formed forest habitats. Individuals carrying the

D-haplotype would have eventually colonized Southeast Alaska and the lineage subsequently radiated into the K, L and T lineages. The presence of Haplotype-D and its decedents in Coastal British Columbia and Southeast Alaska would support this hypothesis.

A second explanation is supported by the hypothesis that ice-free forest refugia were scattered along coastal British Columbia during the Cordilleran glacial period (Pielou 1991, Delcourt and Delcourt 1993). In this scenario, only a few migrants from south of the Ice Sheet made their way up ice free coastal islands, exposed at a time of warming, and colonized coastal British Columbia-Southeast Alaskan islands. With subsequent climatic cooling, some of these island refugia were again isolated, and the D-lineage radiated into the K, L and T lineages. This explanation assumes an earlier colonization event followed by longer isolation, which would have provided more time for lineage divergence. The lack of the K, L and T haplotypes elsewhere in the British Columbia and Alaska would provide support for this hypothesis. In both cases, gene flow northward from Pacific Coast refugia followed a "pioneer model of recolonization" (Hewitt 1996), and the founding event of a single lineage would have resulted in the mini star-like divergence pattern seen in the K, L and T haplotypes.

Haplotypes F and C (Figure 1) suggest historical gene flow into to the Colorado Plateau and Northern Rocky Mountain regions from the Pacific Coast region. Haplotype-A occurs in Coastal British Columbia and Vancouver Island, but outside of California, it is most abundant in the Colorado Plateau populations. Late Pleistocene conifer woodlands spanned across the southwest and southern Great Basin (Betancourt 1990, Thompson 1990), and likely provided a connection from the Colorado Plateau to the

southern Sierra Nevada range. Phylogenetic evidence supports such a connection, where a hypothesized range expansion of Mexican Spotted Owls (*Strix occidentalis lucida*) into the Sierra Nevada, was followed by allopatry, which resulted in the founding of California Spotted Owl populations (*S. o. occidentalis*) (Haig et al. 2004, Barrowclough et al. 2005). Haplotype-P (New Mexico) also shared ancestry within the California haplogroup, and could have diverged from either A or D, as both are present in the Colorado Plateau region.

Southwest refugia - The isolated nature of Haplotype-E in the Southwest suggests a Southwestern refugia origin. The near fixation of the haplotype in the Arizona Sky Islands suggests populations in this desert-mountain archipelago are isolated from nearby populations to the north. Both the Mogollon Rim and Kaibab Plateau are relatively close (~200 km. and ~560 km respectively), but I found little evidence for gene flow from these northern populations into the Sky Islands. As noted, goshawks have the potential for long-distance dispersal, but directionality of dispersal may be important for juvenile goshawks in this fragmented landscape. The Coconino Plateau lies south (~150 km) of the Kaibab Plateau, and may be a stopping point for juveniles dispersing south. Similar haplotype frequency distributions for goshawks on the Coconino and Colorado Plateaus would support the hypothesis of limited southward gene flow. Mexican Spotted Owls, which are distributed across the fragmented forests of Arizona, New Mexico and Southern Utah, showed a genetic break between the Sky Islands and Southern Utah, around the Coconino Plateau and Mogollon Rim (Barrowclough et al. 2006).

Another explanation for the dominance of the E-lineage in the Southwest is that the Arizona Sky Island populations are more demographically connected to goshawk populations in Mexico. The Sky Island Archipelago extends south into Northern Mexico, eventually connecting to the Northern Sierra Madre Occidental range. The Arizona and Mexican Archipelago likely provide a path that facilitates stepping stone gene flow from Northern Mexico into the northern Sky Islands. If such gene flow occurs, the Sky Island populations may represent the northern extent of a larger Mexican population.

I can only speculate about the genetic structure of goshawks in Mexico, but increased logging starting in the Late 1800's eliminated most old growth conifer stands, contributing to the loss of species like the Imperial Woodpecker (*Campephilus imperialis*) (BirdLife International, 2004). Second growth conifer stands now dominate the Sierra Madre Occidental range, but illegal logging continues to be a threat. Severe habitat alteration may have influenced Northern Goshawk populations by reducing suitable breeding sites, increasing competition with open-habitat species like Red-Tailed Hawks (La Sorte et al. 2004, Gatto et al. 2005) and reducing prey densities (e.g., the loss of the Imperial Woodpecker). If severe, such effects could have lead to a population bottleneck, and subsequent genetic drift to few haplotypes, one of which was the E-haplotype. As nothing is known of goshawk populations in Mexico, further work is critically needed to test this hypothesis, and to establish haplotype diversity and genetic structure for goshawks in the region.

The dominance of the G-lineage in New Mexico, suggests this lineage is also Southwestern in origin. This is further supported by its shared history with haplotype-H, which occurs in the Arizona Sky Islands and Colorado Plateau. Like the Arizona Sky

Islands, the Jemez population may be more demographically influenced by populations from Mexico, but since I did not sample Southern New Mexico, it is difficult to tell. Interestingly, the genetic break found among goshawks in New Mexico and Colorado was also seen in Blue Grouse (*Dendragapus obscurus*), where New Mexico Grouse exchanged fewer than one migrant per generation with populations in Northern Colorado (Barrowclough et al. 2004). New Mexico Blue Grouse were also separated into their own phylogenetic clade, indicating divergence from Rocky Mountain populations. A similar phylogeographic pattern was seen in red squirrels (*Tamiasciurus hudsonicus*), which also showed a genetic break between Northern New Mexico and Colorado (Arbogast et al. 2001)

Eastern refugia - The dominance in the East of the B-lineage and the many descendent low frequency haplotypes, suggests an Eastern refugia origin for these lineages. Haplotype-B was found in every population, even when sampling was small (N=3), and it united the northern regions of the Rocky Mountains, the Great Lakes and the Central Appalachian Mountains. These regions also shared the low frequency Haplotypes O and Q. Haplotype-M was also found mostly in the East, but also occurred in Vancouver Island. The presence of haplotype-M in Vancouver Island indicates either a wide-spread occurrence in Canadian populations, or long distance gene flow. Further sampling in Canada would address this question.

Since, I did not sample the northern extent of the goshawks' range, I can only speculate on phylogeographic patterns there. However, the similarities in haplotype structure among goshawks in the Rocky Mountains, the Great Lakes and the Appalachian

Mountains suggest southward gene flow from a large and contiguous Canadian population. Such a large population would have resulted from Early Holocene expansion from Eastern refugia populations as they recolonized newly formed forested habitats following the retreat of the Laurentide Ice Sheet (McLeod and MacDonald 1997, Williams et al. 2004). Pollen and fossil studies support rapid expansion of conifers into Canada from Eastern forest refugia, as do floristic phylogeographic studies (Godbout et al. 2005, Jaramillo-Correa et al. 2006). Mammals (Wooding and Ward 1997, Arbogast 1999, Arbogast et al. 2001) and birds (Kimura et al. 2002, Milá et al. 2007, Mila et al. 2007) also show similar patterns of recolonization across northern Boreal forests from Eastern forest refugia.

Another factor that likely influenced the pattern of haplotype diversity found in the East was recent recolonization (since 1950-1970's) from the north into the Great Lakes and Central Appalachians regions. Goshawks populations in the East were severely reduced as a consequence of heavy logging during the Late 1800's (Ward et al. 2006) and the loss of a probable important food source, the Passenger Pigeon (*Ectopistes migratorius*) (Warren 1890, Schorger 1955). The pattern of haplotype diversity may be reflective of a similar pattern in the north, where Haplotype-B is common and wide-spread.

Population Expansion and Historical Demography - In all cases, neutrality indices, and most mismatch distributions, supported the hypothesis of historical population growth (Table 3, Figures 8-10) and dating well within the Late Pleistocene era, 15,618 BP (12,275-19,595 BP; all samples combined; Table 3). These dates correspond to a time of

Late-Wisconsin climatic warming and forest expansion, especially in the East. In comparing regions, Eastern populations dated to ~10,000 BP, the Rocky Mountain West ~10,000 BP, the Southwest ~17,000 BP, and Cascade-Sierra ~ 21,000 BP (Table 3).

The relatively younger age of goshawk populations in the East may reflect, in part, contemporary expansion into these regions, from northern Canadian forests. In the Great Lakes region, the dominance of the B-lineage coupled with a high number of singleton haplotypes indicates the population is still experiencing rapid growth (Avise 2000). The Central Appalachian population has fewer singleton haplotypes and more ancestral lineages (D, E), but is still dominated by the B-lineage, and shares some low frequency Eastern haplotypes.

This very recent population growth is a result of recent recolonization. Historically, the East was dominated by contiguous old-growth hardwood forests until European settlement (~500 BP). At that time it was believed Eastern forests covered 90-95% of the landscape. By the mid-1800's 54% of New York, 67% of southern New England and 55% of northern New England were converted to pasture (Ward et al. 2006). A second wave of intensive forest clearing occurred in the Late 1800's when the needs of industrialization demanded forest products, which were delivered by the newly built railroads.

Concurrently, the Passenger Pigeon (*Ectopistes migratorius*), was facing its extinction, despite early 1800's abundance estimates around 3-5 billion individuals (Schorger 1955). The species had an Eastern presence during the Late Wisconsin period (Parmalee and Klippel 1982), although they probably occurred at lower numbers (Neumann 1985) Several historical accounts describe the goshawk's use of the

Passenger Pigeon. In Pennsylvania, B.H. Warren notes "Mr. M. M. Larabee, Emporium, Cameron county, says he always met with goshawks about the nesting places of wild pigeons, but when the pigeons left his locality these hawks also departed, and are now seen there only as rare winter visitors" (Warren 1890).

Intensive forest clearing and the loss of an important food source, the Passenger Pigeon, likely contributed to the decline of goshawk populations in the East, and it was not until the 1950's to 1970's that breeding populations were again documented (Spahn 1998). Without, knowledge of genetic structure for goshawk populations in New England and north of the Great Lake region, it is difficult to speculate whether populations there also show a signature of recent expansion. Boreal forested habitats were established 10,000 BP, and fossil evidence supports the presence of avifaunal communities in north-eastern British Columbia 10,000 BP that looked much like that of today, including the Passenger Pigeon (Driver and Hobson 1992). Therefore, it is likely goshawks expanded quickly across the newly formed Boreal forests, and would have exhibited a genetic signature of demographic growth.

It is not surprising that expansion dates for Southwestern and Pacific Coast populations were older, since these populations were likely established since at least the Late Wisconsin period (27,500-14,000 BP). Conifer woodlands and forests were well established during the Wisconsin period in both regions (Delcourt and Delcourt 1993) and fossils provided evidence that goshawks were in California (Miller and DeMay 1942). Late Pleistocene Lake Bonneville, and its surrounding marshes and wetlands, covered ~ 51,000 km^2 of Utah and supported large shorebird and waterfowl communities (Barton et al. 2002), which may have provided prey resources for goshawks.

I found no signature of a mid-Holocene population bottleneck in the West, which was seen in Western Sharp-shinned Hawks *(A. striatus*) (Hull and Girman 2005). Sharp-shinned Hawks, however, use different prey, mostly passerines, which may have been differentially effected by the mid-Holocene drought as compared to medium sized birds and mammals. As well, Western Sharp-shinned Hawks are migratory, and populations may have been negatively impacted by factors on their wintering grounds during the mid-Holocene.

Concordance of Geographic Variation in Morphology and Haplogroups – Evidence of the Apache Goshawk? - The geographic structure in mtDNA found in this study agrees with results pointing to morphological divisions (Whaley and White 1994). Whaley and White (1994) measured several morphological characteristics (e.g., wing chord, tail length, and foot and bill measures) on museum specimens and used principal component analysis to determine differences among Alaskan, Eastern, South East Alaskan (*A. g. laingi*), Western (California and Pacific Northwest), and Southwestern (*A. g. apache*) populations. Among male and female goshawks, they found clinal variation among the morphological traits they measured. Interestingly, the geographic structure of mitochondrial haplogroups found in this study agrees with the morphological differences found by Whaley and White (1994) (Table 11, Figure 14). Such concordance suggests either selection for region-specific habitats (closed, dark, mesic forests vs. open, light, xeric forests) and/or for region specific prey (variable sized prey requires variable hunting strategies possibly facilitated by slight morphology differences). Alternatively,

morphological and mitochondrial differences could have resulted from genetic drift during Pleistocene isolation.

As noted above, it has been suggested that goshawk populations in Mexico and Southeast Arizona constitute a unique subspecies, the Apache Goshawk (*A. g. apache*). Our data do not support or reject sub-specific status; however our data point to geographic isolation of goshawks in the Arizona Sky Islands from sympatric populations to the North. This is supported by several lines of evidence, including significant estimates of pairwise Φ_{ST} (Table 5), and little evidence of immigration from northern populations (Figures 3 and 11, Tables 8-10). The strong presence of the E-haplogroup in the Southwest suggests it has its origin there and thus represents a unique, region specific set of lineages.

The E-lineage, however, is phylogenetically shallow; only a single mutational step from the most common B-haplotype. Subspecific designations are difficult to make, and should be assessed using several lines of evidence, including morphology, behavior, and several markers from various regions of the mitochondrial and nuclear genomes. As such, I have presented results from a single mitochondrial region and because phylogenetic inference can change as more loci and markers are studied (Avise 2000), I recommend more mitochondrial control region loci be sampled, as well as other mitochondrial and nuclear genome markers.

CONCLUSION

Results from this study were concordant with the hypothesis of historical isolation of goshawk populations into three Late-Pleistocene forest refugia: (1) Eastern; (2) Pacific-

Coastal and (3) Southwestern. Several studies of forest species support Pleistocene separation of Eastern and Western populations (Wooding and Ward 1997, Arbogast 1999, Arbogast et al. 2001, Peters et al. 2005), of Southwestern and Pacific-Coastal populations (Spellman et al. 2007), and of Northern-Eastern and Southwestern populations (Moore et al. 1991). Results from this study also mirror that of Arbogast et al. (2001), who found evidence of three *Tamiasciurus* mitochondrial lineages: a Western lineage, which consisted of Pacific Coastal and Sierra Nevada populations; a Southwestern lineage consisting of Arizona and New Mexico populations; and a continental lineage consisting of Rocky Mountain, Great Lakes and Appalachian populations. Interestingly, *Tamiasciurus hudsonicus* (red-squirrel), is an important prey species for goshawks on the Kaibab Plateau, Arizona, where temporal variation in red-squirrel density explained much of the temporal variation in goshawk reproduction (Salafsky et al. 2005, Salafsky et al. 2007) and juvenile post-fledgling survival (Wiens et al. 2006a). Thus, historical fluctuations in climate, and its effect on forest conditions, may have had synchronous demographic and spatial effects on both red-squirrels and goshawks.

I found evidence for demographic expansion from Eastern forest refugia, dating to around 10,000 BP. This date coincides with rapid Holocene forest expansion into the Canadian North and West and correlates with dates for other species like Sharp-shinned Hawk (Hull and Girman 2005). Evidence for demographic expansion of Western populations was likely confounded by the fact that I did not sample the "expanded" population, but rather sampled populations within regions of historical forest refugia. I suspect, this is why dates for the Pacific-Coast and Southwest populations were relatively

old. Lastly, I hypothesized a Rocky Mountain refugial population; however my data did not support the presence of one. Alternatively, a refugial population may have been present in the Rocky Mountains, but the genetic signature (more endemic haplotypes) was lost with lineage sorting coupled with high levels of post-Pleistocene gene flow.

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I was fortunate that the many researchers who contributed samples had the forethought to collect molted feathers and blood from nesting goshawks; this research would have not been possible without their contributions. Laboratory research was funded through a Doctoral Dissertation Improvement Grant (DEB-0508921) provided by the National Science Foundation. Field sampling for genetic samples from the Kaibab Plateau, and laboratory research, was funded through a Rocky Mountain Research Station/Colorado State University Joint Venture Agreement (05-JV-11021607-106). Additional funding was provided by the USDA US Forest Service Southwest Region and the USFS Rocky Mountain Research Station. Approval for field collection of samples was permitted by Colorado State University Animal Care and Use Committee (permit #05-086A-01). I thank A. Campbell and J. Drewy for their assistance with laboratory data collection, and K. Burnham and M. Douglas for comments on early drafts of the manuscript.

Table 1. Sampling locations, number of samples per location and the regional group into which sampling locations were placed.

Regional Groups	Sampled Populations	# Samples and Tissue source		Contributor	US National Forests , Canadian Forest Districts, National Parks
Pacific Northwest	SE Alaska	7	B	K. Titus, R. Ramey	Tongass NF
	Coastal British	3	PF	F. Doyle, S. Hawks	Kispiox Forest District
Vancouver Island, CAN	Vancouver Island	5	PF	E. McClaren, S. Hawks	Coast Forest Region - N. Island, Campbell River, S.Island
Alberta, CAN	Alberta	3	PF	H. Pletz, S. Hawks	Banff, NP
Great Lakes	Wisconsin	13	MF, B	J. Woodford	Nicolet/Chequamegon NF
		11	PF	T. Erdman, S. Hawks	
	Michigan	3	B	R. Ramey	Huron and Manistee NF
	Minnesota	10	PF	C. Boal, S. Hawks	Chippewa NF
Central Appalachians	Maryland	3	B	D. Brinker	
	West Virginia	14	B	D. Brinker	Monongahela NF
	Pennsylvania	9	B	D. Brinker	Allegheny NF
Northern Rockies	Western Montana	10	MF	J. Kirkley	Beaverhead NF
	Eastern Idaho	8	MF	S. Patla	Targhee NF
Rocky Islands	Big Horns, WY	7	MF	J. Warder	Big Horns NF
	Black Hills, SD	3	MF	C. Staab, K. Burns	Black Hills NF
Colorado Rockies	Northern Colorado	16	MF	R. Skorkowsky	Routt, Medicine Bow NF
		3	MF	M. Miller	Yampa
	Southern Colorado	14	MF	C. Ferland	San Juan/Rio Grande NF
Northern Utah	Northern Utah	30	B	S. Sonsthagan, A. Smith	Ashley, Uinita, Wasatch, Cache NF
Colorado Plateau	Southern Utah	28	B	S. Sonsthagan	Dixie, Fishlake, Manti La Sal NF
	Kaibab Plateau, AZ	34	B	R. Reynolds	North Kaibab NF
	Mogollon Rim, AZ	6	B	R. Ramey	Apache-Sitgreves NF
Jemez Mountains, NM	Jemez, Mountains	12	MF	P. Kennedy	Santa Fe NF
Arizona Sky Islands	Arizona Sky Islands	25	B, MF	H. Snyder	Coronado NF – Santa Catalinas, Pinalenos, Patagonia, Huachuca, Chiricahuaus; Chihuahua, Mexico
Cascade-Sierra	Klamath, Ca	22	MF	B. Woodbridge	Klamath NF
	Sierra Nevada, CA	16	MF	J. Keane, S. Rosmos	Modoc, Lassen, Plumas and Tahoe NF's
Europe	Germany, Europe	6	MF, FP	O. Kruger, I Morán	Spenge, Bissendorf; Museo de las Aves de Mexico
Total Samples		321			

B=blood; PF=plucked feathers; MF=molted feathers; FP=foot pad

Table 2. Haplotypes and locus specific mutational changes. Dots represent no change from Haplotype-B.

	Locus position																																
Haplotype	64	72	123	141	195	200	224	238	239	244	245	255	256	276	277	278	281	288	306	308	309	310	317	322	324	327	328	332	338	371	504	514
B	T	T	T	C	T	T	T	C	A	G	T	C	C	C	G	T	G	A	C	C	A	C	C	T	A	C	C	C	T	A	G	A
A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.
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Ger 1	C	C	G	T	C	.	C	T	.	.	C	T	T	T	A	G	A	.	.	T	G	T	.	C	A	.	T	T	.	G	A	G
Ger 2	.	C	G	T	C	.	C	T	.	.	C	T	T	T	A	G	A	.	.	.	G	T	.	C	A	.	T	.	.	G	A	G

Table 3. Indices of haplotype diversity, neutrality and demographic history

Regional Group	*N*	# H	PA	π	*h*	S/d_k	Tajimas's D	Fu & Li's D*	Fu & Li's F*	Fu's F_S	*rg*	τ	Years since expansion: Estimate	Lower 95% bound	Upper 95% bound
Coastal SE AK-BC	10	7	4	0.00419	0.87	4.09	-1.31**	-1.13	-1.32	-3.80*	0.08	1.90	22,093	5,349	40,473
Vancouver Island, BC	5	4	0	0.00343	0.90	2.14	-0.17*	-0.17	-0.18	-1.65	0.35	1.94	22,611	0	46,194
Great Lakes	37	9	3	0.00214	0.58	10.30	-0.48*	-1.71	-2.03	-5.52*	0.05	0.00	0	0	5,473
Central Appalachians	26	7	2	0.00232	0.73	6.33	-0.74**	-1.77	-1.86	-16.49*	0.17*	1.13	13,135	5,653	22,872
Northern Rockies	18	5	0	0.00199	0.61	4.94	-0.05*	-0.70	-0.87	-1.80*	0.08	0.89	10,338	721	19,358
Rocky Islands	10	3	0	0.00196	0.38	5.00	-0.04*	-1.92	-2.08	0.058	0.51	3.00	34,965	5,146	43,435
Colorado Rockies	34	5	0	0.0023	0.53	5.92	-0.82*	1.13	0.64	-0.94	0.09	0.93	10,793	4,797	18,874
Northern Utah	29	6	1	0.00207	0.68	5.52	-0.50*	-0.59	-0.76	-1.99*	0.16	0.98	11,469	4,020	22,342
Colorado Plateau	68	8	1	0.00329	0.81	5.21	-0.20	0.40	0.24	-1.41*	0.10*	1.46	17,005	10,822	23,491
Jemez Mts., NM	12	3	1	0.00331	0.62	2.23	1.17	1.10	1.27	1.33	0.20	3.46	40,268	0	598,209
Arizona Sky Islands	25	5	1	0.0023	0.53	5.32	-0.83*	0.32	-0.01	-1.00	0.09	0.14	1,597	0	37,128
Cascade-Sierra	38	4	0	0.00242	0.65	3.03	0.87	-0.34	0.02	0.60	0.18	1.83	21,329	0	43,727
Southwest	105	10	4	0.00353	0.84	5.31	-0.040	0.44	0.33	-1.93*	0.07	1.59	18,508	11,723	26,351
Rocky Mountain West	91	8	0	0.00201	0.62	12.96	-1.57**	-1.80	-1.56	-4.88*	0.07	0.91	10,559	6,858	15,833
Eastern	66	13	5	0.00211	0.66	13.78	-1.89**	-2.85	-2.99	-9.64*	0.17	0.93	10,793	5,935	17,117
North American Populations	315	26	n/a	0.00303	0.78	13.78	-1.42**	-1.83	-2.02	-20.80*	0.10*	1.34	15,618	12,275	19,595
Expectation if selection				low	low		S	S	S	NS					
Expectation if expansion				low	high	high	S	NS	NS	S					

* $P < 0.05$; ** $P < 0.01$; PA = private alleles; π = nucleotide diversity; *h* = haplotype diversity; S/dk = expansion index; rg = raggedness index;

Table 4. Population and region specific haplotype frequencies

	HAPLOTYPES																											
Regional Groups and Sample Locations	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	*n*
1 PACIFIC NORTHWEST	1	4								1	1	1								1		1					10
SE Alaska		3								1	1	1								1							7
Coastal BC	1	1																				1					3
2 VANCOUVER ISLAND, BC	2	1		1									1														5
3 ALBERTA, CAN		2																						1			3
4 GREAT LAKES		24		2									3		2		2	1				1	1		1		37
Minnesota		5		1											2		1								1		10
Michegan-Wisconsin		19		1									3				1	1				1	1				27
5 CENTRAL APPALACHIANS		12		4	1								6				1				1					1	26
Maryland-West Virginia		5		3	1								2													1	12
Pennsylvania		7		1									4				1				1						14
6 NORTHERN ROCKIES	2	11		3		1											1										18
Beaverhead, W. Montana		8		2																							10
Targhee, E. Idaho	2	3		1		1											1										8
7 ROCKY ISLANDS		8													1				1								10
Big Horns, WY		6													1												7
Black Hills, SD		2																	1								3
8 COLORADO ROCKIES	2	20		7			3												2								34
So. Colorado	1	8		3			1												2								15
No. Colorado	1	12		4			2																				19
9 NORTHERN UTAH	7	15	1	3	2	1																					29
Ashley	3	11	1	2	2																						19
Uinta-Wasatch-Cache	4	4		1		1																					10
10 COLORADO PLATEAU	16	22		5	8	8	7	1	1																		68
Southern Utah	4	11		4	1	5	2	1																			28
Kaibab Plateau	9	10		1	7	3	4																				34
Mogollon Rim	3	1					1		1																		6
11 JEMEZ MTS, NM		2					7									3											12
12 SKY ISLANDS, SE AZ	1	2			17			2						3													25
North Sky Islands		2			10			1																			13
South Sky Islands	1				7			1						3													12
13 CASCADE-SIERRA	19	7		11			1																				38
Klammath, CA	9	5		8																							22
Sierra Nevada, CA	10	2		3			1																				16
TOTAL	50	130	1	36	28	10	18	3	1	1	1	1	10	3	3	3	4	1	3	1	1	2	1	1	1	1	315

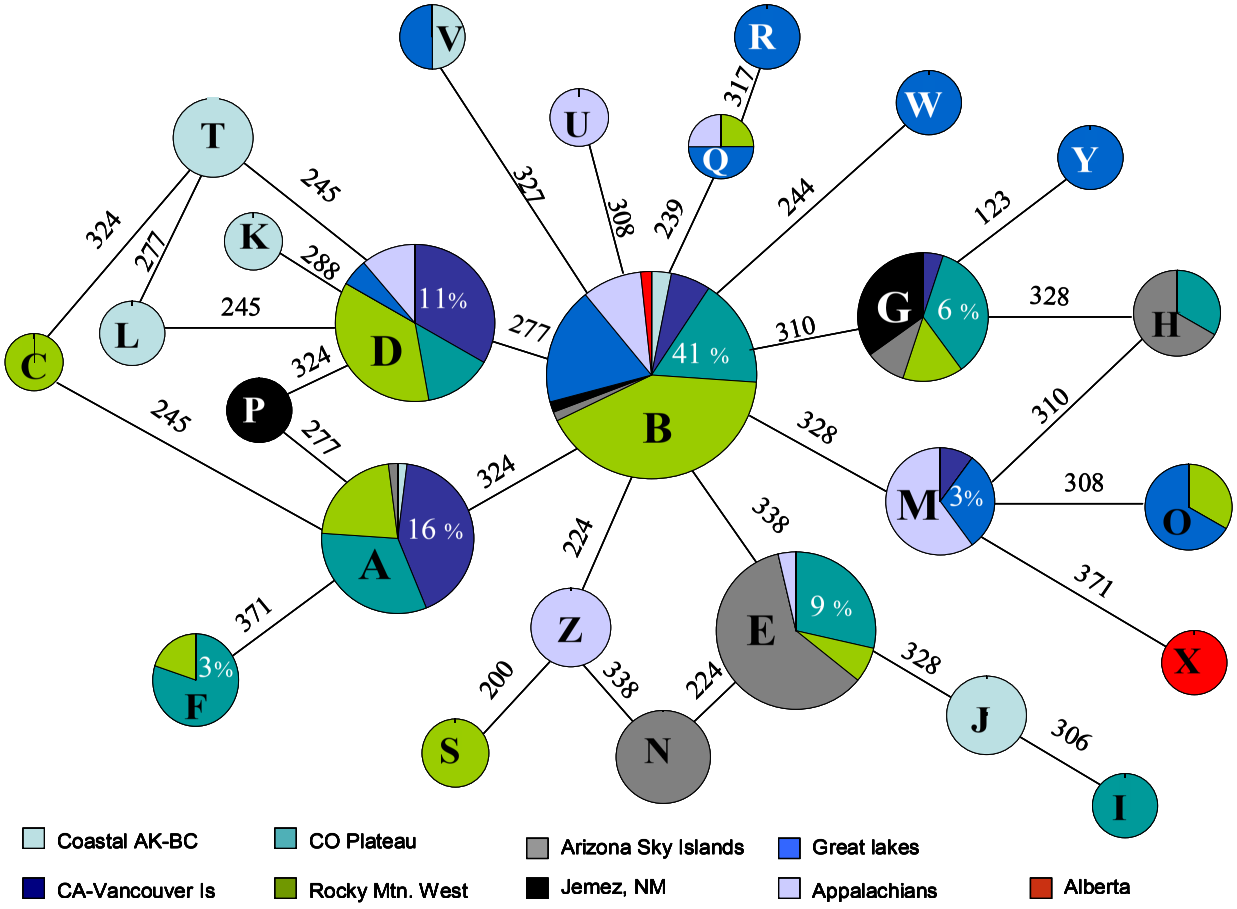


Figure 1. Minimum spanning network of mitochondrial DNA control region haplotypes. Numbered connections represent the locus position for each mutation change Percent values (%) indicate the proportion of all North American samples having that haplotype. Color slices indicate the proportion of each haplotype that occurs in each geographic region.

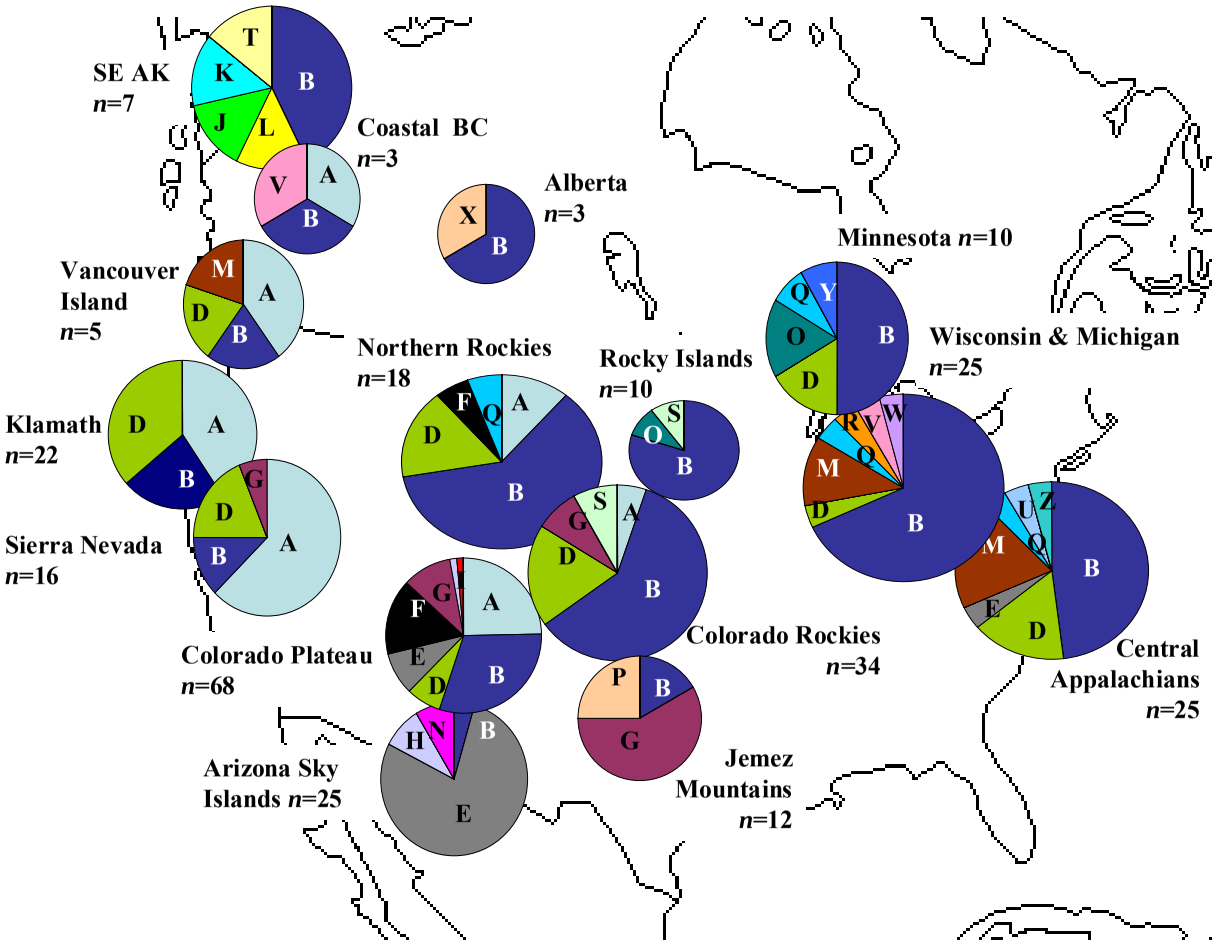


Figure 2 Geographic distribution and relative frequencies of haplotypes

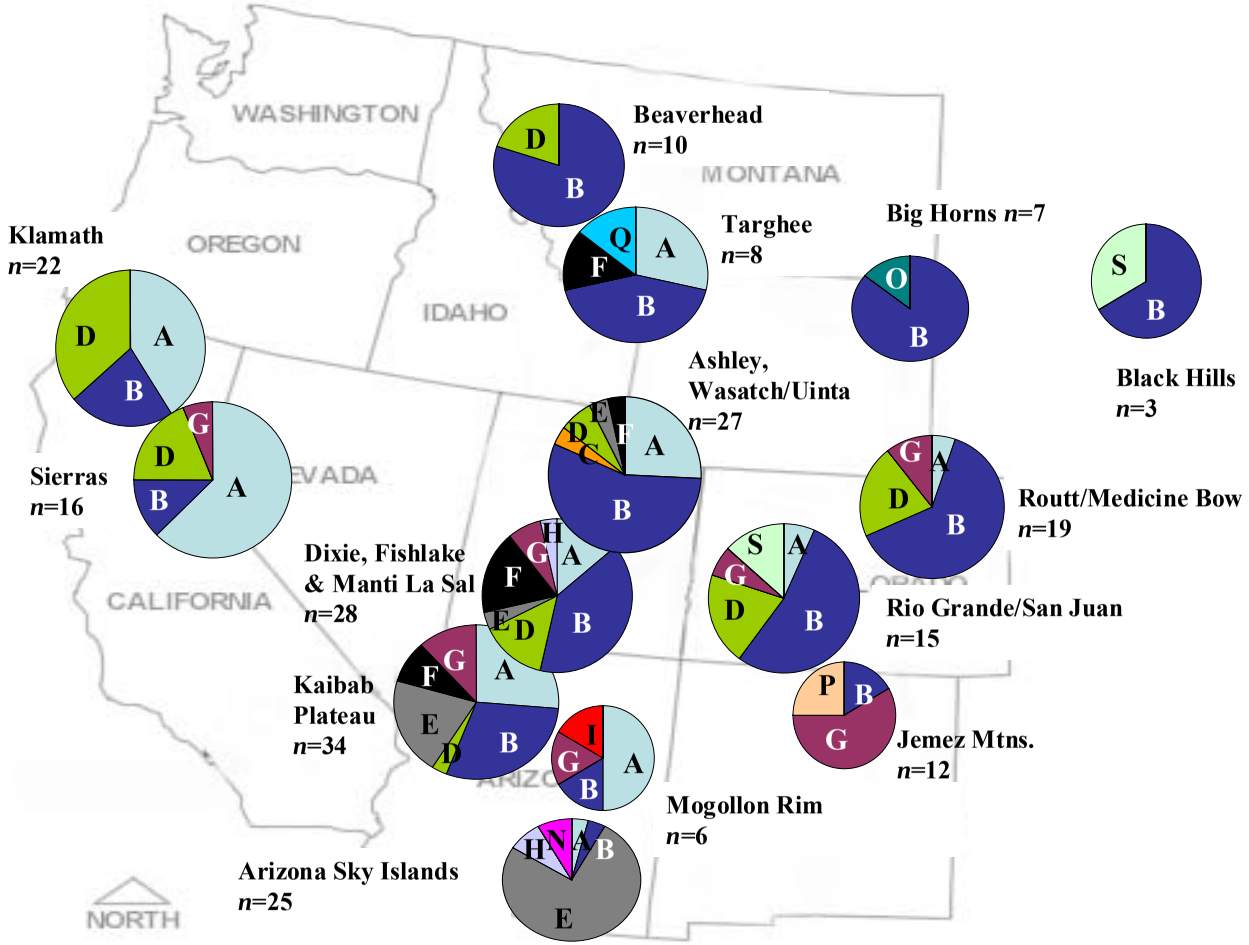


Figure 3 Detail of the geographic distribution and frequencies of haplotypes among Western sampling sites.

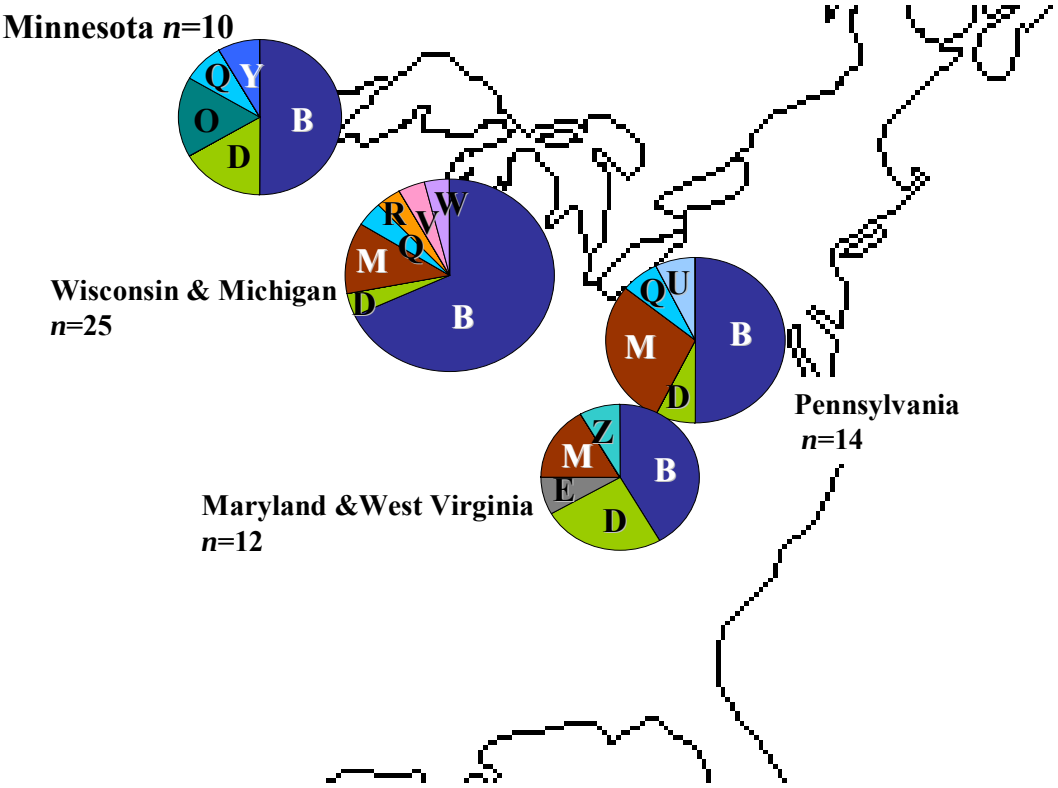


Figure 4. Detail of geographic distribution and frequency of haplotypes for Eastern sampling sites.

Table 5 Pairwise Φ_{ST} and levels of significance below the diagonal, number of female migrants per generation above. Inf = infinite

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
SE AK	1		16.91	71.64	inf	3.64	24.65	13.45	3.34	11.06	16.92	6.79	11.21	3.77	2.89	4.50	4.26	2.01	1.04	2.91	1.41
Coastal BC	2	0.029		inf	109.13	3.66	29.01	3.83	113.92	4.56	134.66	3.34	14.67	inf	inf	inf	inf	2.82	0.71	9.70	14.00
Vancouver Is.	3	0.007	-0.142		inf	3.24	42.04	10.50	inf	6.77	7.30	5.47	16.51	inf	inf	inf	inf	3.33	0.70	inf	inf
Alberta	4	-0.029	0.005	-0.026		7.76	inf	inf	5.52	inf	138.83	2.51	6.81	2.62	4.85	27.58	inf	1.63	0.74	1.56	1.09
WI & MI	5	0.121 *	0.120	0.134 *	0.061		25.79	49.43	11.41	inf	2.85	8.75	7.72	4.20	2.72	1.63	3.63	1.01	0.61	1.55	0.95
Minnesota	6	0.020	0.017	0.012	-0.081	0.019		inf	9.73	inf	27.11	11.09	13.56	3.85	3.29	5.17	5.32	2.45	0.83	2.22	1.44
C. Appalachians	7	0.036	0.115	0.045	-0.030	0.010	-0.011		9.64	inf	4.74	10.74	11.50	3.99	2.63	2.30	3.93	1.31	0.78	2.15	1.17
No. Rockies	8	0.073	0.004	-0.019	0.083	0.042	0.049	0.049		13.79	3.79	inf	inf	inf	8.15	3.91	114.67	1.80	0.67	8.22	2.63
Bighorns	9	0.043	0.099	0.069	-0.018	-0.040	-0.088	-0.032	0.035		4.46	8.53	17.67	5.61	4.30	3.55	6.67	1.40	0.72	1.80	1.11
Blackhills	10	0.029	0.004	0.064	0.004	0.149	0.018	0.095	0.117	0.101		2.53	inf	2.65	3.30	5.95	4.75	1.63	0.77	1.60	1.11
No. Colorado	11	0.069	0.130	0.084	0.166	0.054 *	0.043	0.044	-0.010	0.055	0.165		inf	7.65	3.89	2.01	8.18	2.25	0.62	3.55	1.39
So. Colorado	12	0.043	0.033	0.029	0.068	0.061 *	0.036	0.042	-0.001	0.028	-0.094	-0.016		8.17	4.15	3.20	8.24	2.45	0.77	4.23	1.83
No. Utah	13	0.117 *	-0.033	-0.045	0.160	0.106 **	0.115 *	0.111 **	-0.009	0.082	0.159	0.061 *	0.058 *		30.74	10.15	51.49	1.60	0.75	9.15	4.98
Kaibab	14	0.147 *	-0.041	-0.007	0.093	0.155 ***	0.132 **	0.160 ***	0.058 *	0.104 *	0.131	0.114 ***	0.108 **	0.016			67.70	2.51	1.27	4.26	5.98
Mogollon Rim	15	0.100	-0.119	-0.117	0.018	0.235 **	0.088	0.178 **	0.113 *	0.123	0.078	0.199 **	0.135 *	0.047	-0.033		543.67	4.02	1.02	4.68	97.91
So. Utah	16	0.105 *	-0.057	-0.062	-0.001	0.121 ***	0.086 *	0.113 **	0.004	0.070	0.095	0.058	0.057 *	0.010	0.007	0.001		3.53	0.85	11.27	8.87
Jemez, NM	17	0.199 *	0.151	0.131	0.234	0.330 ***	0.169 *	0.276 ***	0.217 ***	0.264 **	0.234	0.182 **	0.169 **	0.238 ***	0.166 **	0.111	0.124 *		0.56	1.90	1.74
AZ Sky Islands	18	0.325 ***	0.413 *	0.416 **	0.402	0.450 ***	0.377 ***	0.391 ***	0.429 ***	0.410 ***	0.395 *	0.447 ***	0.393 ***	0.401 ***	0.283 ***	0.329 ***	0.370 ***	0.472 ***		0.55	0.48
Klammath	19	0.147 *	0.049	-0.064	0.242	0.244 ***	0.184 **	0.189 ***	0.057	0.217 *	0.239	0.123 *	0.106 **	0.052	0.105 **	0.096	0.042	0.208 **	0.477 ***		16.55
Sierras	20	0.262 **	0.034	-0.043	0.314 *	0.345 ***	0.258 ***	0.299 ***	0.160 *	0.310 **	0.311 *	0.264 ***	0.214 **	0.091 *	0.077 *	0.005	0.053	0.223 *	0.508 ***	0.029	

p-values : * 0.05 – 0.01, ** 0.009 – 0.001, *** 0.0009 – 0.0000

Table 6. Hierarchal Analysis of Molecular Variance (AMOVA) that partitioned the variation in mitochondrial haplotype diversity into three scales; within populations, among populations within 13 regional groups, and among 13 regional groups.

Populations Hierarchy	d.f.	Sum of Squares	Variance	% Variation	*P* <
Among regional groups	12	35.64	0.09964 *Va*	15.63	0.00001
Among populations within regional groups	9	5.86	0.00927 *Vb*	4.45	0.00001
Within populations	292	154.34	0.52858 *Vc*	82.92	0.00001

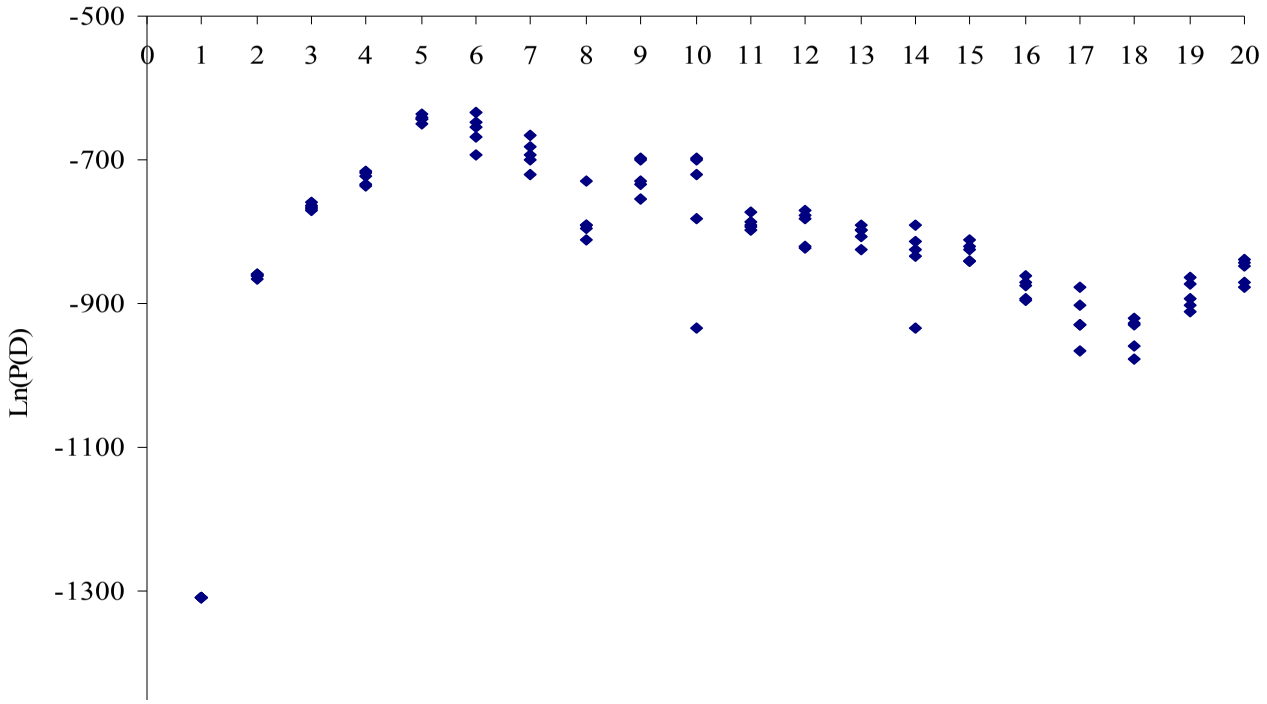


Figure 5. Estimates of the likelihood of *K* genetic clusters (*K* = 1-20). The five points for each value of *K* represent simulations, and likelihood values closer to zero indicate models having the best fit to the data. In this case, a model of *K*=5 genetic clusters provided the best fit to the data

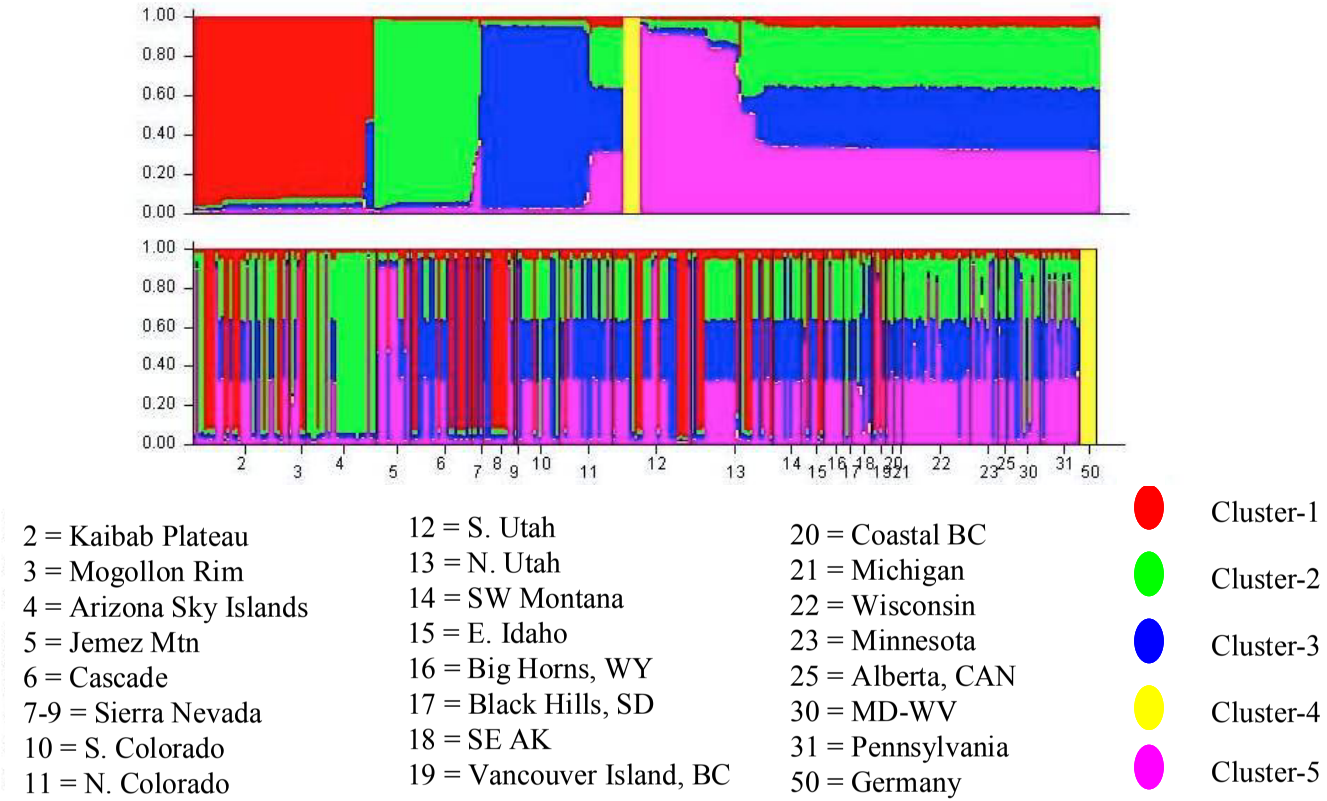


Figure 6. Genetic clusters determined by STRUCTURE. Top figure represents samples sorted according to genetic cluster; bottom figure represents samples (each represented by a single bar) sorted according to populations

Table 7. Proportion of membership of each pre-defined population into five genetic clusters as determined by STRUCTURE.

Pop	Sampling Location	Cluster 1	2	3	4	5	*N*
2	Kaibab Plateau, AZ	0.35	0.29	0.14	0.00	0.22	34
3	Mogollon Rim, AZ	0.47	0.19	0.08	0.01	0.25	6
4	Arizona Sky Islands	0.06	0.77	0.05	0.00	0.13	25
5	Jemez Mountains, NM	0.15	0.08	0.18	0.00	0.59	12
6	Klamath, Ca	0.40	0.09	0.42	0.00	0.10	22
7	Lassen-Modoc, CA	0.69	0.03	0.25	0.00	0.03	4
8	Plumas, CA	0.52	0.08	0.24	0.00	0.16	11
9	Lake Tahoe, CA	0.92	0.03	0.02	0.00	0.02	1
10	Southern Colorado	0.10	0.30	0.36	0.00	0.25	15
11	Northern Colorado	0.09	0.20	0.40	0.00	0.31	19
12	Southern Utah	0.33	0.17	0.27	0.00	0.24	28
13	Northern Utah	0.31	0.23	0.27	0.00	0.18	29
14	Western Montana	0.04	0.25	0.44	0.00	0.26	10
15	Eastern Idaho	0.37	0.17	0.25	0.00	0.20	8
16	Big Horns, WY	0.05	0.27	0.27	0.00	0.41	7
17	Black Hills, SD	0.04	0.52	0.21	0.00	0.23	3
18	SE Alaska	0.04	0.28	0.43	0.02	0.24	7
19	Vancouver Island, CAN	0.39	0.10	0.27	0.00	0.25	5
20	Coastal British Columbia	0.33	0.23	0.20	0.00	0.24	3
21	Michigan	0.05	0.30	0.31	0.00	0.34	3
22	Wisconsin	0.04	0.28	0.28	0.00	0.40	24
23	Minnesota	0.04	0.21	0.27	0.01	0.47	10
24	Alberta	0.10	0.24	0.23	0.02	0.41	3
30	West Virginia-Maryland	0.03	0.30	0.38	0.00	0.29	12
31	Pennsylvania	0.04	0.24	0.25	0.00	0.47	14
50	Germany, Europe	0.00	0.00	0.00	0.99	0.00	6

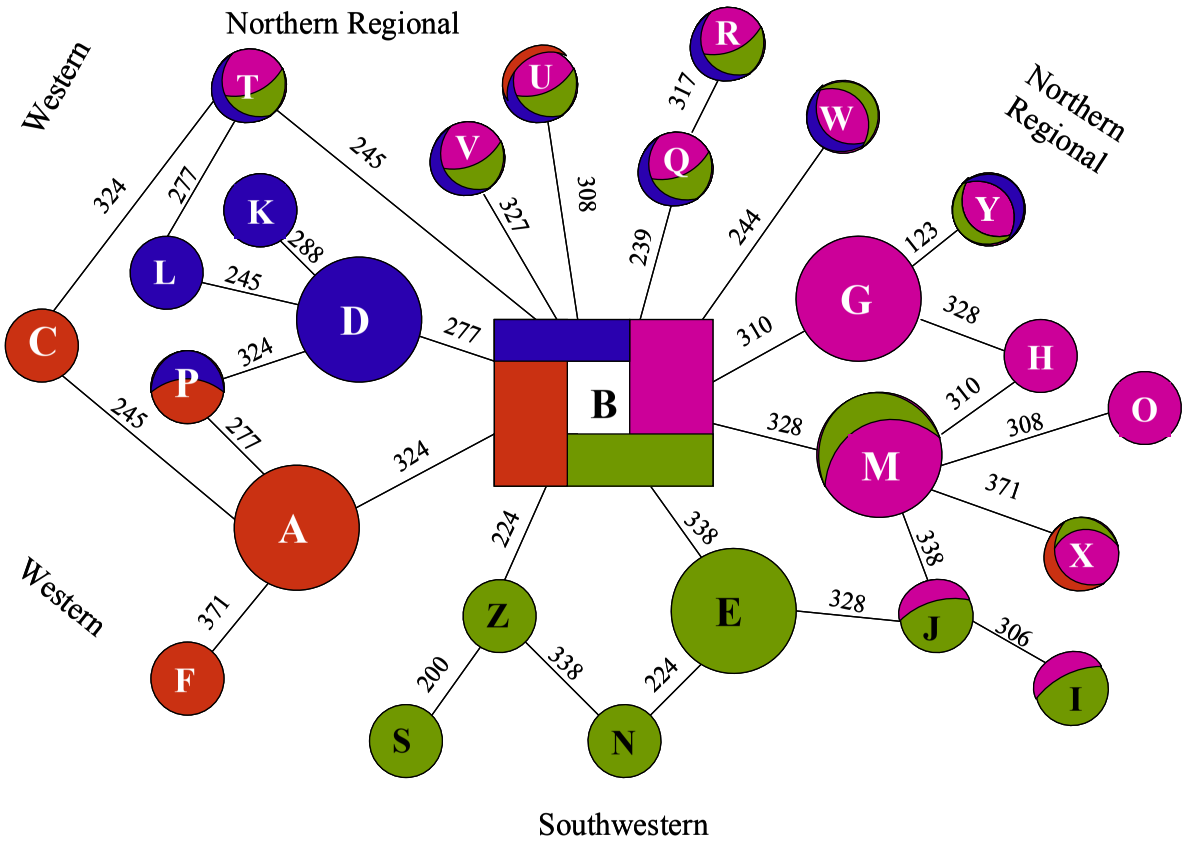
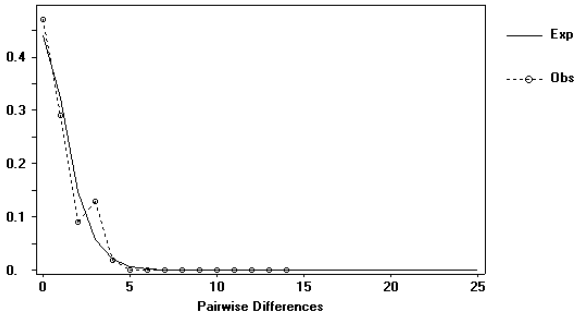
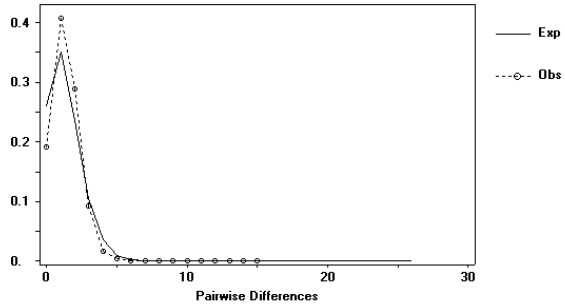


Figure 7. Minimum-spanning network of haplotypes overlaid with genetic clusters (color) as determined by STRUCTURE. The multi-cluster (multiple colors) character of the Haplotype-B resulted from its lack of geographic structure.

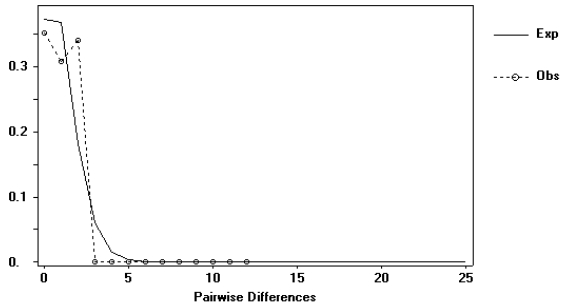
Arizona Sky Islands



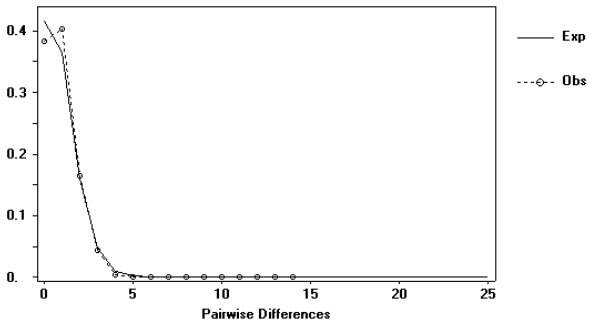
Colorado Plateau



Cascade-Sierra



Rocky Mountain West



Eastern Populations

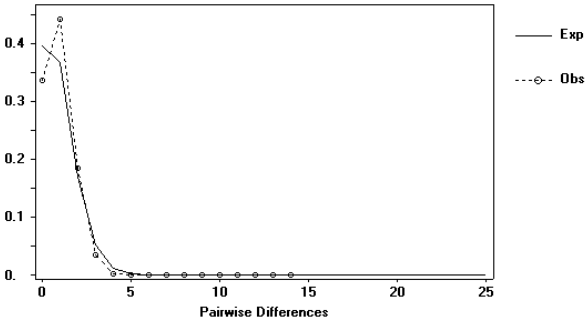
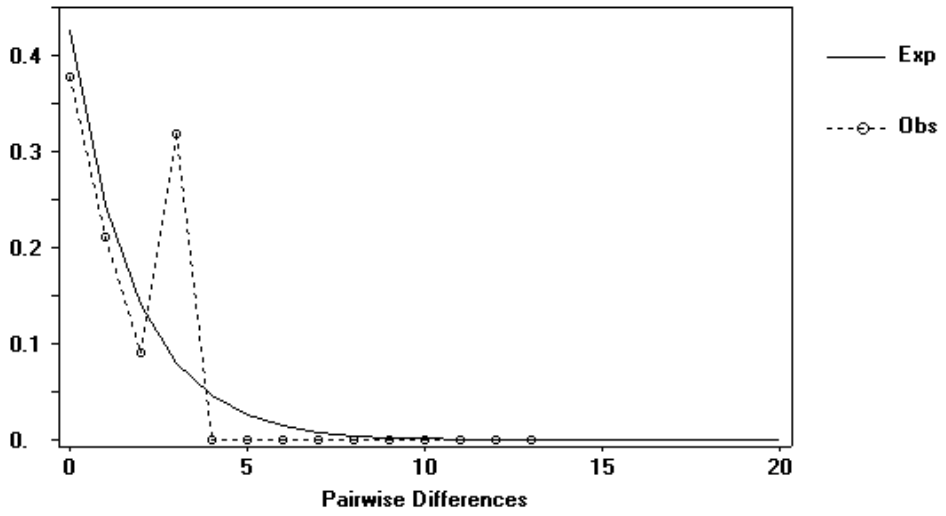


Figure 8: Mismatch distributions under assumptions of population expansion.

Statsis



Expansion

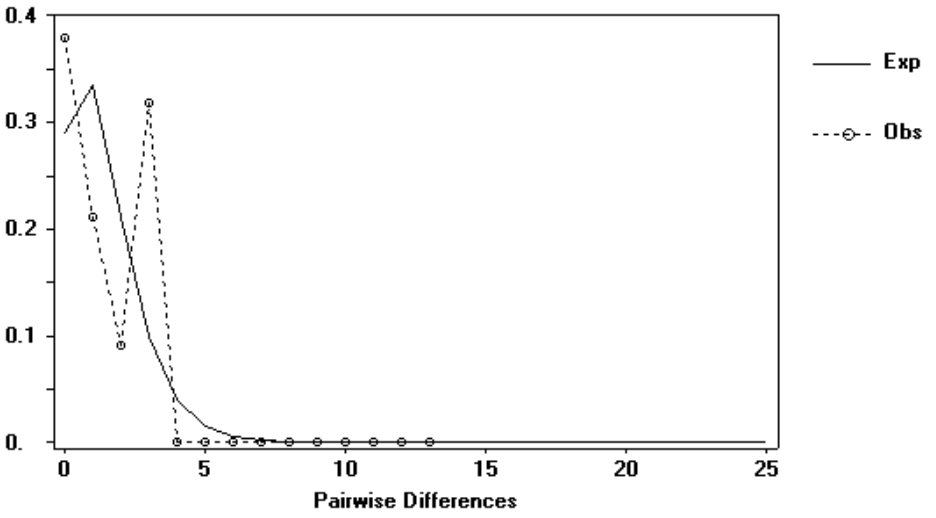
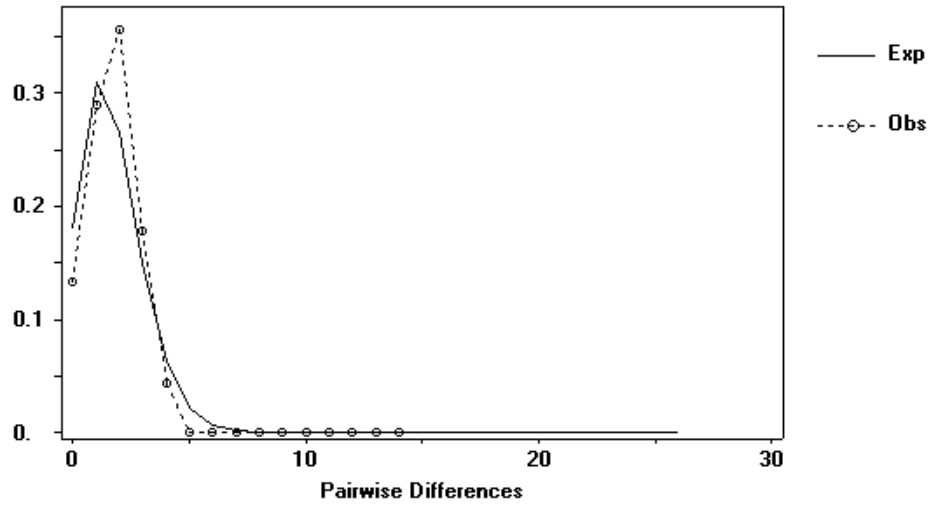


Figure 9. Mismatch distributions for the Jemez Mountain, New Mexico population, under assumptions of stasis and expansion.

SE Alaska-Coastal BC



Vancouver Island, British Columbia

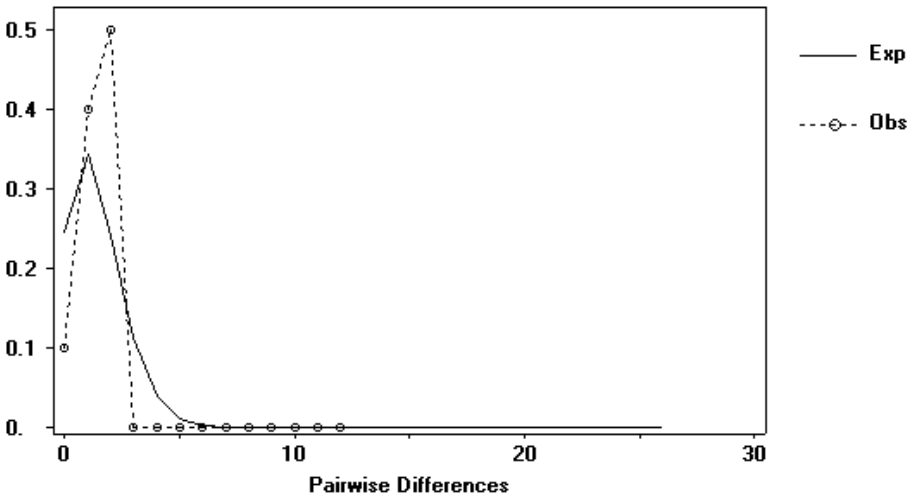


Figure 10. Mismatch distributions under assumptions of population expansion

Table 8. Replicate summary for parameter estimates under the full model (asymmetrical geneflow). Source and receieving population matrix indicates directional gene flow. MIGRATE v 2.4.2.

			Nfm							
			Source Populaions							
Ln(L)		Receiving Populations	Coastal AK-BC	CA-VCIS	Colorado Plateau	AZ Sky Islands	Jemez	Rocky Mtn West	Great Lakes	Appalachians
92.429	0.022	Coastal AK-BC	-------	76	28	0	0	0	0	0
92.429	0.010	CA-VCIS	0	-------	0	0	0	0	0	2
92.429	0.008	Colorado Plateau	5	27	-------	13	0	0	0	0
92.429	0.011	AZ Sky Islands	0	6	0	-------	0	0	0	0
92.429	0.005	Jemez	0	0	0	0	-------	0	2	0
92.429	0.007	Rocky Mtn West	0	37	4	0	0	-------	0	0
92.429	0.008	Great Lakes	0	0	0	0	0	16	-------	59
92.429	0.010	Appalachians	0	0	0	0	0	21	0	-------

$\theta = N_f \mu$, a composite measure of effective population size and mutation rate.

$N_f m$ = Number of female migrants per generation as determined by the product of θ and the M, the rate of migration

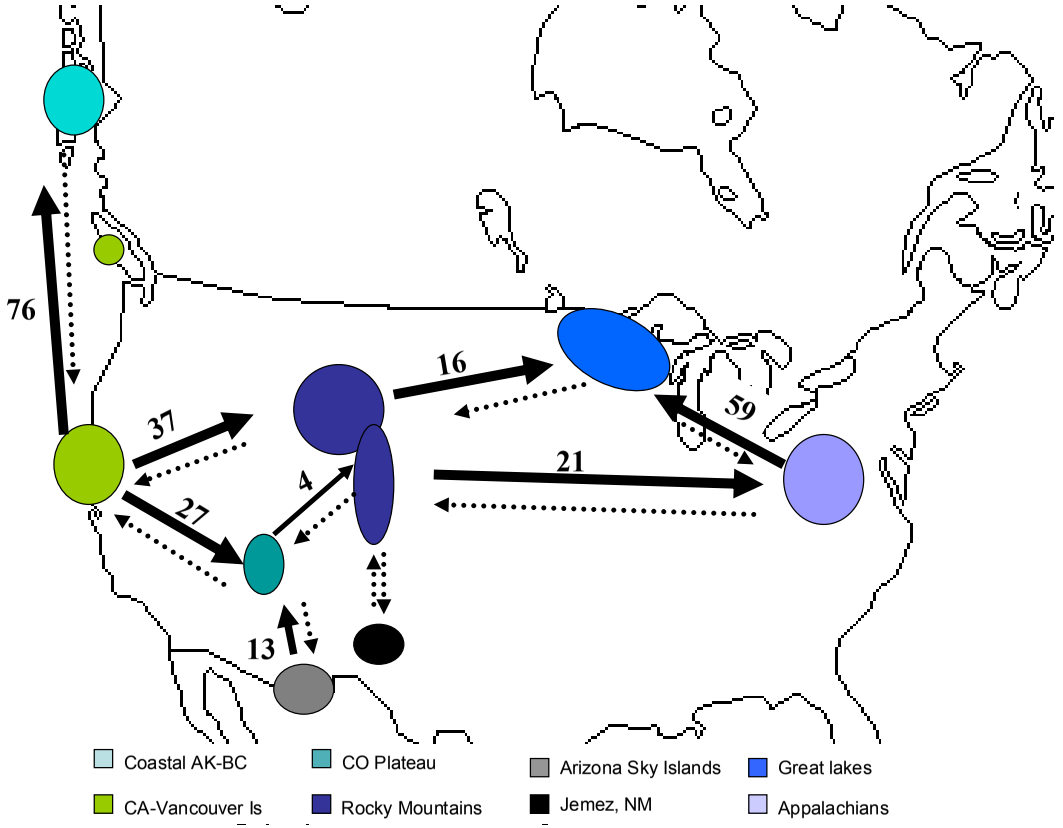


Figure 11 Estimates derived from MIGRATE showing the asymetrical gene flow among regional populations. Solid arrows with numbers indicate directional migration of female migrants ($N_f m$). Broken arrows indicate $N_f m = 0$

Table 9. Pairwise parameter estimates for θ and $N_f m$ assuming asymmetrical gene flow in MIGRATE.

			95% CI			95% CI	
From	To	$N_f m$	LCL	UCL	θ	LCL	UCL
Coastal AK-BC	CA-VCIS	0.0	0.0	0.2	0.022	0.006	0.205
Coastal AK-BC	Colorado Plateau	5.5	1.9	13.7			
Coastal AK-BC	AZ Sky Islands	0.0	0.0	1.0			
Coastal AK-BC	Jemez Mts.	0.0	0.0	1.6			
Coastal AK-BC	Rocky Mtn West	0.0	0.0	1.6			
Coastal AK-BC	Great Lakes	0.0	0.0	6.1			
Coastal AK-BC	Appalachians	0.0	0.0	1.0			
CA-VCIS	Coastal AK-BC	76.4	12.2	1141.3	0.010	0.009	0.011
CA-VCIS	Colorado Plateau	27.3	15.0	49.9			
CA-VCIS	AZ Sky Islands	6.4	2.8	13.9			
CA-VCIS	Jemez Mts.	0.0	0.0	1.6			
CA-VCIS	Rocky Mtn West	36.6	24.3	108.7			
CA-VCIS	Great Lakes	0.0	0.0	6.1			
CA-VCIS	Appalachians	0.0	0.0	1.0			
Colorado Plateau	Coastal AK-BC	27.8	6.7	541.3	0.008	0.006	0.011
Colorado Plateau	CA-VCIS	0.0	0.0	0.2			
Colorado Plateau	AZ Sky Islands	0.0	0.0	1.0			
Colorado Plateau	Jemez Mts.	0.0	0.0	1.6			
Colorado Plateau	Rocky Mtn West	4.0	1.7	16.6			
Colorado Plateau	Great Lakes	0.0	0.0	6.1			
Colorado Plateau	Appalachians	0.0	0.0	1.0			
AZ Sky Islands	Coastal AK-BC	0.0	0.0	88.8	0.011	0.008	0.015
AZ Sky Islands	CA-VCIS	0.0	0.0	0.2			
AZ Sky Islands	Colorado Plateau	12.8	6.0	26.4			
AZ Sky Islands	Jemez Mts.	0.0	0.0	1.6			
AZ Sky Islands	Rocky Mtn West	0.0	0.0	1.6			
AZ Sky Islands	Great Lakes	0.0	0.0	6.1			
AZ Sky Islands	Appalachians	0.0	0.0	1.0			

Table 10. Pairwise parameter estimates for θ and $N_f m$ assuming asymmetrical gene flow in MIGRATE.

			95% CI			95% CI	
From	To	$N_f m$	LCL	UCL	θ	LCL	UCL
Jemez Mts.	Coastal AK-BC	0.0	0.0	88.8	0.005	0.003	0.008
Jemez Mts.	CA-VCIS	0.0	0.0	0.2			
Jemez Mts.	Colorado Plateau	0.0	0.0	1.7			
Jemez Mts.	AZ Sky Islands	0.0	0.0	1.0			
Jemez Mts.	Rocky Mtn West	0.0	0.0	1.6			
Jemez Mts.	Great Lakes	0.0	0.0	6.1			
Jemez Mts.	Appalachians	0.0	0.0	1.0			
Rocky Mtn West	Coastal AK-BC	0.0	0.0	88.8	0.007	0.006	0.018
Rocky Mtn West	CA-VCIS	0.0	0.0	0.2			
Rocky Mtn West	Colorado Plateau	0.0	0.0	1.7			
Rocky Mtn West	AZ Sky Islands	0.0	0.0	1.0			
Rocky Mtn West	Jemez Mts.	0.0	0.0	1.6			
Rocky Mtn West	Great Lakes	15.8	4.5	55.5			
Rocky Mtn West	Appalachians	20.7	11.6	73.3			
Great Lakes	Coastal AK-BC	0.0	0.0	88.8	0.008	0.004	0.015
Great Lakes	CA-VCIS	0.0	0.0	0.2			
Great Lakes	Colorado Plateau	0.0	0.0	1.7			
Great Lakes	AZ Sky Islands	0.0	0.0	1.0			
Great Lakes	Jemez Mts.	2.0	0.4	8.1			
Great Lakes	Rocky Mtn West	0.0	0.0	1.6			
Great Lakes	Appalachians	0.0	0.0	1.0			
Appalachians	Coastal AK-BC	0.0	0.0	88.8	0.010	0.007	0.014
Appalachians	CA-VCIS	2.0	1.1	3.5			
Appalachians	Colorado Plateau	0.0	0.0	1.7			
Appalachians	AZ Sky Islands	0.0	0.0	1.0			
Appalachians	Jemez Mts.	0.0	0.0	1.6			
Appalachians	Rocky Mtn West	0.0	0.0	1.6			
Appalachians	Great Lakes	58.7	23.8	159.4			

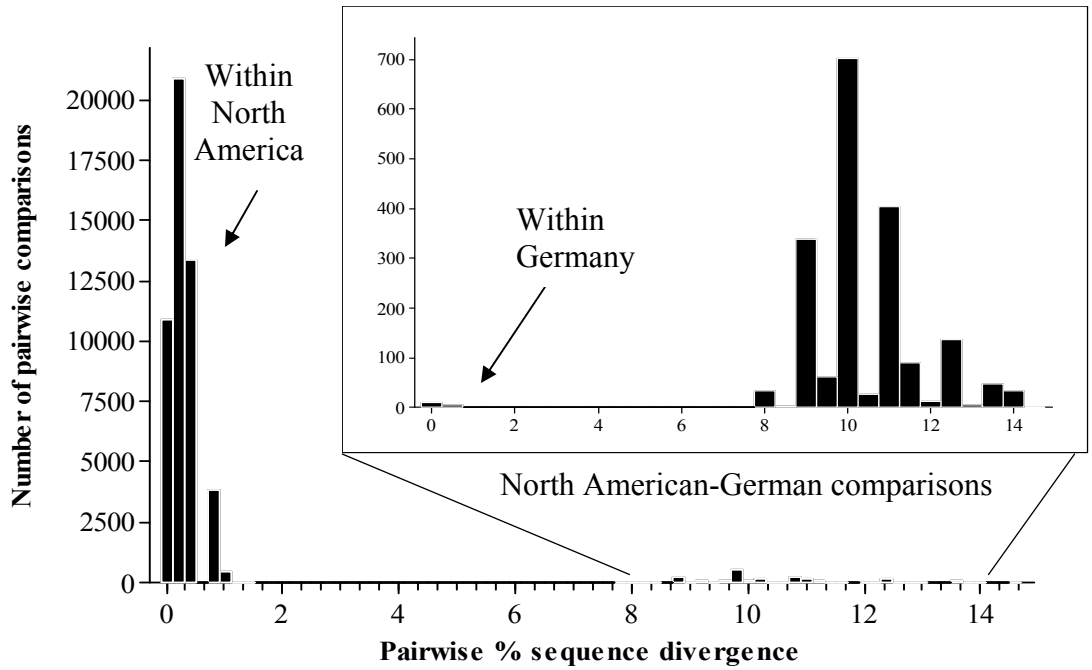


Figure 12. Percent pairwise sequence divergence (pairwise distance) among North American (*A. g. atricapilus*) and German (*A. g. gentilis*) goshawks. Goshawks within continents exhibited little divergence among samples, while divergence among continents was large.

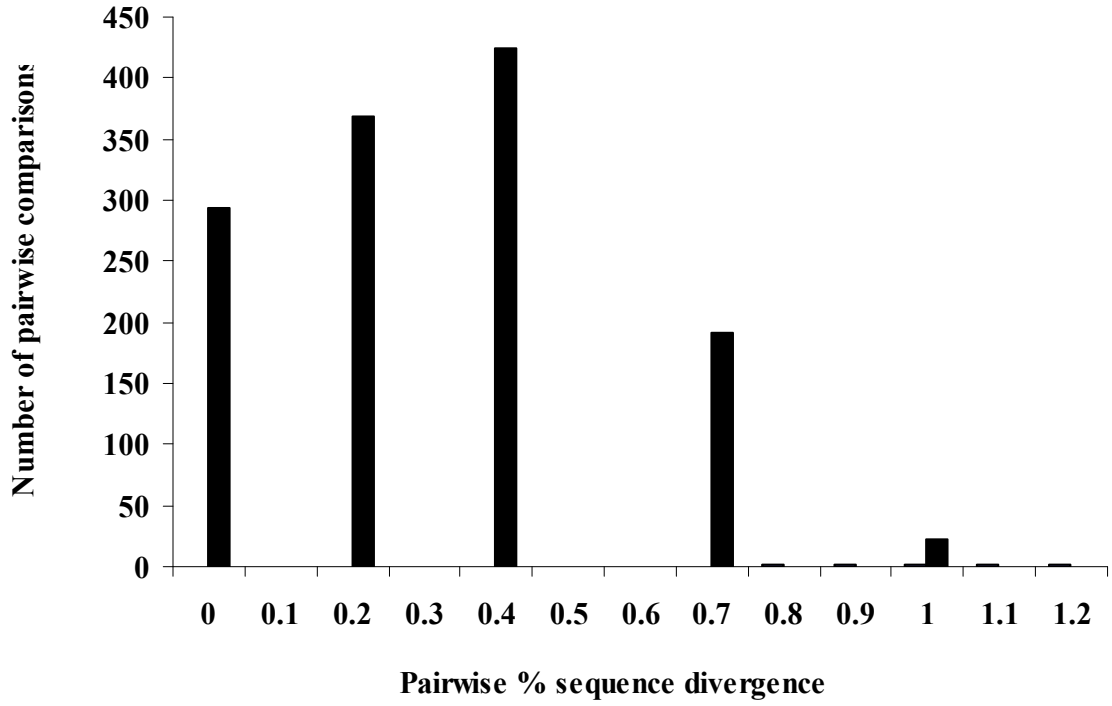


Figure 13. Percent pairwise sequence divergence (pairwise genetic distance) among goshawks in the Arizona Sky Island and all other populations. In all cases, sequence divergence ranged from 0-1.04 % indicating little evolutionary difference among Sky Island goshawks and other North American goshawks.

Table 11. Comparison of geographic variation in size and geographic distribution of haplogroups.

Region	Morphology Whaley and White (1994)	Haplogroup
Alaska	Large - ranges did not overlap	not sampled
Southeast Alaska - *A.g. laingi*	Smallest of all - size ranges overlapped with eastern in females	D descendants
Western (Pacific-Northwest)	Smaller than Interior West	A and D
East	Averaged smaller than West, but size range overlapped with West lower range	B and descendants
Rocky Mountain West	not sampled	B and mix of others
Colorado Plateau	not sampled	Mix of all haplogroups
Jemez Mountains, Northern New Mexico	not sampled	G
Arizona Sky Islands & Mexico (proposed *A.g. apache*)	Largest of all - size ranges did not overlap	E

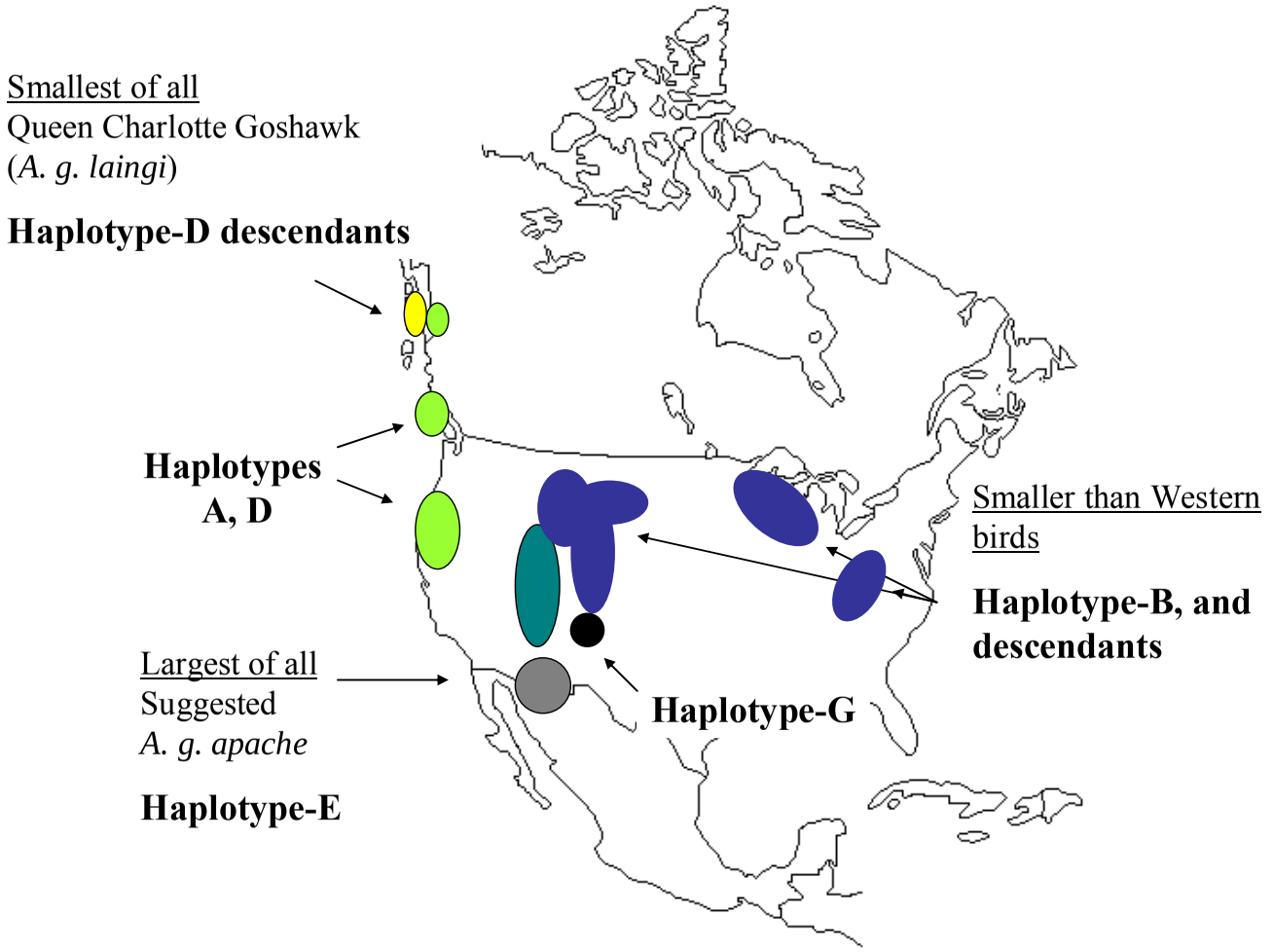


Figure 14. Concordance of clinal variation in size as reported by Whaley and White (1994) and the geographic distribution of genetic lineages (haplogroups).

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