MONTANA FISH, WILDLIFE & PARKS
INTERIM PERFORMANCE REPORT

GRANT TITLE: **Black-backed Woodpecker Study**
GRANT NUMBER: **T - 34 - R - 1**

PROJECT STATEMENT

Objective

To describe the population structure and dispersal dynamics of black-backed woodpeckers, in order to better understand habitat connectivity and provide for improved population monitoring and management of this species.

Approach

Research Questions:

1) What is a breeding population?
2) Which population model best fits genetic population structure observed?
   Hypotheses 1: metapopulation model
   Hypothesis 2: continuous population model
      i. isolation by distance
      ii. apparent panmixia
3) What is the distribution of dispersal distances?
4) How does the observed pattern of genetic variation inform the monitoring and management of populations?
5) Can life history combined with genetic data of a common species inform life history of a rare species, given genetic data?
   Hypothesis 1: BBWO are a highly specialized species that is dependent on post-fire habitat for successful breeding.
   prediction: BBWO are less differentiated than HAWO at the same spatial scale after correcting for population size differences
   Hypothesis 2: BBWO are a moderately specialized species that successfully breeds in both burned and unburned forests.
   prediction: BBWO are equally differentiated as HAWO at the same spatial scale after correcting for population size differences

Study Design and Field Methods:

Very little information exists on BBWO movement. Therefore, woodpeckers will be sampled at several spatial scales to ensure that the ecologically important scale is captured. To control for temporal variation, sampling will be conducted from the same age (2003) wildfires to reduce the chance of dispersal among fires during the study period.

To determine if there is structure at fine scale, we have chosen three large-scale wildfires within 50 km of Missoula and within 50 km of West Glacier, MT. To determine if there is structure at a regional scale, we will compare individuals between the Missoula and Glacier sites (~ 300 km). To determine if there is structure at the landscape scale, we are collaborating with other researchers on several studies in w. North America. These collaborations allow us to collect genetic samples from Oregon, Montana, Idaho, S. Dakota, and N. Alberta.

We obtain genetic samples by locating woodpecker nests to facilitate capture of both male and female adult birds. Woodpeckers are captured at their nest site using modified butterfly nets or target nets (Imbeau and Desrochers 2002, pers. obs.) and spatial coordinates are recorded with a GPS unit. We collect 100 µl of blood from the brachial vein, store the blood in a lysis buffer (Hille 2003) and place a unique color-code band on each bird's leg to ensure resampling does not occur. We plan to collect a minimum of 40 samples from the Missoula and Glacier sites and a minimum of 30 samples from sites in Oregon, South Dakota, Idaho and Alberta, for a minimum of 200 samples over a three-year period. Genetic techniques have been successfully used to determine the population structure of numerous avian species with similar or smaller sample sizes (Caizerguers et al. 2003, Hille et al. 2003, Johnson et al. 2003, Ellegren et al. 1999, McDonald et al. 1999).

Genetic Analysis:
We plan to use both mtDNA and nuclear DNA to examine gene flow on a historical scale and an ecological time scale. The Rocky Mountain Research Station Genetics Lab has screened 17 microsatellite markers that have been developed for use in other avian species. The most closely related species with microsatellite markers available is the white-backed woodpeckers (*Dendrocopos leucotos*). Four of the six loci (*DlU1, DlU3, DlU4, DlU6*) developed for white-backed woodpeckers (Ellegren et al. 1999) amplified and three were polymorphic. An additional two loci, *Lox3, Lox4*, developed for Scottish crossbills (*Loxia scotica*; Piertney et al. 1998) amplified and were polymorphic.

Data Analysis:
Question 1*:*
Population structure at the local scale: We will test the hypothesis that there are family groups within fires, based on the premise that population density increases in the first years after fire due to juveniles establishing breeding territories within the same fires. We will calculate pair-wise measures of relatedness (*r*) among individuals using KINSHIP (Goodnight and Queller 1999) to determine if there are offspring/parent or siblings within fires. I will then test whether there are significantly more individuals that are closely related within fires vs. among fires using permutation tests.

Population structure at the regional scale: To assess patterns of structure across western Montana, we will test for a correlation of geographic and genetic distance among individuals using spatial autocorrelation analysis (Smouse and Peakall 1999). We will use a Bayesian clustering method to determine if there are separate subpopulations within the region (STRUCTURE) (Funk et al. 2005).

Population structure at the landscape scale: To determine if there are separate subpopulations at the landscape scale, we will use a Bayesian clustering method (STRUCTURE) (Funk et al. 2005).

We will calculate F-statistics among subpopulations based on these results. We will assess genetic structuring at different spatial scales using an analysis of molecular variance (AMOVA) (Funk et al. 2005).

Question 2: Once population structure is assessed, we will determine if the pattern fits best into the metapopulation model or the isolation by distance model. Discrete genetic clusters connected by gene flow will be evidence for the metapopulation model. The isolation by distance model will be evident if STRUCTURE is not able to identify genetically distinct clusters and will be further tested using a linear regression model that explains genetic differentiation with geographic distance (Manel et al. 2003). A panmictic population will be evident if STRUCTURE is not able to identify genetically distinct clusters and there is correlation between geographic and genetic distance.

Question 3*:* Dispersal will be assessed using individual-based methods including assignment tests, kinship analysis and spatial autocorrelation methods. Because we are not likely to sample all possible source populations, partial Bayesian assignment methods will be calculated in GENECLASS (Berry et al. 2004). Likelihood methods will be used to determine kinship using microsatellite loci (Goodnight and Queller 1999). We will use the distance between individuals that are parent-offspring and full-siblings to infer dispersal distance. We will use spatial autocorrelation methods to test if there is a correlation between individual relatedness and geographic distance (Peakall et al 2003, Hazlitt et al. 2004). A positive correlation between individual relatedness and geographic distance indicates limited gene flow and the distance class at which there is no longer a positive correlation between relatedness and geographic distance can be interpreted as the average dispersal distance (Peakall et al. 2003, Hazlitt et al. 2004).

Question 4**:** The scale recommended for monitoring and management will be based on the spatial scale and configuration of populations. The scale recommended for management will be based on the population model. In general, a metapopulation would require management at a combination of the subpopulation and metapopulation. The isolation by distance model would require management at the scale of the genetic neighborhood. A regression of the genetic differentiation between pairs of individuals and their geographic distance can be used to estimate neighborhood size (Manel et al. 2003). A panmictic population can be monitored at the scale of the species distribution and managed at a scale that is most practical from local standpoint, such as trends documented by the North American Breeding Bird Survey (BBS; Sauer et al. 2005). Additionally, average dispersal distance can be used in the prioritization of land management treatments. The spatial pattern of BBWO movement can be used to prioritize the spatial arrangement of salvage and prescribed fire treatments.

Question 5: We will compare similar and divergent life history characteristics of these two species in the context of genetic population structure. Similar life history characteristics include foraging patterns (Bull et al. 1986, Woolf 2003), characteristics of microhabitat nest selection (Martin 2004, Dudley and Saab 2003), and parental investment (Martin and Li 1992). A potentially divergent life history characteristic is breeding macrohabitat requirements. We will test hypotheses regarding the strength of breeding macrohabitat specialization of BBWO by comparing the genetic population structure observed in our empirical data between the two species. We will use computer simulations to create several null models of population structure based on patterns of

movement and reproduction (Table 1). Computer simulations can be used to determine if the patterns of genetic structure predicted as a result of different ecological behaviors are correct. We will then compare the empirical data to the null models to determine what model best fits the empirical data.

Accomplishments

BEHAVIORAL BARRIERS TO MOVEMENT: DO MALE AND FEMALE WOODPECKERS RESPOND DIFFERENTLY TO GAPS IN HABITAT ?

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Abstract

Behavioral barriers to movement are characterized by changes in habitat features that an organism is physically capable of crossing yet does not successfully cross for various reasons such as predation risk or lack of foraging resources. We used both population and individual-based genetic approaches to assess barriers to movement in black-backed woodpeckers, a fire-dependent species that occupies mainly the boreal forest in North America. Furthermore, we tested if male and female woodpeckers exhibited the same behaviors in terms of long-distance dispersal patterns. We used both spatially implicit and spatially explicit population – based and individual – based genetic analyses to define population structure and movement patterns of both sexes among populations. Three genetic groups were consistently identified, a large, genetically continuous population that spans from the Rocky Mountains to Quebec, a small isolated population in South Dakota and a separate population in the western portion of their distribution. Patterns of genetic diversity suggest high gene flow mediated by both males and females within the continuous boreal forest. However, male-mediated gene flow is the main form of connectivity between the continuous population and the smaller populations. These smaller populations are separated by large areas of unforested habitat which likely serves as a behavioral barrier to movement.

Introduction

Dispersal is a central process to both evolution and ecology, yet many aspects of dispersal are poorly understood. The movement of individuals and their genes has long-lasting influence on the evolutionary trajectory of a population, as well as on current demographic population dynamics (Clobert et al. 2001). Barriers to dispersal can be characterized as physical or behavioral. Physical barriers are usually large landscape features such as rivers, mountain ranges, or any landscape feature that an organism is incapable of traversing (Gascon et al. 2000 PNAS). Behavioral barriers to movement are characterized by changes in habitat features that an organism is physically capable of crossing yet does not successfully cross for various reasons (Harris and Reed 2002). Individual organisms may be reluctant to enter a certain habitat due to perceived increase in predation risk (Rodriguez et al. 2001), or simply due to a lack of resources (e.g., foraging) to use during the dispersal event (Belisle and Desrochers 2002).

A great deal of research has documented the reluctance of many forest-associated species to move short distances across relatively small gaps in forested habitat (Desrochers and Hannon 1997, St. Clair et al. 1998, Belisle and St. Clair 2001, Belisle and Desrochers 2002, Gobeil and

Villard 2002, Bakker and VanVuren 2004). Many of these studies are based on translocation experiments where organisms are taken from their territory and forced to make decisions on what habitat to travel through to return to their home territory (Desrochers et al. 1999, Gobeil and Villard 2002, Bakker and VanVuren 2004). Ecological models have shown that these behavioral decisions about movement through habitat gaps can affect metapopulation dynamics (Russell et al. 2003, Zollner and Lima 2005).

Short distance movements are different from long distance dispersal events in which an individual may move a long distance before establishing a new territory. However, very few studies have been able to examine patterns of long-distance dispersal events despite the fundamental role it plays in population connectivity (but see Dale et al. 2006). Although studies have documented differential patterns of movement through habitat types at small scales ((Desrochers and Hannon 1997, St. Clair et al. 1998, Belisle and St. Clair 2001, Belisle and Desrochers 2002, Gobeil and Villard 2002, Bakker and VanVuren 2004); patterns of movement documented at one scale may not be the same at a different scale (Morales and Ellner 2002).

Birds are commonly thought to have fewer behavioral limitations to long distance dispersal given their high vagility and migratory nature (With 1997 et al. 1997). However, Harris and Reed (2002) found ecotones, habitat gaps and large water bodies are common behavioral barriers for non-migratory movement of birds. They suggest that birds that are habitat specialists, forest understory species, tropical species, solitary species, and non-migratory species would be sensitive to habitat gaps (Harris and Reed 2002).

An added layer of complexity is whether males and females exhibit similar behaviors regarding the crossing of habitat gaps. Sex-biased dispersal, where one sex is philopatric, is common among a variety of organisms (Lambin et al. 2001). In birds, female-biased dispersal is the most common pattern observed (Greenwood 1980, Clarke et al. 1997). The hypothesis for this pattern is that male birds tend to play a greater role in territory and resource defense and benefit more from being familiar with their natal area and therefore, are the philopatric sex (Greenwood 1980, Perrin and Goudet 2001). However, there are many examples of birds in which both sexes disperse (Lambin et al. 2001).

Long-distance dispersal is hard to measure by directly tracking individuals because most birds are too small to take advantage of advances in GPS technologies and resightings of banded birds in new locations is typically quite low (Dale et al. 2006). In this study, we use genetic techniques to estimate long-distance dispersal patterns. For the purposes of this study, dispersal is defined as the movement of an individual followed by reproduction, which results in gene flow.

Woodpeckers are an excellent species to test hypotheses regarding behavioral barriers to movement because many are non-migratory, habitat specialists, and are often solitary. These characteristics represent three of the five life history characteristics proposed as predictors of species that are likely to perceive ecotones and habitat gaps as barriers (Harris and Reed 2002). Woodpeckers are generally considered sedentary species that disperse short distances due to their non-migratory nature (Paradis et al. 1998), high level of monogamy and territorial fidelity (Mikusiński 2006). Very little is known about the genetic population structure and dispersal patterns of woodpeckers in general (Pasinelli 2006). Only one study has used microsatellite markers to assess intraspecific gene flow in woodpeckers, the white-backed woodpecker (*D. leucotos*; Ellegren et al.1999). This European species has experienced severe range contraction and is endangered in Sweden and declining across Europe (Ellegren et al. 1999). However, the white-backed woodpecker was more continuously distributed previous to intensive logging in

Sweden and Finland in the 1950's and experienced population irruptions in which large numbers of individuals would disperse long distances (Ellegren et al.1999) which likely led to a lack of genetic structure detected among the north European populations (F_{ST} range: 0.000-0.015, R_{ST} = 0.000-0.177; Ellegren et al. 1999).

Our study is focused on black-backed woodpeckers (*Picoides arcticus*), the quintessential example of a fire-dependent species in the spotlight of many land management agency efforts due to conservation concerns regarding fire suppression and post-fire salvage logging (Hutto 200? Consbio). They colonize burned areas within one year after a fire, use burned areas for three to five years, with peak densities three years after fire (Caton 1996, Dixon and Saab 2000). They do breed outside burned areas in low densities leading researchers to hypothesize that burned areas act as source populations and unburned habitat act a sink (Hutto 1995, Huot and Ibarzabal 2006).

Black-backed woodpeckers are a monogamous, resident species that maintains territories year-round (Dixon and Saab 2000). Individuals likely change habitat patches more than once in their lifetime because their life span (six to eight years; Dixon and Saab 2000) is longer than the length of time their habitat is optimal. To date, researchers have been unable to study the dispersal or movement patterns of black-backed woodpeckers due to their natural rarity and unpredictable movement patterns once a burned area is no longer optimal habitat.
Black-backed woodpeckers are continuously distributed across the boreal forest, into Alaska and range down into the northern U.S. (Figure 1.) They also occupy isolated patches in the Black Hills of South Dakota and regions of Oregon and California mainly on the east side of the Cascades and Sierra Nevadas. Due to the northern restrictions of their range, black-backed woodpeckers likely recently colonized most areas from glacial refugia occupied during the Pleistocene. They have been documented making long-distance movements during irruptions outside their normal breeding ranges (Yunick 1985) and therefore long-distance movements are physiologically possible. Given black-backed woodpeckers occupy ephemeral habitats (Dixon and Saab 2000, Saab et al. 2002), both sexes regularly disperse during the course of their lifetime (Huot and Ibarzabal 2006).

Based on the general patterns observed regarding dispersal observed by Harris and Reed (2002), we predict that gaps in habitat will create behavioral barriers to movement to both male and female black-backed woodpeckers. We tested if large gaps in forested areas are behavioral barriers to movement for black-backed woodpeckers and if males and females respond to these potential barriers in the same manner.

Methods

Sampling and DNA extraction

Blood or feather samples were collected in seven sampling locations Idaho, South Dakota Alberta, and genomic DNA from collaborators in Quebec (Figure 1). Blood samples were collected from adults caught at the nest site with either a hoop net or mist net during the 2004-2007 breeding seasons. Individuals were color banded to avoid resampling in concurrent years and to record any dispersal events. We did not sample offspring in the nests to reduce sampling related individuals. A portion of the Idaho samples (n = 29) were feathers collected as part of a radio telemetry study conducted in1998-2000. The samples in Quebec were collected in 2000-2001. The latitude and longitude of individual sample locations was recorded. Blood samples were stored at room temperature in a lysis buffer (Longmire et al. 1988). DNA was extracted from both blood and feather tissues using a DNeasy Tissue Extraction Kit (QIAGEN Inc.).

Blood was incubated for 2 – 24 hours with a final elution of 200 ul and feathers were kept on a rocker for 48 hours with a final elution of 100 ul to increase final DNA concentration.

Genotyping and Sequencing

Mitochondrial DNA (mtDNA) was amplified using the polymerase chain reaction (PCR) and primers (L14841 and H15149) for the cytochrome b region (Kocher et al.1989). The reaction volume (50μl) contained 50-100 ng DNA, 1x reaction buffer (Perkin-Elmer), 2.5 mM $MgCl_2$, 200μM each dNTP, 1μM each primer, 1 U *Taq* polymerase (Titanium Taq; Clontech). The PCR program was 94°C/5 min, [94°C/1 min, 55°C/1 min, 72°C/1 min 30s] x 34 cycles, 72°C/5 min. PCR products (325bp) were purified using ExoSAP-it (USB) and directly sequenced. Both strands were sequenced using the Thermo Sequencase Cycle Sequencing Kit (USB) and run on either a 4300 DNA Analyzer (Li-Cor Biosciences) or a 3730XL (Applied Biosystems). Sequence editing and alignment was completed with *Sequencher* (Genecodes Corp.)

Samples were genotyped at eleven microsatellite loci (C111, C115, D118, Vila et al. 2008; RCW4 (added tail), RCW5, RCW17 (added tail), (Mullins and Haig in review); DIU1, DIU3, DIU4, Ellegren et al. 1999, HrU2, Ellegren 1992, Lox4, Piertney et al. 1998). We added 'GTTTCTT' to the 5' end of the reverse primer of RCW4 and RCW17 to promote the addition of adenine (Brownstein et al. 1996). All PCR amplifications were performed in 10 µl reactions. Three loci (DIU1, DIU3, Lox 4) were analyzed in single PCR reactions containing 2.5 mM $MgCl_2$, 0.2mM of each dNTP's, 2 µM dye-labelled forward primer and 2 µM reverse primer, 1 U *Taq* polymerase (Titanium Taq; Clontech), 1x reaction buffer (Perkin-Elmer), and ~ 15 ng genomic DNA in 10 µL final reaction volume. Samples were amplified with the following profile: initial denaturation at 94 °C for 10 m, followed by 45 cycles of (94 °C for 60 s, 58 °C for 60 s , 72 °C for 60 s). Amplification products were analyzed on 6.5 % polyacrylamide gels and visualized on a Li-Cor DNA Analyser 4300 (Li-Cor Biotechnology). Alleles were visually scored by two individuals based on a ladder and individuals with known genotypes. Eight loci were analyzed in three multiplex reactions (Table 1) using the QIAGEN Multiplex PCR Kit (QIAGEN, Valencia, CA). Samples were amplified with the following profile: initial denaturation at 94 °C for 10 m, followed by 45 cycles of (94 °C for 60 s, 58 °C for 60 s , 72 °C for 60 s). Fragment analysis was performed on an ABI 3130xl Genetic Analyzer (Applied Biosystems Inc.) in the Murdoch DNA Sequencing Facility at the University of Montana. ABI GS600LIZ ladder was used to determine allele sizes and (Applied Biosystems Inc., Foster City, CA) chromatogram output was viewed and analyzed using GeneMapper version 3.7 (Applied Biosystems Inc., Foster City, CA). Genotypes were manually checked by two individuals and if there was disagreement on how to score the sample, we reran the genetic analyses. All feather samples were run a minimum of three separate PCR tubes, a heterozygote genotype was accepted if confirmed a minimum of two times and a homozygote genotype was accepted if confirmed a minimum of three times.

Statistical Analysis

Genetic Variation

Microsatellite markers were tested for departure from Hardy-Weinberg proportions and gametic disequilibrium in GENEPOP (version 1.2; Raymond and Rousset 1995). We calculated observed and expected heterozygosity and average number of alleles/locus in GDA (version 1.1; Lewis and Zaykin 2001). Allelic richness and F_{IS} were calculated in FSTAT. The presence of

null alleles, dropout of large alleles and errors due to stuttering were tested using MICRO-CHECKER (Van Oosterhout et al. 2004). For mtDNA, haplotype diversity (*h*), nucleotide diversity (π) were calculated using DnaSP (version 4.50; Rozas et al. 2003). Haplotype richness was calculated by taking the mean number of haplotypes observed when sampling 21 (minimum number of haplotypes) haplotypes with replacement from the frequency distribution of haplotypes10,000 times.

Population-level Analyses

We calculated pairwise F_{ST} (Weir and Cockerham 1984) among all sampling locations tested for isolation by distance based on $F_{ST}/(1- F_{ST})$ vs. linear geographic distance among sample sites using Mantel tests (Mantel 1967) in the ade4 (Dray et al. 2007) package in the R software environment (http://www.r-project.org/).

Because our study was conducted at such a large spatial scale, we began by assessing hierarchical population structure where individuals at a sampling location (Figure 1) were considered one group. We conducted an analysis of molecular variance (AMOVA) for both marker types using spatial data (SAMOVA; Dupanloup et al. 2002) and without spatial data (ARLEQUIN 3.11; Excoffier et. al. 2005). We tested four different hierarchical groupings (Table 2) and tested for significance of the variance components using 1000 permutations. Populations were identified by maximizing the among group percent of variation. We used principal component analysis (PCA) to visualize how sample sites clustered using PCAGEN (http://www2.unil.ch/popgen/softwares/pcagen.htm).

Individual-based Analyses

We then assessed population structure using individual-based approaches. Specifically, we used a Bayesian clustering approach to determine the number of clusters based on gametic disequilibrium and deviations from Hardy-Weinburg proportions. Again, we used both a spatiallyimplicit and spatially explicit approache to define population clusters and assign individuals to these groups. First, we used the program STRUCTURE (version 2.2; Pritchard et al. 2000; Falush et al. 2003), a widely used approach that does not consider spatial information in the clustering algorithm. Next, we used the program GENELAND (version 3.1.4; Guillot et al. 2005b) which can infers spatial discontinuities in genetic data when incorporating the spatial location of individual samples as well as a user-defined uncertainty around sampling locations. We also employed spatially implicit approach in GENELAND.

In STRUCTURE, we used the admixture model, with correlated allele frequencies and no prior information regarding where individuals were sampled. We used a burn-in period of 300,000 followed by 1,000,000 iterations for $K = 1$ through $K = 10$. We repeated each run four times and averaged log Pr(X|K) across all runs to determine which value of K maximized Pr(X|K). Because STRUCTURE's algorithm uses Hardy-Weinburg proportions to cluster individuals, we used the dataset consisting of the 9 loci that did not show departures from Hardy-Weinburg proportions.

Although the algorithm in GENELAND simultaneously estimates all the parameters, Guillot et al. (2005a), recommend a two-step approach. The first step infers the number of populations (K) and the second step holds K constant to assign individuals to populations.

We began the GENELAND analyses by running 10 replicates with the following parameters: maximum rate of Poisson process of 274 (equal to sample size as recommended by Guillot et al. 2005a), allowed K to vary from 1 to 10, maximum number of nuclei of 825

(roughly three times the sample size as recommended by Guillot et al. 2005a), 500,000 MCMC iterations with a burn-in period of 100,000 iterations, the Dirichlet model in which allele frequencies are assumed to be independent, spatial coordinates with an uncertainty of 5 km. The Dirichlet model has been shown to perform better than the alternative model available in GENELAND (F-model; Guillot et al. 2005a). GENELAND is able to estimate the frequency of null alleles simultaneously with the other parameters, allowing the use of full data set, 11 loci, in the analysis (Guillot et al. 2008).

To test the robustness of our results, we varied several input parameters to see if we obtained the same estimate of *K*. We varied uncertainty on the spatial coordinates from 0 – 50 km. We ran the same analysis as above with the nine loci dataset without using the null allele model to determine if the results would change based on these two different models.

Once *K* was identified, we ran 100 replicates of the model with the same parameters as above and *K* held constant. We ranked the models by mean logarithm of posterior probability and conducted post-processing analyses on the top ten models runs. We used a burn-in period of 100,000 iterations, a spatial domain of 400 pixels along the X axis and 200 pixels along the Y axis and checked the runs visually for consistency.

Sex-biased movement patterns

Sex-biased movements can be estimated using genetic techniques by measuring the proportion of recent immigrants that are male vs. female in a population. However, it is often difficult to sample extensively enough to capture recent immigrants. Another method is examining different patterns of genetic structure in sex-linked markers compared to autosomal markers. We were interested in movements that occur at irregular time intervals and did not anticipate sampling recent immigrants, so we focused on the comparing patterns of genetic structure in mtDNA, which is maternally inherited, to autosomal microsatellites.

We calculated standardized estimates of pairwise estimates of F_{ST} (G_{ST}´) for both marker types (Hedrick 2005, Meirmans 2006). The maximum F_{ST} was calculated by recoding each population to have unique alleles/haplotypes to maximum among population variation, while maintaining observed levels of variation (Hedrick 2005, Meirmans 2006). We also plotted observed and standardized F_{ST} values on plots that show the expected values of F_{ST} for both mtDNA and nuclear markers under island model of migration and following isolation (Zink and Barrowclough 2008).

Results

Genetic Variation

We found 16 variable sites in the 325 base pairs sequenced in the cytochrome b region of the mitochondrial genome. We identified 18 haplotypes, ranging from 12 in Quebec to two in South Dakota (Table 3). Haplotype diversity (*h*) and nucleotide diversity (π) were highest in Idaho ($h = 0.616$, $\pi = .0035$) and lowest in South Dakota ($h = 0.095$, $\pi = 0.0006$). One haplotype was very common (> 60%), a second was relatively common (16%) and eight haplotypes were only detected once (Figure 1).

Ten of the eleven microsatellites were polymorphic in all the populations; Locus DIU1 was monomorphic in South Dakota. After correcting for multiple comparisons (Bonferroni ##) two loci had departures from H-W proportions, DIU1 and RCW17 and four pairwise comparisons were significant for gametic disequilibrium. The average number of alleles per locus ranged from 3.64 in South Dakota to 6.91 in Quebec. Allelic richness was lowest in South

Dakota (3.57) and highest in Alberta (6.36). Observed and expected heterozygosity were fairly equivalent at all sites except Missoula, where the presence of null alleles at two loci decreased the observed heterozygosity. South Dakota had the lowest levels of heterozygosity (H_O = 0.46), other sites ranged from (0.51-0.62) (Table 4).

Null alleles were likely present at three loci: DIU1, RCW17 and C111. Both DIU1 and RCW17 had relatively high estimated frequencies of null alleles (0.20, 0.15 respectively) while the estimated frequency of the null allele at C111 occurred at a relatively low frequency (.06). We conducted most analyses on both a full and reduced data set, with the same general pattern resulting from both datasets. Most analyses presented are from the dataset with nine loci, after removing DIU1 and RCW17 to eliminate any bias caused from high frequencies of null alleles in the data set. GENELAND analyses presented are from the full dataset because the algorithm implemented can estimate frequencies of null alleles.

Population-level Analyses

Samples collected from sites within the continuously distributed areas had lower pairwise F_{ST} values for both mtDNA and microsatellite data (Table 5). For mtDNA, pairwise F_{ST} values for the continuous sites ranged from 0.00-0.11 while the fragmented sites ranged from 0.36-0.75. Overall, pairwise F_{ST} values for microsatellite data was much lower with values in the continuous sites ranging from 0.006 – 0.022 and from 0.035-0.094 in the fragmented sites. The grouping of sites within the continuously distributed locations was supported by AMOVA (Table 2), SAMOVA, and PCA (Figure 2). Due to similar results between the AMOVA and SAMOVA, we are only presenting AMOVA results. For mtDNA, grouping by continuous vs. fragmented sites explained 49.99% of the variation and was the only significant grouping from mtDNA ($P < 0.05$; Table 2). Similarly, the only significant grouping for the microsatellite data was continuous vs. fragmented sites, which explained 3.54% of the variation ($P < 0.05$; Table 2). PCA reveals that, for marker types, all sites within the continuously distributed area cluster tightly together and Oregon and South Dakota cluster very separately from the continuous sites and each other (Figure 2).

Patterns of isolation by distance were more complex. Across all sites there is no pattern of isolation by distance for mtDNA ($r = 0.004$, $P = 0.30$) or microsatellites ($r = 0.03$, $P = 0.30$). However, this pattern is driven mostly by the lack of a relationship between geographic and genetic distance relationship among sites within the continuously distributed region (Rocky Mountains and Quebec; Figure 3).

Individual-based Analyses

Only one population cluster ($K = 1$) was identified by the program STRUCTURE (Figure 4a). However, when three population clusters are enforced ($K = 3$), the proportion of membership for the locations within the continuous population assign fairly even to each cluster (e.g. ~33%), while the Oregon and South Dakota have slightly higher assignment proportions in different clusters (43-47%). The spatially implicit model in GENELAND also consistently identified one cluster (Figure 4b)

When a spatially explicit approach was used, GENELAND consistently identified three populations (K=3), with all ten runs identifying K= 3 with the highest probability (Figure 4c). Individuals assigned to populations with a high probability, with only six individuals ambiguously assigned with probability of assignment =>0.99 (Figure 5). Geographic barriers to gene flow were identified with probability of assignment contours (Figure 5).

Sex-biased movement patterns

Pairwise F_{ST} estimates for microsatellite data between the continuous and fragmented sites were 4 – 5 times lower than you would predict based on island model of migration at mutation-drift equilibrium using the following equation (Brito et al 2007): $F_{ST(msat)} = F_{ST(mtDNA)} /4 - 3*F_{ST(mtDNA)}$ (Figure 6). After standardization, pairwise F_{ST} estimates for microsatellite data between the continuous and fragmented sites were > 2 times lower than expected based on Wright's island model of migration. For example, pairwise $F_{ST(mtDNA)} = 0.49$ between Oregon and the continuous population; under the island model, the expected $F_{ST(msat)} = 0.19$, observed $F_{ST(msat)} = 0.04$. After standardizing, the standardized pairwise $F_{ST(mtDNA)} = 0.72$ between Oregon and the continuous population, the expected $F_{ST(msat)} = 0.39$, observed standardized $F_{ST(msat)} = 0.17$ (Table 6).

Discussion

We found that large gaps among forested sites act as behavioral barriers to the movement of female black-backed woodpeckers and create a higher resistance to movement for male black-backed woodpeckers. Despite the sedentary nature of many woodpeckers, we know black-backed woodpeckers are physiologically capable of long-distance movements based on records of historical irruptions (Yunick 1985). However, these irruptions occurred almost exclusively outside the breeding season (Yunick 1985) and therefore do not represent natal or breeding dispersal, but are more similar to short distance migration events. Given the high levels of gene flow across the Continental Divide within the Rocky Mountains, geographical features such as mountain ranges do not appear to create physical barriers to movement. The complete lack of genetic structure for both microsatellite and mtDNA markers across a vast distance (~3500 KM) in the Canadian boreal forest indicate both males and females are dispersing at equivalent rates and distances when there is continuously distributed habitat (maybe cite simulation chapter/paper since it should be first; Woolf et al. 2020)

Population Structure and Movement

The past few years have seen an explosion of individual-based methods for defining clusters of genetically similar individuals (Manel et al. 2003, Latch et al. 2006, Chen et al. 2007). However, individual-based methods often work best when samples are evenly spaced across the study area. This is because if isolation by distance occurs, these clustering methods can misidentify groups at either end of the spectrum due to a lack of sampling across the distribution of continuously distributed species (Schwartz and McKelvey 2008).

In this study, we sampled in clustered manner, that is, we sampled multiple individuals at several different sites across a large spatial scale. Therefore, we chose to use both traditional population-level based analyses to define groups of individuals (AMOVA, SAMOVA, PCA, F_{ST}) and individual-based analyses (STRUCTURE and GENELAND). In both types of analyses, we used spatially implicit (AMOVA, STRUCTURE) and spatially explicit (SAMOVA, GENELAND) approaches. All of the approaches except the spatially implicit individual-based clustering methods (STRUCTURE, spatially implicit GENELAND). defined the same three populations, a large, genetically continuous population (Rocky Mountains across the boreal forest to Quebec) and two fragmented populations (Oregon and South Dakota).

Both spatially implict individual-based methods identified one population. At low levels of genetic differentiation, spatially implicit models such as STRUCTURE often do a poor job of identifying clusters (Latch et al. 2006). Although the levels of differentiation between the continuous population and fragmented sites (Oregon and South Dakota) were above the level (F_{ST} = 0.03; Table 6) at which STRUCTURE has been able to confidently identify clusters based on simulated data sets (Latch et al. 2006), this approach did not perform well in our study. The spatially explicit approach employed in GENELAND displayed a very low level of uncertainty in estimating the number of populations. All ten runs estimated K= 3, with subsequent identification of population boundaries and assignment of individuals to the three populations incredibly consistent. Very few individuals were assigned to more than one population and all individuals assigned to the "correct" population with a probability > 0.99.

Behavioral barriers to movement

A recent review of patterns of genetic structure in seabirds found that their nonbreeding distribution acted as barriers to dispersal (Friesen et al 2007), a similar pattern to what we found for female black-backed woodpeckers. However, black-backed woodpeckers' distribution is almost identical to the distribution of the boreal forest. These gaps in the boreal forest are likely the ultimate cause of the limited gene flow across these gaps. A great deal of research has shown gaps in forest often present movement barriers to birds reliant on forested habitat (Desrochers and Hannon 1997, other).

Evidence that large gaps in forested habitat are movement barriers for females can be seen in the population structure we detected and the difference in pairwise $F_{STmtDNA}$ values between sites that have large gaps between them (fragmented: Oregon and South Dakota) as compared to sites that have forest between them (continuous: Idaho, Missoula, Glacier, Alberta and Quebec). Hierarchical population structure is a useful tool to detect barriers to gene flow when you have several subpopulations that may be connected by differing levels of gene flow by grouping together subpopulations that are genetically similar and quantifying the amount of differentiation at different levels (Allendorf and Luikart 2007). When sites within the Rocky Mountains are grouped with Quebec, we see a large amount of genetic variation among the groups and almost no genetic variation among the sites within group (Table 2). When we tried including Oregon with the Rocky Mountains and Quebec, the variation among sites increased 15 –fold, confirming a barrier likely exists between Oregon and the Rocky Mountains. Additional evidence can be seen in the high pairwise $F_{STmtDNA}$ values (0.36 – 0.75) between sites with large habitat gaps between them. These values are similar to what have been documented among subspecies or separate clades in other birds occupying similar ranges (Gibbs et al. 2000, Mila et al. 2007). Black-backed woodpeckers do not have any subspecies designated and only one clade exists. The pattern of haplotype distribution is consistent with one common ancestral population and current differences due to genetic drift since colonization after the Pleistocene (Figure 1).

The inclusion of spatial data in GENELAND identified the general location of barriers to gene flow among the three populations (Figure 5). Sharp discontinuities match the break in large forested areas between the Rocky Mountains and Oregon and the Rocky Mountains and South Dakota. The probability contour plot around the South Dakota is likely correct at high levels of assignment probability (Figure 5c), however, the lack of sample in the boreal forest between Alberta and Quebec does not allow GENELAND to do a good job of assessing probability plots across the boreal forest.

Black-backed woodpeckers may not be a classic forest species due to their proclivity for burned forests in which most of the standing trees are dead, but it has been well documented that these birds prefer dense stands of dead trees (Saab et al. 2002). Organisms usually avoid dispersing through certain habitat types for three reasons, to avoid predators, lack of resources during travel and the inability to see the next patch (Bélisle and Desrochers 2002). When birds are dispersing long distances, they are never likely to see the next patch. However, the risk of predation and the amount of resources available for foraging will be much more similar in burned and live forests than between any forest type and non-forest type (e.g., grassland, etc.). Therefore, it makes sense that black-backed woodpeckers would be averse to travelling long distances through non-forested habitat.

Sex-biased movement patterns

Males successfully travel long distances across inhospitable habitat at a much higher rate than females. We conclude this by looking at the comparison between the nuclear and mtDNA data. While both types of genetic markers support the same pattern of population structure relatively little genetic variation is explained among the groups in the AMOVA analysis of the microsatellite data (Table 2) and pairwise $F_{ST(msat)}$ are low (Table 6).

To assess if differential amounts of gene flow were present across the large gaps, we plotted the expected pairwise values of F_{STnuc} : $F_{STmtDNA}$ under Wright's island model of migration at equilibrium and under a model of isolation, along with our observed values (Figure 6; adapted from Zink and Barrowclough 2008). Generally, points above both lines would indicate female-biased dispersal and points below both lines indicate male-biased dispersal. The pairwise estimates for sites within the continuous population fall within the range of what is expected under a model of gene flow or a model of isolation with extremely large effective population sizes (eg. Ne > 325,000 after 5,000 generations of isolation) which is much larger than the estimates of long term effective population size of other more common warblers (Ne ~ 10,000 Milot et al. 2000) and downy woodpeckers ($N_{e(f)}$ ~ 6,500; Ball and Avise 1992). Conversely , the pairwise values for the sites separated by large habitat gaps fall well below expected values under either model (Figure 6). Finally, the standardized estimates of F_{ST} are plotted and fall well below expected values under either model, indicating male-biased movement after correcting for potential bias due to high levels of heterzygosity when using microsatellite markers.

Possible Alternative Explanations

There are four potential explanations for the observed departure from the expected pairwise values of F_{STnuc} : $F_{STmtDNA}$: lack of equilibrium, high rate of homoplasy in the microsatellite markers compared to mtDNA (Zink and Barrowclough 2008), large amount of heterozygosity due to highly polymorphic microsatellite markers (Hedrick 1999), and differential gene flow between males and females. We will discuss the likelihood of these three possibilities below.

An ice sheet covered most of the boreal forest until approximately 10,000 years ago, so most of the habitat currently occupied by black-backed woodpeckers was likely colonized within the last 10,000 years (Hewitt 2000). The generation interval for black-backed woodpeckers (e.g., average age of reproduction; Allendorf and Luikart 2007) is likely 2 – 4years, based on the estimates for red-cockaded woodpecker and the lifespan of black-backed woodpeckers (Dixon and Saab 2000 and cite RCWO). Therefore, black-backed woodpeckers have likely occupied

their current habitat for 2,500 to 5,000 years and it is possible the populations have not reached equilibrium. However, the time it takes a population to reach equilibrium is a function of the effective population size, with smaller population reaching equilibrium faster (Hedrick 2005 book). If a lack of equilibrium was responsible for the differences between estimates of F_{STnuc} : $F_{STmtDNA}$, then we would expect a pattern opposite of what we observed. The larger, continuous population in the Canadian boreal forest would have very low F_{STnuc} compared to a disproportionately higher $F_{STmtDNA}$. The population in South Dakota is likely quite small (low Ne), given they only occupy the Black Hills, a relatively small area (15,500 km^2) and black-backed woodpeckers are a relatively rare bird that occupies large territories (50 – 250 ha; Dixon and Saab 2000). The South Dakota population has also likely been established a longer time given the southern location relative to the other sites and should be the closest to equilibrium. So, we would expect the pairwise comparison between F_{STnuc} : $F_{STmtDNA}$ between South Dakota and other sites to be closest to the predicted pairwise F_{STnuc} : $F_{STmtDNA}$ values under an equilibrium scenario, and in fact, these sites are the furthest from predicted values (Figure 6).

Homoplasy, two alleles in a population that are the same but have different origins, are common in microsatellites because they often follow a step-wise mutation model in which each mutation is 'one step' or repeat different (eg., 'CA', 'CACA'). For example, an allele with four repeats can be a mutant from an allele from either three or five repeats (Allendorf and Luikart 2007). Homplasy can cause two populations to appear more genetically similar than they are because they may share the same alleles from different origins. If homoplasy caused an underestimate of F_{STnuc}, it would likely be driven by one to two loci having high amounts of homoplasy. In our case, we have nine loci that all had relatively similar F_{STnuc} values. Additionally, homoplasy would not explain the high $F_{STmtDNA}$ between sites with large habitat gaps and the low $F_{STmtDNA}$ between sites with continuous habitat between them, which is a main driver of our pattern.

Because estimates of F_{ST} depend on how much variation there is in a population, estimates based on microsatellites are often biased low due to the large number of alleles per locus and high amount of heterozygosity (Hedrick 2005 paper). We used recently suggested techniques (Hedrick 2005, Meirmans 2006) to standardize our estimates of both F_{STnuc} and $F_{STmtDNA}$ and found that F_{STnuc} estimates were still lower than expected under an island model of gene flow, where $F_{STnuc} = F_{STmtDNA} / (4 - 3\ F_{STmtDNA}$; Brito et al. 2007). Based on standardized estimates of $F_{STmtDNA}$, we would expect estimates of genetic divergence between South Dakota and the continuous population in the boreal forest to be $F_{STnuc} = 0.335$ and our standardized estimate of $F_{STnuc} = 0.167$, a value twice as low as expected. Therefore, highly variable loci deflating estimates of genetic divergence does not explain the pattern we observed.

This leaves differential gene flow between males and females as the best explanation for the pattern in our data. Sex-biased dispersal is a common phenomena in birds, with a majority having female-biased dispersal (Clarke et al. 1997). However, a review of patterns of avian sex-biased dispersal found 11% (6/53) of birds to have male-biased dispersal and 15% (8/53) showed equal dispersal between sexes (Clarke et al. 1997, Gibbs et al. 2000). Our findings differ from a simple pattern of male-biased dispersal. Females and males both regularly disperse because their habitat is ephemeral and there is evidence for gene flow attributed to both sexes over large areas with continuous forest.

Conclusions or Conservation Implications

We are unaware of any other studies to find evidence for males and females making different decisions regarding crossing large gaps in habitat for long-distance dispersal events, given both sexes disperse. Black-backed woodpeckers are a solitary, non-migratory specialist habitat specialist in which females perceived large gaps in habitat as a behavioral barrier to movement and males perceived large gaps in habitat as a higher resistance landscape to long-distance dispersal. Future studies examining behavioral barriers to movement should consider different decision-making by males and females. This is especially important give the common use of nuclear genetic data to define populations.

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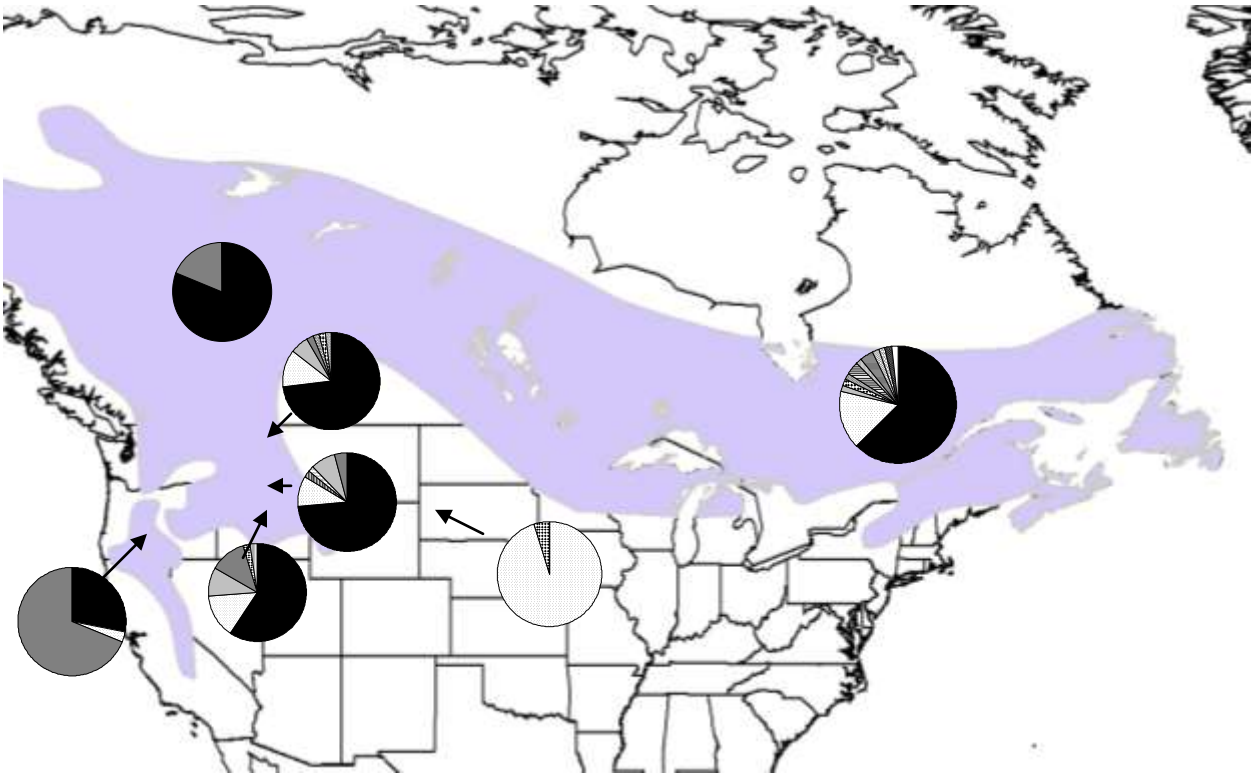

Figure 1. The distribution of black-backed woodpeckers (Natureserve) with the seven sampling locations: Oregon, Idaho, Missoula, Glacier, Alberta and Quebec. The frequency of observed mtDNA cytochrome b haplotypes at each sampling location is represented by pie charts at each location.

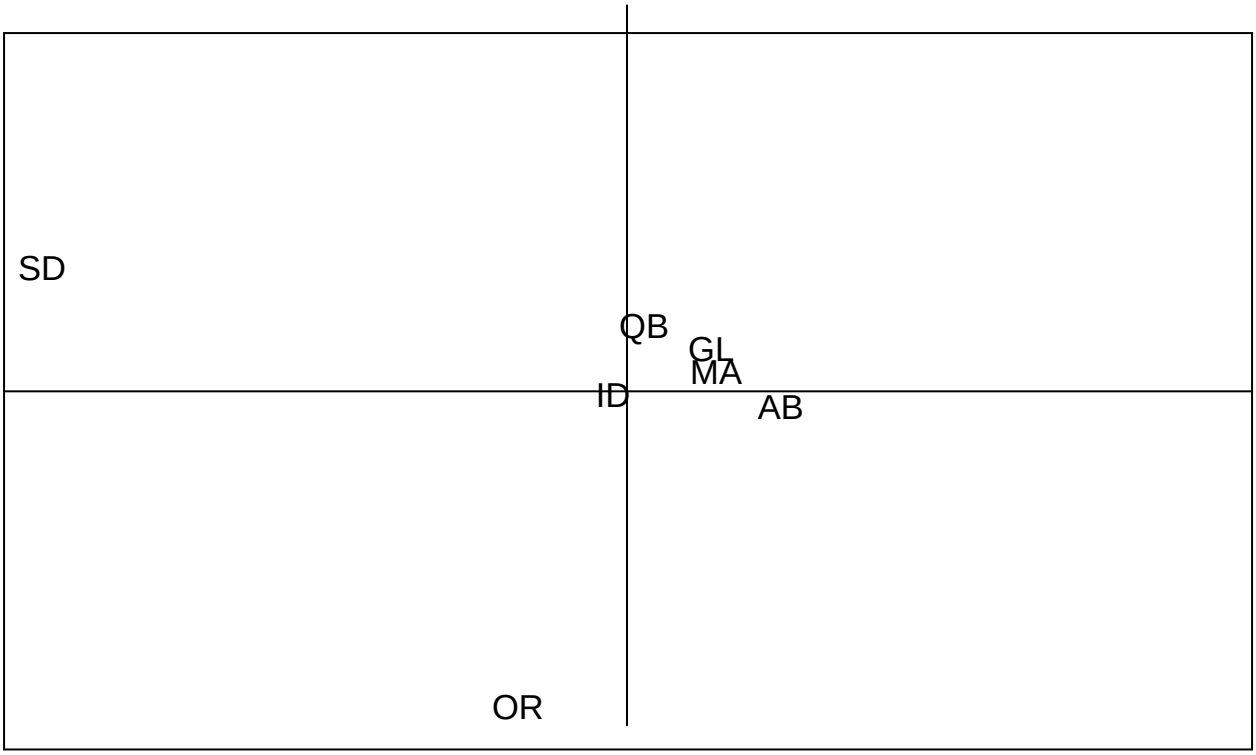

Figure 2. Principal Components Analysis visualizing clustering of sampling locations based on mtDNA; PC 1 = 59%, PC 2 = 39%; SD = South Dakota, OR = Oregon, ID = Idaho, MA = Missoula, GL = Glacier, AB = Alberta, QB = Quebec. PCA results for microsatellite data were nearly identical and therefore, are not shown (microsatellite data; PC 1 = 59%, PC 2 = 39%).

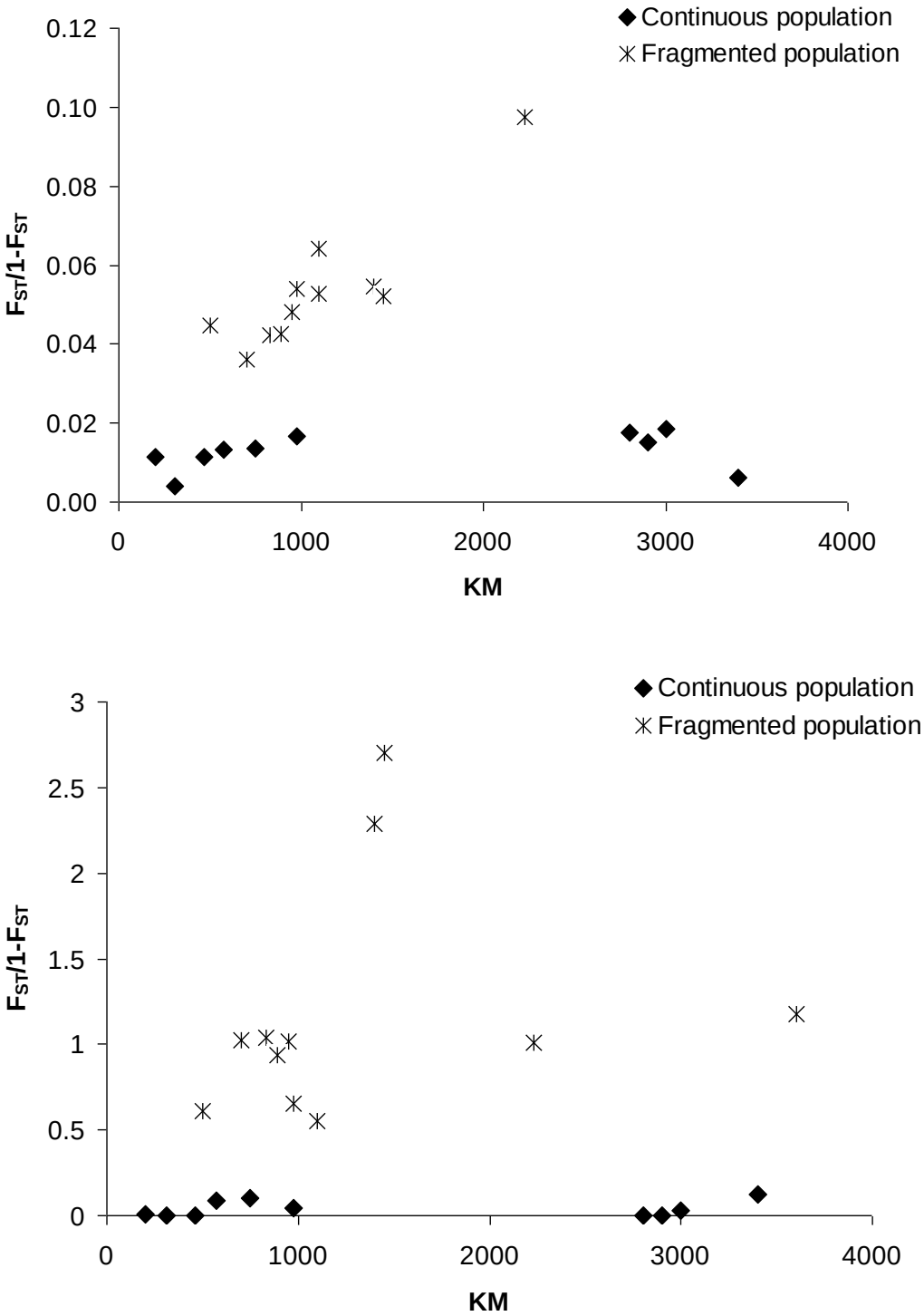

Figure 3. Scatterplot of genetic distance (F_{ST}/1-F_{ST}) vs. linear geographic distance (km) for top:. microsatellite data and bottom: mtDNA data. Sites sampled within the continuous portion of the distribution are represented by black diamonds and sites sampled in the fragmented portions of the distribution are represented by asterisks

a
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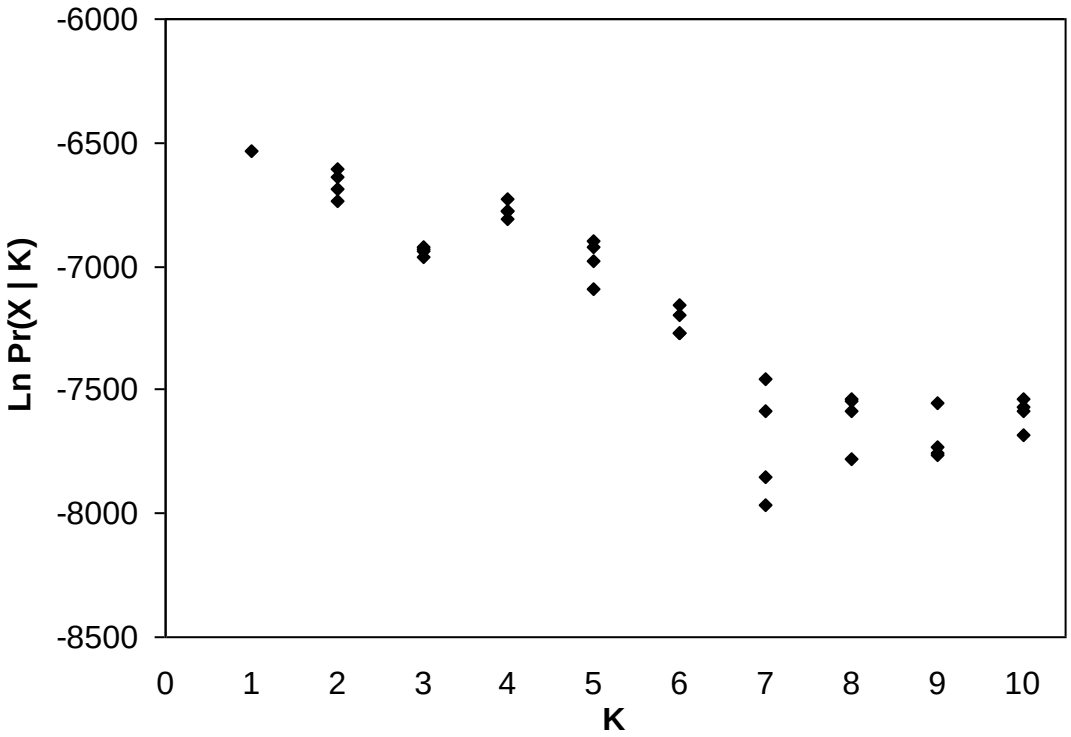

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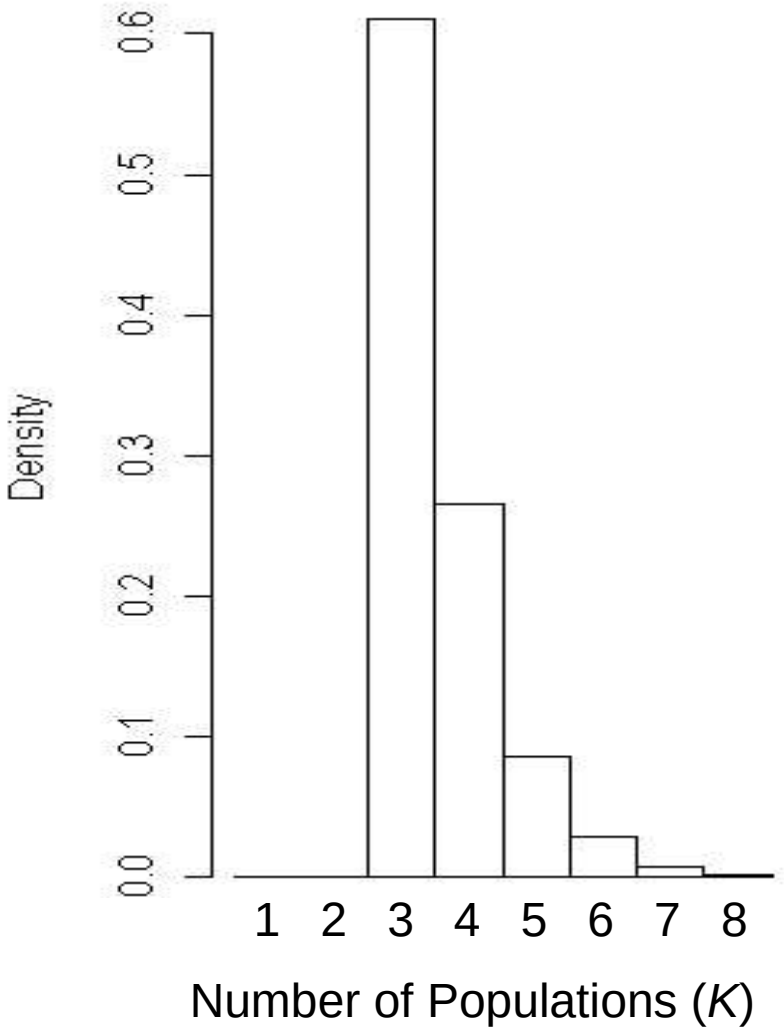

Figure 4. (a) The Ln Pr(X|K) for the number of populations (*K*) estimated in the Bayesian clustering program STRUCTURE, based on four replications. (b) The posterior density distribution of the number of populations (*K*) estimated using the spatially implicit model in GENELAND. All ten replicates of analyses in GENELAND shared similar distributional plots. (c) The posterior density distribution of the number of populations (*K*) estimated using the spatially explicit model in GENELAND. All ten replicates of analyses in GENELAND shared similar distributional plots.

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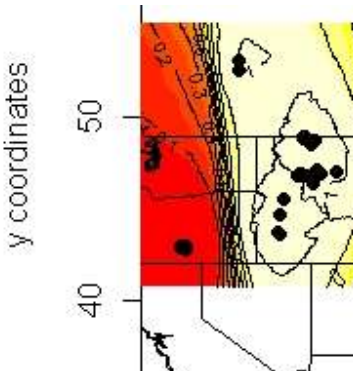
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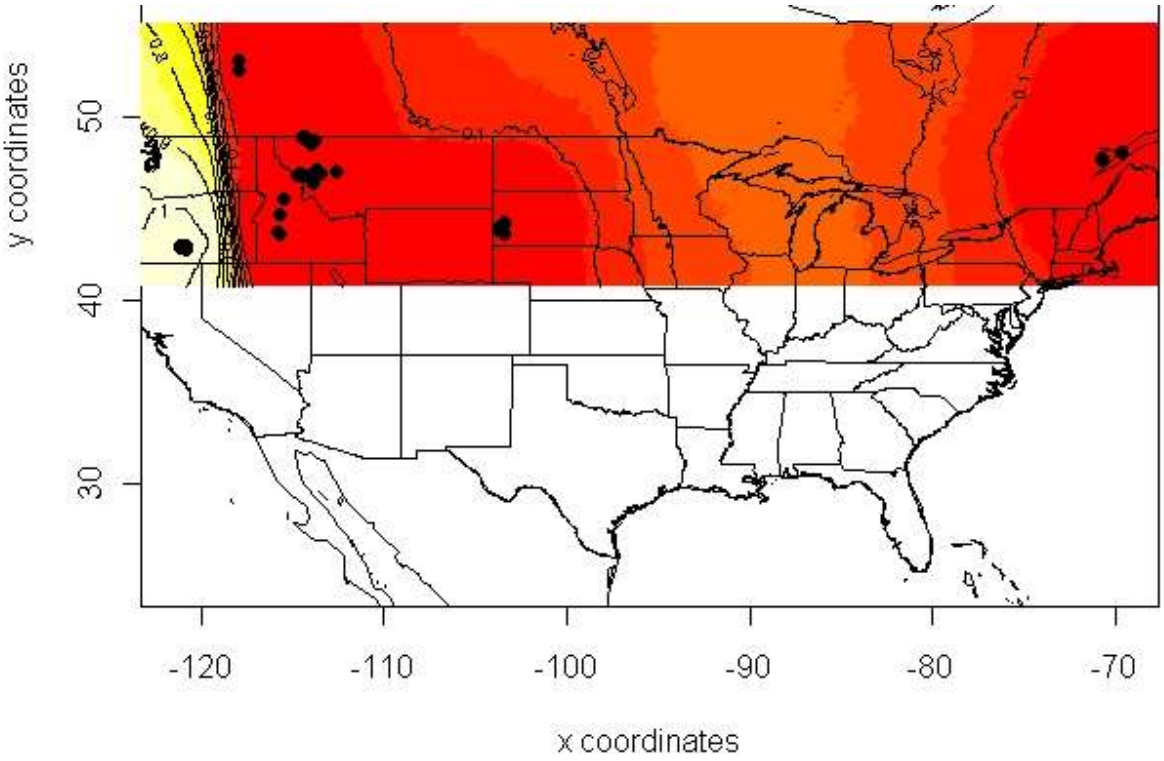
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c)

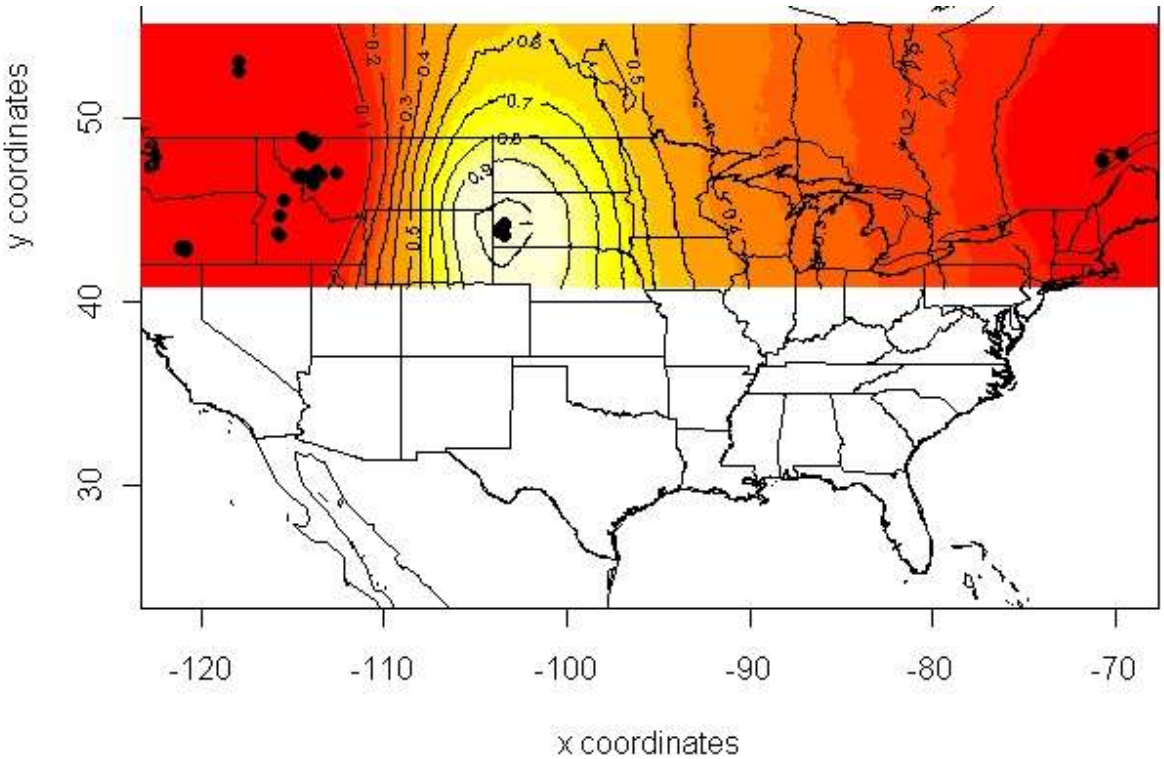

Figure 5. Maps showing the three clusters identified in the spatially explicit analysis conducted in GENELAND. The three clusters are a) western population, b) continuous population extending from the Rocky Mountains to Quebec, c) South Dakota population. The contours represent probability of assignment to the clusters and display where barriers to gene flow exist. The appearance of a partial barrier to gene flow within (a) is likely an artifact of the lack of samples between Alberta and Quebec given samples at each end of this cluster assign with a high probability to the same population.

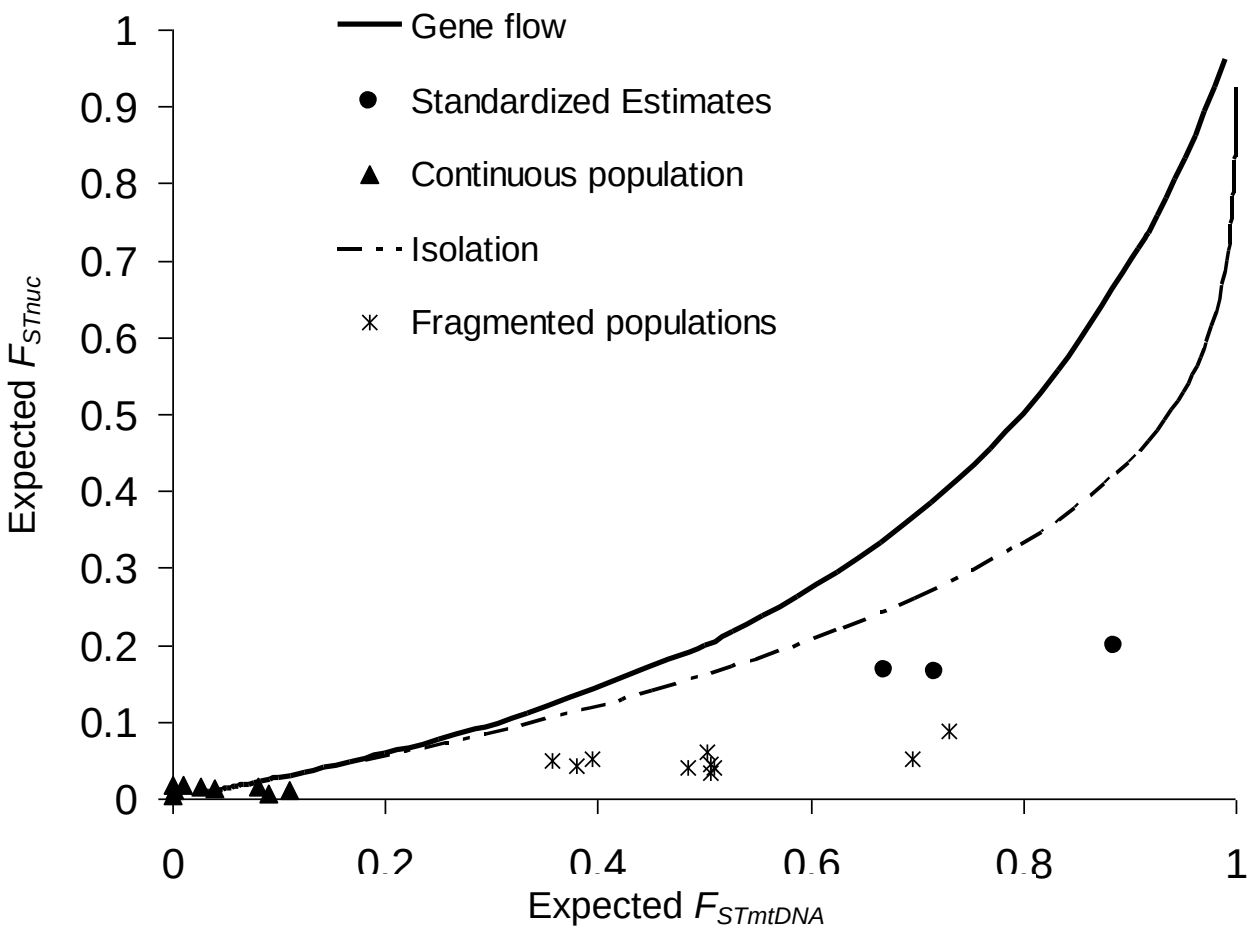

Figure 6. The expected relationship between F_{STnuc} and $F_{STmtDNA}$ at mutation-drift equilibrium under Wright's island model of migration (solid black line) and under a model of isolation (dashed line). Observed pairwise values of F_{STmsat} and $F_{STmtDNA}$ for black-backed woodpeckers are plotted; black triangles are sites within the continuous distribution, asterisks are pairwise values where at least one of the pair are in the fragmented sites and solid black circles are standardized estimates between the three populations inferred from both hierarchical population analyses and individual-level clustering in GENELAND.

Table 1. The primers, number of alleles, length range, annealing temperature and primer concentrations used in either multiplexed or single PCR reactions. Primer sequences can be found in the reference listed; 'GTTTCTT' was added to the 5' end of the reverse primer of RCW4 and RCW17 to promote the addition of adenine (Brownstein et al. 1996).

Locus	No. Alleles	Length	Annealing Temp.	Primer concentration	Reference
Multiplex 1					
RCW5	2	287-289	60-50 TD	2 µm	Haig and Mullins (in press?)
RCW17	9	258-280	60-50 TD	2 µm	Haig and Mullins (in press?)
DIU4	28	114-182	60-50 TD	2 µm	Ellegren et al. 1999
Multiplex 2					
HRU2	4	119-125	60-50 TD	0.75 µm	Primmer et al. 1996
C111	6	224-252	60-50 TD	0.75 µm	Vila et al. 2007
C115	12	271-295	60-50 TD	3 µm	Vila et al. 2007
D118	13	188-236	60-50 TD	1 µm	Vila et al. 2007
Single PCR					
RCW4	8	144-170	68-48 TD	2 µm	Haig and Mullins (in press?)
DIU3	8	139-153	58	2 µm	Ellegren et al. 1999
DIU1	4	142-148	58	2 µm	Ellegren et al. 1999
LOX4	4	150-156	58	2 µm	Piertney et al. 1998

Table 2. Analysis of molecular variance (AMOVA) results of four different groupings of black-backed woodpecker sampling sites for mtDNA and microsatellite loci. Significance values are based on 1000 permutations using ARLEQUIN 3.11. Results from spatial analysis of molecular variance (SAMOVA) are nearly identical and therefore, are not shown.

Group	No. of groups	Variance component	mtDNA % of variance	Microsatellites % of variance
(Rocky Mountains[1] + Quebec+ Oregon) (South Dakota)	2	Among groups	35.26	3.18
		Among sites	15.24**	2.22**
		Within sites	49.5**	94.61**
(Rocky Mountains[1] + Quebec) (Oregon) (South Dakota)	3	Among groups	49.99*	3.54*
		Among sites	1.07*	1.38**
		Within sites	48.95**	95.08**
(Rocky Mountains[1]) (Quebec) (Oregon) (South Dakota)	4	Among groups	37.51	2.33
		Among sites	1.4	1.27**
		Within sites	61.08**	96.4**
(Missoula + Glacier) (Idaho) (Alberta) (Quebec) (Oregon) (South Dakota)	6	Among groups	34.13	1.9
		Among sites	-1.03	1.18**
		Within sites	66.90**	96.93**

[1]Rocky Mountains = Idaho, Missoula, Glacier, Alberta; * P , 0.05; ** $P < 0.0001$

Table 3. Genetic diversity of mtDNA (325 bp cyt b) for all sampling locations, including the number of individuals sampled (n), number of haplotypes observed at each location, haplotype diversity (h), nucleotide diversity (π), haplotype richness (HR); standard errors are in parentheses.

	n	No. of haplotypes	h	π	HR
Idaho	42	6	0.616 (0.012)	0.004	4.57
Missoula	49	6	0.450 (0.012)	0.002	4.00
Glacier	48	7	0.457 (0.012)	0.002	4.12
Alberta	21	2	0.324 (0.024)	0.002	1.98
Quebec	56	12	0.589 (0.010)	0.002	5.58
Oregon	32	3	0.462 (0.013)	0.003	2.47
S. Dakota	27	2	0.074 (0.013)	0.001	1.55
All locations	275	18	0.613 (0.029)	.003	

Table 4. Genetic diversity of microsatellite data for all sampling locations including *n*, number of individuals sampled, F_{IS}, fixation index; H_E, expected heterozygosity; H_O, observed heterozygosity; NA, average number of alleles; AR, allelic richness; standard errors are in parentheses.

Sampling location	*n*	F_{IS}	H_E	H_O	NA	AR
Idaho	42	-0.01	0.58	0.59	6.18 (1.64)	5.46 (1.35)
Missoula	49	0.12	0.58	0.51	6.36 (1.48)	5.52 (1.18)
Glacier	48	0.01	0.58	0.58	6.36 (1.48)	5.69 (1.15)
Alberta	21	0.02	0.63	0.62	6.36 (1.30)	6.36 (1.30)
Quebec	56	0.05	0.60	0.57	6.91 (1.87)	5.76 (1.38)
Oregon	32	0.08	0.58	0.54	5.46 (0.96)	5.13 (0.90)
S. Dakota	27	0.01	0.46	0.46	3.64 (0.54)	3.57 (0.52)
All locations	275	0.05	0.60	0.55	8.91 (2.23)	6.03 (1.33)

Table 5. Pairwise F_{ST} values for mtDNA (below diagonal) and microsatellite (above diagonal). Significant values are indicated in bold and with asterisks

	Idaho	Missoula	Glacier	Alberta	Quebec	Oregon	S. Dakota
Idaho		**0.007*** **	**0.015** **	**0.022*** **	**0.019*** **	**0.048*** **	**0.057*** **
Missoula	0.000		**0.012*** **	**0.014*** **	**0.014*** **	**0.035*** **	**0.044*** **
Glacier	0.001	0.000		**0.012* **	**0.017*** **	**0.042*** **	**0.049*** **
Alberta	0.040	**0.092** **	**0.08*** **		0.006	**0.050*** **	**0.050*** **
Quebec	0.028	0.000	0.000	**0.11** **		**0.049*** **	**0.056*** **
Oregon	**0.38*** **	**0.51*** **	**0.51*** **	**0.36*** **	**0.54*** **		**0.094*** **
S. Dakota	**0.43*** **	**0.51*** **	**0.54*** **	**0.73*** **	**0.53*** **	**0.75*** **	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 6. Observed and standardized pairwise F_{ST} estimates between inferred populations ; mtDNA (below diagonal) and microsatellite (above diagonal).

Observed F_{ST}				Standardardized F_{ST}			
	Continuous	Oregon	S. Dakota		Continuous	Oregon	S. Dakota
Continuous		0.039	0.043	Continuous		0.165	0.167
Oregon	0.490		0.095	Oregon	0.716		0.200
S. Dakota	0.452	0.754		S. Dakota	0.669	0.885	

Funding

Proposed:

	Federal Share	Non-Federal Share	Totals
Direct Costs:	39,599.00	15,316.89	54,915.89
Indirect @16.04%:	6,351.68	0.00	6,351.68
Total:	45,950.68	15,316.89	61,267.57

Actual:

	Federal Share	Non-Federal Share	Totals
Direct Costs:	39,037.13	15,316.89	54,915.89
Indirect:	6,913.55	0.00	6,351.68
Total:	45,950.68	15,316.89	61,267.57

Variance is due to increased indirect rate over the period of the grant.

Project Personnel

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