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Genetic population structure and dispersal of two North American woodpeckers in ephemeral habitats

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GENETIC POPULATION STRUCTURE AND DISPERSAL OF TWO NORTH AMERICAN WOODPECKERS IN EPHEMERAL HABITATS

by

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Abstract

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Genetic population structure and dispersal of two North American woodpeckers in ephemeral habitats

Co-Chairs: Fred W. Allendorf, Michael K. Schwartz

Disturbance-dependent species regularly colonize ephemeral habitat patches. In this research, I used patterns of genetic variation to estimate the dispersal dynamics of black-backed woodpeckers (*Picoides arcticus*), a fire specialist, and compared these patterns to hairy woodpeckers (*Picoides villosus*), a generalist. I then examined how frequent colonization of ephemeral habitat patches versus stable migration among static habitat patches shapes the genetic structure of species.

I examined patterns of genetic variation in mtDNA and microsatellites in both black-backed and hairy woodpeckers to determine large-scale spatial structure. Black-backed woodpeckers have high genetic connectivity across the boreal forest and lower genetic connectivity among sites separated by large gaps in forested habitat. Across the boreal forest, hairy woodpeckers have low genetic differentiation in mtDNA that lacks spatial structure, but moderate genetic differentiation in an isolation by distance pattern in microsatellite data. These results suggest that large gaps in forest act as a movement barrier to black-backed woodpeckers; movement patterns of hairy woodpeckers are primarily driven by geographic distance as opposed to landscape composition.

Once I understood the primary mechanisms driving large-scale patterns, I determined the fine-scale spatial structure in both species. Black-backed woodpeckers apparently disperse twice as far as hairy woodpeckers based on patterns of fine-scale geneticstructure. Female black-backed woodpeckers have limited dispersal, with long-distance dispersal being male-biased. A weak pattern of female-biased dispersal was observed in hairy woodpeckers.

I used simulations to evaluate how effective population size and dispersal distance interact with two models of dispersal, frequent colonization of ephemeral patches and stable migration, to shape large-scale genetic structure. Frequent colonization of ephemeral habitats resulted in lower spatial structure and higher genetic differentiation among patches in comparison to stable migration. Low genetic differentiation with little spatial structure occurred at an intermediate dispersal distance in the frequent colonization model, the pattern observed in black-backed woodpeckers. Stable migration with short dispersal distance results in isolation by distance, the pattern observed in hairy woodpeckers.

Disturbance-dependent species have evolved with a natural mosaic of shifting habitat patches. As anthropogenic disturbance increasingly changes this mosaic, ecologists need to consider how this shift may affect connectivity for disturbance-dependent species.

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Chapter 1 - Introduction

Until the recent past, the role of natural disturbance in creating habitat has been underappreciated (Hutto 2008). Historically, wildfire has been a dominant disturbance that shaped western landscapes in North America. As such, many species are adapted to postfire habitat. The role of fire in creating habitat for numerous avian species, such as certain woodpeckers, flycatchers and ground-foraging birds in western North America has been fairly well documented (Hutto 1995, Brawn et al. 2001). However, a more complete understanding of population dynamics and movement patterns of fire-associated species is necessary to take an ecosystem management approach to postfire ecosystems.

An important element of population dynamics is dispersal or gene flow, which is also referred to as migration in the genetics literature. The distribution of genetic variation in space is an important factor to consider in the management and conservation of a species (Torres et al. 2003). Both theory and empirical evidence have shown that habitat patches connected by very little movement will be highly differentiated. Conversely, habitat patches with high levels of gene flow among them will show very little genetic differentiation. However, it is not known what patterns of genetic population structure will look like when organisms are continually colonizing new patches as opposed to, say, seasonal movement back and forth between patches.

My dissertation research examines how the frequent colonization of ephemeral habitat patches shapes the genetic structure of species. I combine empirical data collected on species that use ephemeral habitat patches and simulations to understand the dispersal dynamics of disturbance-dependent species. The empirical component of this thesis focused on black-backed woodpeckers (*Picoides arcticus*), a fire specialist, compared to hairy woodpeckers (*Picoides villosus*), a species that exploits burned forests but also occupies a variety of forest habitats.

From a theoretical perspective, we can learn a great deal by contrasting the genetic structure of closely related species that differ in specific life history characteristics of interest (McDonald et al. 1999, Whiteley et al. 2004).

Black-backed woodpeckers are a fire specialist that has been in the spotlight of many land management agency efforts and the target of several litigation efforts surrounding land use. Until recently, black-backed woodpeckers were considered the management indicator species for postfire habitat and as such were designated a Sensitive Species by the U.S. Forest Service. Black-backed woodpeckers are classified as a species of conservation concern in most western states in which they are present (Montana Fish, Wildlife and Parks 2005, Oregon Department of Fish and Wildlife 2005, South Dakota Natural Heritage Program). Black-backed and hairy woodpeckers are broadly sympatric in the northern part of their range. However, on a fine-scale they occur closely together only in burned areas. Both woodpeckers share a wide overlap in many habitat selection characteristics for nesting and foraging resources (Murphy and Lehnhausen 1998, Saab et al. 2007, Vierling et al. 2008, Saab et al. 2009). Thus, studying both species simultaneously can inform us regarding the differences in the dispersal dynamics of a fire specialist compared to a generalist.

1.1 Research objectives and findings

Large-scale genetic structure of black-backed woodpeckers

The objective of chapter 2 was to define population structure and movement patterns of male and female black-backed woodpeckers. Black-backed woodpeckers colonize postfire habitats within one year after a fire and occupy burned forests in peak numbers for approximately three to five years (Saab et al. 2007). The majority of their range is the boreal forest which consists of fairly

continuously distributed forested habitat with fire as an important disturbance shaping landscape patterns (Cyr et al. 2009). Black-backed woodpeckers distribution extends south into Oregon and California, and a small area in the Black Hills of South Dakota; both areas are isolated from the boreal forest by large non-forested habitat. These large gaps in habitat, while physically close to the main distribution, may act as barriers to woodpecker movement. To determine the movement patterns of black-backed woodpeckers across North America, I estimated their landscape-scale genetic structure using both mtDNA (325 bp *cyt b*) and nine nuclear microsatellite loci. The following specific research questions were addressed:

- What is the large-scale genetic structure of black-backed woodpeckers?
- Do large gaps in forested habitat act as barriers to movement?
- Do males and female woodpeckers respond to potential movement barriers equally?

At a large spatial scale, I found high genetic connectivity across the boreal forest; there was low genetic differentiation among sites within the boreal forest. Sampling locations outside the boreal forest (Oregon, S. Dakota) were more genetically differentiated than locations within the boreal forest, and hence had lower genetic connectivity to sites within the boreal forest. Both mtDNA and microsatellite data shared the same pattern of genetic differentiation. These results suggest that non-forested habitat acts as a barrier to movement for black-backed woodpeckers. Males and females appear to respond differently to these barriers. Non-forested habitat may be a more resistant landscape than forested habitat for males, with less movement among sites separated by gaps in forest compared to sites with forest between them. Females appear to cross non-forested habitat less than males, with large gaps in forest acting as a barrier to movement. This Chapter is currently in press in *Evolutionary Applications* entitled 'Do male and female woodpeckers respond differently to gaps in habitat?' coauthored with Fred. W. Allendorf, Pierre

Drapeau who contributed samples from Quebec and expertise on the ecology of woodpeckers, Victoria Saab who collaborated on field sites in Oregon and Idaho as well as contributing expertise on the ecology of woodpeckers and Michael K. Schwartz.

Large-scale genetic structure of hairy woodpeckers

The objective of Chapter 3 was to determine the large-scale genetic structure of hairy woodpeckers and compare it to the large-scale genetic structure of black-backed woodpeckers. The hairy woodpecker is a widely distributed species that exploits burned forests for their plentiful resources, but also occupies a variety of forest habitats. Despite a large overlap in resource selection for both nesting and foraging resources (Murphy and Lehnhausen 1998, Saab et al. 2009), black-backed woodpeckers are nearly restricted to burned forests in the Rocky Mountain region (Hutto 2008) and occupy unburned forests in low density in the boreal forest (Nappi and Drapeau 2009). In chapter 3, I found that non-forested habitat acted as a barrier to movement for black-backed woodpeckers. I tested the hypothesis that the hairy woodpecker, as a generalist species, is less sensitive than the black-backed woodpecker, a specialist, to habitat heterogeneity in the landscape matrix. I used both mtDNA (319 bp *cyt b*) and eight nuclear microsatellite loci to estimate the large-scale genetic structure of hairy woodpeckers. The following specific research questions were addressed

- What is the large-scale genetic structure of the hairy woodpecker across northern North America?
- Do hairy and black-backed woodpeckers have similar patterns of genetic structure across the same spatial scale?

Mitochondrial DNA and microsatellite data had different patterns of large-scale spatial genetic structure in the hairy woodpecker. Patterns of genetic differentiation based on mtDNA were similar to patterns observed in the black-backed woodpecker, but likely are a result of different evolutionary forces. Hairy woodpeckers have low genetic differentiation among sites within the boreal forest as a result of a single common shared haplotype, suggesting that an ancestral polymorphism is responsible. Low genetic differentiaton across the boreal forest in black-backed woodpeckers is likely due to gene flow as several haplotypes are shared among sites. Based on microsatellite data, hairy woodpeckers have a strong signal of isolation by distance. This pattern suggests geographic distance is the primary driver of genetic structure as opposed to habitat characteristics of the landscape matrix. This differs from black-backed woodpeckers, where microsatellite data support a pattern of low genetic differentiation across vast distances in the boreal forest (3500 km) and higher genetic differentiation among sites that are more geographically proximate but separated by non-forested habitat. As a habitat generalist, hairy woodpeckers appear to be less sensitive than black-backed woodpeckers, a specialist, to habitat heterogeneity in the landscape matrix. This Chapter is being submitted to the Auk, with coauthors Fred W. Allendorf, Michael K Schwartz and Victoria Saab.

Fine-scale genetic structure of black-backed and hairy woodpeckers

The objective of Chapter 4 was to determine the dispersal patterns and resulting fine-scale genetic structure of black-backed and hairy woodpeckers. Several studies have suggested that burned forests act as source habitats for black-backed woodpeckers (Hutto 1995, Nappi and Drapeau 2009). Specific data on dispersal distance is required to determine the scale at which burned forests may act as source habitats by providing emigrants. I used spatial autocorrelation

analyses based on nine microsatellite loci in the black-backed woodpecker and eight microsatellite loci for the hairy woodpecker to estimate fine-scale structure. The comparison between two closely related species that differ in their reliance on burned habitat allowed me to make the following predictions: 1) black-backed woodpeckers disperse farther than hairy woodpeckers resulting in fine-scale genetic structure at a larger scale; 2) a signature of kin groups within burned areas would occur for both species if burned habitat is acting as source habitat due to delayed juvenile dispersal which would likely increase juvenile survival due to a higher habitat quality during the transition from juvenile to adult. The following specific research questions were addressed:

- Given frequent colonization events, is there fine-scale genetic structure in black-backed and hairy woodpeckers?
- Do male and females have the same patterns of fine-scale genetic structure in both species?
- Are there family groups within burned areas as a result of delayed juvenile dispersal?

I found a strong pattern of fine-scale genetic structure in both black-backed and hairy woodpeckers. As predicted, black-backed woodpeckers had positive spatial genetic structure at a larger spatial scale (90 km) than hairy woodpeckers (45 km), likely as a result of longer dispersal distances. Sex-specific analyses in black-backed woodpeckers supported a pattern of male-biased dispersal and suggested female-biased dispersal in hairy woodpeckers. A temporal increase in genetic correlation was detected within burned patches for black-backed woodpeckers, but not hairy woodpeckers, supporting the hypothesis that juvenile black-backed woodpeckers may delay dispersal to exploit plentiful resources. This Chapter is being submitted to Molecular Ecology with coauthors Fred W. Allendorf, Michael Schwartz, and Pierre Drapeau

(who contributed samples from Quebec and expertise on source-sink dynamics in black-backed woodpeckers).

Frequent colonization effects on genetic population structure

The objective of Chapter 5 was to determine how frequent colonization of ephemeral habitats shapes large-scale genetic structure. In this chapter, I use life history parameters similar to a fire specialist and a generalist to simulate frequent colonization of newly created habitat patches compared to stable migration among static habitat patches. The simulation results are then used to interpret the empirical genetic structure of these species. The following specific questions were addressed:

- How does effective population size (N_e) influence patterns of spatial genetic structure in a model of frequent colonization compared to stable migration?
- How does dispersal distance influence patterns of spatial genetic structure in a model of frequent colonization compared to stable migration?
- Can theoretical expectations from the simulation model explain the empirical results observed in black-backed and hairy woodpeckers?

In general, the frequent colonization model resulted in higher genetic differentiation among subpopulations with less spatial structure than the stable migration model. Genetic drift had a stronger influence in the frequent colonization model due to repeated founder events of new subpopulations as opposed to migration among established subpopulations. At larger dispersal distances, the effect of founder events was reduced because a larger number of subpopulations contributed emigrants for colonization of new patches. The black-backed woodpecker has low genetic differentiation that is not spatially structured in the boreal forest.

The hairy woodpecker has similar estimates of genetic differentiation, with a strong pattern of isolation by distance. The simulation results indicate that frequent colonization of ephemeral habitat by a species with intermediate dispersal distance can produce the pattern observed in the black-backed woodpecker.

ECOS Fellowship

I was supported by a NSF funded ECOS (Ecologists, Educators and Schools) fellowship during the 2004-2005 academic year. The ECOS program paired a team of graduate and undergraduate students in biology with teachers in local K-12 schools to conduct hands-on science in 'outdoor classrooms'. Fellow graduate student Andrew Whiteley and undergraduate Frank Janes and I were paired with Dave Oberbillig and Kathleen Kennedy at Big Sky High School. Our responsibilities included developing and leading outdoor ecological investigations for eight high school classes (~200 students) on a biweekly basis. Our tenure culminated in a large-scale prescribed fire experiment that tested the effects of two types fuel augmentation on a variety of response variables such as the plant and insect community. This project successfully taught students about the scientific process by having them develop and participate in a field experiment.

In Appendix B, I describe the demonstration project, where we conducted an experimental prescribed burn in a field dominated by invasive weeds. We had three main goals in conducting our experiment: 1) teach the process of science using hands-on learning, 2) teach field ecology as science and 3) use locally relevant issues to engage students. The project focused on two primary ecological themes: disturbance and invasive organisms, both of which are extremely relevant locally because residents often burn fields to reduce invasive weeds.

Montana Department of Natural Resources Fire Department graciously volunteered their equipment and professional fire fighters to conduct the burn. Students collected pre- and post-treatment that addressed eight different questions that ranged from the effect of fire on insects to plant biomass. The students were able to actively engage in the design of this experiment including development of questions through examination of results, thereby directly engaging in the process of science as a way of learning as opposed to simple knowledge gained from a textbook. An inquiry entitled 'Classroom mark-recapture with crickets' was published in American Biology Teacher, coauthored by Andrew Whiteley, Kathleen Kennedy, Dave Oberbillig and Carol Brewer.

1.2 Synthesis and significance

The boreal forest and forests in the western U.S. evolved with fire creating a mosaic of different aged patches of forest in a constantly changing pattern (Hutto 2008, Cyr et al. 2009). The dynamic nature of ephemeral habitats makes it challenging to understand habitat connectivity and resulting metapopulation structure among habitat patches. This research has shown that fire-adapted species disperse farther than generalist species, but still have limited dispersal. My modeling exercise demonstrates that frequent colonization of new patches provides a mechanism to explain these empirical results. Specifically, it showed that frequent colonization of ephemeral habitat patches can result in low genetic differentiation that is not spatially structured across large spatial scales.

Microsatellite and mtDNA data suggest black-backed woodpeckers have had high genetic connectivity across the boreal forest for a long time period. The social change from fire to timber harvest as the primary disturbance is a concern for fire-adapted species for several reasons

(Belleau et al. 2007). Timber harvest removes the nesting and foraging resources that many fire-associated species (including woodpeckers and wood-boring beetles) exploit in postfire stands. Additionally, this ecological change is shifting the spatial context of disturbance (Cyr et al. 2009) which may affect habitat connectivity for species that have adapted to the natural mosaic.

My data suggest that female black-backed woodpeckers do not cross large gaps in forested habitat and that these gaps also act as a higher resistance landscape to long-distance dispersal for male black-backed woodpeckers. Therefore, small isolated populations, such as the one in South Dakota, may deserve higher priority for conservation. For example, land management actions that affect black-backed woodpecker habitat, such as salvage logging, should be considered in a spatial and temporal context because fewer habitat patches are available to birds in South Dakota. Furthermore, biologists cannot rely on dispersal from the boreal forest to rescue these isolated populations. Within the boreal forest, management actions should strive to maintain forested connectivity between burned patches to maintain gene flow.

My data suggest that changes in the landscape matrix are likely more detrimental to specialists than to habitat generalists. While large gaps in forest appear to be a barrier to black-backed woodpeckers, for hairy woodpeckers, dispersal patterns appear to be driven mainly by geographic distance regardless of the habitat heterogeneity in the matrix. This supports previous experimental research on model systems that suggesting that habitat specialists may be more sensitive to crossing suboptimal habitats, especially over large distances (Haddad 1999).

From an ecological and evolutionary perspective my results are important because I show that species dependent on ephemeral habitat do not necessarily disperse unlimited distances to colonize new habitat patches. Species adapted to early successional habitats have evolved with natural disturbance regimes that have created spatial and temporal patterns of habitat. As

anthropogenic disturbance increasingly changes this mosaic, ecologists will need to consider how this shift may affect connectivity for disturbance-dependent species.

Chapter 2 - Do male and female black-backed woodpeckers respond differently to gaps in habitat?

2.1Abstract

We used population and individual-based genetic approaches to assess barriers to movement in black-backed woodpeckers (*Picoides arcticus*), a fire specialist that mainly occupies the boreal forest in North America. We tested if male and female woodpeckers exhibited the same movement patterns using both spatially implicit and explicit genetic analyses to define population structure and movement patterns of both sexes among populations. Three genetic groups were 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 (Oregon). Patterns of genetic diversity suggest extensive 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 continuously distributed group and the smaller populations of South Dakota and Oregon that are separated by large areas of unforested habitat, which apparently serves as a behavioral barrier to movement of female woodpeckers.

2.2 Introduction

Dispersal is a central process of interest in evolution and ecology, yet many aspects of dispersal are poorly understood. The movement of individuals and their genes has a 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). 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, 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 et al. 1997). However, Harris and Reed (2002) found ecotones, habitat gaps and large water bodies are common behavioral barriers to non-migratory movements of birds. They predict that birds that are habitat specialists, forest understory species, tropical species, solitary species, and non-migratory species can be sensitive to habitat gaps (Harris and Reed 2002).

Woodpeckers are an excellent family to test predictions regarding behavioral barriers to movement because many are non-migratory, habitat specialists, and they are often solitary. Woodpeckers are typically 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), including patterns of sex-biased dispersal. Sex-biased dispersal, where one sex is philopatric or one sex typically disperses more often and/or farther than the other, 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). Female-biased dispersal can be seen in both red-cockaded (*Picoides borealis*; Daniels and Walters 2000) and acorn woodpeckers (*Melanerpes formicivorus*; Hannon et al. 1985), which are cooperative breeders with males that tend to stay in the natal territory as helpers.

Dispersal is difficult 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). Genetic techniques can be used to estimate successful movement that results in reproduction and thus gene flow. Few studies have used genetic techniques to assess movement patterns in woodpeckers (Ellegren et al. 1999), a taxon with 214 recognized species in the family Picidae (Winkler et al. 1995).

My study focused on black-backed woodpeckers (*Picoides arcticus*), a fire-dependent species. This species colonizes burned areas within one year after a fire, occupies burned areas for three to five years, with peak densities three years after fire (Caton 1996; Saab et al. 2007). 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 a habitat patch 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. They have been documented making long-distance

movements during irruptions outside their normal breeding ranges (Yunick 1985), indicating long-distance movements are physiologically possible. Given black-backed woodpeckers occupy ephemeral habitats (Dixon and Saab 2000; Saab et al. 2009), both sexes regularly disperse during the course of their lifetime (Huot and Ibarzabal 2006). The objective of our study was to test 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.

2.3 Methods

2.3.1 Sampling and DNA extraction

Blood or feather samples were collected in seven sampling locations: Alberta, Idaho, Oregon, west-central (W.C.) Montana, northwest (N.W.) Montana, South Dakota, and 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. Blood samples were stored at room temperature in a lysis buffer (Longmire et al. 1988). 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 in 1998-2000 (Dudley and Saab 2007); Quebec samples were collected in 2000-2001. The latitude and longitude of individual sample locations was recorded. 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.

2.3.2 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 Sequenase 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 personal communication); *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). Eight loci were analyzed in three multiplex reactions (Supplementary 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.), 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 in 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. We reanalyzed any samples with discrepancies in scoring of alleles to confirm the correct genotype.

2.3.3 Genetic variation

Microsatellite markers were tested for departure from Hardy-Weinberg (H-W) 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, where the number of alleles is standardized to the smallest sample size, 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) and 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) haplotypes with replacement from the frequency distribution of haplotypes created by sampling 10,000 times.

2.3.4 Population-based analyses

We calculated pairwise F_{ST} (Weir and Cockerham 1984) among all sampling locations and 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; ARLEQUIN 3.11; Excoffier et al. 2005) and a spatial analysis of molecular variance (SAMOVA; Dupanloup et al. 2002) for both marker types. We tested five different hierarchical groupings (Table 1) and tested for significance of the variance components using 1000 permutations. Populations were identified by maximizing the among group percent of variation (F_{CT}) as recommended by Dupanloup et al. (2002). We used principal component analysis (PCA) to visualize how sample sites clustered using PCAGEN (http://www2.unil.ch/popgen/softwares/pcagen.htm).

2.3.5 Individual-based analyses

We 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-Weinberg proportions. 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), a spatially explicit approach which can infer spatial discontinuities in genetic data when incorporating the spatial location of individual samples as well as a user-defined uncertainty around sampling locations.

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).

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 in the Poisson-Voronoi tessellation set to 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 (which has been shown to perform better than alternate models available in GENELAND; Guillot et al. 2005a) in which allele frequencies are assumed to be independent, spatial coordinates with an uncertainty of 5 km.

To test the robustness of our GENELAND 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.

2.3.6 Phylogeographic analyses

We used several approaches to assess historical versus contemporary processes, which can be difficult to separate when interpreting sequence data (Edwards and Bensch 2009). To visualize the relationship among mtDNA haplotypes, we used NETWORK 4.5 (http://www.**fluxus**-engineering.com) to create a median-joining network. We estimated Tajima's D and Fu's F to test whether patterns of sequence divergenece in mtDNA followed the pattern expected under a model of neutral evolution. We calculated both metrics in DnaSP and significance was tested using coalescent simulations (Rozas et al. 2003). Departures from neutrality in these metrics are often interpreted as evidence for population expansion (Tajima 1989; Fu 1997).

2.3.7 Sex-biased movement patterns

Sex-biased movements can be estimated by using highly variable autosomal markers, such as microsatellites, to compare estimates of population structure between males and females or differences in assignment indices between males and females. Methods based on autosomal markers require the sampling of recent immigrants or that bias in dispersal is strong (Goudet et

al. 2002). When dispersal rates are greater than 10%, the metric that performs best is the comparison of pairwise F_{ST} estimates between males and females (Goudet et al. 2002). Sex-biased dispersal should result in pairwise F_{ST} estimates that are lower in the dispersing sex (Goudet et al. 2002). We estimated F_{ST} among the inferred populations for each sex separately because rates of dispersal in black-backed woodpeckers are likely much greater than 10% given the ephemeral nature of their habitat.

Another method to estimate sex-biased dispersal is to examine different patterns of genetic structure in sex-linked or sex-transmitted markers (i.e., mtDNA) compared to autosomal markers. Estimates of F_{ST} based on microsatellite markers can be biased low due to their highly variable nature (Hedrick 2005a). To account for this potential bias, we calculated standardized estimates of pairwise estimates of F_{ST} (G_{ST}') for both marker types (Hedrick 2005a; 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 2005a; 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).

2.4 Results

2.4.1 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 two in South Dakota to 12 in Quebec (Table 2). Haplotype diversity (h) and nucleotide diversity (π) were highest in Idaho (h = 0.616, π = .0035) and lowest in South Dakota (h = 0.095, π = 0.0006). One haplotype was

very common (> 60%), a second was relatively common (16%), and eight haplotypes were only detected once (Figure 1; Table 3).

Ten of the eleven microsatellites were polymorphic in all the populations; Locus *DIU1* was monomorphic in South Dakota. After correcting for multiple comparisons (Rice 1989), two loci had significant departures from H-W proportions, *DIU1* and *RCW17*; 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). South Dakota had the lowest levels of heterozygosity (H_O = 0.46), other sites ranged from (0.51-0.62; Table 2).

Null alleles were apparently present at three loci: *DIU1*, *RCW17* and *C111*. Both *DIU1* and *RCW17* had relatively high estimated frequencies of null alleles (0.20, 0.15) while the estimated frequency of the null allele at *C111* occurred at a relatively low frequency (0.06). We conducted most analyses on both a full and reduced data set, with the same general pattern resulting from both datasets; most results presented are from the dataset with nine loci, after removing *DIU1* and *RCW17* (Chapuis and Estoup 2007). GENELAND results are from the full dataset because the algorithm implemented can estimate frequencies of null alleles.

2.4.2 Population-based analyses

Samples collected from sites within the continuously distributed areas had lower pairwise F_{ST} values for both mtDNA and microsatellite data (Table 4). 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 were much lower than mtDNA, with values among the continuous sites ranging from 0.006 – 0.022 and from 0.035-0.094 among the

fragmented sites. The grouping of sites within the continuously distributed locations as one population was supported by AMOVA (Table 1), SAMOVA, and PCA (Figure 2). Due to similar results between the AMOVA and SAMOVA, we only present AMOVA results. The hierarchical grouping of the Rocky Mountain sites (Glacier, Missoula, Idaho, Alberta) with Quebec, with Oregon and South Dakota as separate groups, explained the most variation among groups for both mtDNA and nuclear DNA (Table 1). PCA reveals that, for both marker types, all sites within the continuously distributed area cluster tightly together and Oregon and South Dakota cluster 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, the lack of isolation by distance is driven mostly by the absence of a correlation between geographic and genetic distance among sites within the boreal forest (Rocky Mountains and Quebec).

2.4.3 Individual-based analyses

Both STRUCTURE and a spatially implicit approach in GENELAND identified one population cluster ($K = 1$). We focus on results from GENELAND due to the similarity between the results found from STRUCTURE and the spatially implicit option available in GENELAND. When a spatially explicit approach was used, GENELAND identified three populations ($K=3$), with all ten runs identifying $K= 3$ with the highest probability (Figure 3). Individuals assigned to populations with a high probability, with only six individuals ambiguously assigned with probability of assignment =>0.99 (Figure 4). Geographic barriers to gene flow were identified with probability of assignment contours (Figure 4).

2.4.4 Phylogeographic analyses

The haplotype network displayed a star-like pattern, which is often attributed to a recent population expansion (Avise 2004; Figure 5). However, eight of 13 rare (<0.055%) haplotypes that created this pattern were found in Quebec. Accordingly, only Quebec showed a significant departure from expectations from a neutral model of evolution (Tajima 1989; Fu 1997; Table 2; Figure 5). Nucleotide diversity among haplotypes was low with one haplotype common to populations within the boreal forest and all other haplotypes differing by few nucleotides (Figure 5, Table 3). The most common haplotypes in Oregon and South Dakota differed from the most common haplotype within the boreal forest, but were present in the boreal forest population. Locations across the boreal forest population shared several common haplotypes (28%: Hap 1, 2, 5, 6, 8; Figure 5, Table 3).

2.4.5 Sex-biased movement patterns

Pairwise F_{ST} estimates between the continuous population found across the boreal forest (boreal forest) and the fragmented sites (Oregon and S. Dakota) were consistently lower for males compared to females (Table 5b), a pattern indicating males are dispersing at a higher rate than females. Pairwise F_{ST} estimates for microsatellite data between the continuous and fragmented sites were 4 – 5 times lower than would be predicted 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 boreal forest 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 boreal forest population, the expected $F_{ST(msat)} = 0.39$, observed standardized $F_{ST(msat)} = 0.17$ (Table 5a).

2.5 Discussion

We found evidence that large gaps among forested sites apparently act as 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 outside their normal distribution into areas south of the boreal forest (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.

Shared ancestry and current gene flow can result in similar patterns of genetic structure, especially in species that have recently colonized areas and undergone population expansion. The star-like phylogeny of mtDNA variation (Figure 5) suggests that black-backed woodpeckers have undergone a fairly recent population expansion. However, private haplotypes in Quebec are a major contributor to this pattern. Samples collected in Quebec have significant departures from a neutral model of evolution (Table 2); since this location is the only one to have significant departures from neutrality, it is unlikely that mtDNA is under selection in this site. A more probable explanation is a recent population expansion in the eastern boreal forest (Fu 1997). The western portion of the boreal forest population shows a pattern of high haplotype diversity in the

south (Idaho) that decreases in a northerly direction, indicating a south to north pattern of colonization. High genetic diversity on the east (Quebec, h= 0.589) and west side (Idaho, h= 0.616) of the boreal forest population and the sharing of all common haplotypes (Table 3), as opposed to a single common haplotype, suggest that current gene flow may be responsible for the weak genetic structure observed. Lower genetic diversity within both fragmented populations (Oregon, h = 0.462; S. Dakota, h = 0.074) based on a subset of haplotypes found in the boreal forest suggest shared ancestry without much current gene flow.

Geographical features such as mountain ranges do not appear to create physical barriers to movement given gene flow across the Continental Divide within the Rocky Mountains is the most likely explanation for the weak genetic structure observed. The complete lack of genetic structure for both microsatellite and mtDNA markers across a vast distance (~3500 Km) in the Canadian boreal forest suggest both males and females are dispersing equally when there is continuously distributed habitat.

2.5.1 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 2009; Frantz et al. 2009).

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 (GENELAND). All of the approaches except the spatially implicit individual-based clustering methods defined the same three populations, a large, genetically continuous population (boreal forest) and two fragmented populations (Oregon and South Dakota).

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 highly consistent. Two individuals were assigned to more than one population, but they both assigned to the "correct" population with a probability > 0.99.

2.5.2 Behavioral barriers to movement

A recent review of patterns of genetic structure in seabirds found that areas between their breeding and nonbreeding distribution indicated potential barriers to dispersal (Friesen et al 2007), a similar pattern to what we found for female black-backed woodpeckers. However, black-backed woodpeckers' distribution closely follows the distribution of the boreal forest. There are large areas without contiguous forested habitat between the boreal forest population, the Cascade region, and the Black Hills of South Dakota. These gaps in forested habitat are likely the ultimate cause of the limited gene flow across these geographic regions.

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 without forest between them (fragmented: Oregon and South Dakota) as compared to sites that have forest between them (boreal forest: 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 (Allendorf and Luikart 2007). When we considered three groups: boreal forest, Oregon, and S. Dakota, a large amount of genetic variation was explained among groups and almost no genetic variation was explained among sites within group (Table 1). When we included Oregon with the boreal forest group, the variation among sites increased 15 –fold, confirming a barrier likely exists between Oregon and the boreal forest. Additional evidence can be seen in the high pairwise $F_{STmtDNA}$ values (0.36 – 0.75) between sites with large gaps in forest between them. These values are similar to those documented among subspecies or separate clades in other birds occupying similar ranges (Gibbs et al. 2000; Mila et al. 2007).

The inclusion of spatial data in GENELAND identified the general location of barriers to gene flow among the three populations (Figure 4). Sharp discontinuities in gene flow match the break in large forested areas between the Rocky Mountains and Oregon and the Rocky Mountains and South Dakota. However, the lack of samples in the boreal forest between Alberta and Quebec does not allow GENELAND to do a good job of assessing connectivity 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. 2009). Organisms usually avoid dispersing through certain habitat types to avoid predators or a lack of resources during travel (Bélisle and Desrochers 2002). The risk of predation and the amount of resources available for foraging will be more similar in burned and live forests than between any forest type and non-forest type (e.g., grassland, etc.). Non-forested habitat may be somewhat of a physical barrier to

movement as it does not provide foraging or roosting resources. Yet, historic records show black-backed woodpeckers traveling far south of their normal distribution in the eastern boreal forest and arriving in areas such as Ohio and Illinois, which would require crossing large expanses of non-forested habitat (Yunick 1985).

2.5.3 Sex-biased movement patterns

Males apparently successfully travel long distances across inhospitable habitat at a much higher rate than females. We propose this because pairwise estimates of F_{STmale} are lower than $F_{STfemale}$ and the F_{STnuc} are lower than $F_{STmtDNA}$ (Table 5). Dispersal rates between males and females must differ substantially to detect differences using microsatellite markers (Goudet et al. 2002). Pairwise estimates of $F_{STfemale}$ were 1.3 – 2.1 times higher than F_{STmale} (Table 5b), a considerable difference given these methods have a difficult time detecting differential dispersal rates between sexes.

We plotted the expected pairwise values of F_{STnuc} versus $F_{STmtDNA}$ under Wright's island model of migration at equilibrium and under a model of complete 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. N_e > 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 ($N_e \approx$ 10,000; Milot et al. 2000) and downy woodpeckers, *P. pubescens* ($N_{e(f)} \approx$ 6,500; Ball and Avise 1992). Conversely, the pairwise values of 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 heterozygosity when using microsatellite markers.

2.5.4 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 dispersal rates of males and females. We will discuss the likelihood of these possibilities below.

An ice sheet covered most of the boreal forest until approximately 10,000 years ago (Hewitt 2000). Consequently, most habitat currently occupied by black-backed woodpeckers was likely colonized within the last 10,000 years (Hewitt 2000). 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 was observed. The population in South Dakota is likely quite small (low N_e), given black-backed woodpeckers only occupy the Black Hills, a relatively small area (15,500 km^2) and they are a rare bird that occupies large territories (50 – 700 ha; Dixon and Saab 2000; Dudley and Saab 2007). 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. In fact, these sites are the farthest from predicted values (Figure 6). An alternative reason for the pattern observed in South Dakota could be lack of equilibrium due to a recent bottleneck. While this is plausible, it does not explain why the

largest population of black-backed woodpeckers (boreal forest) follows the expected pattern at equilibrium and the two fragmented sites (Oregon and S. Dakota) fall far from expectations.

Homoplasy occurs when two alleles in a population are identical in state but have different origins. Homoplasy is common in microsatellites because they often follow a step-wise mutation model in which each mutation is 'one step' or repeat different. Homoplasy can cause two populations to appear more genetically similar than they are because they may share the same alleles from different origins. While homoplasy is certainly common in microsatellite markers and can prevent F_{STnuc} from going to fixation ($F_{STnuc} = 1.0$), it is unlikely to cause as severe an underestimate in F_{STnuc} as we observed. More importantly, 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 downward due to the large number of alleles per locus and high amount of heterozygosity (Hedrick 2005a). Based on standardized estimates of $F_{STmtDNA}$, we would expect estimates of genetic divergence between South Dakota and the boreal forest population 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.

Differential gene flow between males and females is the best explanation for the pattern in our data. Sex-biased dispersal is a common phenomenon in birds, with a majority having female-biased dispersal (Clarke et al. 1997). A review of patterns of avian sex-biased dispersal found 11% (6/53) of bird species to have male-biased dispersal and 15% (8/53) showed equal dispersal between sexes (Clarke et al. 1997; Gibbs et al. 2000). Several studies have documented

patterns of sex-biased dispersal based on different patterns of genetic structure observed in mtDNA as compared to nuclear DNA (Gibbs et al. 2000; Helbig et al. 2001; Johnson et al. 2003; Bouzat and Johnson 2004; Gay et al. 2004; Tiedemann et al. 2004; Jones et al. 2005). Although female-biased dispersal dominates in birds, waterfowl often show a pattern of male-mediated gene flow (Greenwood and Harvey 1982; Lecomte et al. 2009). Red-crested pochards (*Netta rufina*; Gay et al. 2004), common eiders (*Somateria mollissima*; Tiedemann et al. 2004), sandhill cranes (*Grus canadensis*), as well the yellow warbler (*Dendroica petechia*; Gibbs et al. 2000) have patterns of genetic structure consistent with male-mediated gene flow (i.e., highly structured mtDNA and weakly structured nuclear DNA). Lekking species tend to have the common pattern of female mediated gene flow with high site fidelity by males that is evidenced by mtDNA structure that is weak compared to nuclear structure (Johnson et al. 2003; Bouzat and Johnson 2004). Most of these species are migratory species with one sex that is philopatric to the natal territory.

The leading hypothesis attributes sex-biased dispersal to advantages to the philopatric sex of obtaining higher quality territories and the dispersing sex leaving to avoid inbreeding (Greenwood 1980). Black-backed woodpeckers' life history is quite different than most migratory species because they are a resident species that occupy a territory year-round, and then both males and females disperse when their habitat becomes suboptimal (three to five years postfire). Thus, we would predict equal rates of dispersal for males and females because neither sex can occupy sites near the natal territory long term and the frequent colonization of new habitats should provide a mechanism to avoid inbreeding.

Our findings differ from a simple pattern of male-biased dispersal because females and males both must regularly disperse due to ephemeral nature of their habitat. Additionally, we

found evidence that suggests gene flow attributed to both sexes; that is, an absence of genetic structure for both mtDNA and nuclear DNA, over large areas across the boreal forest. Differential gene flow between males and females was confirmed by different estimates of pairwise F_{ST} estimates.

2.5.5 Conservation implications

Our data suggest that females do not cross large gaps in habitat and that large gaps in habitat act as a higher resistance landscape to long-distance dispersal for males. Therefore, small isolated populations, such as the one in South Dakota, may deserve higher priority for conservation. For example, land management actions that affect black-backed woodpecker habitat, such as salvage logging, would need to be considered in a spatial and temporal context because fewer habitat patches are available to birds through time. If the population in South Dakota declines due to habitat degradation, recolonization of the areas by females is unlikely. Additionally, it is important to determine if the Oregon population is connected to California or Washington populations when planning management actions that affect populations. Our data suggests there has been gene flow across the boreal forest for a long period of time. Management actions should strive to maintain forested connectivity between burned patches to maintain these levels of gene flow.

We are unaware of any other studies finding evidence for males and females making different decisions regarding crossing large gaps in habitat for long-distance dispersal events. Future studies examining behavioral barriers to movement should consider that patterns of movement of males and females can differ across various types of habitats. This is especially important given the common use of nuclear genetic data to define populations.

Table 2-1 Analysis of molecular variance (AMOVA) results of five 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**
(W. Montana + N.W. Montana + Idaho + Quebec) (Alberta); (Oregon); (South Dakota)	4	Among groups	45.01*	2.71*
		Among sites	0.12	1.41**
		Within sites	54.87**	95.88**
(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, W. Montana, N.W. Montana, Alberta; * P , 0.05; ** $P <$ 0.0001

Table 2-2 Genetic diversity and neutrality tests 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); F_{IS}, fixation index; AR, allelic richness; H_E, expected heterozygosity; Fu's F, test for neutrality and DTaj, Tajima's D test for neutrality; standard errors are in parentheses.

	n	No. of haplotypes	h	π	HR	F_{IS}	H_E	AR	Fu's *F*	D_{Taj}
Idaho	42	6	0.616 (0.012)	0.004	4.57	-0.01	0.58	5.46 (1.35)	-0.92	-0.54
Missoula	49	6	0.450 (0.012)	0.002	4.00	0.12	0.58	5.52 (1.18)	-1.90	-1.11
Glacier	48	7	0.457 (0.012)	0.002	4.12	0.01	0.58	5.69 (1.15)	-3.25	-1.45
Alberta	21	2	0.324 (0.024)	0.002	1.98	0.02	0.63	6.36 (1.30)	1.94	0.38
Quebec	56	12	0.589 (0.010)	0.002	5.58	0.05	0.60	5.76 (1.38)	-10.60**	-1.87*
Oregon	32	3	0.462 (0.013)	0.003	2.47	0.08	0.58	5.13 (0.90)	1.54	0.64
S. Dakota	27	2	0.074 (0.013)	0.001	1.55	0.01	0.46	3.57 (0.52)	-0.07	-1.51
All locations	275	18	0.613 (0.029)	0.003		0.05	0.60	6.03 (1.33)	-11.46*	-1.50

* $P < 0.05$; ** $P < 0.0001$

Table 2-3 Haplotype frequencies in each of the seven sampling locations (see Figure 1)

Haplotype	Missoula	Glacier	Alberta	Idaho	S. Dakota	Oregon	Quebec
1	0.74	0.73	0.81	0.60	0.00	0.28	0.63
2	0.10	0.13	0.00	0.14	0.96	0.03	0.16
3	0.02	0.00	0.00	0.00	0.00	0.00	0.00
4	0.02	0.00	0.00	0.00	0.00	0.00	0.00
5	0.08	0.06	0.00	0.10	0.00	0.00	0.02
6	0.04	0.02	0.19	0.12	0.00	0.69	0.00
7	0.00	0.02	0.00	0.00	0.00	0.00	0.00
8	0.00	0.02	0.00	0.02	0.04	0.00	0.02
9	0.00	0.02	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.02	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00	0.00	0.02
12	0.00	0.00	0.00	0.00	0.00	0.00	0.04
13	0.00	0.00	0.00	0.00	0.00	0.00	0.02
14	0.00	0.00	0.00	0.00	0.00	0.00	0.04
15	0.00	0.00	0.00	0.00	0.00	0.00	0.02
16	0.00	0.00	0.00	0.00	0.00	0.00	0.02
17	0.00	0.00	0.00	0.00	0.00	0.00	0.02
18	0.00	0.00	0.00	0.00	0.00	0.00	0.02

Table 2-4 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 2-5 A comparison of estimates of pairwise F_{ST} between inferred populations between autosomal and sex-transmitted markers (a) and pairwise F_{ST} estimates for males and females based solely on autosomal markers to test for sex-biased movement patterns among populations. (a) Observed and standardized pairwise F_{ST} estimates are based on mtDNA (below diagonal) and microsatellite markers (above diagonal). (b) Male and female estimates are based on microsatellite markers for males (below diagonal) and females (above diagonal).

a)

Observed F_{ST}				Standardardized F_{ST}			
	Boreal Forest	Oregon	S. Dakota		Boreal Forest	Oregon	S. Dakota
Boreal Forest	-	0.039	0.043	Boreal Forest	-	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	-

b)

Sex-specific F_{ST}			
	Boreal Forest	Oregon	S. Dakota
Boreal Forest	-	0.035	0.048
Oregon	0.027	-	0.088
S. Dakota	0.024	0.042	-

Figure 2-1 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 2-2 Principal Components Analysis visualizing clustering of sampling locations based on a) mtDNA; PC 1 = 59%, PC 2 = 39%; b) microsatellite data, PC 1 = 38%, PC 2 = 29%; SD = South Dakota, OR = Oregon, ID = Idaho, MA = Missoula, GL = Glacier, AB = Alberta, QB = Quebec.

Figure 2-3 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.

Figure 2-4 Maps showing the three clusters identified in the spatially explicit analysis conducted in GENELAND. Figure (a) identifies which cluster each sample was grouped with in assignment tests conducted in GENELAND (circles = continuous population extending from the Rocky Mountains to Quebec; squares = Oregon; triangles = South Dakota)The three clusters are b) continuous population extending from the Rocky Mountains to Quebec, c) western population, d) 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 2-5 Median-joining network constructed using NETWORK 4.5.1.0 (Fluxus Technology Ltd 2005) from 325 bp of *cytochrome b* region of mtDNA for 275 black-backed woodpeckers sampled in seven different geographic locations across their range. The size of the circles are proportional to the frequency of the haplotype; the number of individuals that share a haplotype in each geographic region is represented by a different color and/or pattern pie slice within each circle.

Figure 2-6 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 complete 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.

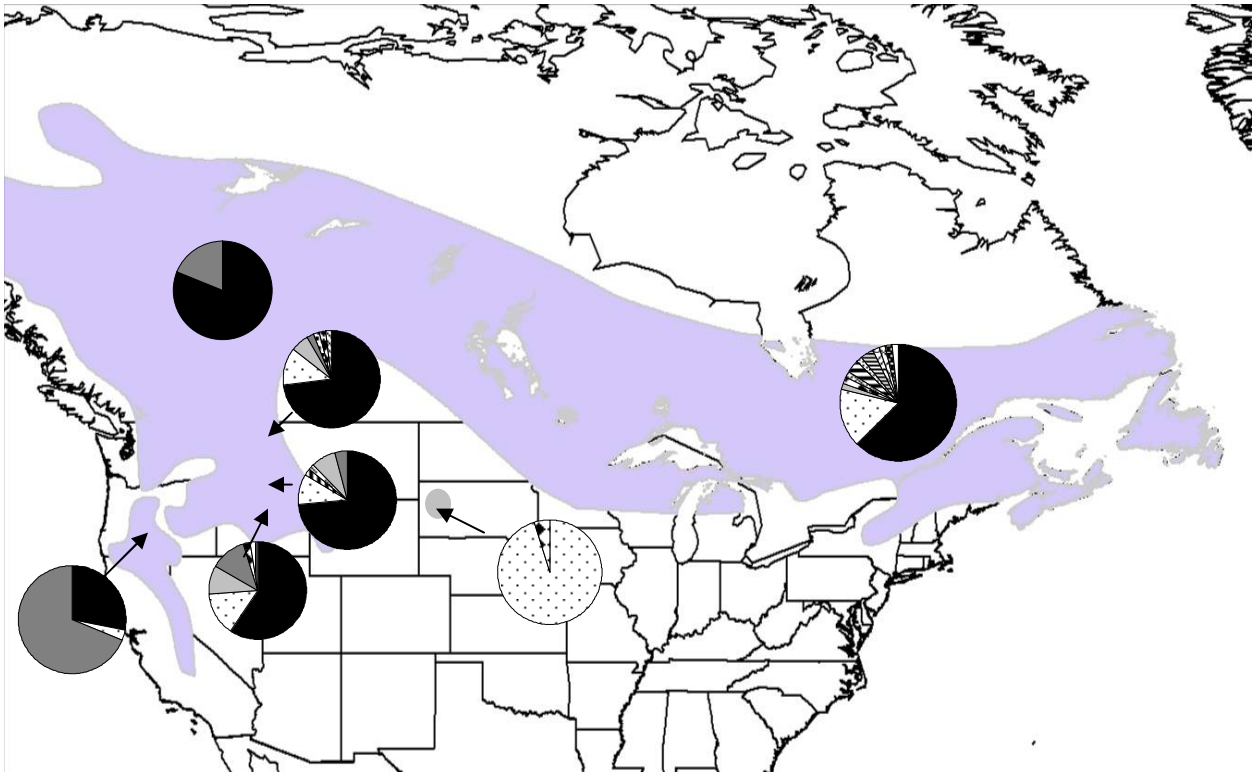
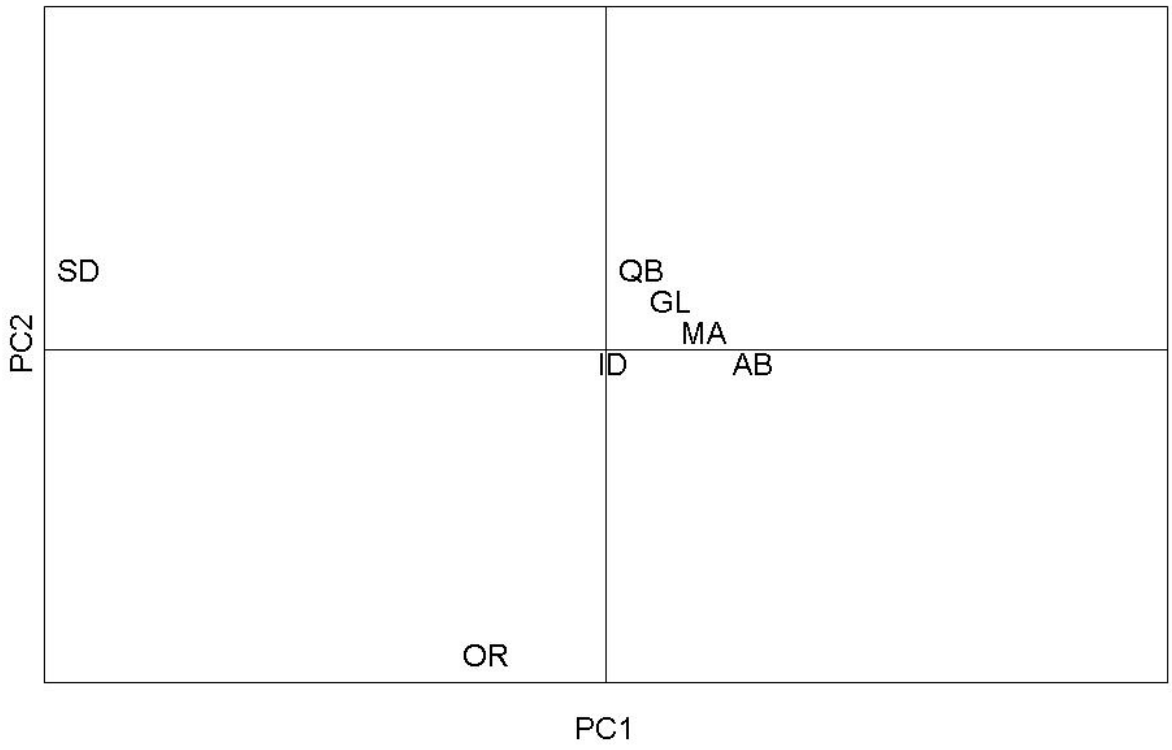


Figure 2-1

a)



b)

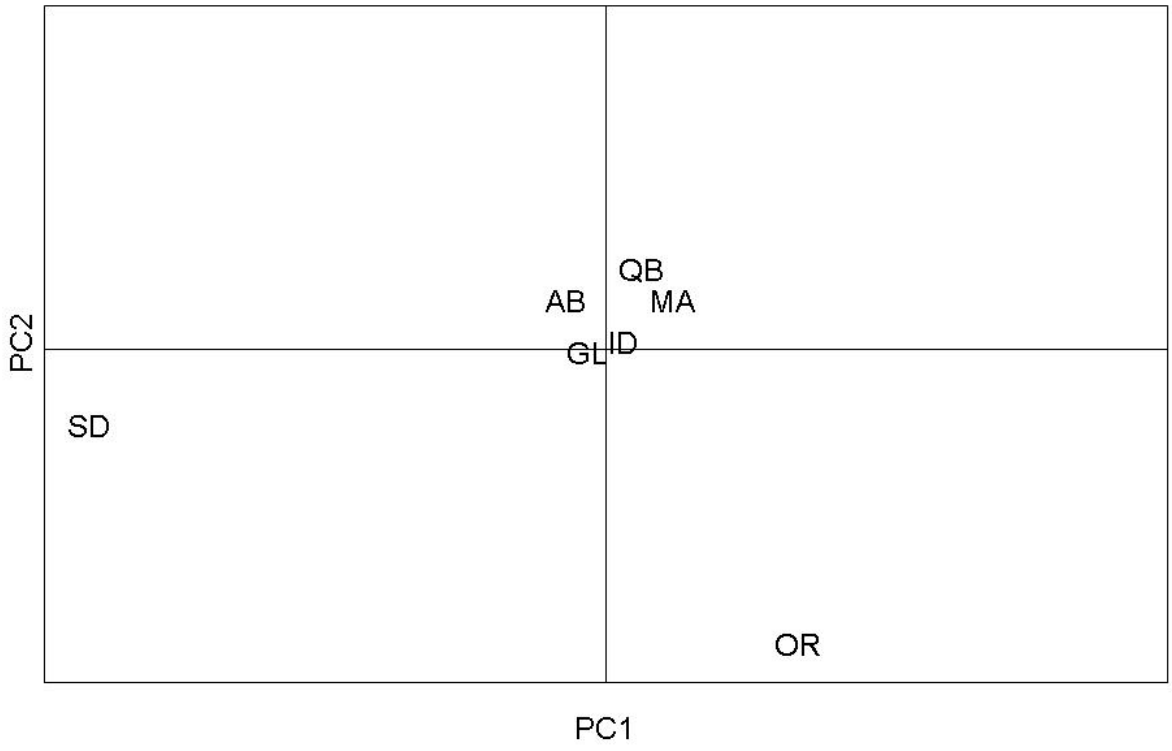


Figure 2-2

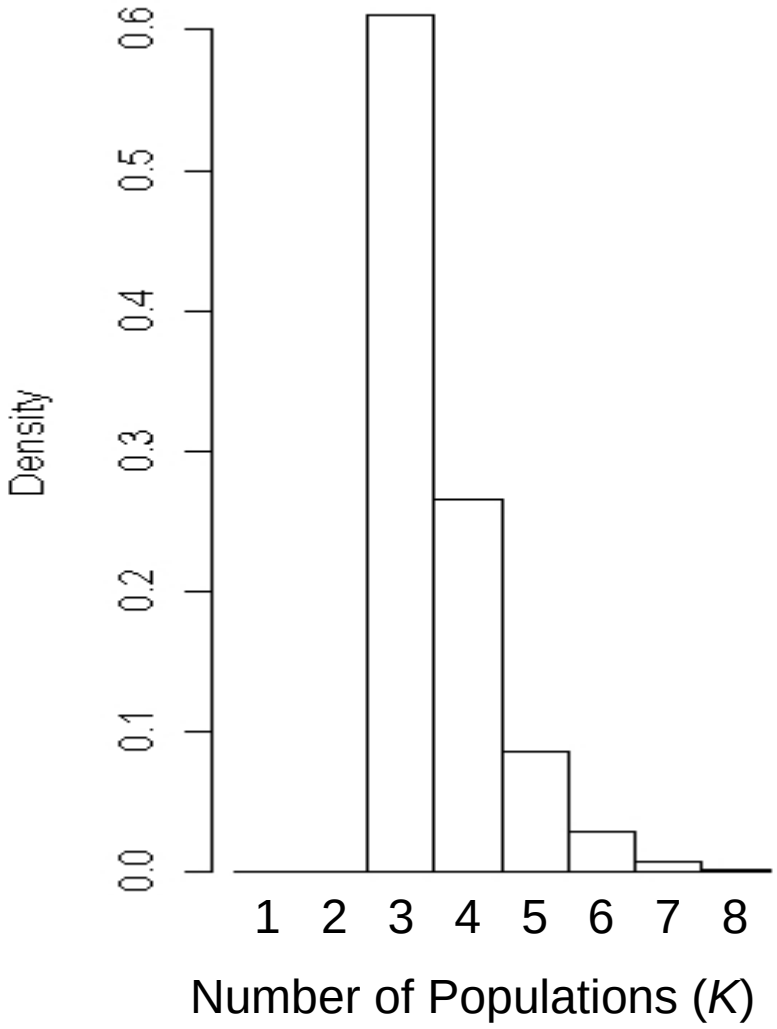
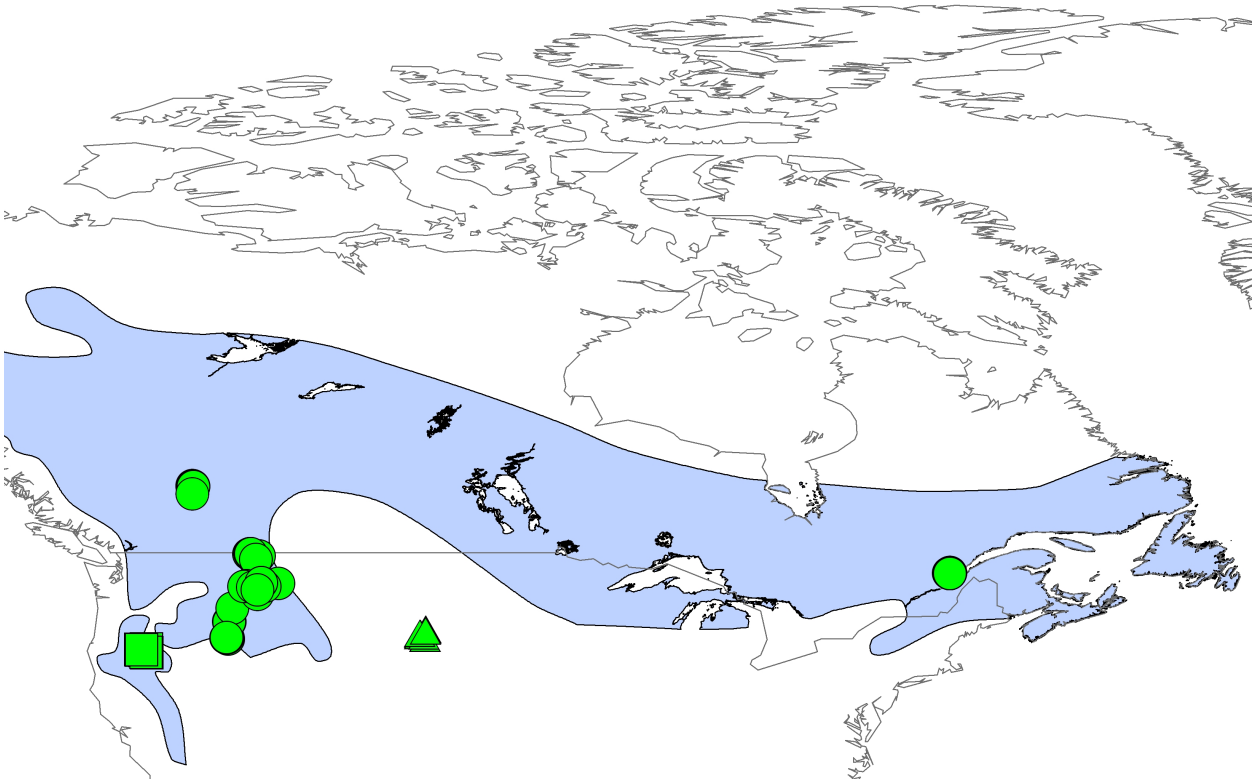
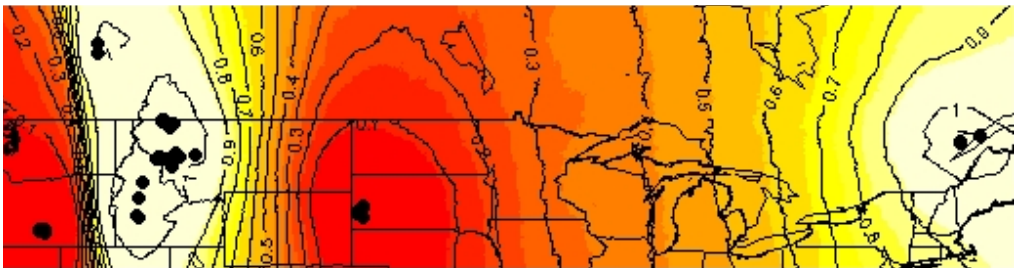


Figure 2-3

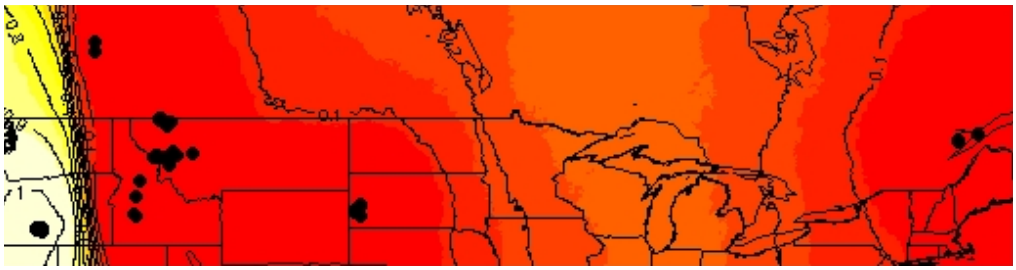
a)



b)



c)



d)

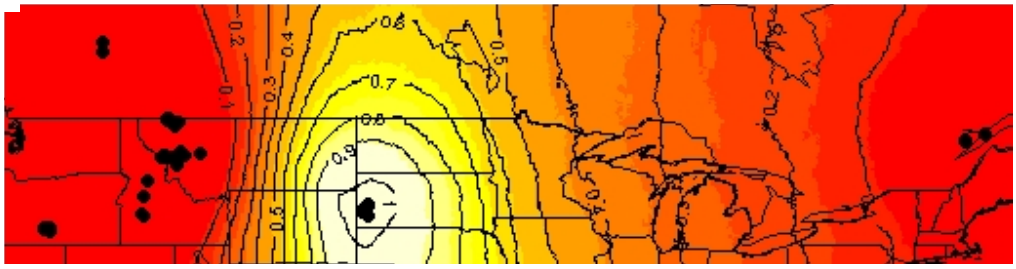


Figure 2-4

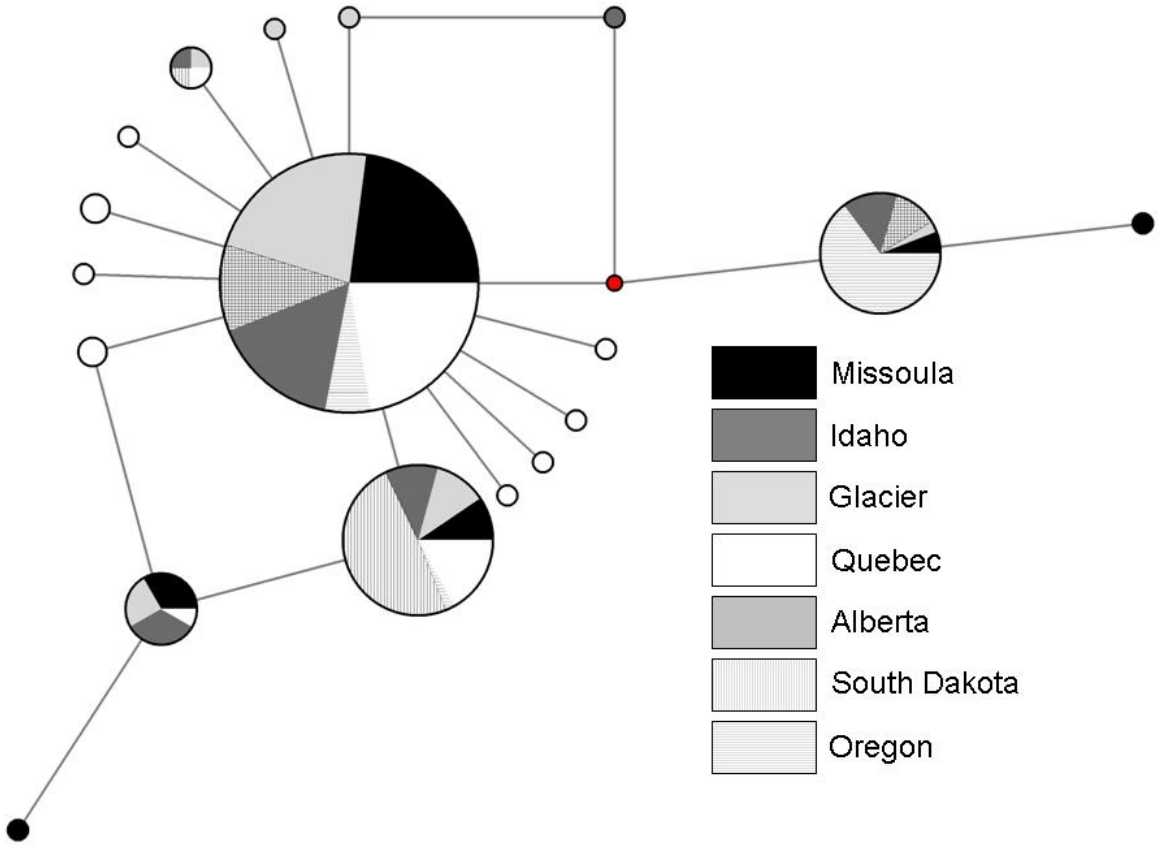

Missoula
Idaho
Glacier
Quebec
Alberta
South Dakota
Oregon

Figure 2-5

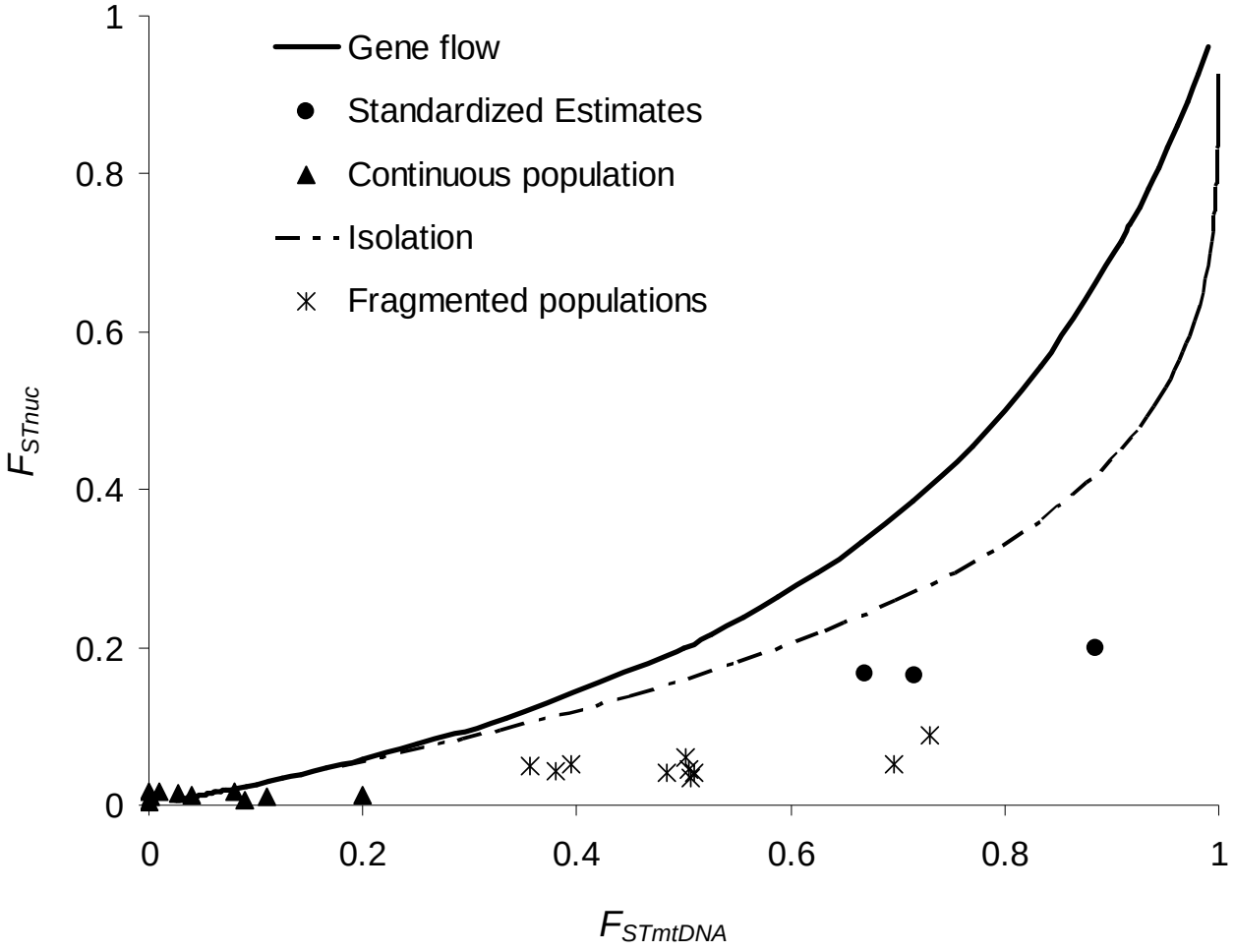

Gene flow
Standardized Estimates
Continuous population
Isolation
Fragmented populations
1
0.8
0.6
0.4
0.2
0
0
0.2
0.4
0.6
0.8
1
F_{STnuc}
$F_{STmtDNA}$

Figure 2-6

Chapter 3 - Discordant patterns in mtDNA and microsatellite markers reveal historic and contemporary processes shaping spatial structure of hairy woodpeckers (*Picoides villosus*).

3.1 Abstract

The spatial genetic structure of populations at the landscape scale can provide information about the permeability of different habitat types. In this study, I used both microsatellite and mitochondrial DNA (mtDNA) data to determine large-scale genetic structure of the hairy woodpecker (*Picoides villosus*), a common species continuously distributed over much of North America. I then compare the patterns observed with that of the black-backed woodpecker (*P. arcticus*), a sympatric species that shares several life history traits but is more restricted in the types of habitats occupied. Microsatellite data show a strong pattern of isolation by distance that is completely absent in mtDNA data. In mtDNA, genetic divergence is lower among sites within the boreal forest and higher among sites that are geographically closer but outside the boreal forest. This apparent lack of structure is likely due to an ancestral polymorphism shared at high frequency, as opposed to historical or ongoing gene flow. Based on isolation by distance in the microsatellite data, gene flow is primarily influenced by geographic distance, regardless of the composition of the landscape matrix at the large-scale. This differs from the congeneric black-backed woodpecker, where the spatial pattern of genetic structure appears to be shaped by the landscape matrix, with large areas of non-forested habitat being less permeable to gene flow. This research supports the hypothesis that habitat

generalists are less sensitive than specialists to habitat heterogeneity in the landscape matrix.

3.2 Introduction

Population genetics can provide important insights into both historical and contemporary processes, such as gene flow and demography, in relation to landscape features. The landscape matrix plays an important role in shaping the genetic structure of many species by limiting or encouraging gene flow through dispersal behavior. But for highly vagile species such as birds, gene flow may simply be related to distance between patches regardless of the intervening habitat. Habitat generalists may be willing to cross many types of habitat in the landscape matrix. Habitat specialists, on the other hand, are thought to be more sensitive to the types of habitat in the landscape matrix, especially over large distances (Haddad 1999, Tischendorf et al. 2003, Gillies and St. Clair 2008). In this study, I compare the landscape-scale genetic structure of two species that share many life history characteristics, the black-backed woodpecker and hairy woodpecker. The black-backed woodpecker is considered a habitat specialist, whereas the hairy woodpecker is much more cosmopolitan in the habitats occupied.

Generalizations among species with common life history characteristics are appealing due to the challenging nature of collecting demographic data, such as dispersal patterns, on many species (Walters 1998, Koopman et al. 2007). Black-backed and hairy woodpeckers are both resident, cavity-nesting, bark-foraging species that exploit burned forests for their plentiful nesting and foraging resources (Dixon and Saab 2000, Jackson et al. 2002, Vierling et al. 2008, Saab et al. 2009). Black-backed woodpeckers are nearly restricted to early postfire habitat in the Rocky Mountain region and are present in very low densities outside burned areas in the boreal forest (Hutto 2008, Nappi and Drapeau 2009, Russell et al. 2009). Hairy woodpeckers are the most widespread of the *Picoides*

woodpeckers and use a wide variety of habitats, including large forested areas, small woodlands in grasslands, and are common in urban areas (Jackson et al. 2002).

In a previous study, I found that black-backed woodpecker populations have high genetic connectivity across the boreal forest where the habitat matrix is continuous forest. Populations separated by areas without forest, such as the open grasslands between the boreal forest and the Black Hills of South Dakota have lower genetic connectivity (Pierson et al. in press). If the reason black-backed woodpeckers do not cross the large openings between the boreal forest and southern sites is due to a physical lack of resources such as foraging and roosting sites, then the same pattern of genetic structure should be present in hairy woodpeckers due to their shared use of these resources. Alternatively, if hairy woodpeckers are less sensitive to the types of habitats in the landscape matrix because they are a generalist, then I expect genetic structure to be determined by geographic distance among sites regardless of the intervening habitat. My objectives were to determine 1) patterns of genetic structure for hairy woodpeckers across northern North America using both mtDNA and microsatellite markers and 2) compare patterns observed to a previous study of the landscape-scale genetic structure of the black-backed woodpeckers.

3.3 Methods

3.3.1 Sampling

Blood or feather samples were collected from 119 hairy woodpeckers in five sampling locations: Oregon, west-central (W.C.) Montana, northwest (N.W.) Montana, South Dakota, and Eastern Canada (Table1, Fig. 1). A detailed description of sampling

protocols has been described elsewhere (Pierson et al. in press). Briefly, adult woodpeckers were captured at the nest site, blood was collected from the brachial vein, birds were marked with a unique color-band combination and a spatial location was recorded. Samples from E. Canada were provided by a collaborative study (T. Burg, personal communication)

3.3.2 Genotyping and sequencing

A full description of DNA extraction and genotyping protocols and analyses can be found in Pierson et al. (in press). 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). Samples were genotyped at ten microsatellite loci: *C111*, *C115*, *D118*, (Vila et al. 2008); *RCW4* (added tail), *RCW5*, *RCW17* (with an added tail), (Mullins and Haig personal communication); *DIU1*, *DIU4*, (Ellegren et al. 1999); *HrU2*, (Ellegren 1992); *Lox4*, (Piertney et al. 1998). I added 'GTTTCTT' to the 5' end of the reverse primer of *RCW4* and *RCW17* to promote the addition of adenine which produces a clearer genotype (Brownstein et al. 1996).

3.3.3. Genetic variation

Microsatellite markers were tested for departure from Hardy-Weinberg (H-W) proportions and gametic disequilibrium in GENEPOP (version 1.2; Raymond and Rousset 1995). I calculated expected heterozygosity and average number of alleles/locus in GDA (version 1.1; Lewis and Zaykin 2001). Allelic richness, where the number of alleles is standardized to the smallest sample size, 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*) and nucleotide diversity (π) were calculated using DnaSP (version 4.50; Rozas et al. 2003).

3.3.4 Population-based analyses

I calculated pairwise F_{ST} (Weir and Cockerham 1984) in GENEPOP (version 1.2; Raymond and Rousset 1995) among all sampling locations and 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/). I conducted an analysis of molecular variance (AMOVA; ARLEQUIN 3.11; Excoffier et al. 2005) for both marker types. I tested three different hierarchical groupings (Table 2) and tested for significance of the variance components using 1000 permutations.

3.3.5 Phylogeographic analyses

To visualize the relationship among mtDNA haplotypes, I used NETWORK 4.5 (http://www.fluxus-engineering.com) to create a median-joining network. I estimated Tajima's D and Fu's F to test whether patterns of sequence divergence in mtDNA followed the pattern expected under a model of neutral evolution. Departures from neutrality in these metrics are often interpreted as evidence for population expansion (Tajima 1989; Fu 1997). Mismatch distributions were estimated for further testing to see if there was a signal of recent population expansion (Rogers and Harpending 1992).

Mismatch distributions calculate the expected frequency of nucleotide differences between DNA sequences under a model of constant population size compared to a change in population size. I calculated these metrics in DnaSP and significance was tested using coalescent simulations (Rozas et al. 2003).

3.4 Results

3.4.1 Genetic variation

I found 16 variable sites in the 319 base pairs sequenced in the *cytochrome b* region of the mitochondrial genome, with 9 haplotypes identified. Haplotype diversity (*h*) and nucleotide diversity (π) were highest in Oregon (h = 0.699, π = .0046) and lowest in E. Canada (h = 0.442, π = 0.00175). Null alleles were apparently present at *DIU1* and *RCW6* and these loci were omitted from further analyses. The remaining eight loci conformed to H-W proportions and I did not detect gametic disequilibrium. Allelic richness and expected heterozygosity were similar across all sampling locations (AR: 6.70 – 7.26, H_E: 0.71 – 0.77; Table 1).

3.4.2 Population-based analyses

For mtDNA, pairwise F_{ST} values among sites connected by the boreal forest (W. Montana, N.W. Montana, E. Canada) were lower than among the isolated sites (Oregon, S. Dakota; Table 3). Furthermore, the hypothesis of isolation by distance was rejected (Figure 2; r = -0.09, P = 0.40). A hierarchical analysis of population structure supported the lack of genetic structure across the boreal forest, revealing three groups (Table 2; boreal forest: N.W. Montana + W.C. Montana + E. Canada; Oregon; S. Dakota). In

contrast, pairwise F_{ST} values for microsatellite data followed an isolation by distance pattern, where genetic differentiation increased with geographic distance (Table 3; Figure 2). A hierarchical analysis of population structure based on microsatellites defined four different population groupings with only sites in close geographic proximity being the only ones grouping together (Table 2; N.W. Montana + W.C. Montana; E. Canada; Oregon; S. Dakota)

3.4.3 Phylogeographic analyses

The haplotype network revealed little spatial structure in the pattern of mtDNA diversity (Figure 3). Most sites shared a common haplotype and all other haplotypes differed by one to three nucleotides. Locations within the boreal forest (W. Montana, N.W. Montana, E. Canada) showed significant departures from a neutral model based on Fu's F. No departures from a neutral model were detected with Tajima's D (Table 1). I am reporting only the mismatch distributions for all sampling locations grouped together because there was little power to detect expected differences on an individual site basis. Mismatch distributions for all sites support a signal of population expansion (Figure 4). Low genetic divergence within the boreal forest was driven by the sharing of a common, centrally located haplotype; all rare haplotypes in E. Canada were detected only in that site (Table 4, Figure 3).

3.5 Discussion

Spatial patterns of genetic variation present in mtDNA and microsatellite data convey different patterns of population structure in hairy woodpeckers. MtDNA analysis shows low genetic differentiation within the boreal forest sites and higher genetic differentiation

among sites outside the boreal forest (Oregon, S. Dakota). Genetic data from microsatellites suggest a stepping stone model of dispersal based on a strong pattern of isolation by distance. This disparity is likely the result of a single shared, likely ancestral, haplotype found in high frequency in N.W. Montana, W.C. Montana and E. Canada reducing genetic differentiation based on mtDNA across the boreal forest.

3.5.1 Mitochondrial DNA variation

There is no correlation between genetic and geographic distance, suggesting geographic distance is not a primary factor influencing mtDNA structure in hairy woodpeckers. Pairwise F_{ST} values indicate high genetic divergence among Oregon, South Dakota and boreal forest sites (N.W. Montana, W.C. Montana, and E. Canada) and low genetic divergence within the boreal forest. The lack of spatial structure within the boreal forest could be a result of current gene flow or a shared ancestral polymorphism. The high frequency and central position of the single shared haplotype among sites within the boreal forest suggest the pattern is driven by an ancestral polymorphism. Of the six haplotypes detected in E. Canada, five are present only in that region, suggesting that gene flow is limited between E. Canada and all other sites (Table 4). Oregon and South Dakota each have a different common haplotype that is only one or two base pairs different from the probable ancestral haplotype and these haplotypes are present in other sampling locations. Oregon shares several rare haplotypes with N.W. Montana and W.C. Montana, suggesting that some gene flow may be present among these sites.

Across the entire study area, sequence variation in *cytochrome b* provides some evidence for recent range expansion. The median joining network consists of a few

common haplotypes that are closely related and many rare haplotypes. In addition, range expansion produced low nucleotide diversity combined with high haplotype diversity, and a significant Fu's F. Mismatch distributions provide additional support for recent range expansion (Figure 4). All values of Tajima's D were negative, suggesting a historic change in population size; however no values were significant.

This pattern of haplotype diversity is common in many temperate bird species that have undergone postglacial expansions across the boreal forest (Milá et al. 2006). However, many species show an east-west division due to fragmentation of the boreal forest into eastern and western refugia (Weir and Schluter 2004, Peters et al. 2005, Hull 2008). Weir and Schuter (2004) suggest the Pacific Coast and Rocky Mountains were further divided into separate refugia by more recent glacial advances (0.7 million years before present) based on common splits in the lineages of many boreal species. My data support the presence of a historic population in Oregon that was previously connected to the Rocky Mountain sites. The presence of ancestral polymorphisms often complicates the interpretation of contemporary patterns and may be the reason for a lack of an east-west division across North America in hairy woodpeckers.

3.5.2 Microsatellite variation

The hairy woodpeckers is a continuously distributed species with limited dispersal and whose genetic structure is primarily influenced by geographic distance regardless of the intervening habitat. Banding data on hairy woodpeckers suggest that most birds disperse less than 40 km (Jackson et al. 2002). The magnitude of genetic differentiation across my study area was moderate (max $F_{ST} = 0.07$) compared to other bird species across large

areas in North America (Boreal owls (*Aegolius funereus*) 0.004 Koopman 2007; yellow warbler (*Dendroica petechia*) 0.014 Gibbs 2000). The red-tailed hawk (*Buteo jamaicensis*), a common species with a similar continuous distribution has lower genetic divergence between recognized eastern and western subspecies (F_{ST} = 0.03; Hull et al. 2008).

Hierarchical grouping of sampling locations defined four groups with the two geographically proximate sites in Montana grouping together and all other sites being separate. In my study, hierarchical analysis of population structure provides insight into the amount of genetic variation among groups compared to within groups. However, my sampling design and analysis did not allow the defining of populations based on these analyses. Given the strong pattern of isolation by distance in the microsatellite data, populations that are a large distance apart are likely to be defined as separate groups. Sampling of intermediate locations would likely show a large continuous population (Schwartz and McKelvey 2009).

The hairy woodpeckers has the largest distribution of all the *Picoides* woodpeckers, ranging from Alaska to Central America. It shows a large amount of geographic variation in morphologic characteristics across its range (Jackson et al. 2002). As many as 21 subspecies have been recognized, with fourteen subspecies recognized most recently (Jackson et al. 2002). Across my study area, up to five recognized subspecies may have been sampled including *Picoides villosus monticola, Picoides villosus septentrionalis, Picoides villosus villosus, Picoides villosus terraenovae, Picoides villosus harrisi*. All of these recognized subspecies are thought to intergrade at their boundaries and do not likely reflect genetic discontinuities (Jackson et al. 2002).

3.5.3 Comparison between species

In a previous study, I found concordant patterns in microsatellite data and mtDNA within the black-backed woodpecker that supports high genetic connectivity across the boreal forest and lower connectivity among sites with non-forested matrix (Pierson et al. in press). Despite many commonalities among the black-backed and hairy woodpecker (Russell et al. 2009), patterns of genetic variation differ between the two species. Patterns of mtDNA structure in the hairy woodpecker are concordant with that of the black-backed woodpeckers; however, the factors responsible for low genetic differentiation within the boreal forest differ between the two species. High gene flow is the most likely reason for low genetic differentiation in the black-backed woodpecker because E. Canada sites share several high and moderate frequency haplotypes with sites in the western boreal forest (Pierson et al. in press). As mentioned previously, hairy woodpeckers in E. Canada share only the most common haplotype with sites in the western boreal forest, which suggests that ancestral polymorphism is the most likely reason for low differentiation.

Microsatellite data in black-backed woodpeckers also provide support for higher gene flow within the boreal forest compared to sites isolated from the boreal forest. Pairwise F_{ST} values between the Rocky Mountains and E. Canada ($F_{ST} < 0.02$) are markedly lower than sites geographically closer (e.g., Rocky Mountains to S. Dakota $F_{ST} = 0.05$), and there is a lack of isolation by distance (Mantel's $r = 0.03$, $P = 0.3$, Pierson et al. in press). Conversely, the hairy woodpecker has a strong pattern of isolation by

distance, with geographic distance appearing to be the primary influence on genetic differentiation.

Genetic variation within sites supports the different patterns of spatial structure observed in each species described above. The black-backed woodpecker populations in S. Dakota has extremely low genetic diversity (h = 0.074, AR = 3.57, Pierson et al. in press), which is typical in a small isolated population. In contrast, hairy woodpeckers had similar amounts of genetic variation in all sampling locations (Table 1), typical of a continuously distributed species spread over large areas.

This study supports the hypothesis that black-backed woodpeckers, a specialist, are more sensitive than hairy woodpeckers, a generalist, to the composition of the landscape matrix. Recent work by Gillies and St. Clair (2008) documented markedly different movement patterns through corridors by barred antshrikes (*Thamnophilus doliatus*), a forest specialist compared to rufous-naped wrens (*Campylorhynchus rufinucha*), a forest generalist. The barred antshrike relied on high quality habitat (i.e., riparian forest versus fencerow) within a corridor to successfully cross gaps between habitat. The rufous-naped wren crossed gaps in habitat twice as often and was willing to use lower quality habitat (fencerows) as a movement corridor.

The black-backed woodpecker is a fire specialist (Hutto 2008, Russell et al. 2009) that prefers to forage and nest in dense stands of dead trees (Saab et al. 2007). The hairy woodpecker nests and forages in many forest types (Jackson et al. 2002, Ripper et al. 2007). Within burned forests, Saab et al. (2007) found a lower density of hairy woodpecker nests in partially logged versus unlogged sites, with lower nest survival in logged sites. In unburned forests, Hayes et al. (2003) found hairy woodpecker density

increased in mixed conifer stands that had been moderately or heavily thinned. Although hairy woodpeckers may prefer older forest stands (over 60 years), they nest and forage in stands of various ages and size (Ripper et al. 2007). Nest success in second growth forests near clear cuts was high (88%; Ripper et al. 2007), and edge habitats were regularly used for foraging.

The hairy woodpecker's use of a wide range of habitats, including forest edges, may explain the differences in large-scale genetic structure observed between the black-backed and hairy woodpecker. Black-backed woodpeckers may find large gaps in contiguous forest a higher resistance landscape to cross than hairy woodpeckers because they are more associated with dense stands of burned forest. The hairy woodpecker's tendency to occupy such a wide variety of forest types, particularly edge habitats near clear cuts, may explain why geographic distance as opposed to intervening habitat type, is the primary influence on genetic connectivity across large spatial scales.

3.5.4 Conclusions

My results demonstrate the importance of using both mtDNA and microsatellite markers when assessing population structure because they provide insights into different time scales of genetic connectivity. Metrics of population differentiation based on mtDNA must be interpreted with caution given the presence of ancestral polymorphisms, especially in areas that have been colonized relatively recently such as the boreal forest. Together they provide the opportunity to identify historical versus contemporary processes influencing current estimates of population structure.

Contemporary movement patterns of hairy woodpeckers are determined primarily by geographic distance, supporting the hypothesis that habitat generalists are less sensitive to habitat heterogeneity in the landscape matrix. Despite many other life history similarities, black-backed and hairy woodpeckers have different population genetic structure; which is likely a result of differential movement patterns through the landscape matrix. However, caution should be exercised when making generalizations about movement patterns based on any life history characteristics (i.e., habitat generalist) because generalized patterns often have limited predictive power. Species specific data may be necessary when making conservation plans.

Table 3-1 A summary of genetic diversity statistics for all sampling locations, including the number of individuals sampled (*n*), number of haplotypes, haplotype diversity (h), nucleotide diversity (π), Fu's F, test for neutrality (significant values $P < 0.05$ in bold); DTaj, Tajima's D test for neutrality; P, significance values; AR, allelic richness; F_{IS}, measure of departure from H-W proportions within subpopulations; H_E, expected heterozygosity; standard errors are in parentheses.

	n	No. of haplotypes	h	π	Fu's Fs	Tajima's D	AR	F_{IS}	H_E
Missoula	30	7	0.510 (0.110)	0.00238	**-3.793**	-1.424	6.79 (0.36)	0.020	0.74
Glacier	25	7	0.587 (0.110)	0.00242	**-4.068**	-1.800	7.26 (0.37)	0.074	0.77
S. Dakota	10	3	0.600 (0.130)	0.00209	-0.272	-0.184	7.13 (0.44)	0.087	0.71
E. Canada	20	5	0.442 (0.130)	0.00175	**-2.677**	-0.909	6.70 (0.37)	0.028	0.73
Oregon	34	7	0.699 (0.059)	0.00460	-1.231	-0.408	6.70 (0.29)	0.037	0.73
All locations	119	19	0.701 (0.042)	0.00388	**-13.988**	-1.604	7.34 (0.36)	0.040	0.71

Table 3-2 Analysis of molecular variance (AMOVA) results of three different groupings of hairy woodpecker sampling sites for both mtDNA and microsatellite loci. Significance values are based on 1000 permutations using ARLEQUIN 3.11.

Group	No. of groups	Variance component	mtDNA % of variance	Microsatellites % of variance
(N.W. Montana + W. Montana + E. Canada + Oregon) (South Dakota)	2	Among groups	27.79	1.04
		Among sites	17.61**	2.83**
		Within sites	54.6**	96.13**
(N.W. Montana + W. Montana + E. Canada) (Oregon) (South Dakota)	3	Among groups	38.04	-0.19
		Among sites	0.79*	3.18**
		Within sites	61.18**	97**
(N.W. Montana + W. Montana) (E. Canada) (Oregon) (South Dakota)	4	Among groups	33.56	2.9
		Among sites	-0.68	0.54
		Within sites	67.13**	96.56**

* P , 0.05; ** $P < 0.0001$

Table 3-3 Pairwise F_{ST} values for mtDNA (below diagonal) and microsatellite data (above diagonal). NWM: N.W. Montana, WM: W. Montana, OR: Oregon, SD: S. Dakota, EC: E. Canada. Significant values are indicated in bold and with asterisks

	NWM	WM	OR	SD	EC
NWM	-	0.005	**0.014****	**0.026****	**0.038****
WM	0.00	-	0.003	**0.046****	**0.057****
OR	**0.310****	**0.262****	-	**0.053****	**0.070****
SD	**0.545****	**0.526****	**0.270****	-	**0.023***
EC	**0.043***	**0.052***	**0.354****	**0.629****	-

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

Table 3-4 Haplotype frequency in hairy woodpeckers in each sampling location

Haplotype	N.W. Montana	W. Montana	Oregon	S. Dakota	E. Canada
1	0.64	0.70	0.29	0.00	0.71
2	0.08	0.03	0.00	0.60	0.00
3	0.04	0.07	0.03	0.00	0.00
4	0.12	0.03	0.00	0.00	0.00
5	0.04	0.00	0.00	0.00	0.00
6	0.04	0.00	0.00	0.00	0.00
7	0.04	0.00	0.00	0.00	0.00
8	0.00	0.07	0.00	0.00	0.00
9	0.00	0.03	0.03	0.00	0.00
10	0.00	0.07	0.47	0.00	0.00
11	0.00	0.00	0.03	0.00	0.00
12	0.00	0.00	0.06	0.00	0.00
13	0.00	0.00	0.09	0.00	0.00
14	0.00	0.00	0.00	0.30	0.00
15	0.00	0.00	0.00	0.10	0.00
16	0.00	0.00	0.00	0.00	0.05
17	0.00	0.00	0.00	0.00	0.05
18	0.00	0.00	0.00	0.00	0.10
19	0.00	0.00	0.00	0.00	0.05
20	0.00	0.00	0.00	0.00	0.05

Figure 3-1 The distribution of hairy woodpeckers (from Natureserve) with the five sampling locations: Oregon, W. Montana, N. W. Montana, S. Dakota and E. Canada.

Figure 3-2 A scatterplot with trend lines showing the relationship between genetic distance (F_{ST}/1-F_{ST}) and linear geographic distance (km) for both mtDNA (left axis; black diamonds, solid line) and microsatellite data (right axis; open squares, dashed line).

Figure 3-3 A median joining network visualizing the relationship among haplotypes in hairy woodpeckers. The size of each pie chart is proportional to the relative frequency of each haplotype and the colors within each pie chart represent different sampling locations. Branch length is proportional to the number of base pair differences among haplotypes. Black: W. Montana, dark gray: Oregon, light gray: N.W. Montana, white: E. Canada, striped: S. Dakota

Figure 3-4. A mismatch distribution showing the number of expected base pair differences under a historic model of constant population size (short dashed line), changing population size (long dashed line) and observed pattern (solid line).

Figure 3-1

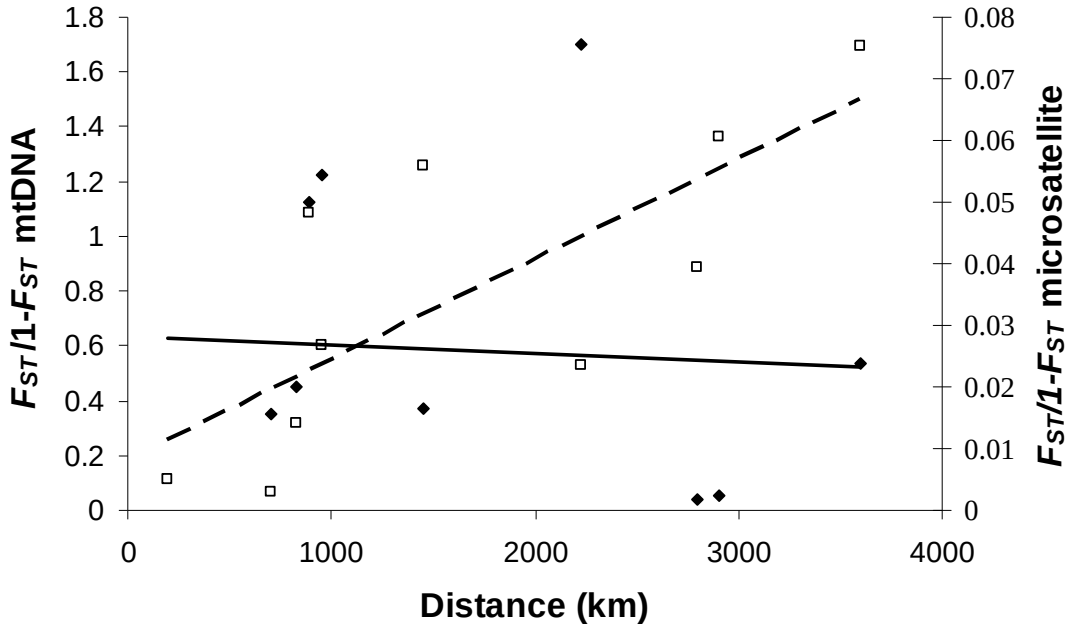

1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
1000
2000
3000
4000
Distance (km)
FST/1-FST mtDNA
FST/1-FST microsatellite

Figure 3-2

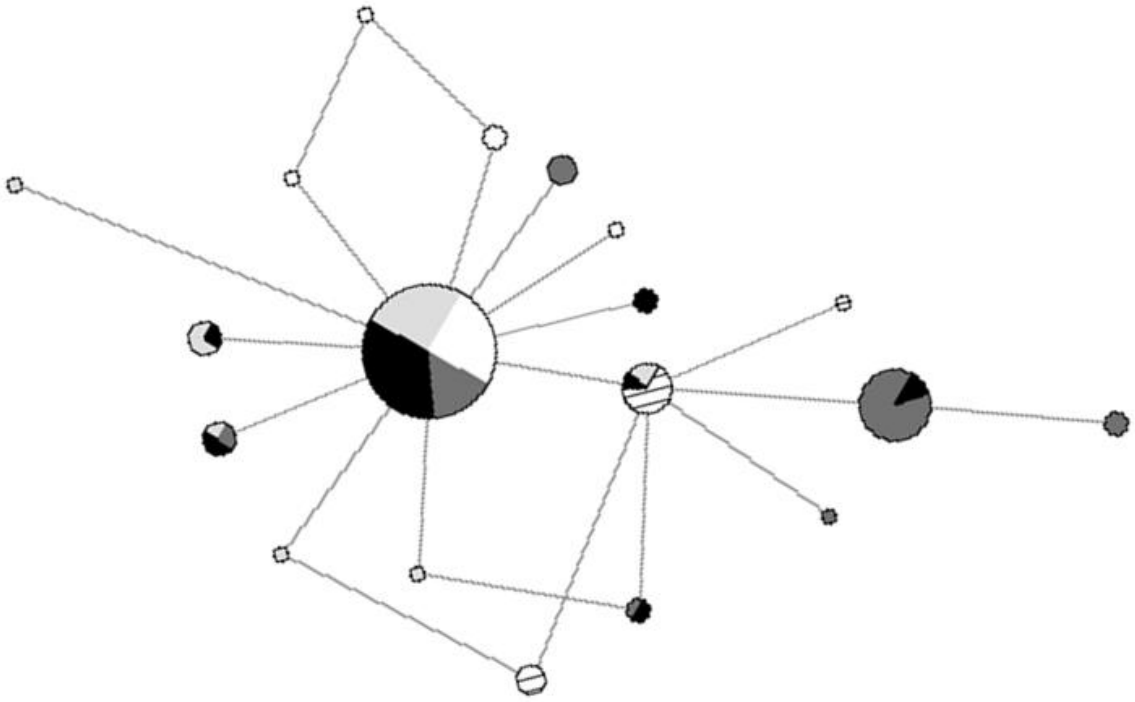


Figure 3-3

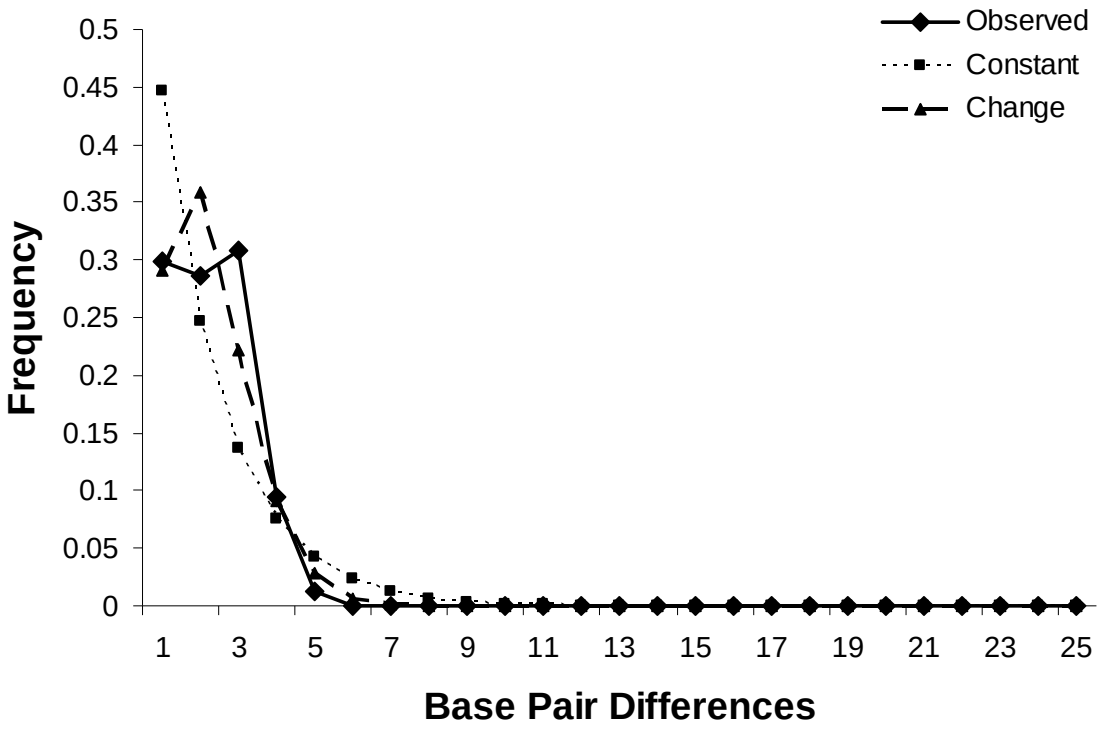

Observed
Constant
Change
Frequency
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
3
5
7
9
11
13
15
17
19
21
23
25
Base Pair Differences

Figure 3-4

Chapter 4 - Temporal changes in fine-scale genetic structure of fire-specialized species reveals source dynamics

4.1 Abstract

Frequent colonization of habitat patches can increase or decrease the amount of divergence among occupied patches depending on the source of the propagules. Highly ephemeral habitats, such as burned forest, provide a situation where both the source and destination of colonists vary in space and time. In this study, I documented the effect of frequent colonization of highly ephemeral habitats on the fine-scale genetic structure of a fire specialist, the black-backed woodpecker (*Picoides arcticus*), compared to a more generalist species, the hairy woodpecker (*P. villosus*). Despite high levels of habitat patch turnover, I detected a strong signal of fine-scale genetic structure in both species. The black-backed woodpecker displayed positive spatial genetic structure at a larger spatial scale (90 km) than the hairy woodpecker (45 km), which was likely due to its need to disperse farther distances to search for suitable habitat patches. I tested for differences in spatial structure between sexes in both species and detected a pattern consistent with male-biased dispersal in the black-backed woodpecker and female-biased dispersal in the hairy woodpecker. Finally, I detected a temporal increase (over a three year time span) in genetic correlation among black-backed woodpeckers, but not among hairy woodpeckers, within habitat patches. This pattern provides support for the hypothesis that juvenile black-backed woodpeckers may delay dispersal to exploit habitat patches while they are optimal, but hairy woodpeckers likely disperse from the natal territory. Burned forests have long been thought to be source habitat for highly specialized woodpeckers, such as

the black-backed woodpecker. My work used patterns of temporal stability in genetic structure to provide insight into the source dynamics of burned forests and it provides the first estimates on the spatial scale at which burned forests can provide migrants.

4.2 Introduction

The genetic population structure of organisms is driven by both habitat quality and dispersal (Hanski and Gaggiotti 2004). Habitat quality has a strong influence on the demographic (survival and reproductive) success of a species, and dispersal patterns determine the connectivity among habitat patches. Highly ephemeral habitats add a layer of complexity that leads to unclear predictions regarding the population structure of disturbance-dependent species because the spatial context of habitat patches of varying quality is constantly changing.

Wildfire has historically been the dominant force responsible for shaping numerous landscapes in both the western and boreal forests of North America. Consequently, many species are adapted to and some are even dependent on living in burned forests. The role of fire in creating highly suitable habitat has been documented for numerous avian species, such as certain woodpeckers, flycatchers, and ground-foraging birds in western North America (Hutto 1995, Brawn et al. 2001, Saab and Powell 2005). Despite this recognition, we continue to have a poor understanding of the spatial and population dynamics of fire-associated species and almost no information on movement patterns of species in this habitat. A common assumption is that species associated with early successional habitats, such as burned forests, have good dispersal capabilities because they must be readily able to colonize newly created habitats (Brotons et al. 2005). However, a recent empirical study revealed that several avian species relied on short distance dispersal to colonize early postfire habitat (Brotons et al. 2005). My goal was to evaluate the dispersal patterns and the resulting fine-scale population structure of species that commonly colonize these highly ephemeral habitats.

In North America, the black-backed woodpecker (*Picoides arcticus*) is perhaps the most commonly cited example of a fire specialist (Dixon and Saab 2000, Brawn et al. 2001, Hutto 2008, Nappi and Drapeau 2009). Black-backed woodpeckers live six to eight years, yet they only occupy fire-disturbed areas for three to five years after fire (Murphy and Lehnhausen 1998, Dixon and Saab 2000, Saab et al. 2007, Vierling et al. 2008). Peak densities occur two to four years following a burn (Saab et al. 2007, Nappi and Drapeau 2009), which corresponds to high wood-boring beetle (Coeloptera: Buprestidae and Cerambycidae) densities, their primary prey (Otvos 1979), in postfire habitats (Murphy and Lehnhausen 1998). After four to five years, the ephemeral and highly dynamic postfire habitat becomes less suitable due to a reduction in food resources.

While black-backed woodpeckers have been documented in unburned areas such as beetle-killed stands, nest success tends to be extremely high (80-100%) in areas that have burned at moderate to high severity (Saab et. al. 2007; Vierling et al. 2008) and tends to be much lower in unburned areas (44-78%; Bonnot et al. 2008). This leads to the assumption that unburned areas are not optimal habitat for black-backed woodpeckers to nest. In fact, Hutto (1995) suggested that burned areas might be necessary to maintain black-backed woodpecker populations, with moderate to high severity burned areas serving as source habitats and unburned areas acting as sink habitats. Concordantly, Hoyt and Hannon (2002) proposed that the long-term persistence of black-backed woodpecker populations may depend on the frequency of recently burned patches within their dispersal range. More recently, Nappi and Drapeau (2009) used a combination of empirical reproductive success data within burned areas and source-sink models to test if

productivity is above mortality rates in burned areas. They concluded that burned forests may serve as a source one year after fire and that pre-fire forest conditions (burned mature versus burned young forests) had an important effect on this source-sink status.

The majority of research looking at source-sink dynamics, especially in birds, is based solely on reproductive success (Saab and Vierling 2001, Gentry and Vierling 2007, Nappi and Drapeau 2009) measured through nest success and the number of young fledged. Postfire habitats may act as source habitats by increasing reproductive rates and reducing mortality rates resulting in more individuals to contribute to immigration and emigration. However, details are lacking on mechanisms that may increase survival in high quality postfire habitats. In particular, juvenile survival may be markedly higher if juveniles delay dispersal and remain near their natal territory while the habitat patch has plentiful resources. Delayed juvenile dispersal may increase survival by providing juveniles access to high quality food and nesting resources prior to incurring the cost of long-distance dispersal. While many studies have been able to estimate nest success of black-backed woodpeckers in a variety of postfire habitat types (Saab et al. 2007, Vierling et al. 2008, Nappi and Drapeau 2009), information on other vital rates remain elusive due to the difficult nature of obtaining such estimates in the field.

For black-backed woodpeckers, the spatial scale of dispersal determines the scale at which source dynamics can contribute to population structure. Nappi and Drapeau (2009) suggest that regions with high fire frequency can serve as 'regional sources' for areas with lower fire frequency. Thus, estimating dispersal distance is central to determining the scale at which regional sources can contribute to emigration and immigration. Because black-backed woodpeckers are so highly specialized to postfire

habitat (Hutto 2008), connectivity among 'older' habitat patches (~ 4 year-old) and 'young' (~ 1 year-old) postfire patches may be necessary for population persistence. While Pierson et al. (in press) found a lack of genetic structure in black-backed woodpeckers at a large spatial scale, a signature of fine-scale genetic structure produced by limited dispersal distance may persist at a small spatial scale (Lecomte et al. 2009).

Comparative studies between closely related species that differ in a life history trait of interest provide an opportunity to make specific predictions regarding patterns observed in empirical data (Whiteley et al. 2004, McDonald et al. 1999). The hairy woodpecker (*P. villosus*) exploits burned forests for their plentiful resources yet reproduces in a large variety of habitats (Saab et al. 2005, Ripper et al. 2007). Both black-backed and hairy woodpeckers are medium sized (60-80g), resident species that maintain territories year-round. Very little information exists on dispersal patterns of juvenile or adult black-backed woodpeckers (Pasinelli 2006). Hoyt and Hannon (2002) hypothesized black-backed woodpeckers likely move approximately 75 km to colonize burned areas based on a field study examining occupancy of burned and unburned areas. Banding data on over 800 hairy woodpeckers indicate that most birds (> 90%) disperse less than 40 km (Jackson et al. 2002).

My goals in this study were to use molecular genetic data to assess the fine-scale genetic structure of black-backed and hairy woodpeckers, and to examine the scale at which burned habitats may serve as source habitats. I also explore the dynamics of how burned habitats may act as population sources. Specifically, I set out to answer the following questions: 1) given frequent colonization events, is there fine-scale genetic structure as a result of limited dispersal and 2) are there family groups within burned

areas as a result of delayed juvenile dispersal? The comparison between these two species allows me to make clear predictions about what I expect if black-backed woodpeckers are a fire-dependent species relying on postfire habitat as sources. First, I predict that black-backed woodpeckers disperse farther than hairy woodpeckers, resulting in fine-scale genetic structure at a larger scale. Second, a signature of kin groups within burned areas would occur for both species if burned habitat is acting as source habitat due to delayed juvenile dispersal. This would likely increase juvenile survival due to a higher habitat quality during the transition from juvenile to adult.

4.3 Methods

4.3.1 Field methods

I designed my study in a hierarchical manner to assess genetic structure at multiple scales. I had two field locations in western Montana with three burned areas within 50 km of each other to assess fine-scale structure within and among burned areas (Figure 1b). These areas ranged in size from approximately 4,000 to 16,000 hectares. All areas burned in 2003, and sampling occurred between 2004 and 2007. In addition, I collected samples from both species of woodpeckers as part of a larger scale study in Oregon, South Dakota, and Eastern Canada (Figure 1b). Additional black-backed woodpecker samples were collected from Alberta, and Idaho. Only samples collected within five years after an area burned are included in these analyses.

A detailed description of sampling protocols has been described elsewhere (Pierson et al. in press). Briefly, adult woodpeckers were captured at the nest site, blood was collected from the brachial vein (for a few birds I collected only feathers as a

sample), marked with a unique color-band combination and latitude and longitude locations (collected as WGS 84 data in decimal degrees) was recorded.

4.3.2 Microsatellite Analyses

I analyzed samples from 264 black-backed woodpeckers and 119 hairy woodpeckers (Table 1). For black-backed woodpeckers, I used the following nine microsatellite loci in my analyses: *C111*, *C115*, *D118*, (Vila et al. 2008); *RCW4* (added tail), *RCW5*, (Mullins and Haig pers. comm.); *DIU3*, *DIU4*, (Ellegren et al. 1999); *HrU2*, (Ellegren 1992); *Lox4*, (Piertney et al. 1998). For hairy woodpeckers, I used the following eight microsatellite loci on my analyses: *C111*, *C115*, *D118*, (Vila et al. 2008); *RCW4* (added tail), *RCW5*, *RCW17* (added tail), (Mullins and Haig pers. comm.); *DIU4*, (Ellegren et al. 1999); *HrU2*, (Ellegren 1992). A full description of DNA extraction and genotyping protocols and analyses can be found in Pierson et al. (in review). These loci were highly variable, conform to Hardy-Weinberg proportions, and are not in gametic disequilibrium (Table 1).

4.3.3 Global spatial autocorrelation analyses

I performed global spatial autocorrelation analyses to test within patch dispersal patterns and rates, (Smouse and Peakall 1999, Double et al. 2005) in GenAlEx6 (Peakall and Smouse 2006). Global autocorrelation analysis is a multivariate approach which can detect a spatial pattern generated by multiple loci simultaneously (Smouse and Peakall 1999). This approach calculates a genetic autocorrelation coefficient (r) for a specified set of distance classes from both a genetic and geographic distance. Significant spatial

structure is measured using both bootstrapping and permutation tests as described in Peakall et al. (2003). Specifically, I used bootstrapping (999) to calculate 95% error bars around the estimate of *r* and assumed significance when the error bar did not cross zero, which is considered a conservative approach (Peakall and Smouse 2006). Permutation tests (999) calculate a 95% confidence envelope and significance is assumed when the estimate of *r* falls outside the confidence envelope around the null hypothesis of $r = 0$. Permutation tests provide a robust estimate of significance when sample sizes are small because they use the entire data set (Peakall and Smouse 2006).

The genetic correlation matrix contains pairwise individual to individual genetic distances using the distance statistic of Smouse and Peakall (1999). The geographic distance matrix was calculated from spatial locations of the nest site where the bird was captured.

My first step was to conduct a global spatial autocorrelation that included all samples across the study and divided the sample into bins of even sample sizes. This allowed me to determine the largest spatial scale that genetic structure (autocorrelation between genetic and geographic distance) among individuals could be detected. Next, I performed a global spatial autocorrelation analysis at the spatial scale the initial step indicated was the maximum scale of autocorrelation, which was 225 km with 15-km distance classes. The 15-km distance class was based on the maximum distance that woodpeckers were captured within a particular wildfire.

To test for positive spatial autocorrelation due to limited dispersal, I used a one-tailed test to determine if the estimated $r >$ was significantly greater than the permuted *r* based on a significance level of 0.05. I tested for sex-biased dispersal patterns by

performing global spatial autocorrelation on males and females separately at this 225-km scale.

4.3.4 Local autocorrelation analyses

I used two different approaches to assess if a genetic correlation among individuals was higher within burned areas as compared to among burned areas. I limited these analyses to burned areas in which I was able to collect samples from > 10 individuals over the course of the study. First, I employed a two-dimensional local spatial autocorrelation (2D LSA) that calculates a local autocorrelation (*lr*) for each focal point and a specified subset of *n* neighboring points. I calculated a 2D LSA for the five nearest neighbors and permutation tests were used to calculate significance. While multiple comparisons are involved in this type of analyses, Bonferroni corrections are not necessary because I am only looking at a small, specific subset of points (Peakall and Smouse 2006), therefore used a P = 0.05 to indicate significant *lr* values. I used *SigmaPlot 11* to create bubble plots to visualize significant *lr* values.

To test if relatedness increased over time, as expected if juveniles were remaining near the natal territory, I calculated the genetic correlation coefficient (*r*) within each burned area and used both random permutations (1000) and bootstrap (1000) methods to calculate 95% confidence limits in order to assess if *r* was greater than expected. I calculated *r* within each area for the first, second and third years. The second and third years include cumulative samples, that is, year two includes samples from both year one and two. I was unable to conduct this temporal analysis for hairy woodpeckers due to smaller sample sizes per year.

4.4 Results

4.4.1 Global spatial autocorrelation

For black-backed woodpeckers, I detected significantly positive genetic correlation (*r*) at less than 229 km (Figure 2a) when conducting global spatial autocorrelation at the largest spatial scale (3500 km). When examining smaller distance classes (15 km), black-backed woodpeckers displayed significantly positive *r*-values in distance classes up to 90 km (Figure 3a; Table 2). Female black-backed woodpeckers had significantly positive *r*-values in distance classes up to 75 km, a similar pattern to the entire population (Figure 3c; Table 2). Male black-backed woodpeckers had significantly positive *r*-values at the smallest distance class (15 km; Figure 3b, Table 2), although results from the one-tailed test were significant in the 60-km class as well (Table 2).

Hairy woodpeckers showed significantly positive *r*-values at less than 51 km (Figure 2b). In the smaller-scale analysis (15-km distance classes), hairy woodpeckers had significantly positive *r*-values up to 45 km (Figure 3d; Table 2). Several of the larger distances classes (e.g. 90 km, 105 km, 120 km) also revealed significant *r*-values, but there is not a pattern in the data. These distance classes have small sample sizes. Female hairy woodpeckers have significantly positive *r*-values in the first distance class (15 km; Figure 3f; Table 2), whereas males have significantly positive *r*-values in the 15- and 45-km distance classes and at larger distance classes with small sample sizes (Figure 3e; Table 2).

4.4.2 Local spatial autocorrelation analysis

I performed 2D LSA on samples in eight burned areas for black-backed woodpeckers and three burned areas for hairy woodpeckers. In general, black-backed woodpeckers had a higher percentage of individuals within burned areas with significantly positive *lr*-values based on a one-tailed test (Table 3). The *lr*-values tended to be larger and more significant for black-backed woodpeckers than hairy woodpeckers (Table 3). Black-backed woodpeckers had a higher percentage of clusters with stronger, more significant genetic correlations (Table 3).

The results from my temporal local spatial autocorrelation revealed a pattern of increased relatedness over time for black-backed woodpeckers (Figure 5a-c). Two burned areas (BM and OR) had genetic correlation values (*r*) that were significant in the first year the area was sampled (Figure 5a). Four burned areas (BM, OR, QB, WC) had significant genetic correlations in the second and third year the areas were sampled (Figure 5bc). Estimates of genetic correlation increased in four of the seven (57%) burned areas between year 1 and year 2. For hairy woodpeckers, I was able to calculate only a genetic correlation (*r*) for all years combined due to small sample sizes per year and did not detect any pattern of genetic correlation within burned areas (Figure 5d).

4.5 Discussion

4.5.1 Fine-scale genetic structure

Black-backed woodpeckers showed a signal of positive genetic structure at twice the spatial scale (90-120 km) of hairy woodpeckers (45 km), suggesting they may disperse twice as far. This is not surprising given black-backed woodpeckers high degree of specialization on burned areas. Hairy woodpeckers can disperse to many habitat types

within range of their natal territory (Jackson et al. 2002), whereas black-backed woodpeckers rarely colonize unburned patches (Hutto 2008). Therefore, one would expect black-backed woodpeckers to disperse a larger distance from their natal territory in an attempt to locate optimal habitat patches.

Across the range of black-backed woodpeckers, fire has been a part of the landscape for thousands of years (Bergeron et al. 2006, Cyr et al. 2009), and many species, including black-backed woodpeckers, have evolved life history characteristics important for persisting in postfire habitats (Hutto 2008). The black-backed woodpecker is the most specialized bird in the conifer forests of the Rocky Mountains, with over 95% of detections occurring in postfire habitat (Hutto 2008). In the Canadian boreal forest, black-backed woodpeckers are also highly specialized to early postfire habitat (Koivula and Schmiegelow 2007). Although hairy woodpecker densities can be up to 15 times greater in burned areas versus unburned areas (Smucker et al. 2005, Covert-Bratland 2006), they occupy and reproduce in unburned habitats (Kriesel and Stein 1999, Jackson et al. 2002, Ripper et al. 2007). Although some evidence suggests that the hairy woodpecker has lower reproductive success in unburned areas compared to burned areas (Saab et al. 2005). Densities of both species peak two to five years after an area has burned (Saab et al. 2007, Vierling 2008, Nappi and Drapeau 2009), leading to frequent colonization of newly created habitat patches across the landscape. Huot and Ibarzabal (2006) found black-backed woodpeckers present in both recently colonized burned areas and unburned forests, indicating all age classes are likely moving throughout the landscape.

Frequent colonization events combined with a high rate of population turnover in source-sink systems usually leads to a lack of genetic structure among subpopulations (Gaggiotti 1996). Black-backed woodpeckers within the boreal forest adhere to this pattern when measuring among population genetic structure at large spatial scales (Pierson et al. in press). Yet, I detected a strong signal of fine-scale genetic structure in both black-backed and hairy woodpeckers despite the highly ephemeral habitat patches they were occupying. Fine-scale genetic structure due to an individual's limited dispersal distance can exist despite high gene flow across large spatial scales (Lecomte 2009). My study reiterates the importance of considering the appropriate spatial scale for the biological question of interest (Wiens 1989).

The hierarchical design of my study allowed me to detect the largest spatial scale that genetic correlation among individuals was pmresent, and conduct a finer scale analysis to more precisely determine the distance classes at which positive genetic correlation dissipates. The scale, intensity of sampling, and distance classes analyzed are important to correctly interpret correlograms (Double et al. 2005). By sampling both woodpecker species in the same areas, I could readily compare the signatures of fine-scale structure. I also used a biologically relevant distance class based on the maximum distance apart birds occurred within a particular fire (15 km), (Double et al. 2005), which allowed me to make biologically relevant inferences.

4.5.2 Dispersal distance

I would expect to see a significant positive genetic correlation among individuals when dispersal is limited (Peakall and Smouse 2006). Peakall et al. (2003) found that the scale

at which positive genetic correlation persists in bush rats generally matched demographic data on dispersal distance. In birds, two studies have evaluated the usefulness of spatial autocorrelation techniques in assessing dispersal patterns by comparing demographic data to correlograms based on individually based genetic data (Double et al. 2005, Temple et al. 2006). These studies found a high level of concurrence between data sets. Double et al. (2005) concluded that to fully exploit the power of spatial autocorrelation, highly variable markers, appropriate sampling, and detailed ecological data such as age, sex and social status were needed. I was able to obtain all of these variables in this study. I sampled individuals both within and among fires at varying scales, used microsatellite markers, and sampled only breeding adults that were easily sexed based on morphological differences at the time of capture.

4.5.3 Sex-biased dispersal pattern

When sex-biased dispersal is present, fine-scale structure may be due to restricted dispersal in only one sex even though fine-scale structure is observed when the sexes are pooled. When examined alone, only the sex with restricted dispersal will display fine-scale structure and the dispersing sex will show a lack of fine-scale structure (Temple et al. 2006). Black-backed woodpeckers had a clear signal of male-biased dispersal and hairy woodpeckers had a weak, but present pattern of female-biased dispersal common in most bird species (Greenwood 1980).

In black-backed woodpeckers, when the sexes are combined, there was a positive signal of genetic correlation up to 90-120 km. When females are examined alone, there was a positive genetic correlation up to 75 km. Males had a positive genetic correlation

in the smallest distance class, which is the scale within fires. This signal is likely due to delayed dispersal of juvenile males as opposed to differences in dispersal distance or rates between sexes.

In contrast, female hairy woodpeckers had a genetic correlation at the smallest spatial scale, and males have a positive genetic correlation in the 15- and 45-km distance classes. Because I detected such a weak signal of sex-biased dispersal in hairy woodpeckers, more intensive research addressing this question needs to be conducted.

4.5.4 Kin groups/genetic clusters

I predicted that juveniles would delay dispersal to exploit habitat that is high in food and nesting resources. Indeed, all eight burned areas assessed had a relatively large number of genetic clusters of black-backed woodpeckers (Figure 4) with strong signals of genetic correlation (Table 3). Hairy woodpeckers had few individuals with genetic clusters around them, which could be an artifact of incomplete sampling. I do not think this is the case because the burned area with the largest number of samples (OR) had a lower proportion of individuals with genetic clusters than any of the burned areas examined for black-backed woodpeckers.

Black-backed woodpeckers did show evidence of an increase in genetic relatedness over time in 57% of the burned areas I sampled (Figure 5, 6). While this is not an overwhelming majority of the sites, hairy woodpeckers did not show any signal of genetic correlation within any of the burned areas (Figure 5d). The temporal accumulation of genetic relatedness in black-backed woodpeckers in a subset of the study sites provides initial support for the hypothesis that juveniles stay near their natal territory

while postfire habitat has plentiful resources. Additional anecdotal support includes a black-backed woodpecker banded as a nestling that was documented breeding in the same wildfire the following season (Saab and Dudley, unpublished data). Although I was unable to test for a temporal increase in genetic correlation in hairy woodpeckers, the lack of a pattern in the pooled samples suggests there is a different dynamic occurring within burned areas for hairy woodpeckers as compared to black-backed woodpeckers. It appears juvenile dispersal may be delayed in black-backed woodpeckers which likely increases juvenile survival, further explaining how burned areas act as source habitat. Juvenile hairy woodpeckers may disperse from their natal territory prior to breeding regardless of the habitat type in which they hatch. These results confirm the importance of not assuming temporal stability in genetic structure (Nussey et al. 2005). In fact, they illustrate how changes in the patterns of genetic structure over a short time period can answer questions regarding the demography of populations.

Fine-scale genetic structure can be the result of family groups that exist when there is a high rate of natal philopatry or delayed juvenile dispersal. For example, many lekking species, such as the red grouse (*Lagopus lagopus scotica*), white-bearded manakin (*Manacus manacus*), and peacock (*Pavo cristatus*) cluster in groups of related individuals (Petrie et al. 1999, Piertney et al. 1999, Shorey et al. 2000). Sex-biased dispersal can also lead to genetic clusters as a result of single-sex groups clustered if one sex tends to stay in or near the natal territory (Coltman 2003, Nussey 2005, Double et al. 2005, Lecomte et al. 2009). I wanted to test if family groups were present in burned areas as a result of delayed juvenile dispersal. Additional benefits to delayed dispersal are the lack of aggression directly towards kin from individuals in neighboring territories. I

predicted I would see genetic clusters within burned areas as evidenced by the 2D LSA analysis and an increase of genetic relatedness (*r*) through time if this were indeed the case. Although this is a short time scale to assess changes in structure, Nussey et al. (2005) found spatial genetic structure can change rapidly through time as a result of changes in population size and decreasing polygyny.

4.5.5 Conclusion

Early postfire habitat may provide source habitat for some woodpecker species because abundance is higher in burned areas versus unburned areas for species that occupy both habitat types (Hutto 1995). Nappi and Drapeau (2009) found that burned forests provide source habitat for black-backed woodpeckers in Quebec. Burned forest may provide source habitat for both woodpecker species I studied by providing emigrants due to high reproduction. I found black-backed woodpeckers likely disperse less than 100 km, providing the first details on the spatial scale that these burned areas may act as a source. My results confirm banding studies that show hairy woodpeckers only disperse ~40 km (Jackson et al. 2002). Black-backed woodpeckers likely move longer distances because they are more reliant on burned forest for habitat, whereas hairy woodpeckers can reproduce in many habitat types (Ripper et al. 2007). It may be that unburned forests are a more detrimental sink to black-backed populations than hairy woodpecker populations, forcing black-backed woodpeckers to search farther for burned forest (but see Saab et al. 2005).

This research has provided insight into the dynamics of how burned forest may function as source habitat through increased juvenile survival. The temporal increase in

genetic clusters in 57% (four of seven) of burned areas sampled provides initial support for my hypothesis that juvenile black-backed woodpeckers are delaying dispersal and may enjoy a higher rate of survival. Although burned patches may provide excellent short term resources for hairy woodpeckers, the same dynamics are not occurring within burned forest for these two species. Demographic data in both burned and unburned forests would confirm whether burned areas act as sources for both species. Yet demographic data remains incredibly difficult to collect, especially in unburned forests. Given the relative ease of collecting genetic data in comparison, the best approach may be to test for asymmetrical migration from sources to sinks using genetic techniques (Manier and Arnolf 2005, Hänfling and Weetman 2006, Peery et al. 2008), if sampling is possible in both source and sink habitats.

Table 4-1 The number of individuals included in global spatial autocorrelation analyses and summary statistics of genetic diversity for each location. GL: Glacier National Park; MSLA: Missoula, MT; OR: Silver Lake, Oregon; EC: Eastern Canada; SD: Black Hills, South Dakota; ID: central Idaho; AB: Jasper National Park, Alberta; N_{female}: number of females N_{male} : number of males N_{total}: total individuals; AR: allelic richness based on 7 loci common to both species; H_E: expected heterozygosity

Species	Location	N_{female}	N_{male}	N_{total}	F_{IS}	AR	H_E
Black-backed woodpecker	GL	23	24	48	-0.05	5.00	0.57
	MSLA	22	27	49	0.018	4.86	0.57
	OR	12	17	29	0.054	4.97	0.57
	EC	20	32	52	-0.014	5.26	0.59
	SD	11	10	21	-0.107	3.22	0.48
	ID	6	8	42	-0.065	4.97	0.57
	AB	12	9	21	-0.029	5.45	0.61
Hairy woodpecker	GL	12	13	25	0.074	6.89	0.76
	MSLA	13	17	30	0.02	6.54	0.74
	OR	16	17	33	0.037	6.39	0.76
	EC	7	14	21	0.028	7.10	0.73
	SD	5	5	10	0.087	6.57	0.71

Table 4-2 Results from a one-tailed test for positive genetic autocorrelation (*r*) which is expected when there is limited dispersal for black-backed (BBWO) and hairy woodpeckers (HAWO) including both sexes, then for males and females separately. The number of pairwise comparisons (n) per distance class (km) and the probability the estimated *r* is greater than expected based on 1000 permuations (P); significant values are indicated in bold

	Distance class (km)	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**	**135**	**150**	**165**	**180**	**195**	**210**	**225**
BBWO BOTH	**n**	2976	526	599	617	121	69	24	321	31	8	157	190	597	583	429
	Prob r > perm. r	0.001	0.001	0.001	0.013	0.001	0.038	0.180	0.001	0.739	0.105	0.004	0.908	0.599	0.899	0.608
BBWO MALE	**n**	960	152	177	71	39	225	67	846	168	195	260	89	46	79	69
	Prob r > perm. r	0.001	0.726	0.173	0.025	0.550	0.996	0.769	0.852	0.390	0.910	0.985	0.870	0.405	0.242	0.956
BBWO FEMALE	**n**	492	134	147	57	49	125	65	417	105	161	166	52	20	70	30
	Prob r > perm. r	0.001	0.001	0.001	0.006	0.014	0.141	0.997	0.122	0.995	0.709	0.387	0.983	0.399	1.000	0.871
HAWO BOTH	**n**	726	156	161	229	52	4	10	11			53	22	141	128	184
	Prob r > perm. r	0.001	0.005	0.005	0.586	0.408	0.009	0.033	0.001			0.033	0.158	0.005	0.816	0.971
HAWO MALE	**n**	180	55	48	68	15	2	3	7			19	7	47	37	50
	Prob r > perm. r	0.001	0.249	0.004	0.745	0.474	0.268	0.269	0.034			0.038	0.033	0.029	0.786	0.876
HAWO FEMALE	**n**	161	21	34	47	11		1				9	5	25	27	42
	Prob r > perm. r	0.015	0.084	0.562	0.668	0.149		0.281				0.549	0.839	0.134	0.689	0.548

Table 4-3 The number of individuals included (N) in 2D LSA for each location, the percent of individuals that had significant genetic clusters surrounding them, including the range of significance values and local genetic autocorrelation values (*lr*). AB: Alberta, BLM: Black Mountain fire in Missoula, MT, BM: Boles Meadow fire in Missoula, MT, RB: Robert fire in Glacier National Park, MT, WC: Wedge Canyon fire in Glacier National Park, MT, ID: central Idaho, OR: Oregon.

	Location	N	N significant	% significant	P-value range	*lr* range
BBWO	AB	21	3	14	0.005 - 0.011	0.20 - 0.24
	BLM	21	5	24	0.003 - 0.026	0.17 - 0.25
	BM	11	5	45	0.003 - 0.045	0.15 - 0.24
	RB	24	1	4	0.018	0.18
	WC	16	3	19	0.004 - 0.032	0.16 - 0.25
	ID	10	1	10	0.041	0.14
	OR	29	4	14	0.002 - 0.02	0.17 - 0.26
	EC	49	5	10	0.003 - 0.041	0.14 - 0.27
HAWO	BLM	13	3	23	0.01 - 0.03	0.14 - 0.24
	WC	14	0	0	NA[1]	NA[1]
	OR	33	3	9	0.01 - 0.04	0.14 - 0.15

[1] NA: not applicable because there were no significant clusters.

Figure 4-1 (a) A map of the United States and Canada showing the hierarchical sampling design including (a) the location of the seven broad-scale study sites: GL: Glacier National Park; MSLA: Missoula, MT; OR: Silver Lake, Oregon; EC: Eastern Canada; SD: Black Hills, South Dakota; ID: central Idaho; AB: Jasper National Park, Alberta and (b) the two study sites within western Montana that each have three areas that burned in 2003: Missoula – BLM: Black Mountain fire; BM: Boles Meadow fire; FC: Fish Creek fire and Glacier National Park – WC: Wedge Canyon fire; RB: Robert fire; TR: Trapper fire

Figure 4-2 Correlogram plots based on global spatial autocorrelation analyses conducted at the broadest spatial scale using the even sample size per distance class option. The y-axis is the genetic correlation coefficient (*r*) and the x-axis is the distance class (km). 95% confidence intervals were calculated using bootstrapping (error bars) and permutation tests (dashed lines). (a) black-backed woodpeckers (b) hairy woodpeckers (bottom).

Figure 4-3. Correlogram plots based on global spatial autocorrelation analyses conducted with 15km distance classes up to 225 km The y-axis is the genetic correlation coefficient (*r*) and the x-axis is the distance class (km). 95% confidence intervals were calculated using bootstrapping (error bars) and permutation tests (dashed lines). (a) black-backed woodpeckers, (b) male black-backed woodpeckers, (c) female black-backed woodpeckers, (d) hairy woodpeckers, (e) male hairy woodpeckers, and (f) female hairy woodpeckers.

Figure 4-4 Bubble plots of the two-dimensional local spatial autocorrelation (2D LSA) based on the five nearest neighbors for (a) black-backed woodpeckers and (b) hairy woodpeckers. Each bubble plot displays significant genetic clusters, based on one-tailed permutation tests, detected around individual woodpeckers within each burned area with more than 10 individual woodpecker samples. The size of the circle represents the strength of the genetic correlation detected using 2D LSA analysis in Genalex. Axis are latitude and longitude locations of individuals. AB: Alberta; BLM: Black Mountain fire in Missoula, MT; BM: Boles Meadow fire in Missoula, MT; RB: Robert fire in Glacier National Park; MT, WC: Wedge Canyon fire in Glacier National Park; MT, ID: central Idaho; OR: Oregon; EC: Eastern Canada

Figure 4-5 Temporal estimates of genetic correlation coefficients (*r*) within each burned area with at least 10 individuals sampled (see Figure 4 for location descriptions). The 95 % confidence intervals are based on both bootstrapping (error bars surrounding estimates) and permutations (error bars surrounding null expectation of 0); (a) year one of sampling black-backed woodpeckers (b) year one and two pooled for black-backed woodpeckers, (c) year one, two and three pooled for black-backed woodpeckers. (d) year one, two and three pooled together for hairy woodpeckers; samples sizes were too small to do a temporal analysis.

Figure 4-6 A plot of temporal change in estimates of genetic correlation coefficients (*r*) within each burned area with at least 10 black-backed woodpeckers sampled (see Figure 4 for location descriptions).

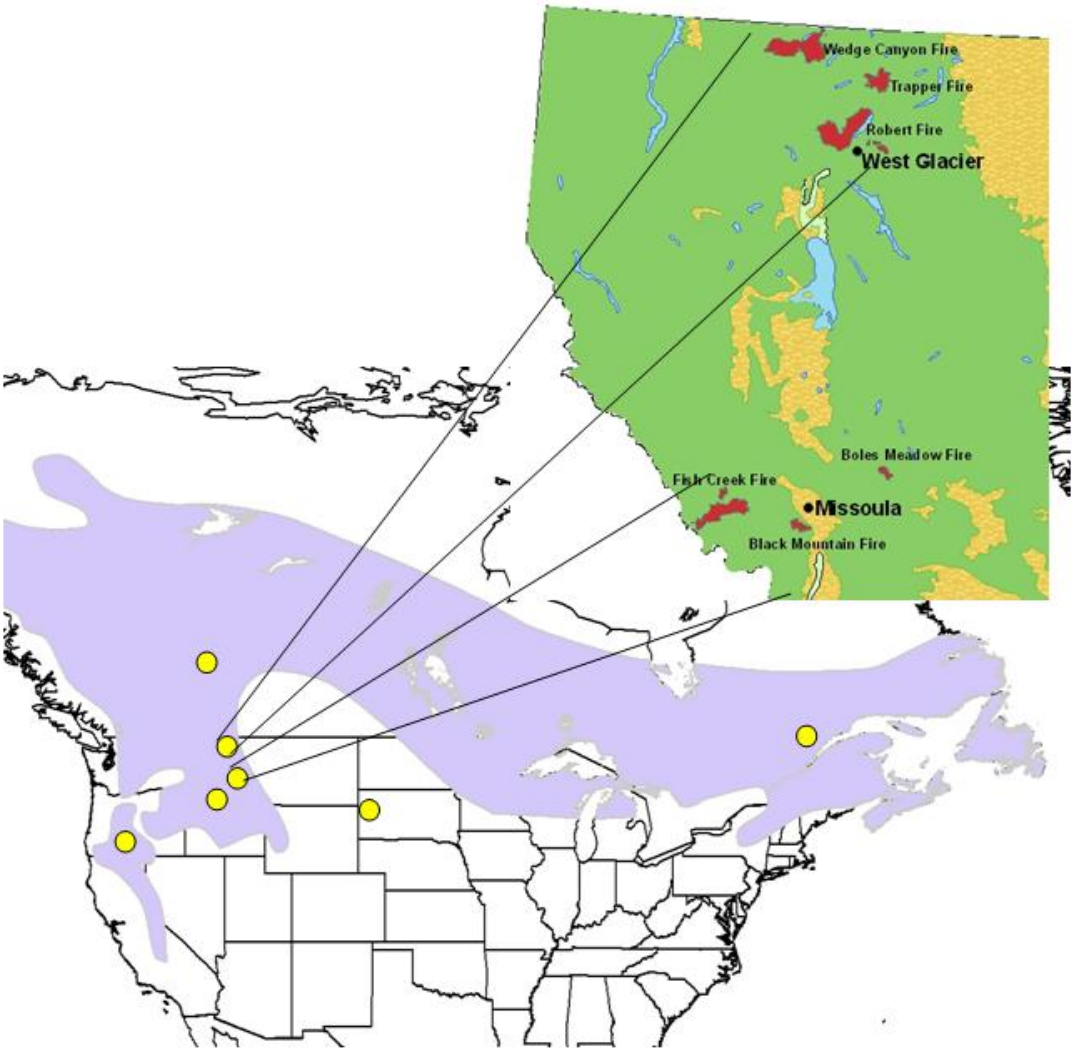
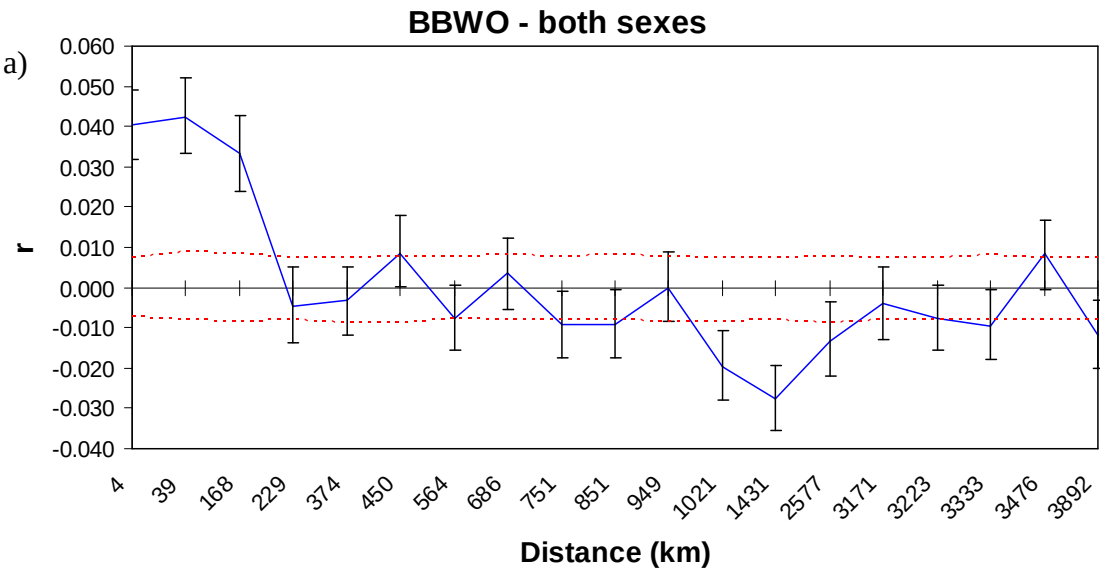

Wedge Canyon Fire
Trapper Fire
Robert Fire
West Glacier
Boles Meadow Fire
Fish Creek Fire
Missoula
Black Mountain Fire

Figure 4-1

a)


BBWO - both sexes
r
0.060
0.050
0.040
0.030
0.020
0.010
0.000
-0.010
-0.020
-0.030
-0.040
4
39
168
229
374
450
564
686
751
851
949
1021
1431
2577
3171
3223
3333
3476
3892
Distance (km)

b)

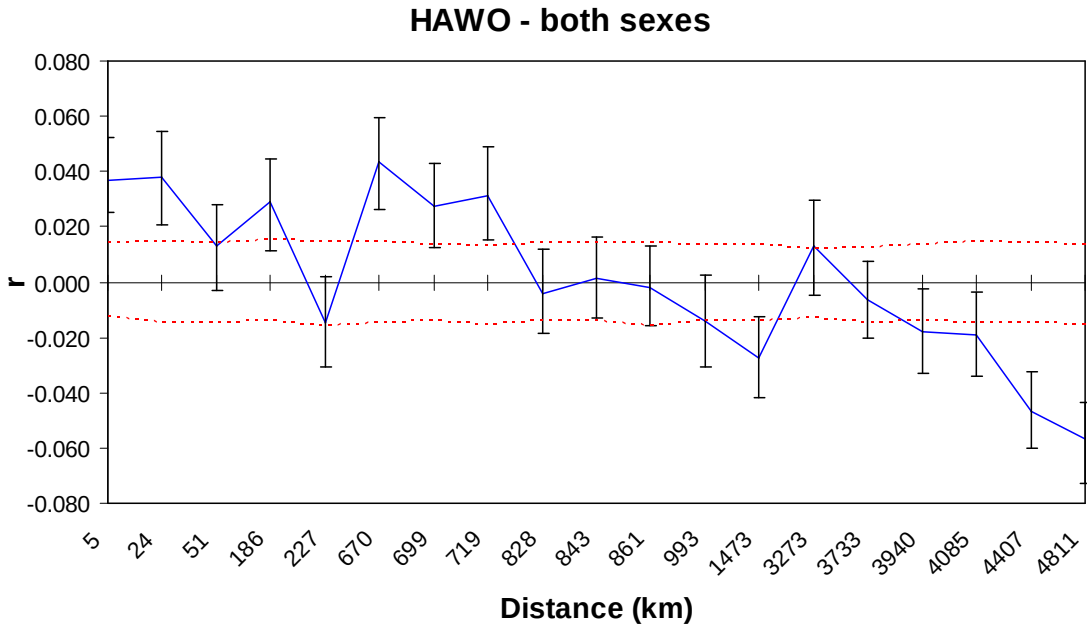
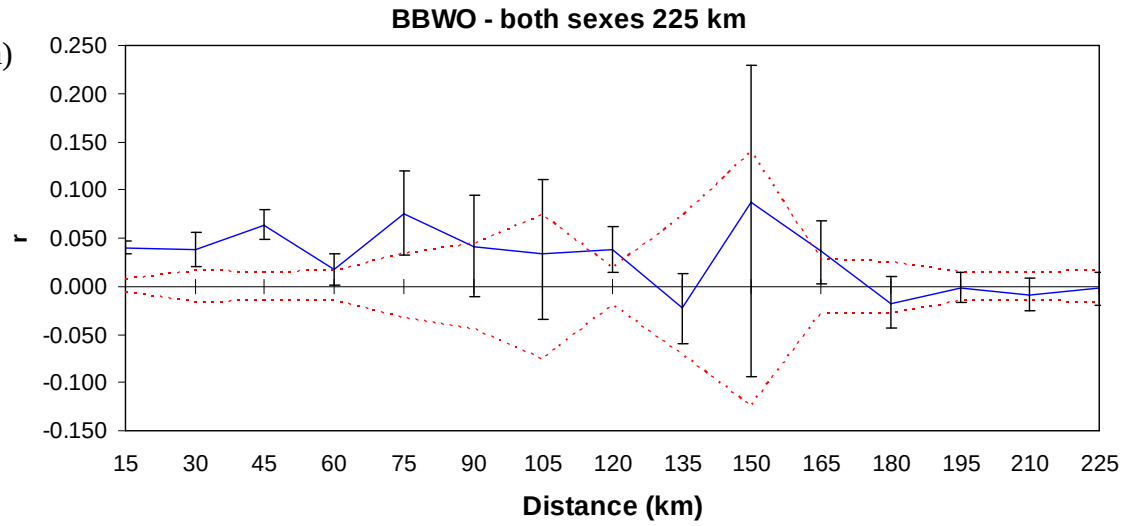
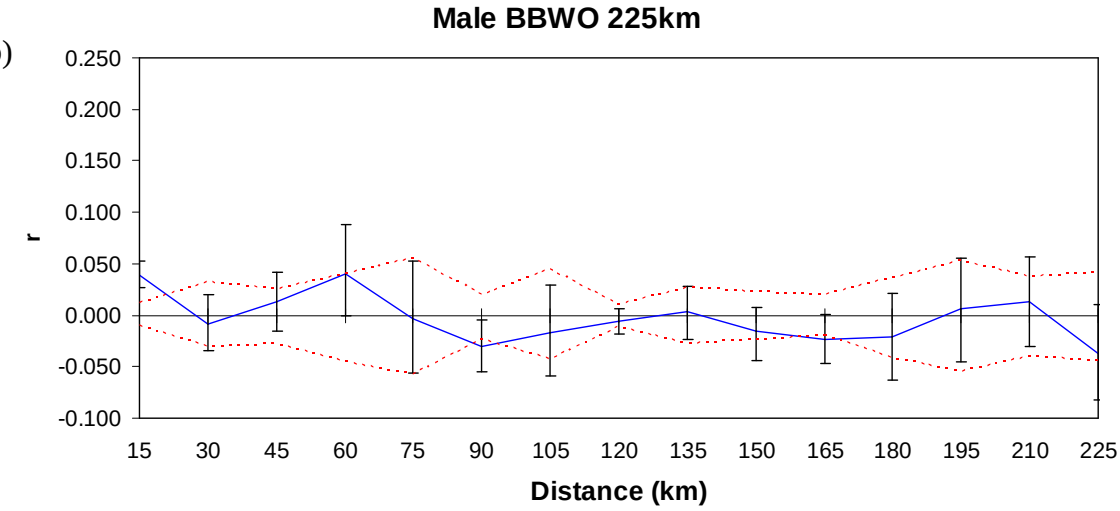

HAWO - both sexes
r
0.080
0.060
0.040
0.020
0.000
-0.020
-0.040
-0.060
-0.080
5
24
51
186
227
670
699
719
828
843
861
993
1473
3273
3733
3940
4085
4407
4811
Distance (km)

Figure 4-2

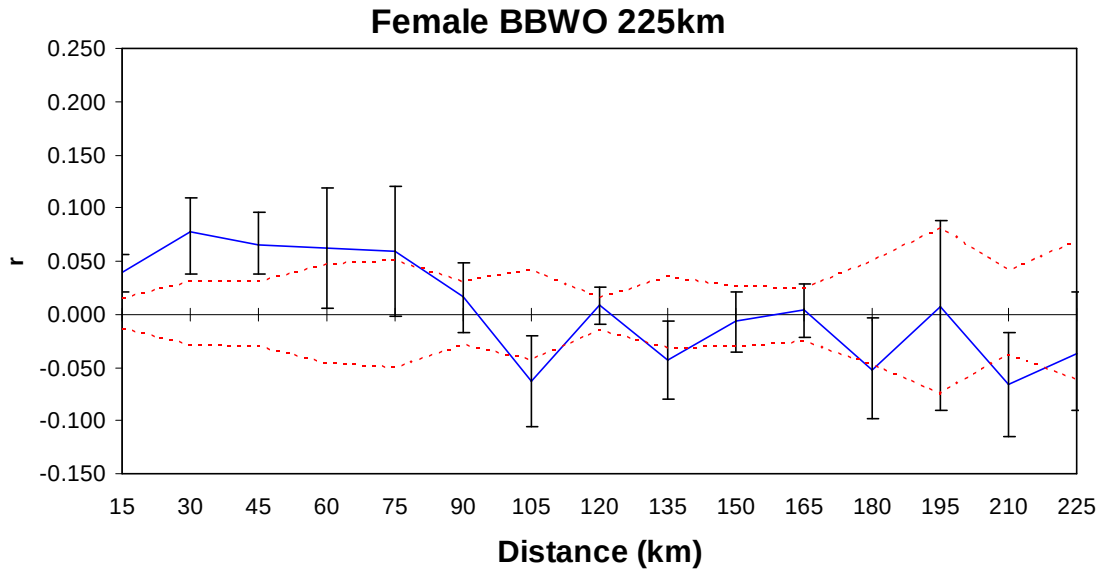
a)


BBWO - both sexes 225 km
r
0.250
0.200
0.150
0.100
0.050
0.000
-0.050
-0.100
-0.150
15
30
45
60
75
90
105
120
135
150
165
180
195
210
225
Distance (km)

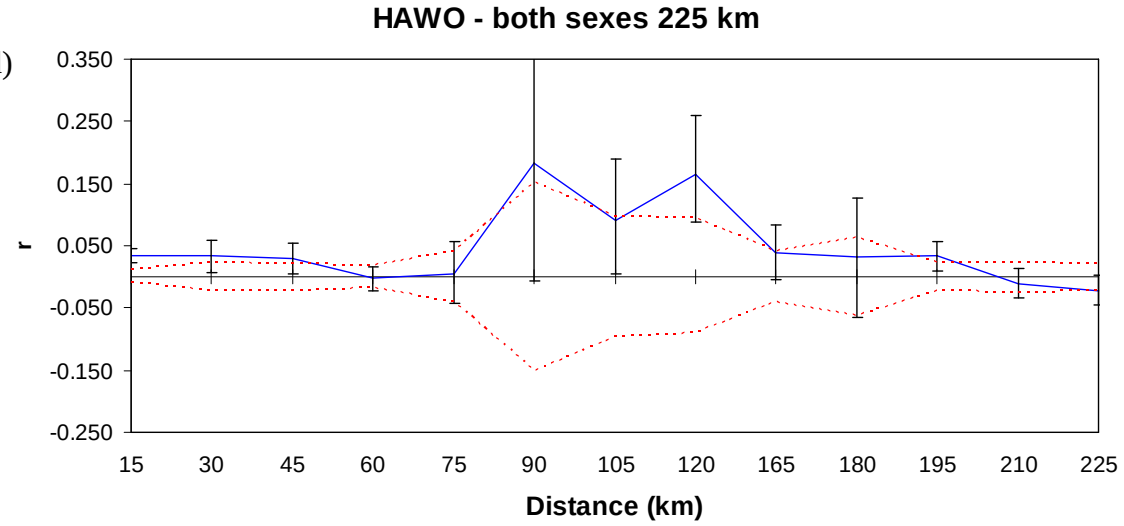
b)


Male BBWO 225km
r
0.250
0.200
0.150
0.100
0.050
0.000
-0.050
-0.100
15
30
45
60
75
90
105
120
135
150
165
180
195
210
225
Distance (km)

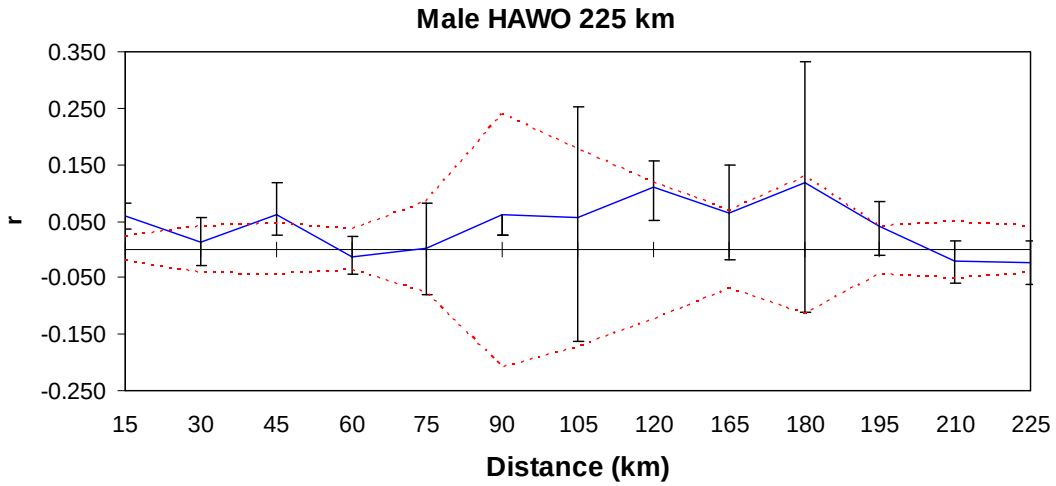
c)


Female BBWO 225km
r
0.250
0.200
0.150
0.100
0.050
0.000
-0.050
-0.100
-0.150
15
30
45
60
75
90
105
120
135
150
165
180
195
210
225
Distance (km)

d)


HAWO - both sexes 225 km
r
0.350
0.250
0.150
0.050
-0.050
-0.150
-0.250
15
30
45
60
75
90
105
120
165
180
195
210
225
Distance (km)

e)


Male HAWO 225 km
0.350
0.250
0.150
0.050
-0.050
-0.150
-0.250
r
15
30
45
60
75
90
105
120
165
180
195
210
225
Distance (km)

f)

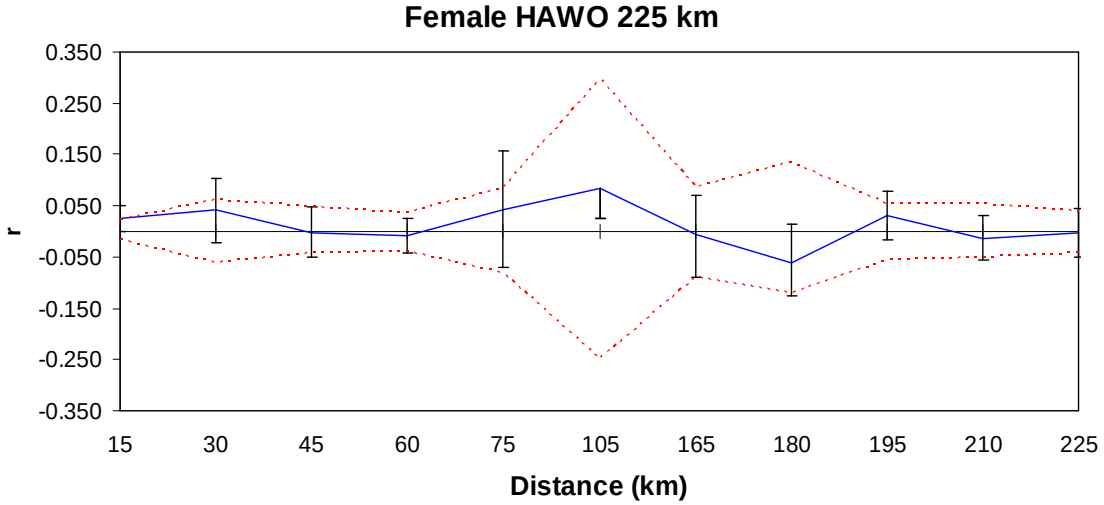
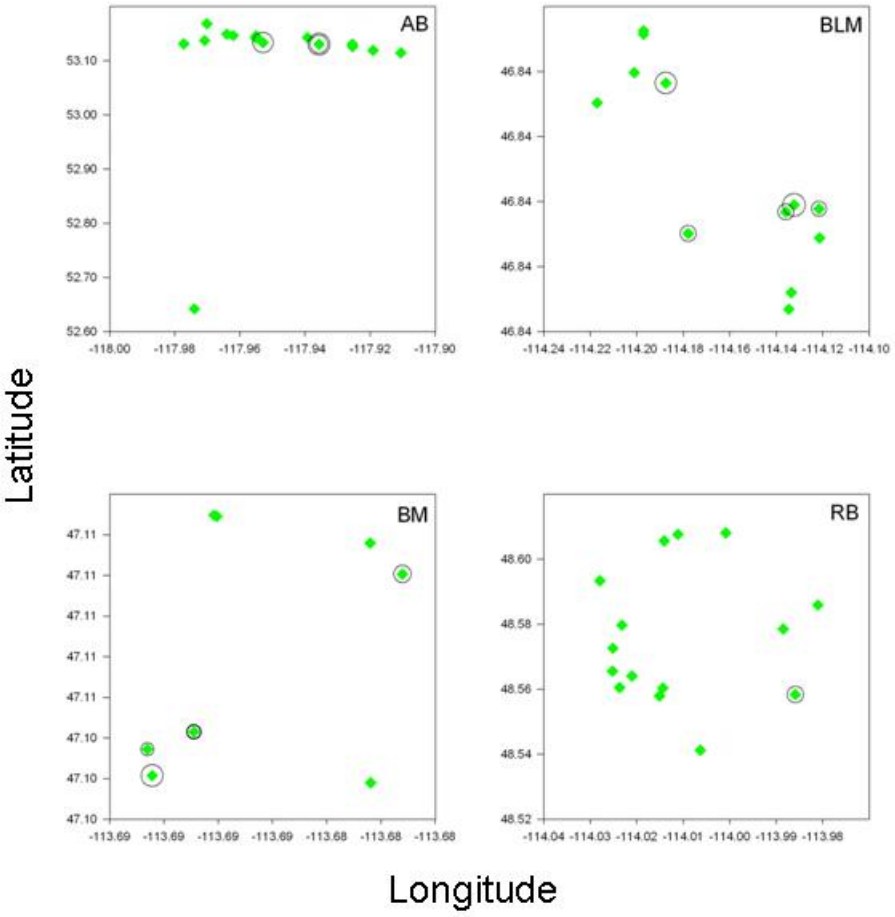

Female HAWO 225 km
0.350
0.250
0.150
0.050
-0.050
-0.150
-0.250
-0.350
r
15
30
45
60
75
105
165
180
195
210
225
Distance (km)

Figure 4-3

a


AB
BLM
BM
RB
Latitude
Longitude
53.10
53.00
52.90
52.80
52.70
52.60
-118.00 -117.98 -117.96 -117.94 -117.92 -117.90
46.84
-114.24 -114.22 -114.20 -114.18 -114.16 -114.14 -114.12 -114.10
47.11
47.10
-113.69 -113.68
48.60
48.58
48.56
48.54
48.52
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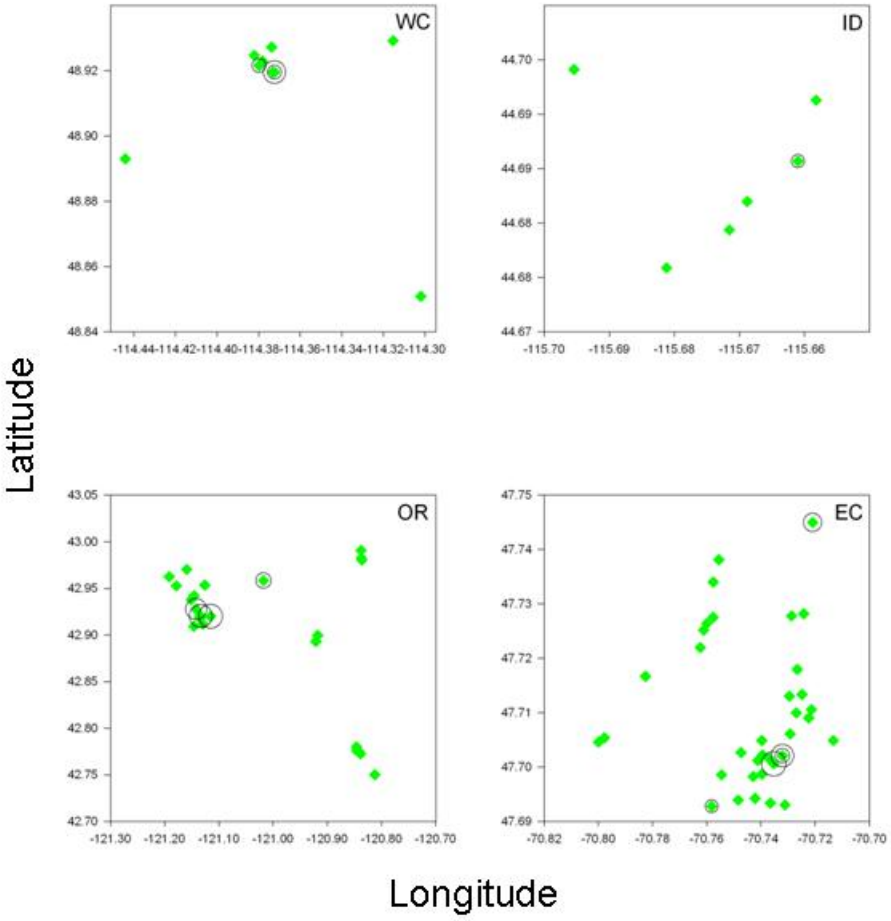

WC
ID
OR
EC
Latitude
Longitude
48.92
48.90
48.88
48.86
48.84
-114.44-114.42-114.40-114.38-114.36-114.34-114.32-114.30
44.70
44.69
44.69
44.68
44.68
44.67
-115.70
-115.69
-115.68
-115.67
-115.66
43.05
43.00
42.95
42.90
42.85
42.80
42.75
42.70
-121.30
-121.20
-121.10
-121.00
-120.90
-120.80
-120.70
47.75
47.74
47.73
47.72
47.71
47.70
47.69
-70.82
-70.80
-70.78
-70.76
-70.74
-70.72
-70.70

b

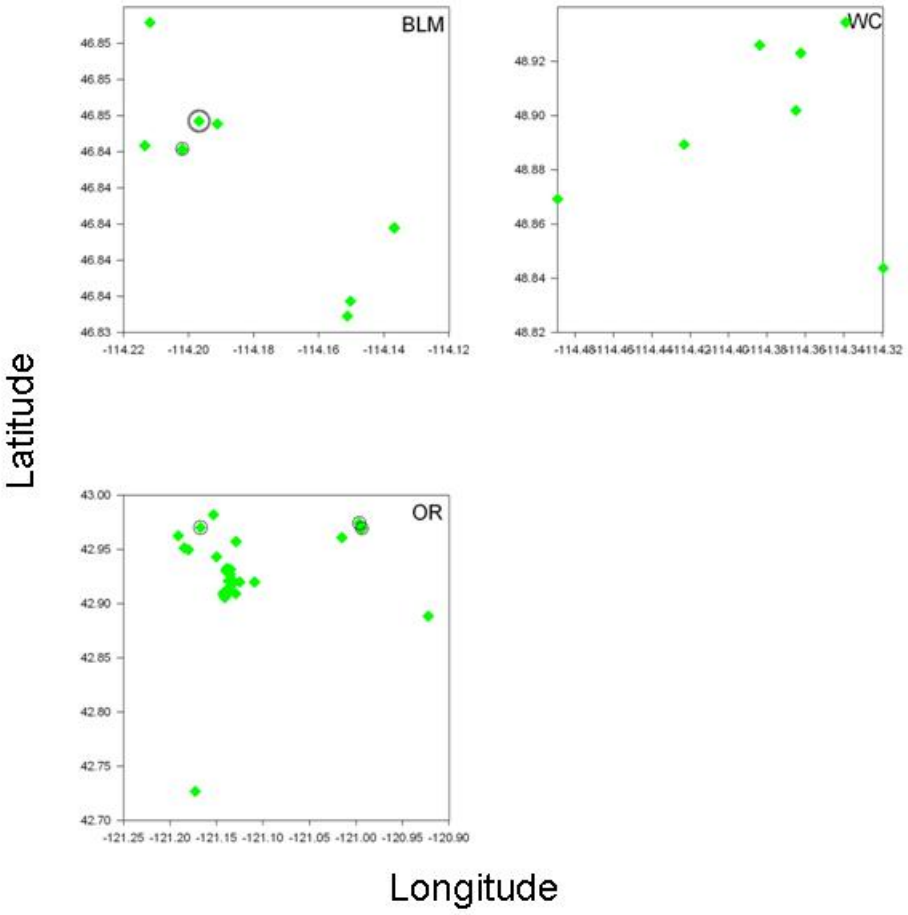
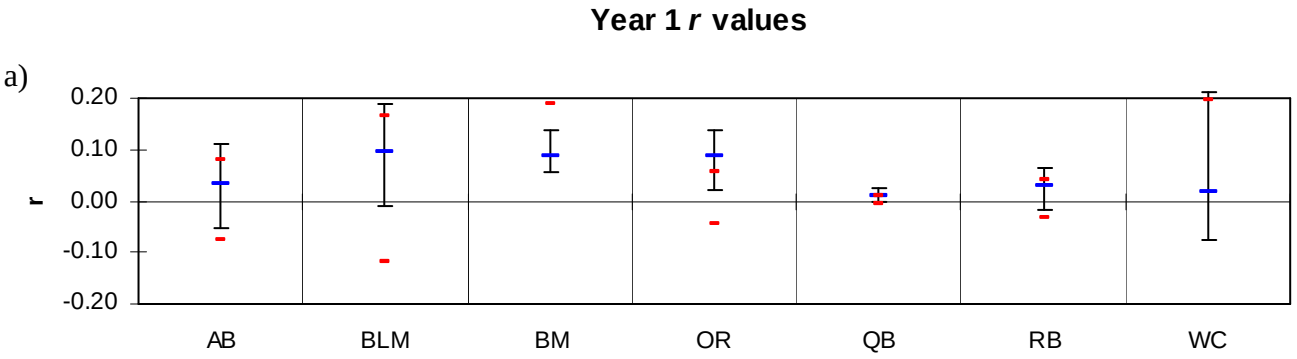
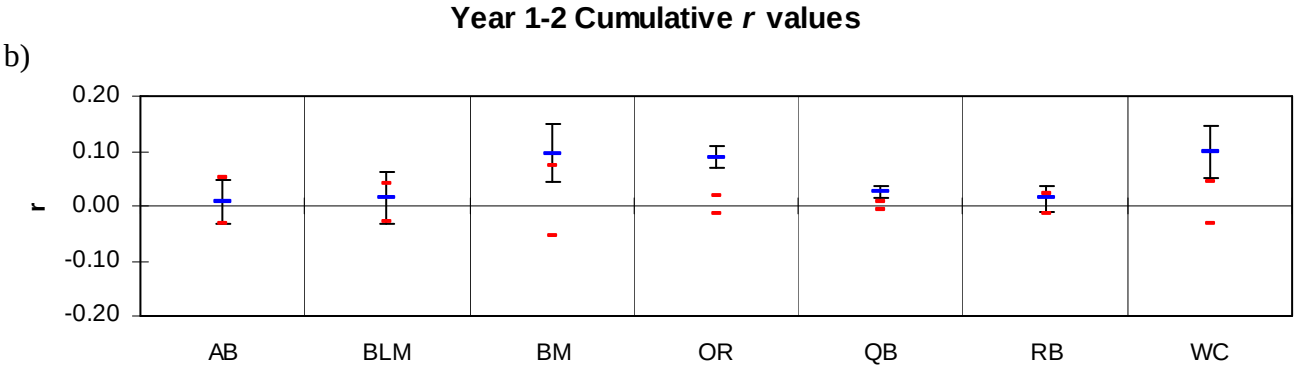

BLM
WC
OR
Latitude
Longitude
46.85
46.85
46.85
46.84
46.84
46.84
46.84
46.84
46.84
46.83
-114.22
-114.20
-114.18
-114.16
-114.14
-114.12
48.92
48.90
48.88
48.86
48.84
48.82
-114.48
-114.46
-114.44
-114.42
-114.40
-114.38
-114.36
-114.34
-114.32
43.00
42.95
42.90
42.85
42.80
42.75
42.70
-121.25
-121.20
-121.15
-121.10
-121.05
-121.00
-120.95
-120.90

Figure 4-4

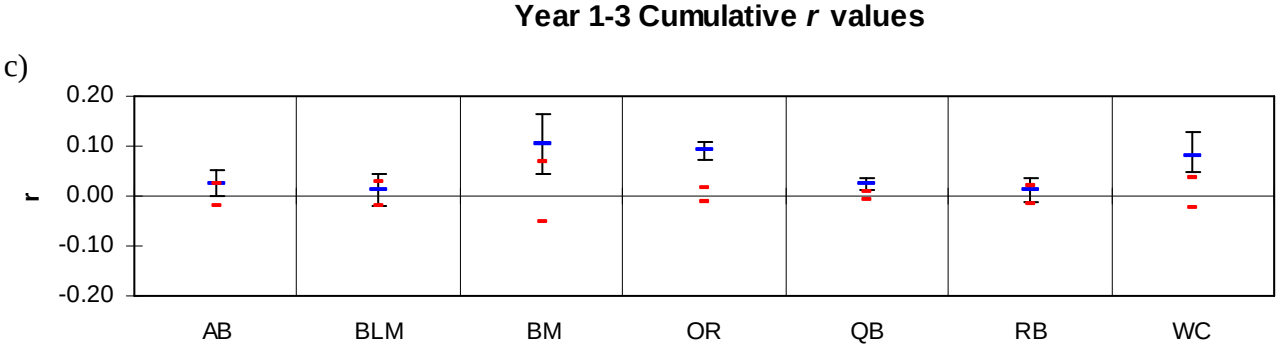
a)


Year 1 r values
0.20
0.10
0.00
-0.10
-0.20
r
AB
BLM
BM
OR
QB
RB
WC

b)


Year 1-2 Cumulative r values
0.20
0.10
0.00
-0.10
-0.20
r
AB
BLM
BM
OR
QB
RB
WC

c)


Year 1-3 Cumulative r values
0.20
0.10
0.00
-0.10
-0.20
r
AB
BLM
BM
OR
QB
RB
WC

d)

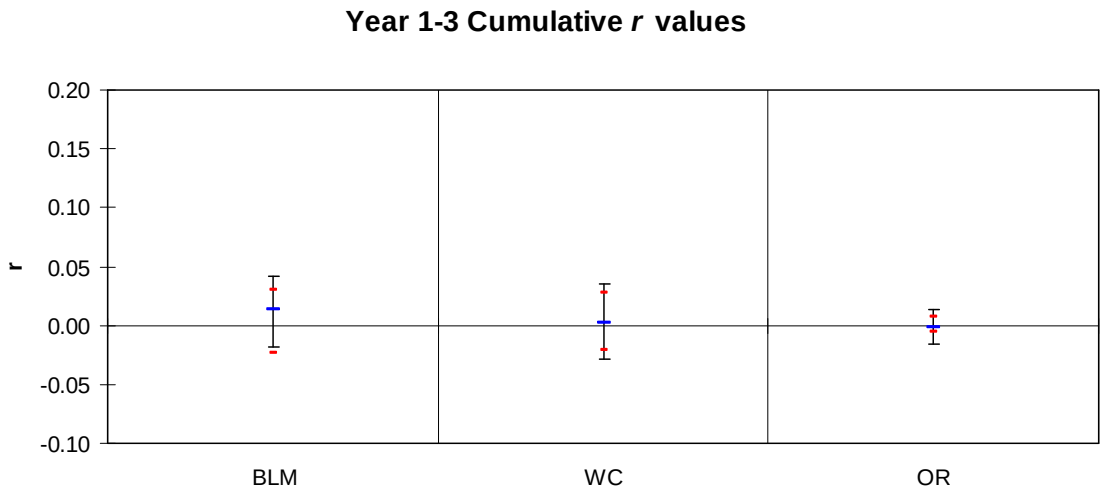

Year 1-3 Cumulative r values
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
r
BLM
WC
OR

Figure 4-5

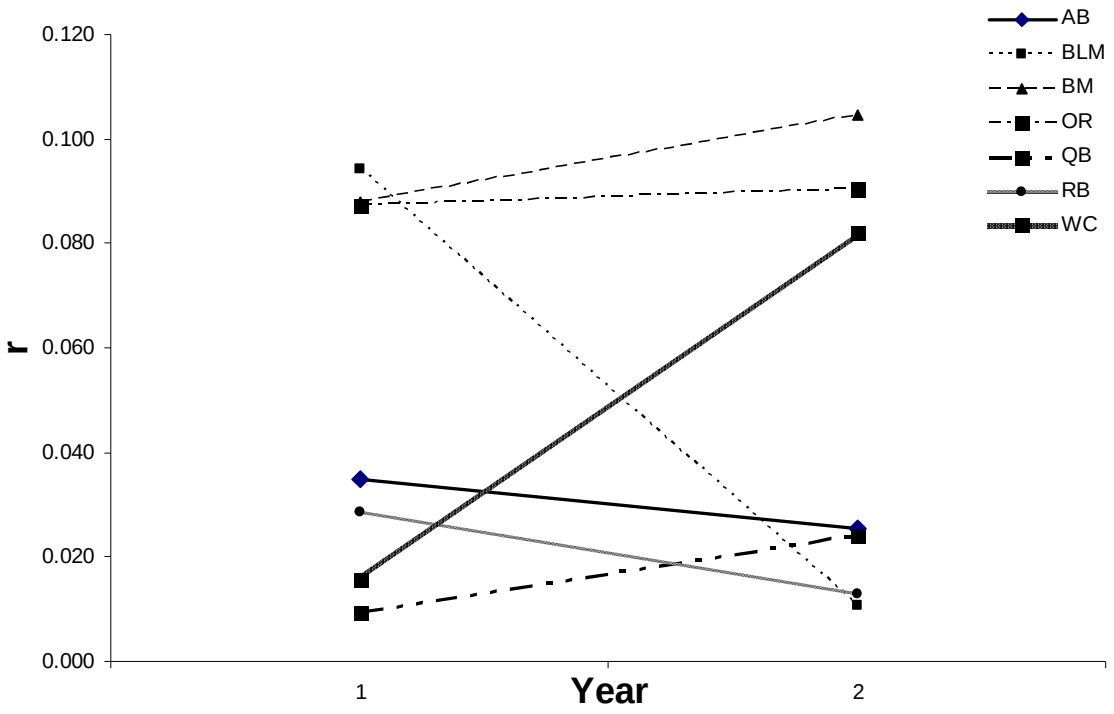

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Figure 4-6

Chapter 5 – Frequent colonization of ephemeral habitats reduces spatial structure

5.1 Abstract

The genetic characteristics of classical metapopulations, where extinction and colonization of subpopulations occur in static habitat patches, have been studied extensively. However, genetic characteristics of a "habitat-tracking" metapopulation, where a species tracks early successional habitat patches that vary through space and time, are virtually unknown. In this study, I used life history and dispersal parameters characteristic of a rare fire specialist and a common generalist to estimate patterns of genetic structure in a habitat-tracking metapopulation. This led me to simulate two basic models of dispersal: frequent colonization of new patches and stable migration among static patches. In each model, I evaluated the effect genetic drift compared to gene flow by varying subpopulation size and dispersal distance. I then compare the simulation results to empirical patterns of genetic structure of the black-backed woodpecker (*Picoides arcticus*), a fire specialist, and the hairy woodpecker (*P. villosus*), a generalist, to understand the mechanisms that create the observed patterns of genetic structure. Using a simulation framework, I found the non-intuitive result that frequent colonization generally results in greater genetic differentiation with less spatial structure compared to a stable model of dispersal. Simulation results also suggest that a combination of frequent colonization of new habitat patches combined with an intermediate dispersal distance can result in low genetic differentiation without spatial structure at large scales, the pattern observed in a fire specialist, the black-backed woodpeckers. The black-backed woodpecker shows no spatial structure across a vast spatial scale (3500 km)

whereas the hairy woodpecker, a common generalist, has a strong pattern of isolation by distance.

5.2 Introduction

Metapopulation theory has been well developed over the last century (Hanski and Gaggiotti 2004). Sewall Wright (1931, 1940) first recognized the influence that spatial structure may have on the evolution of populations, especially when local extinction and recolonization is common. Andrewartha and Birch (1954) suggested that extinction of local populations was in fact quite common, however this idea did not gain wide acceptance until island biogeography theory gained popularity. The basis of island biogeography is that a large, mainland source area supports a species and the small island populations of different sizes and distances from the source are commonly colonized and suffer extinction (MacArthur and Wilson 1963, 1967).

Levins (1969) developed the metapopulation concept we are familiar with today; in the idealized form there are many patches equidistant and of the same size that regularly go extinct and are recolonized. We do not often see examples of this idealized concept in natural populations. More often, we see mainland-island metapopulations that are similar to island biogeography theory, where the islands commonly go extinct and are recolonized by the mainland source. Alternatively, organisms exist in patchy populations that either have enough movement that extinction is rare or too little movement among them to allow recolonization. But what about situations where the habitat patches themselves are ephemeral and move? Snäll et al.(2003) called this type of metapopulation a habitat-tracking or patch-tracking metapopulation and currently we have very little theoretical or empirical understanding of systems such as this.

Disturbance-dependent species occupy early successional habitats and may fit better into a habitat-tracking metapopulation framework (Snäll et al.2003) where organisms track habitat through space and time (Thomas 1994, Harrison and Taylor 1997). The critical difference between a classical metapopulation and a habitat-tracking metapopulation is how the processes of extinction and colonization occur. In a classical metapopulation, subpopulation colonization and extinction is a stochastic process that occurs in habitat patches that are constant in space and time. In a habitat-tracking metapopulation, subpopulation extinction is a deterministic process where a habitat patch goes 'extinct' by aging through time. New habitat patches are 'created' by disturbance and available for colonization. That is, the habitat patches are dynamic and vary through space and time.

A great deal of theoretical work has been conducted on the effect of metapopulation structure on patterns of genetic variation in space (Harrison and Hastings 1996). The genetic population structure of classical metapopulations is complex, and has been found to vary based on the effective population size (N_e) of each subpopulation, and the amount and pattern of gene flow (m) among the subpopulations (Slatkin 1977, Slatkin 1985, Wade and McCauley 1988, Whitlock and McCauley 1990, Whitlock 2001, Hanski and Gaggiotti 2004). Extinction and recolonization can enhance or diminish the amount of genetic differentiation among subpopulations depending on the rates of subpopulation extinction, patch recolonization, migration among existing subpopulations, and subpopulation size (Wade and McCauley 1988). In general, extinction and recolonization promote genetic differentiation except under a narrow set of ecological scenarios (Wade and McCauley 1988). Fundamentally, the source of colonizing

individuals and the relationship between colonization and migration will determine patterns of genetic differentiation among subpopulations (Wade and McCauley 1988).

Wildfire is the primary disturbance that shapes landscapes in the Canadian boreal forest and forests in the western United States. Wildfires vary in their size, shape, and severity, creating a mosaic of different-aged patches of forest (Cyr et al. 2009). Primary cavity-nesting birds are closely tied to early postfire habitat because of the plentiful snags available for nest sites (Drapeau et al. 2002). Bark-foraging woodpeckers, such as the black-backed woodpecker (*Picoides arcticus*) and hairy woodpecker (*P. villosus*), are found in particularly high density in early postfire habitat because of the high density of wood-boring beetles found there during the first few years after a fire (Vierling et al. 2008). I used the simulation results to understand the mechanisms responsible for creating the observed population structure of both woodpecker species (Pierson et al. in prep, Chapter 3)

Black-backed and hairy woodpeckers are medium sized (60-80g), resident species that maintain territories year round. The black-backed woodpecker is a naturally rare fire specialist (Dixon and Saab 2000, Brawn et al. 2001, Hutto 2008, Nappi and Drapeau 2009) that colonizes burned forests within one year after fire and occupies burned patches for three to five years (Murphy and Lehnhausen 1998, Dixon and Saab 2000, Saab et al. 2007, Vierling et al. 2008). Black-backed woodpeckers live approximately eight years, thus they likely colonize more than one fire during their lifespan (Dixon and Saab 2002). The hairy woodpecker occupies a variety of habitat types, including burned and unburned forests (Jackson et al. 2002, Warren et al. 2005, Ripper et al. 2007) thus over their lifespan have a wider variety of nesting habitat available.

In this study, I address how frequent aging and colonization of new habitat patches affects genetic differentiation among habitat patches. Three alternative hypotheses are: 1) frequent colonization will result in high genetic differentiation without a spatial pattern due to frequent founder events, 2) frequent colonization with restricted dispersal distance will result in a spatial pattern of isolation by distance, 3) frequent colonization will result in low genetic differentiation across the species range, an essentially panmictic signature. To address my question, I simulate the effects of frequent colonization of newly created habitats compared to dispersal among static habitats on large-scale genetic structure. I then compare empirical estimates of genetic population structure across the range of the black-backed woodpecker, a fire specialist, to the hairy woodpecker, a generalist species.

5.2 Methods

5.2.1 Simulation

I built two basic simulation models, a frequent colonization model (FC) and a stable migration model (S), in the R software environment (http://www.r-project.org/). The package Rmetasim (Strand 2002), an individual-based model that simulates demographic and genetic parameters simultaneously, was embedded into the models. Rmetasim is an individual-based model that allows the incorporation of different age structure and demographic parameters within habitat patches and different migration rates among habitat patches (Figure 1). Individuals are the primary discrete object with a life history stage, birth date, and multilocus genotype (Strand 2002). Within a patch, an individual

survives within a demographic stage or transitions to a different demographic stage, followed by reproduction and movement.

Effective population size (N_e) and gene flow (m) are the two parameters that affect genetic differentiation among subpopulations. I varied these parameters to understand their effects under frequent colonization versus stable migration scenarios. I tested two subpopulation sizes and five dispersal distances (Table 5-1) to quantify the effects of short-, medium-, and long-distance dispersal among small and large subpopulations. The scenarios are named based on the dispersal model (FC versus S), subpopulation size (N_e = 50 versus 500) and dispersal distance (dd: 2, 4, 10, 15, 20 cells; e.g., FC.500.dd2 is the frequent colonization model with a subpopulation size of 500 and dispersal distance of two cells).

The basic landscape includes 450 habitat patches arranged in a 15 by 30 grid, based on the shape and size of the boreal forest, the main range of the black-backed woodpecker (1500 x 3000 km; each cell approximates 100 x 100 km). Subpopulations in the landscape are initiated with 10 microsatellite loci with 10 alleles per locus. Initial conditions are maximal genetic diversity resulting in a panmictic signature across the landscape at time step one. The demography within patches is a two-stage model consisting of juveniles and adults with vital rates based on average reported vital rates from other woodpeckers in the genus *Picoides* (Figure 1; Pasinelli et al. 2006): adult survival (A_S = 0.64), juvenile survival and transition to adult stage (J_A = 0.64), and fecundity (maternal contribution of offspring; F = 1.76). Adult and juvenile survival are assumed to be the same, population growth is deterministic and exponential until carrying capacity (N_e) of the patch is reached. Each simulation was initialized with 100 juveniles

and 50 adults per patch, run for 5000 time steps, allowing for a mutation rate of mutation rate of 1×10^{-6} per site, and replicated a minimum of ten times. Given the small standard errors around estimates and the length of time taken to run simulations, ten replicates were deemed adequate.

5.2.2 Dispersal

For each occupied habitat patch, the model determines which patches are available for colonization within a given dispersal distance, which is the defined number of cells in the grid in all four directions. Patches on the edge of the landscape send all of their emigrants to internal patches. The probability of moving and surviving is defined by dividing the total survival and movement probability for a particular life stage (Figure 5-1) by the number of patches available for colonization within dispersal distance of the occupied patch (Figure 5-2).

5.2.3 Frequent Colonization model

The frequent colonization model (FC) is designed to mimic the process of wildfire creating habitat patches for black-backed woodpeckers. I used data from past wildfire occurrence in the boreal forest to parameterize this model. On average, approximately 15% of the boreal forest is burned in a five-year time period (Alberta Forest Protection 2004). I used a five-year time period because black-backed woodpeckers occupy burned forests for approximately five years. The model is initialized with 68 habitat patches occupied (~ 15% of 450 patches) and survival and reproduction occurring within patches for four time steps. On the fifth time step, 68 new patches were randomly selected to

'burn' and become available for colonization (based on ~ 15% of 450 patches). The model determines which of these newly 'burned' habitat patches are within dispersal distance of occupied patches and divides the probability of an adult surviving and moving (A_{SM}) to a new patch by the number of patches available for colonization (Figure 5-2a). For example, when dispersal distance is two cells, the average number of patches 'newly available for colonization' within dispersal distance is three, when dispersal distance is ten, the average number of patches 'newly available for colonization' within dispersal distance is 28 (Table 5-1). In this model, each patch has a certain number of patches to send emigrants and A_{SM} = 0.64/ the number of 'burned' patches within the allotted dispersal distance (Figure 5-2a). Because all juveniles transition to adults in this simulation model, all dispersal is by adults. Based on black-backed woodpeckers occupying burned forests for three to five years, the next four time steps have no dispersal, and survival and reproduction occur within patches based on the demographic parameters mentioned above. This cycle is repeated for 5000 timesteps based on the approximate time the boreal forest has been present (10,000 years; Hewitt 2000) and the approximate generation time of black-backed woodpeckers (2 years).

5.2.4 Stable model

The stable model (S) is designed to mimic a species occupying stable habitat with a specified dispersal distance similar to that of the hairy woodpecker. Demographic parameters within patches are constant (Figure 1; A_S= 0.64; J_A = 0.64) and dispersal is by juveniles, as is typical of species with stable habitat. To standardize the number of patches that are colonized between the FC and S models, I calculated the average number

of patches available for colonization within each dispersal distance in the FC model and randomly selected that number of patches within the defined dispersal distance in the S model (Figure 5-2b). In this way, the only variable that was changed was the location of the patches, not the number of patches. Both survival and movement parameters were held constant (Table 1) and the model was run for 5000 time steps.

5.2.5 Simulation Scenarios and Analysis

The relevant output from the simulation consists of individuals in subpopulations occupying a cell in the landscape grid and their associated genotypes. I calculated pairwise geographic distances (i.e., number of cells), global G_{ST} (Nei 1973) and pairwise Euclidean distances among all patches. I performed Mantel tests based on a matrix of pairwise G_{ST} and a pairwise matrix of distances among patches. Given the minimal variation per run, I chose one replicate to visualize the relationship between genetic and geographic distance, (e.g., FC.50.dd2.001) and plotted G_{ST} versus geographic distance. A regression line was added using standard linear regression. All analyses were performed in R software environment (http://www.r-project.org/).

To test model performance, I simulated the extreme scenarios of no movement and panmixia (dd30). At time step one under all scenarios, the models produced a landscape without any genetic differentiation among patches. Under a scenario without movement, the model resulted in a landscape without a signature of isolation by distance because the subpopulations quickly attained maximal differentiation (G_{ST} = 1.0). The panmictic model was tested by setting dispersal distance to 30 and the results produced a pattern without any isolation by distance and no genetic differentiation among patches. I

began with a minimum dispersal distance of two cells. All individuals in the landscape in the FC model went extinct within several time steps when dispersal was limited to one cell because patches were set to go extinct if newly created habitats were not within dispersal distance in time step five.

5.2.6 Historical fire patterns

I used historic wildfire data from the Canadian boreal forest to determine if wildfires occur in a spatial and temporal context that is ecologically realistic for frequent colonization by black-backed woodpeckers. I used data from 1980-2006 because GIS layers documenting the area of wildfires (i.e., polygons) are available for this time period (Alberta Forest Protection 2004, Mike Flannigan, personal communication). I calculated the minimum distance from the center of each fire to the nearest edge of all fires. I then calculated the median minimum distance between fires that occur within a five year span. I used a five-year span because this is the average time period a burned forest is occupied by black-backed and hairy woodpeckers (Saab et al. 2007).

5.3 Results

5.3.1 Simulation

My primary goal in this simulation was to determine how the frequent colonization of habitat patches influenced the spatial genetic structure of a metapopulation compared to a stable model of dispersal. Within this context, I examined the effect of genetic drift by varying subpopulation size (N_e) and gene flow (m) by varying dispersal distance.

Overall, genetic differentiation among subpopulations (global G_{ST}) was larger in the FC model than the S model and declined as dispersal distance increased in both models (Figure 5-3). On average, the frequent colonization model with a small population size and small dispersal distance (FC.50.dd2) had the largest amount of genetic divergence among subpopulations (mean G_{ST} = 0.15) and the stable model with a large population size and large dispersal distance (S.500.dd20) had the smallest amount of genetic divergence among subpopulations (mean G_{ST} = 0.001).

The FC model generally had a weaker pattern of isolation by distance than the S model as evidenced by lower average Mantel's r correlation coefficient (Figures 5-4, 5-5). The exception was at short dispersal distances in the N500 series where S.500.dd2 and S.500.dd4 scenarios had smaller average correlation coefficients than FC.500.dd2 and FC.500.dd4 respectively (Figure 5-5).

5.3.2 Effect of dispersal distance

As dispersal distance increased, total genetic differentiation and the average correlation between genetic and geographic distance decreased in both the FC and S model of dispersal (Figure 5-3 – 5-5). Again, the exception to this pattern was the S.500 series in which the average Mantel's *r* increased to a peak value (mean *r* = 0.24) at intermediate dispersal distance then decreased with increasing dispersal distance (Figure 5-5).

5.3.3 Effect of N_e

The effective population size of subpopulations had a large effect on estimates of genetic differentiation and spatial structure in both the FC and S models. Estimates of mean

global G_{ST} were generally an order of magnitude larger when subpopulation size was N_e = 50 compared to N_e = 500 under both dispersal scenarios (Figure 5-3). In the FC model, average estimates of the correlation between genetic and geographic distance were markedly lower in the FC.50 series than the FC.500 series (Figure 5-6). As mentioned above, the S.500 series resulted in a different pattern of average correlation coefficients than the other simulation scenarios modeled. At the shortest dispersal distances (dd2), the S.500 series had a smaller average correlation coefficient between genetic and geographic distance than the S.50 model. Once dispersal distance reached four cells (dd4), the S.50 model had smaller average correlation between genetic and geographic distance than the S.500 series (Figure 5-7).

I examined spatial patterns in wildfire occurrence in the boreal forest to relate dispersal distances in the FC model to empirical patterns. Based on the fire history in the boreal forest from 1980-2006, the minimum distance between the center of a fire to the nearest edge of the closest fire in a five year time period ranged from 2-290 km apart (Figure 5-8). The minimum distance among fires was less than 50 km for 55% of the years examined.

5.4 Discussion

In this study, I focused on how genetic drift and gene flow interacted to shape genetic structure under two models of dispersal. I used life history characteristics similar to those of the black-backed woodpecker, a fire specialist (Hutto 2008), and the hairy woodpecker, a generalist (Jackson et al. 2002, Ripper et al. 2007), to test how frequent colonization of newly created habitat patches affected patterns of genetic differentiation

across space. In general, the frequent colonization model (FC) resulted in higher genetic differentiation among subpopulations with less spatial structure than the stable model (S). These results provide a possible mechanism for the low genetic differentiation observed in black-backed woodpeckers across the boreal forest.

5.4.1 Simulation

To examine the influence of genetic drift, I modeled the population size of each patch as $N_e = 50$ and $N_e = 500$. Not surprisingly, population size had a major influence on the outcome of both the FC and S model of dispersal; yet the resulting patterns were substantially different between models. All scenarios with small N_e had higher estimates of genetic differentiation compared to the respective scenarios with large N_e, regardless of the model (Figure 5-6, 5-7).

Perhaps the most striking result is the differential effect subpopulation size had on patterns of spatial structure in the FC model compared to the S model. The FC.50 series displayed markedly weaker patterns of isolation by distance than the comparative FC.500 series models. Genetic drift is likely the dominant force creating the pattern of genetic distances across space given the high global G_{ST} in the FC.50 series (Figure 5-3) and the large range of pairwise G_{ST} (Figure 5-9; Hutchison and Templeton 1999, Holder 2000). The pattern of residuals in a regression plot of genetic versus geographic distance can provide an indication of whether gene flow or genetic drift is the dominant force restricting a pattern of isolation by distance from developing, with genetic drift causing a large spread of residuals because subpopulations diverge from one another regardless of their spatial context (Figure 5-10, Hutchison and Templeton 1999). If genetic drift was

the primary force restricting a pattern of isolation by distance from developing at $N_e = 50$ compared to $N_e = 500$, then there should be considerably more spread in the residuals around the regression line between genetic and geographic distance at the same dispersal distance. There is more variance in the estimates of G_{ST} across space at small population sizes (Figure 5-9). For example, in a FC.50.dd2 scenario, values of G_{ST} ranged from 0.0-0.6 compared to the same scenario but with a large effective population size (FC.500.dd2), where values ranged from 0.0-0.035 (Figure 5-9).

Genetic drift apparently had a stronger influence in the FC model than the S model (Figure 5-9), likely as a result of repeated founder events of new subpopulations as opposed to migration among established subpopulations populations. Dispersal distance also influenced the variance of G_{ST} across space, with larger dispersal distances resulting in smaller variance in the residuals around the regression line (Figure 5-9). In the FC model, the founder effect is likely reduced at larger dispersal distances because new patches are colonized by emigrants from a larger number of subpopulations (i.e., the migrant pool model of Wade and McCauley 1988).

The S model resulted in extremely different patterns for the two different population sizes considered (Figure 5-7). The S.50 series followed the expected pattern of larger correlations between genetic and geographic distance at shorter dispersal distances. In contrast, the S.500 series resulted in a smaller correlation values between genetic and geographic distance at shorter dispersal distances, with peak values at a dispersal distance of 10 cells. A possible explanation for this pattern is the S.500 series did not reach equilibrium. Across the entire landscape of 450 subpopulations, the S model has a larger landscape N_e (sum of subpopulations' N_e) than the FC model because

all the habitat patches are occupied, whereas only ~ 68 of the habitat patches are occupied at once in the FC model. The S.500 series has a landscape N_e of 225,000, compared to the S.50 series landscape N_e of 22,500 or the FC.500 series landscape N_e of 34,000, and consequently will take longest to reach equilibrium across the landscape. Isolation by distance will be maximal at equilibrium, developing at short distances initially (Slatkin 1993). Isolation by distance at short distances can be obscured by a lack of genetic spatial structure at larger distances (Figure 5-10d, Hutchinson and Templeton 1999). To test if a lack of equilibrium created the weaker pattern of isolation by distance at short dispersal distance, I ran S.500.dd2, S.500.dd10 and S.500.dd15 for 15,000 time steps, with the prediction that the correlation between genetic and geographic distance would increase in the S.500.dd2 scenario. Correlation estimates did not shift with increased time; after 5,000 timesteps S.500.dd2 mean Mantel's $r = 0.975$, and after 15,000 time steps the mean Mantel's $r = 0.0898$. The other two scenarios had similar correlation coefficients after 15,000 versus 5,000 timesteps, indicating a lack of equilibrium is not the reason for the observed pattern.

Further exploration with the simulation environment is needed to determine what is causing this unexpected pattern. The pivotal point is the intermediate dispersal distance of 10 cells where the peak in correlation between geographic and genetic distance occurs (S.500.dd10 mean $r = 0.24$, $P = 0.001$). The dispersal distance of ten cells represents a situation where long-distance dispersal predominates but not all cells in the landscape can be reached by individuals. A logical next step is to simulate intermediate subpopulation size to determine at what subpopulation size the pattern emerges and explore how variation in survival and transition values affect the consistency

of the pattern. These unexpected results demonstrate the value of using detailed genetic and demographic simulation models to evaluate how genetic drift and gene flow interact under different demographic scenarios.

5.4.2 Simulations explain empirical differences in structure among species

In a previous study, I found black-backed woodpeckers show little genetic divergence among sites sampled across their distribution (Figure 5-11) and no significant correlation between genetic and geographic distance (Figure 5-12). Sampling locations within the boreal forest (Idaho to Quebec, Figure 5-11) have particularly low genetic differentiation among them (i.e., all pairwise $F_{ST} < 0.02$) despite spanning a large geographic area (~ 3000 km; Pierson et al. in press). Hairy woodpeckers have a similar range of genetic differentiation among sampling locations (i.e, all pairwise $F_{ST} < 0.07$;), yet show a strong correlation between genetic and geographic distance (isolation by distance; Figure 5-12; Chapter 3), which is expected when dispersal distance is less than the maximal distance between sampled populations, a distance that spans 3500 km. Hairy woodpeckers have larger population sizes than black-backed woodpeckers and tend to disperse fairly short distances (~ 40 km; Jackson et al. 2002, Chapter 4). The empirical genetic results for the hairy woodpecker are generally in concordance with the results of the simulation as the stable dispersal series resulted in low genetic differentiation across space with a strong pattern of isolation by distance, regardless of effective population size.

Black-backed woodpeckers have low genetic differentiation among sampling localities with a lack of isolation by distance across their range. Because it is unlikely black-backed woodpeckers regularly disperse this distance, I discuss the possible

mechanisms that may have resulted in a lack of isolation by distance at such a large geographic scale.

My simulation modeled the frequent colonization of new habitats in a spatial and temporal context that mimicked wildfire creating new habitat patches for black-backed woodpeckers across the boreal forest. Given the rarity of black-backed woodpeckers, I will focus on the FC.50 series because black-backed woodpeckers likely occur in low numbers (e.g., 50 versus 500) within particular patches or wildfires. Although the mean Mantel's *r* suggests isolation by distance at short dispersal distances in the FC.50 scenario (Figure 5-4), these scenarios had at least one replicate without a significant correlation between genetic and geographic distance. Conversely, all replicates in the S.50.dd2 and S.50.dd4 scenarios resulted in highly significant ($P = 0.001$) correlations between genetic and geographic distance (Figure 5-4). Thus, frequent colonization of ephemeral patches provides a mechanism for a lack of isolation by distance at large spatial scales. Given the same dispersal distance in a stable model of migration, isolation by distance develops at a large spatial scale.

The simulation results suggest an intermediate dispersal distance is required to produce low genetic differentiation across large spatial scales. The shortest dispersal distance that results in low genetic differentiation in the FC model is four cells (dd4; minimum global $G_{ST} = 0.042$). If I extrapolate my simulation landscape to the boreal forest, each cell equals 100 by 100 km based on the 1500 by 3000 km boreal forest area sampled. Therefore, black-backed woodpeckers would need to regularly colonize new patches up to 400 km to have low genetic differentiation that is not spatially structured. In a previous study, I found female black-backed woodpeckers regularly disperse ~ 100

km, and male black-backed woodpeckers disperse farther (Chapter 5-4). If a dispersal distance of 400 km was required to explain the empirical results, male black-backed woodpeckers must be as likely to disperse 400 km as 100 km. The spatial and temporal context of historical fire patterns in the boreal forest support this hypothesis given the closest fires usually occurred less than 200 km apart. While this seems plausible, below I explore other possible explanations.

5.4.3 Theoretical expectations for isolation by distance

Hutchison and Templeton (1999) describe four cases of how isolation by distance develops through time since colonization and how the relative influences of genetic drift and gene flow create patterns of genetic structure across space. In Case I, limited dispersal results in isolation by distance; .the pattern present in the stable model of migration (S) and the hairy woodpecker (Figure 5-10a, Figure 5-12) In Case II, gene flow dominates over genetic drift at given distances, which produces little scatter of points around the regression line of genetic versus geographic distance (Figure 5-10b). In Case III, genetic drift dominates over gene flow, resulting in a wide spread of points around the regression line (Figure 5-10c); a pattern observed in the frequent colonization model when population size was small (FC.50). The pattern observed in black-backed woodpeckers is similar to Case II, where there is little variation in the estimates of genetic differentiation, especially among sites within the boreal forest (i.e. all pairwise $F_{ST} < 0.02$). Case II often results from historic colonization of patches from a homogenous source population and is the pattern observed immediately after invasion. If

gene flow remains strong relative to drift, then Case II will persist; if habitat patches are fragmented into small isolated populations, then Case III will develop.

If gene flow is restricted by limited dispersal distance, a pattern of isolation by distance will develop in nearby habitat patches over time (Case IV; Figure 5-10d) until eventually a pattern of isolation by distance develops across the entire region connected by dispersal (Case III). In Case IV, the spatial scale of sampling can greatly influence whether isolation by distance is detected. If too large a scale is sampled, a pattern that exists at a small scale may not be detected. Both woodpecker species have had the same amount of time to develop isolation by distance across the region sampled. Under restricted dispersal, isolation by distance should take longer to develop among hairy woodpecker subpopulations because they have a larger N_e than black-backed woodpecker subpopulations. Given hairy woodpeckers have a strong signal of isolation by distance, time since colonization is not likely the reason for the lack of isolation by distance in black-backed woodpeckers (e.g., Case IV). In a meta-analysis of empirical studies, Crispo and Hendry (2005) found time since colonization was a weak factor in predicting isolation by distance, and factors such as dispersal distance are much stronger forces in determining the strength of isolation by distance that develops. Bradbury and Bentzon (2007) confirmed that dispersal distance was the main factor determining maximum isolation by distance.

Wade and McCauley (1988) found that the genetic structure of a metapopulation is primarily a function of how patches are colonized. In the propagule pool model, colonists come from a few sources and genetic differentiation tends to be high. In the migrant pool model, colonists come from many sources, which prevents genetic

differentiation among patches. My simulation results can be explained in this general context. In the FC model, short dispersal distances function similar to the propagule model, where there are few source subpopulations providing colonists (Table 5-1). Models with longer dispersal distances function like the migrant pool model where many subpopulations contribute colonists.

Gaggiotti (1996) examined population structure in a simple source-sink metapopulation model and found that when there is a high rate of population turnover, genetic differentiation among populations is low because the sink populations are composed primarily of migrants from the source population. He found that stochastic migration resulted in higher genetic differences compared to deterministic migration because drift had a larger effect on patches that sustained long periods without colonists. The frequent colonization model has a combination of high population turnover and subpopulations that sustain periods without migration. Several studies suggest black-backed woodpeckers may have source-sink population dynamics (Hutto 1995, Nappi and Drapeau 2009, Chapter 4). A next step in exploring the dynamics of this system may be to incorporate source-sink dynamics into the model where the sources are ephemeral in nature. Currently, I have modeled the life of each 'burned' patch deterministically. Additional insight may be gained by incorporating variation into a 'burned' patch lifespan to test the effects on the spatial structure of genetic differentiation. Variation in vital rates also will likely affect the simulation results. Specifically, the effect of the assumption that juvenile and adult survival rates are equal needs further investigation.

5.4.4 Empirical evidence of isolation by distance

Several species show a lack of isolation by distance at large spatial scales. Turgeon and Bernatchez (2001) found that lake cisco (*Coregonus artedi*), a species that colonized lakes relatively recently (< 12,000 years before present), had a strong pattern of isolation by distance across their range (3500 km) that disappeared within watersheds (1500 km). The lack of isolation by distance within watersheds was attributed to colonization from a homogenous source, with a large N_e preventing drift from erasing the historic signal. However, they acknowledged that lake cisco had the potential for long-distance dispersal. Statistical power can also be an issue when using Mantel tests to test for correlation with small sample sizes. Castric and Bernatchez (2004) did not detect isolation by distance in brook trout (*Salvelinus fontinalis*) among 15 subpopulations, but were able to detect isolation by distance among 59 subpopulations.

Rock ptarmigan (*Lagopus mutus*) in the Aleutian archipelago, an area similar to the boreal forest in spatial and post-glacial scale (4000 km and ~ 11,000 years before present; Holder 2000) show no isolation by distance. Genetic drift is apparently responsible for this pattern as estimates of the number of migrants per generation are evenly spread among populations and range from 1 – 100 (Holder 2000). Similarly, McDonald (1999) found a lack of isolation by distance in the Florida scrub jay (*Aphelocoma coerulescens*) as a result of genetic drift. Other studies of bird species that found a lack of isolation by distance at large spatial scales combined with low estimates of genetic differentiation conclude that gene flow is the predominant force precluding a correlation between genetic and geographic differentiation (Clegg et al. 2003, Burg et al. 2006).

The most similar comparison to my work examined three snake species that shared a recent colonization history (~ 8,000 years before present) and many life history characteristics (King and Lawson 2001). Isolation by distance was strong for two of the three species brown (*Storeria dekayi*) and water snakes (*Nerodia sipedon*), but absent for the garter snake (*Thamnophis sirtalis*). King and Lawson (2001) concluded the lack of isolation by distance was likely most similar to Case II described above (Hutchison and Templeton 1999) where there was a common source population followed by high gene flow.

5.4.5 Conclusions

The simulation suggested that frequent colonization of newly created habitats at intermediate dispersal distances (four cells ≈ 400 km) provide a mechanism for producing a pattern of low genetic differentiation and no isolation by distance across vast spatial scales, consistent with my empirical results on black-backed woodpeckers. Black-backed and hairy woodpeckers share many life history characteristics and recent colonization of the boreal forest. The most likely explanation for the lack of isolation by distance in black-backed woodpeckers is colonization of the boreal forest post-glaciation by a common source population followed by high gene flow. Dispersal across my entire study area is unlikely given the large spatial scale (3500 km). Previous studies conducted on black-backed woodpeckers confirm that intermediate distance dispersal by male woodpeckers is a plausible scenario (Chapter 4). Finally, an analysis of fire history in the boreal forest shows that fires have historically occurred in a spatial and temporal context

conducive to frequent colonization by black-backed woodpeckers if they are able to disperse at least 250 km.

This research exemplifies the need to consider dispersal behavior when interpreting empirical patterns of genetic structure across large spatial scales. Detailed simulations provide researchers with a tool to understand how different ecological scenarios influence patterns of genetic structure, thereby allowing for more accurate interpretations of empirical patterns.

Table 5-1 Simulation scenarios and associated parameters in the simulation of frequent colonization of new habitats (FC) compared to stable migration among habitats (S). The basic simulation model consists of a 15 by 30 grid of habitat patches that can be occupied by subpopulations (maximum 450 subpopulations). N_e: effective population size within subpopulations; dd: dispersal distance defined by number of cells in the landscape grid; #subpop within dd: in the FC model, the mean number of patches available for colonization within dispersal distance (see Figure 5-2), in the S model, the number of subpopulations within dispersal distance was subsampled based on the average number colonized in the FC model to standardize dispersal rates between models; landscape N_e: product of the number of habitat patches occupied and subpopulation N_e; mean adult and juvenile dispersal rates were calculated by dividing the probability of movement (A_{SM} = 0.64; F = 1.76) by the number of subpopulations within dispersal distance.

Simulation Scenario	N_e	**dd**	**# subpop within dd**	**landscape N_e**	**mean adult dispersal rate**	**mean juvenile dispersal rate**
FC.50.dd2	50	2	3.0	3,400	0.21	0.59
FC.50.dd4	50	4	7.6	3,400	0.08	0.23
FC.50.dd10	50	10	28.2	3,400	0.02	0.06
FC.50.dd15	50	15	48.8	3,400	0.01	0.04
FC.50.dd20	50	20	58.3	3,400	0.01	0.03
FC.500.dd2	500	2	3.0	34,000	0.21	0.59
FC.500.dd4	500	4	7.6	34,000	0.08	0.23
FC.500.dd10	500	10	28.2	34,000	0.02	0.06
FC.500.dd15	500	15	48.8	34,000	0.01	0.04
FC.500.dd20	500	20	58.3	34,000	0.01	0.03
S.50.dd2	50	2	3.0	22,500	0.00	0.59
S.50.dd4	50	4	8.0	22,500	0.00	0.22
S.50.dd10	50	10	28.0	22,500	0.00	0.06
S.50.dd15	50	15	49.0	22,500	0.00	0.04
S.50.dd20	50	20	58.0	22,500	0.00	0.03
S.500.dd2	500	2	3.0	225,000	0.00	0.59
S.500.dd4	500	4	8.0	225,000	0.00	0.22
S.500.dd10	500	10	28.0	225,000	0.00	0.06
S.500.dd15	500	15	49.0	225,000	0.00	0.04
S.500.dd20	500	20	58.0	225,000	0.00	0.03

Figure 5-1 A schematic representing the within and among patch vital rates visualized in matrix form. In this example, there are three habitats and within patch demographic rates are represented on the diagonal (e.g., juvenile transition to juvenile: J_J, juvenile survival and transition to adult: J_A, maternal contribution of offspring: F, and adult survival: A_S). Demographic rates can vary within patches and are found in the shaded cells on the diagonal; the within patch rates for the FC model are in the center and the within patch demographic rates for the S model are in the bottom right matrix. Movement probabilities are above and below the diagonal and can be varied bidirectionally among all sites (e.g., $A_1 – A_2$ = adult movement from patch 1 to patch 2, $A_2 – A_1$ = adult movement from patch 2 to patch 1). In this example, the matrix is 6 x 6 because there are two life stages and three patches. The combined movement and within patch demographic matrix used in both the FC and S simulation models was 900 x 900 because there was two life stages and 450 patches.

Figure 5-2 a) Schematic showing an example of a 5 x 6 grid landscape with 30 habitat patches for the simulation mimicking frequent colonization of burned habitat patches. In time step 0, patch 15 is occupied and survival and reproduction occurs for four times steps (Ja = 0.64, As = 0.64, F = 1.76). In the fifth time step, five habitat patches (8, 10, 25, 28, 30) are randomly selected to 'burn' and become available as habitat. In this example, dispersal distance is set to one cell, as indicated by the cells outlined in bold. In time step 5, individuals from patch 15 disperse to patch 8 and patch 10 (Asm = 0.64/# patches; Asm 15 to 8 = 0.32; Asm 15 to 10 = 0.32). In time steps 6 – 9, survival and reproduction occurs within patches 8 and 10. In time step 10, five new patches are

randomly selected and the model determines which patches are within dispersal distance and available for colonization. Habitat patch 10 has one 'burned' patch (9) within dispersal distance therefore the Asm = 0.64; habitat patch 8 has two 'burned patches (2, 9) within dispersal distance, therefore Asm = 0.32 to each one respectably. In this time step, habitat patch 9 receives colonizers from two different patches.

b) Schematic example of a 5 x 6 grid landscape with 30 habitat patches for the simulation mimicking a stable migration model of dispersal. In time step 0, habitat 15 is occupied. In time step one, the model determines all cells within dispersal distance (one cell in this example). Based on the average number of cells available for colonization in the frequent colonization model for each specified dispersal distance (see Table 1), the appropriate number of patches are randomly selected for colonization by juveniles (two cells in this example, patch 8 and 10). Demographic rates are held constant for 5000 time steps (J_A = 0.64, A_S = 0.64; F =1.76/ number of patches, or 0.88 in this example).

Figure 5-3 Average Global G_{ST} of each landscape for the different simulation scenarios. Global G_{ST} was calculated in the R software environment and the average of ten replicate simulation runs was calculated. solid triangles: FC.50 series; open squares: FC.500 series; open diamonds: S.50 series; solid circles: S.500 series

Figure 5-4 The average Mantel's *r* coefficient from ten simulation runs for a range of dispersal distances for both the FC and S model at a N_e = 50 within occupied habitat patches: solid triangles: FC.50 series; open diamonds: S.50 series; error bars are standard error of the ten replicates

Figure 5-5 The average Mantel's *r* coefficient from ten simulation runs for a range of dispersal distances for both the FC and S model at a N_e = 500 within occupied habitat patches: open squares: FC.500 series; solid circles: S.500 series; error bars are standard error of the ten replicates

Figure 5-6 The average Mantel's *r* coefficient from ten simulation runs for a range of dispersal distances for the FC model at a N_e = 50 compared to N_e = 500 within occupied habitat patches. solid triangles: FC.50 series; open squares: FC.500 series; error bars are standard error of the ten replicates.

Figure 5-7 The average Mantel's *r* coefficient from ten simulation runs for a range of dispersal distances for the S model at a N_e = 50 compared to N_e = 500 within occupied habitat patches. open diamonds: S.50 series; solid circles: S.500 series; error bars are standard error of the ten replicates.

Figure 5-8 The median minimum distance from the center of fires that occurred in a year to the nearest edge of a fire that occurred in the next five year period. Historical fire data was obtained from Alberta Forest Protection 2004 (http://www.srd.gov.ab.ca/wildfires/).

Figure 5-9 Plots of pairwise G_{ST} versus geographic distance from a replicate of selected simulation scenarios.

Figure 5-10 Conceptual plots of isolation by distance adapted from Hutchison and Templeton (1999). a) Case I: regional equilibrium between gene flow and genetic drift among populations with restricted dispersal; b) Case II: a lack of isolation by distance with gene flow as the dominant force, evidenced by the little variation among genetic distances; b) Case III: a lack of isolation by distance with genetic drift as the dominant force, evidenced by high variation among genetic distances; c) Case IV: nonequilibrium isolation by distance where a correlation between genetic and geographic distance can be seen at short distances (increasing slope) due to restricted dispersal, and genetic drift dominates at larger distances (slope of line = 0)

Figure 5-11 Sampling locations of black-backed and hairy woodpeckers across the range of the black-backed woodpecker.

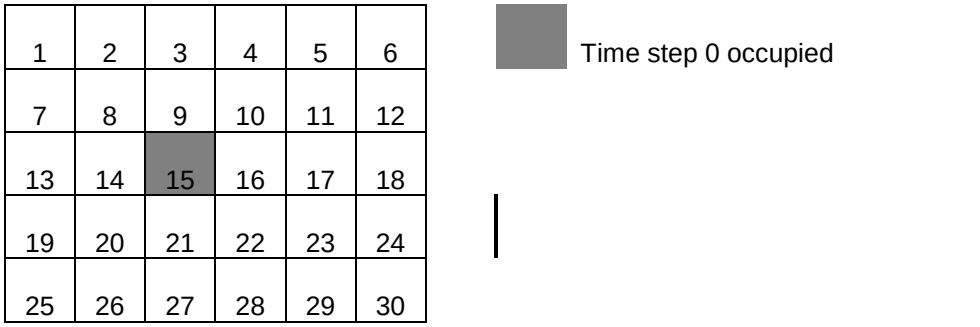
Figure 5-12 Patterns of isolation by distance for black-backed woodpeckers, a fire specialist, compared to hairy woodpeckers, a generalist species that exploits burned areas when available. Hairy woodpeckers have a classic pattern of isolation by distance with genetic differentiation, based on $F_{ST}/1\text{-}F_{ST}$, increasing with geographic distance. Black-backed woodpeckers have much less increase genetic differentiation over the same spatial scale. Isolation by distance was tested using Mantel tests, black-backed woodpeckers ($r = 0.028$, $P = 0.30$); hairy woodpecker ($r = 0.097$, $P = 0.05$).

J_J	F	$J_2 - J_1$	$A_2 - F_1$	$J_3 - J_1$	$A_3 - F_1$
J_A	A_S	$J_2 - A_1$	$A_2 - A_1$	$J_3 - A_1$	$A_3 - A_1$
$J_1 - J_2$	$A_1 - F_2$	0	1.76	$J_3 - J_2$	$A_3 - F_1$
$J_1 - A_2$	$A_1 - A_2$	0.64	0.64	$J_3 - A_2$	$A_3 - A_2$
$J_1 - J_3$	$A_1 - F_3$	$J_2 - J_3$	$A_2 - J_3$	0	0
$J_1 - A_3$	$A_1 - A_3$	$J_2 - A_3$	$A_2 - A_3$	0.64	0.64

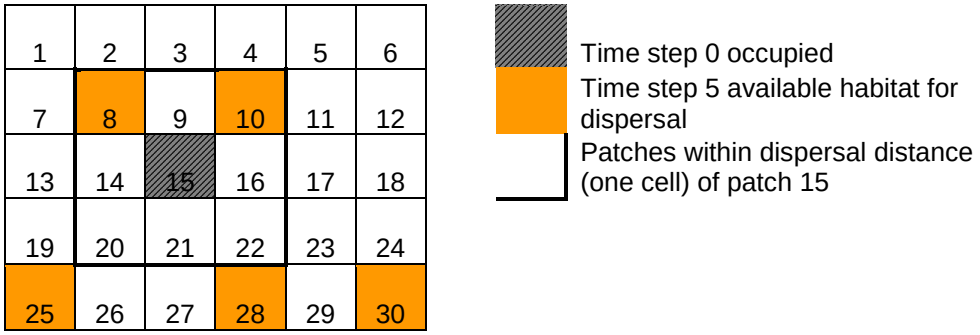
Figure 5-1

a)

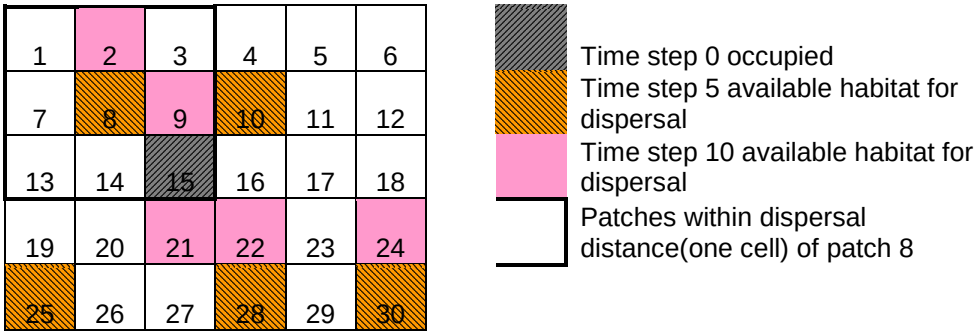
Time step 0



Time step 5



Time step 10



b)

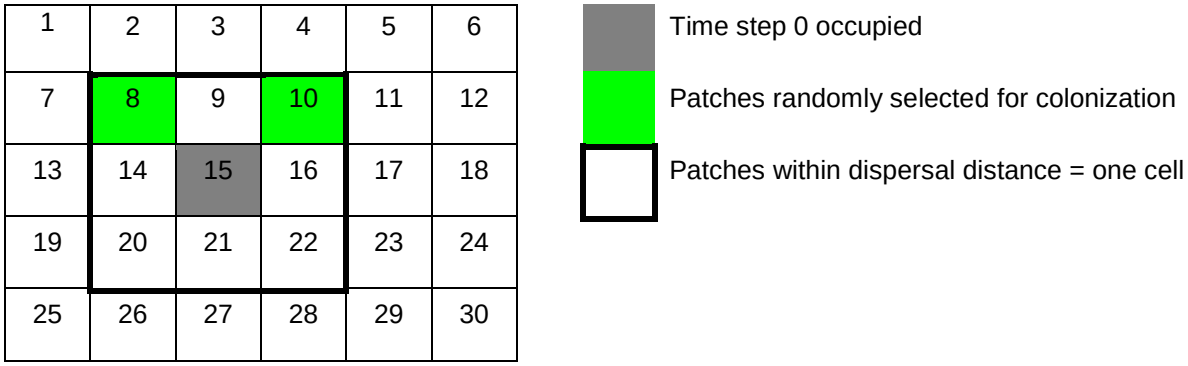

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
Time step 0 occupied
Patches randomly selected for colonization
Patches within dispersal distance = one cell

Figure 5-2

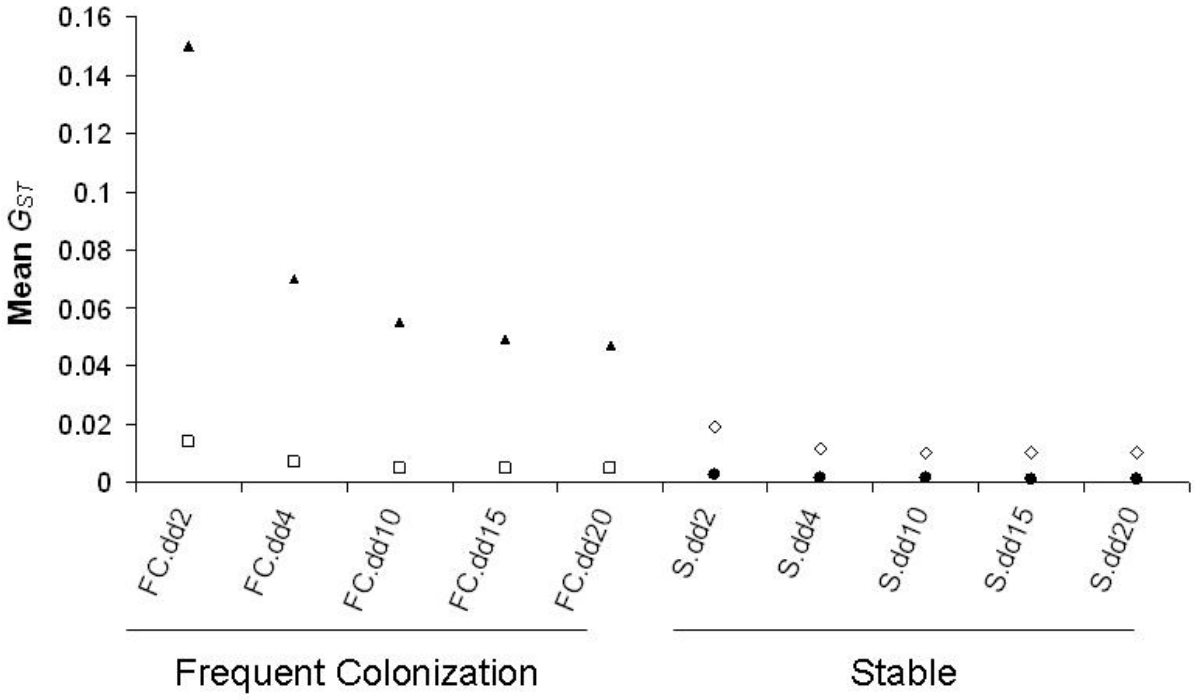

0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Mean G_{ST}
FC.dd2
FC.dd4
FC.dd10
FC.dd15
FC.dd20
S.dd2
S.dd4
S.dd10
S.dd15
S.dd20
Frequent Colonization
Stable

Figure 5-3

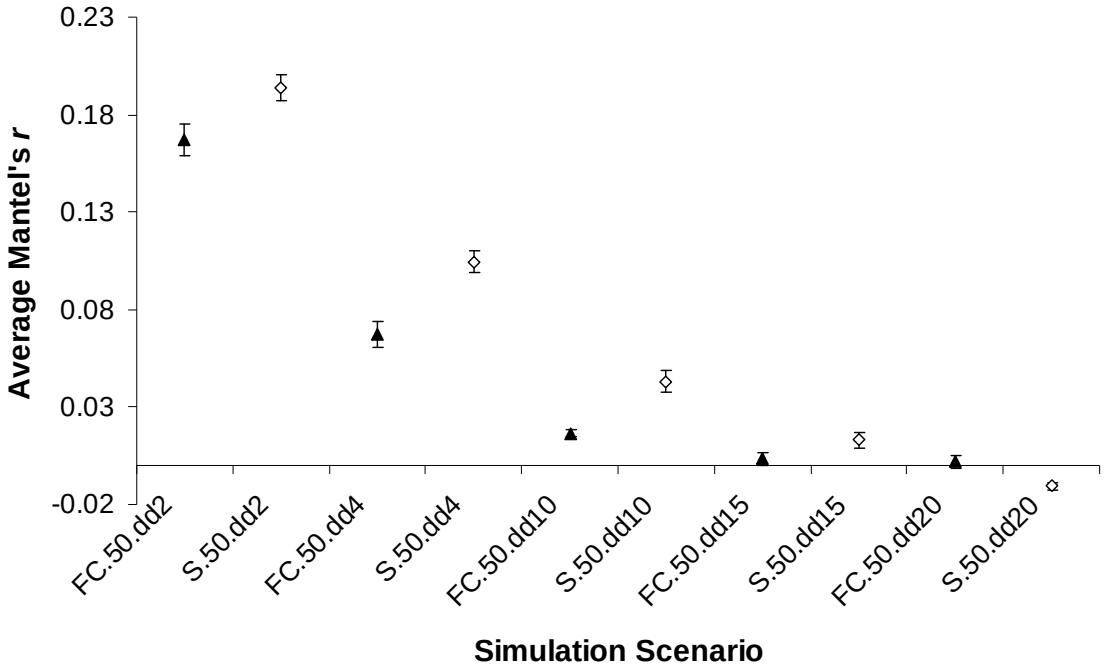

0.23
0.18
0.13
0.08
0.03
-0.02
Average Mantel's r
FC.50.dd2
S.50.dd2
FC.50.dd4
S.50.dd4
FC.50.dd10
S.50.dd10
FC.50.dd15
S.50.dd15
FC.50.dd20
S.50.dd20
Simulation Scenario

Figure 5-4

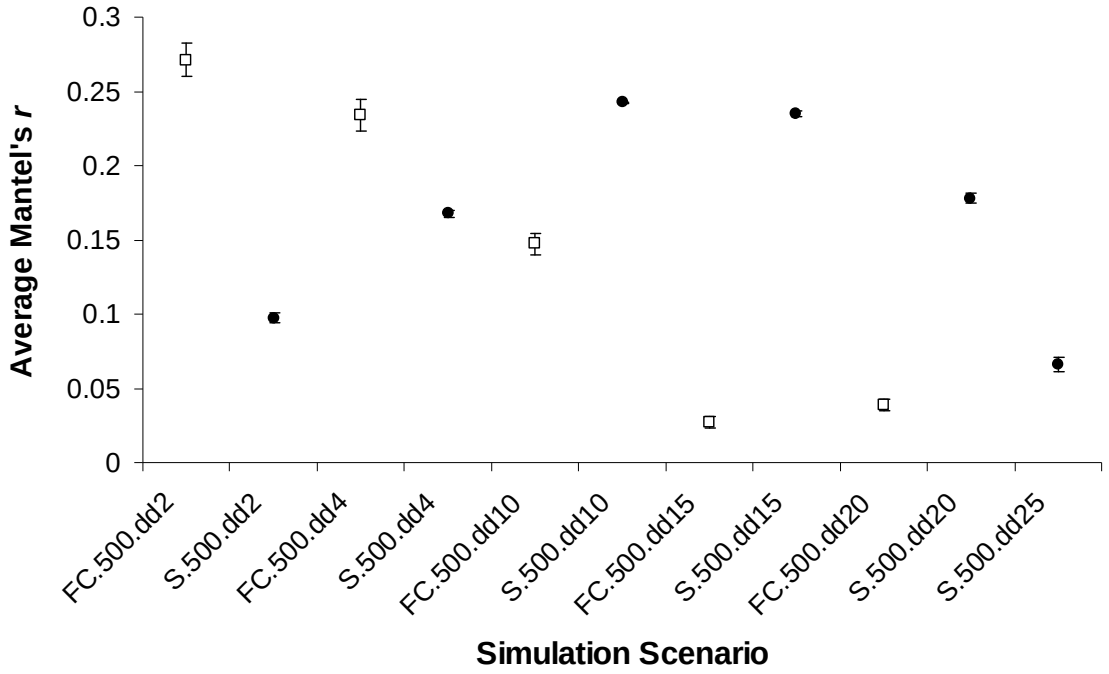

0.3
0.25
0.2
0.15
0.1
0.05
0
Average Mantel's r
FC.500.dd2
S.500.dd2
FC.500.dd4
S.500.dd4
FC.500.dd10
S.500.dd10
FC.500.dd15
S.500.dd15
FC.500.dd20
S.500.dd20
S.500.dd25
Simulation Scenario

Figure 5-5

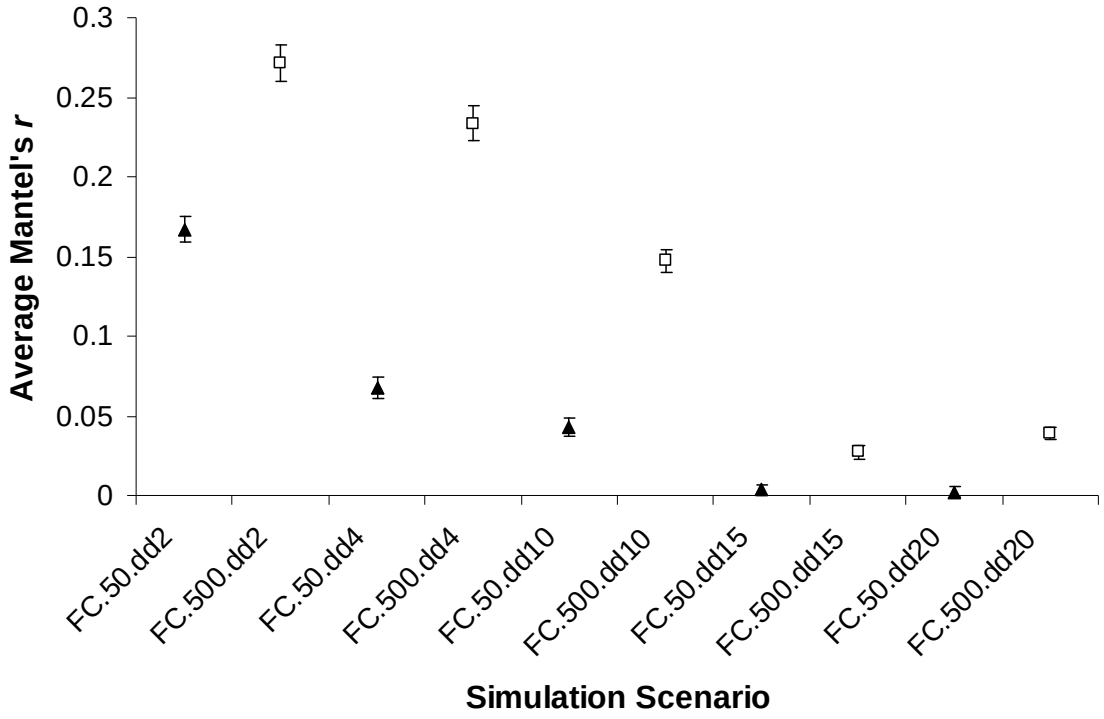

0.3
0.25
0.2
0.15
0.1
0.05
0
Average Mantel's r
FC.50.dd2
FC.500.dd2
FC.50.dd4
FC.500.dd4
FC.50.dd10
FC.500.dd10
FC.50.dd15
FC.500.dd15
FC.50.dd20
FC.500.dd20
Simulation Scenario

Figure 5-6

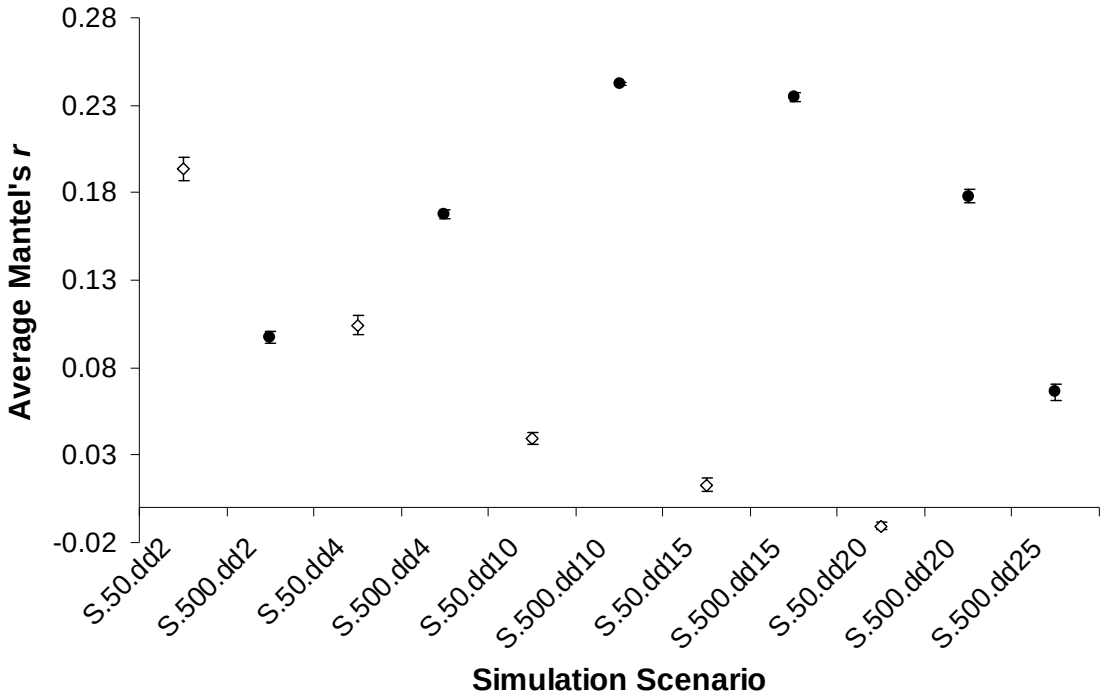

0.28
0.23
0.18
0.13
0.08
0.03
-0.02
Average Mantel's r
S.50.dd2
S.500.dd2
S.50.dd4
S.500.dd4
S.50.dd10
S.500.dd10
S.50.dd15
S.500.dd15
S.50.dd20
S.500.dd20
S.500.dd25
Simulation Scenario

Figure 5-7

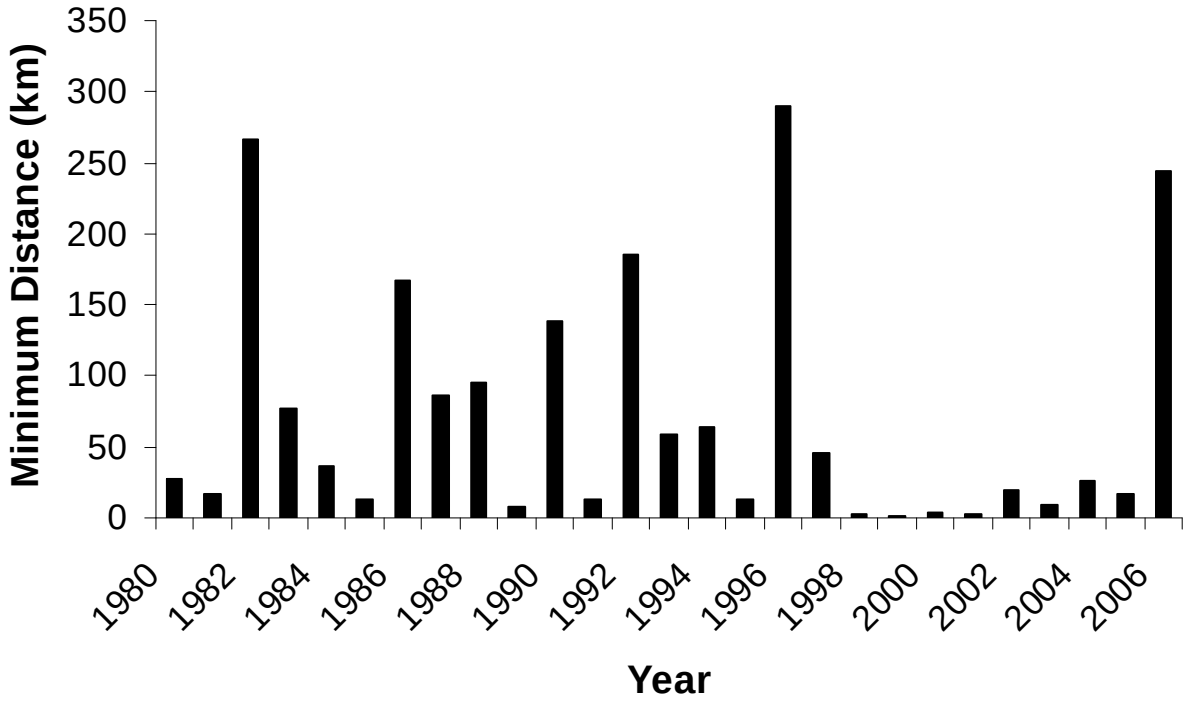
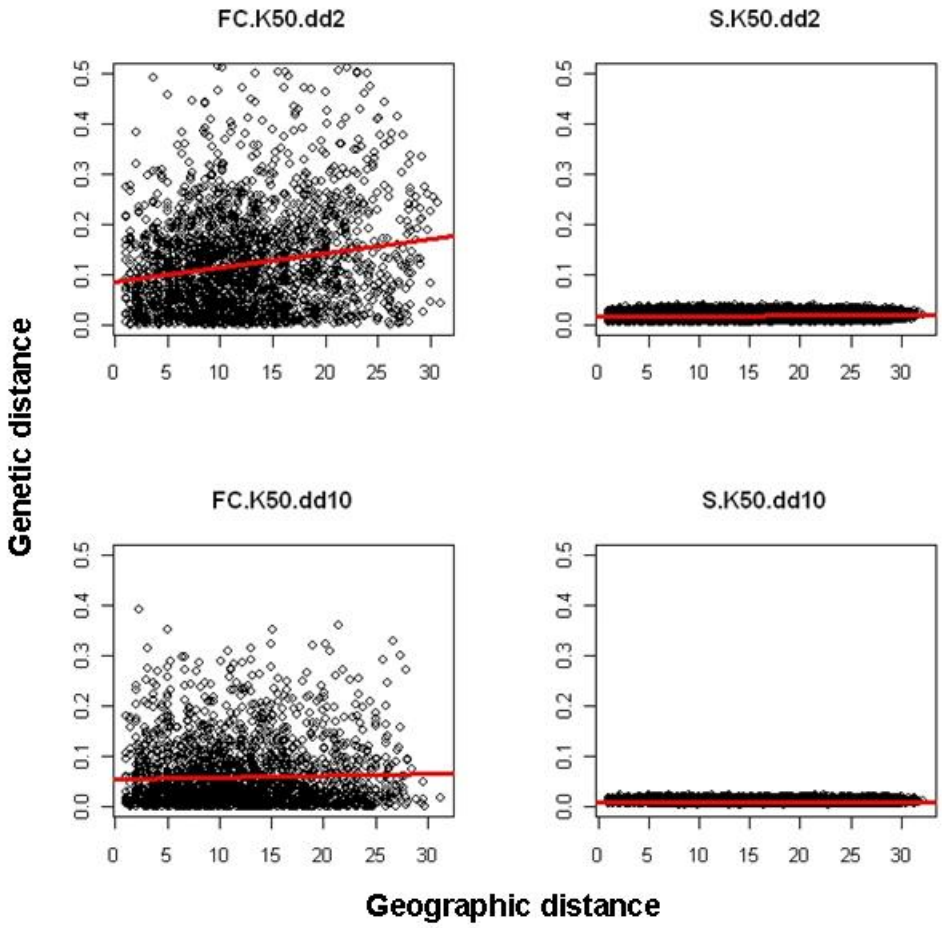

Minimum Distance (km)
350
300
250
200
150
100
50
0
1980
1982
1984
1986
1988
1990
1992
1994
1996
1998
2000
2002
2004
2006
Year

Figure 5-8


FC.K50.dd2
S.K50.dd2
FC.K50.dd10
S.K50.dd10
Genetic distance
Geographic distance
0.0
0.1
0.2
0.3
0.4
0.5
0
5
10
15
20
25
30

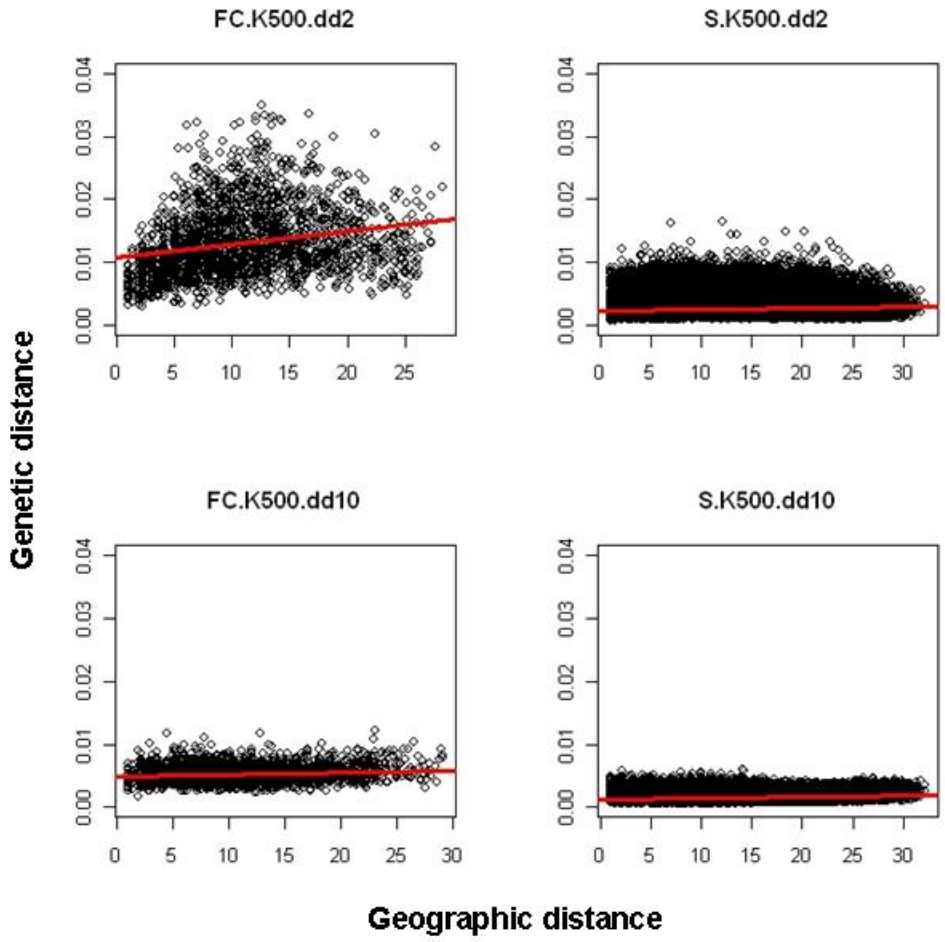
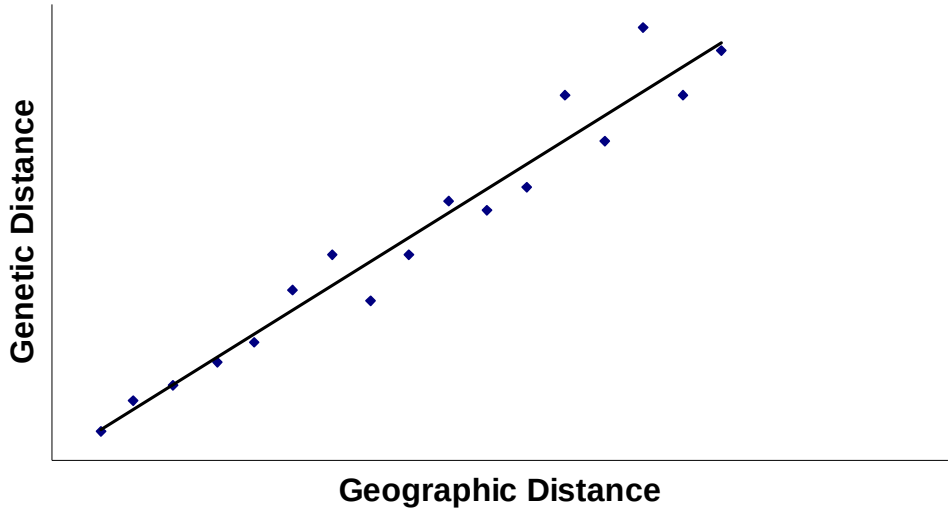
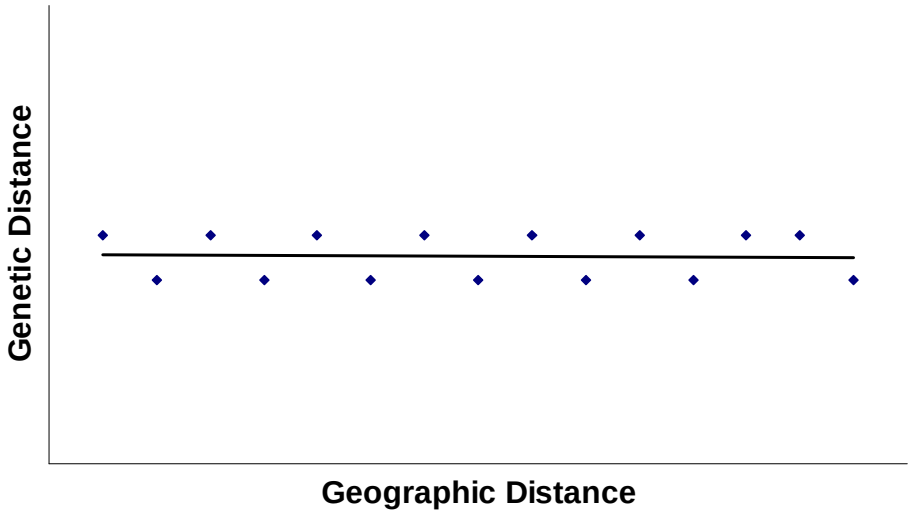

FC.K500.dd2
S.K500.dd2
FC.K500.dd10
S.K500.dd10
Genetic distance
Geographic distance
0.00
0.01
0.02
0.03
0.04
0
5
10
15
20
25
30

Figure 5-9

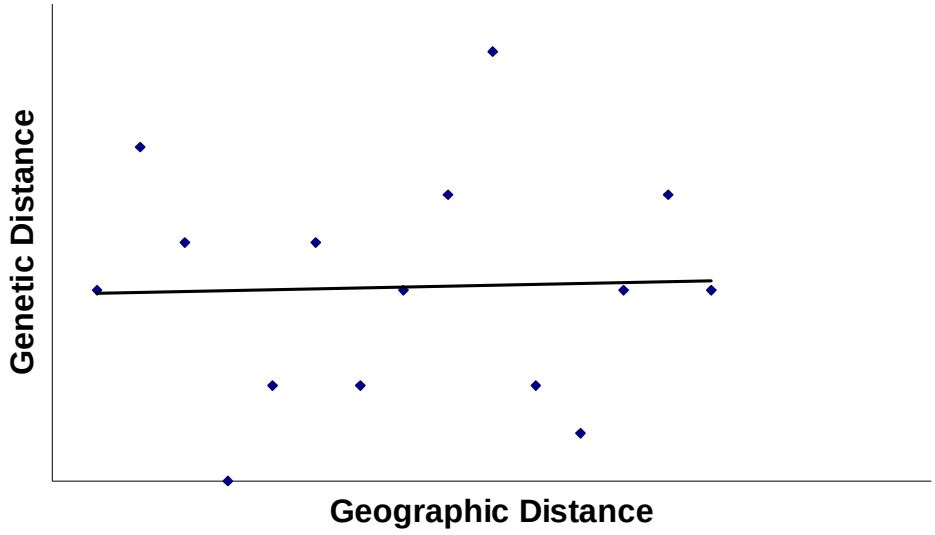
a)


Genetic Distance
Geographic Distance

b)


Genetic Distance
Geographic Distance

c)



d)

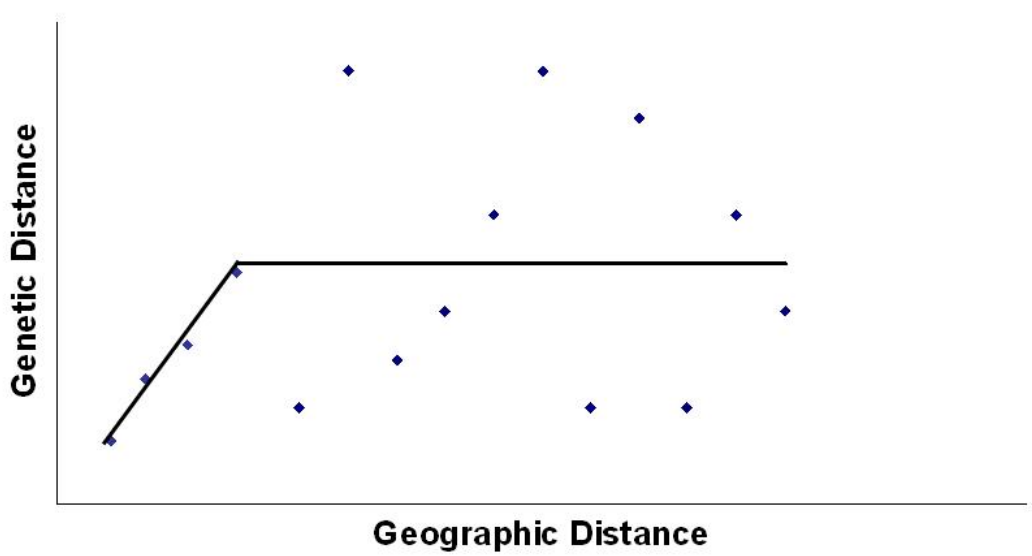


Figure 5-10

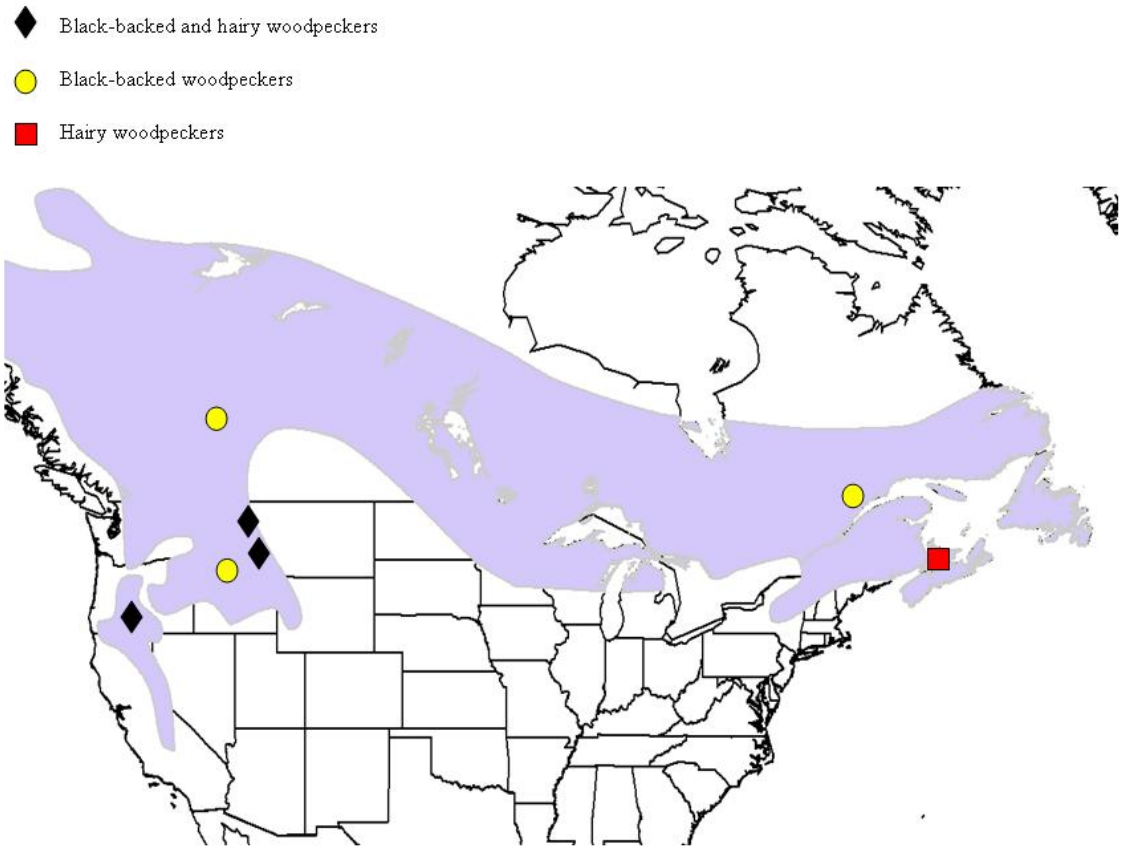

Black-backed and hairy woodpeckers
Black-backed woodpeckers
Hairy woodpeckers

Figure 5-11

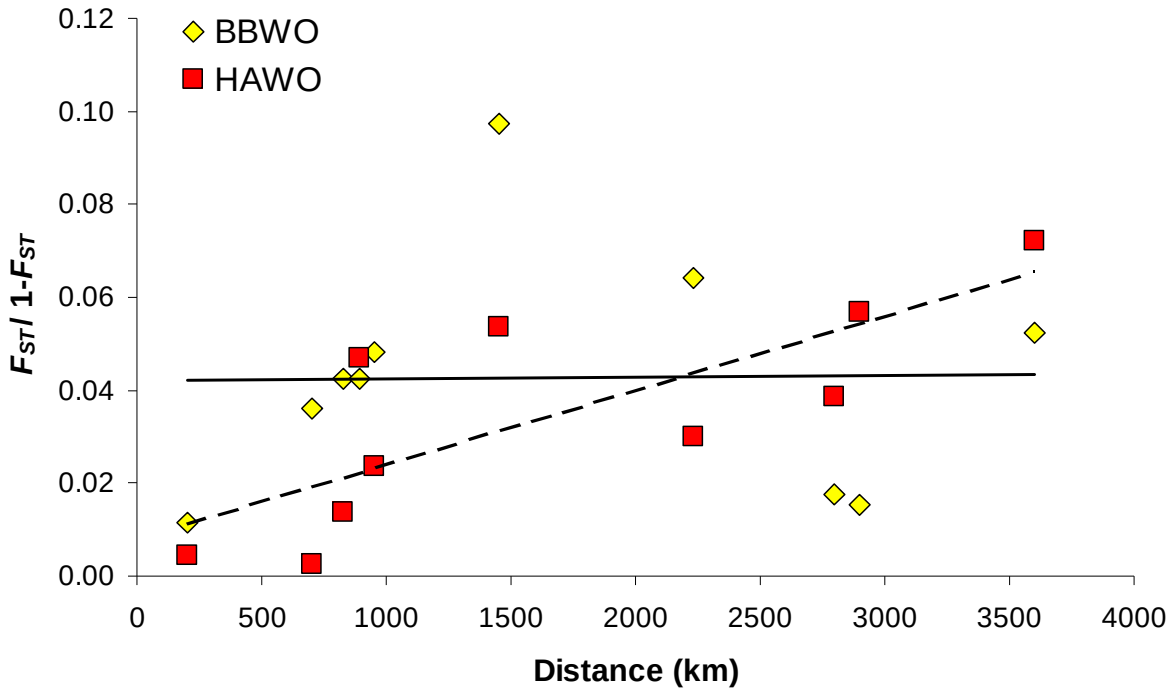

BBWO
HAWO
0.12
0.10
0.08
0.06
0.04
0.02
0.00
F_{ST}/ 1-F_{ST}
0
500
1000
1500
2000
2500
3000
3500
4000
Distance (km)

Figure 5-12

Appendix A. 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 (personal communication)
RCW17	9	258-280	60-50 TD	2 µm	Haig and Mullins (personal communication)
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 (personal communication)
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

Appendix B: To burn or not burn: what is the question?

B.1 Abstract

The ECOS Program is a partnership between the University of Montana's Division of Biological Sciences and College of Forestry and Conservation and Missoula County Schools Curriculum Consortium. The goal of ECOS is to contribute to an adaptable model of how locally based ecological research can be introduced to improve the teaching and learning of science in K-12 environments. A primary objective of ECOS is to develop science demonstration projects related to local ecology and conservation biology. As a demonstration project at a local high school, we conducted an experimental prescribed burn in a field dominated by invasive weeds. The project focused on two primary ecological themes: disturbance and invasive organisms, both of which are extremely relevant locally because residents often burn fields to reduce invasive weeds. This project successfully taught students about the scientific process and about ecology as science by having them develop and participate in a field experiment. We also designed and implemented other outdoor exercises throughout the school year to ensure the students fully participated in the experiment, including lessons on sampling, population biology and data collection. This demonstration project can be used as an international model for teaching science through hands-on schoolyard ecology.

B.2 Introduction

Conservation biology can be advanced through a greater understanding of locally relevant environmental issues and how science plays a role in these issues. By investing in education of the general public, it is possible to make a large difference in understanding critical conservation issues such as invasive species and biodiversity crises. To focus on the general public's increased understanding of locally relevant issues, high school science classes are the perfect target for this type of education. Many high school students will never take a college level biology course, let alone ecology, even if they acquire an advanced degree. As a demonstration project at a local high school, we conducted an experimental prescribed burn in a field dominated by invasive weeds. Our goal was to teach: 1) process of science using hands-on learning; 2) field ecology as science; 3) locally relevant ecological issues: disturbance and invasive weeds.

The ECOS program is a partnership between the University of Montana's Division of Biological Sciences and College of Forestry and Conservation and Missoula County Schools Curriculum Consortium. The goal of ECOS is to contribute to an adaptable model of how locally based ecological research can be introduced to improve the teaching and learning of science in K-12 environments.

This type of approach is not possible without direct involvement of scientists both in the classroom setting and in the development of an experiment. The benefit to scientist is the ability to directly increase the general public's understanding of ecological issues. The benefit to teachers is learning how to implement experiments and the ability to use the demonstration sites for various future teaching topics. A substantial short term investment in the project must be made by both groups. However, long term investment

from scientists will be minimal in comparison to the benefit from a conservation and education perspective. Here we describe a case study of this type of approach we implemented with eight sophomore classes (200 students) at Big Sky High School in Missoula, MT, USA.

B.2.1 Case Study

As a demonstration project at a local high school, we conducted an experimental prescribed burn in a field dominated by invasive weeds. The project focused on two primary ecological themes: disturbance and invasive organisms. These are locally relevant issues because residents often burn fields to reduce invasive weeds.

To ensure the students were able to fully participate in the experiment, the students needed to have knowledge of experimental design, population biology and importance of good scientific questions. Therefore we implemented outdoor experiential learning activities throughout the year that addressed these issues.

We taught the students the importance of sampling design using an inquiry we developed, 'sampling safari' (http://www.bioed.org/ecos/inquiries/Inquiries/sampling_safari.pdf). The goal of this activity was to demonstrate to students how to sample a small area and use the number of animals counted in the small area to estimate the number in the larger area. We then introduced population biology with a semi-guided inquiry that teaches students how to define a population in a real ecological situation (http://www.bioed.org/ecos/inquiries/Inquiries/Population_activity.pdf). We conducted the population activity in the field where the burn experiment was planned and used

invasive species as the plant populations to define. As a final preparation for the experiment, we taught students how to ask a good scientific question based on observations using bird skins (web location).

B.3 Methods

The first step in our experiment was to introduce the idea of fire research in relation to invasive weeds to the students. After interacting with the students throughout the year, we knew they had exposure to both the idea of invasive weeds and prescribed fire. We introduced the two topics in the classroom using examples of current research taking place in the Missoula Valley. We then took the students to the field site to make observations. The field site is a 15 acre field adjacent to the high school that is owned and managed by the Montana Department of Natural Resources. We developed a memorandum of understanding among Big Sky High School, the University of Montana and Montana Department of Natural Resources to use the land for this experiment.

The students were divided into groups of three to work together for the spring semester on this project. Each group was required to record five observations and develop three good scientific questions based on these field observations. We compiled a list of questions that were repeatedly suggested and then adjusted them to be answerable in the context of our experiment. Each class had approximately 24 students, so we selected the following eight questions to answer so questions would not be replicated within a class:

> 1) How will different levels of fuel augmentation followed by prescribed fire affect insect composition and density?

2) How will different levels of fuel augmentation followed by prescribed fire affect biotic soil factors (bacteria)?

3) How will different levels of fuel augmentation followed by prescribed fire affect abiotic soil factors (moisture, nutrients)?

4) How will different levels of fuel augmentation followed by prescribed fire affect cheatgrass and bunchgrass density?

5) How will different levels of fuel augmentation followed by prescribed fire affect plant growth rates (biomass)?

6) How will different levels of fuel augmentation followed by prescribed fire affect individual plant vigor and growth?

7) How will different levels of fuel augmentation followed by prescribed fire affect plant species composition?

8) How will different levels of fuel augmentation followed by prescribed fire affect moss density?

We conducted this exercise with eight different classes, so each question would be answered up to eight times.

B.3.1 Experiment

We designed the experiment without direct input from the students because we did not have enough time to teach about the principles of experimental design necessary for the students to actively participate in this process. We worked directly with Mick Harrington, a fire scientist at the USFS Fire Science Laboratory in Missoula, MT to design this experiment. We used a Before-After Control-Impact (BACI) design with three replicate a 20 x 20 m plots of each treatment. Dr. Harrington recommended we test two different types of fuel augmentation, as the fuel loading on our sites was quite low.

We tested the effect of dried leaves as a fuel augmentation compared to weed-free straw. We also had three replicate control plots, for a total of nine plots (Figure 1).

Students collected pretreatment data, witnessed the burn, collected post treatment data and then interpreted the results. The sampling design was based on the sampling safari exercise conducted earlier in the year and was familiar to the students. The specific methodology for each question was developed either by an ECOS fellow or with the assistance of a local expert at the University of Montana. For example, the following protocol was provided to students for Question 1:

> Q1: How will different levels of fuel augmentation followed by prescribed fire affect insect composition and density?
>
> We will base our sampling design on sampling safari, except these plots are twice as big so the cells are 2 x 2 m
>
> Step 1: choose 10 random numbers between 1-100 using a random number (stopwatch) generator, record these numbers on your data sheet and shade them in on your gridmap.
>
> Step 2: locate your first random cell in your grid
>
> Step 3: locate a corner of the cell
>
> Step 4: in this corner, bury a pitfall trap (solo cup) in the ground even with the surface of the soil.
>
> Step 5: pour 1 inch of soapy water into the cup
>
> Step 6: on a ziploc bag, record group name, date, class period, teacher and grid cell #.

Step 7: place a small piece of paper inside ziploc bag with group name, date, class period, teacher and grid cell #.

Step 8: repeat for each random cell in your grid

Step 9: return to traps the 3 days later and collect insects found in each trap in the correctly marked ziploc bag.

Step 10: make sure your all the blanks are filled in on your data sheet and your writing is neat, we will be sharing your data with other groups doing the same question in other classes

In early spring, we set aside a day to collect pre-treatment data for each question. We wanted each group to have personal attention while collecting this data, so we solicited volunteers from the University to come to the High School for a 90 minute commitment and assist the students if following a detailed protocol (Figure 2). The volunteers did not need to have any experience in the field of the question they were addressing, but simply were present to assist the students in following protocols. Many groups were required to revisit the site or take additional measurements later in the week to complete pre-treatment data collection on insects, bacteria, and soil moisture.

We coordinated the prescribed burn with the Montana Department of Natural Resources Fire Department. They graciously volunteered their equipment and professional fire fighters to conduct the burn. In our local area, prescribed burning is dependent on various local conditions such as green up, rain, wind, and airshed quality. Therefore, we planned six tentative dates with the fire crew and teachers to conduct the burn. Immediately prior to the burn, we took samples of fuel moisture and placed temperature sticks designed to melt at certain temperatures in the plots to determine how

hot it burned. We placed two of each temperature (109, 113, 250, 500) randomly throughout the plot. All 6 plots were burned on March 31,2005; the fire crew staggered the burns so different classes could observe (Figure 3).

The students collected post-treatment data by following the same protocols as before. Because the students had previous experience with the protocol, we did not enlist the help of any volunteers.

B.4 Results

We obtained quantifiable results for five of the eight questions. Because of our time constraints, data collection was not effective for questions regarding the effects of prescribed fire on soil factors and plant growth. Overall, the number of insects increased (Table 1) and there was mixed results for cheatgrass, biomass, moss and number of plants (Figure 4).

B.5 Discussion

The prescribed fire was an excellent lesson in science as a process and a way of learning as opposed to knowledge gained from a textbook. The students gained an appreciation for the natural variation present in ecological studies (Figure 4). We used this variation as an exercise for the students to refine questions and methodology to better answer questions the next time an experiment is conducted. We used the questions in which the data collection was unsuccessful as a lesson in how experiments often do not work the first time. This is especially true in field biology, where scientists often spend an entire field season learning what data is actually possible to collect.

B.5.1 Conclusions

Although this project required an extensive time commitment from both ecologists and teachers, all participants gained valuable skills and insights. Ecologists learned how to communicate to students from expert teachers. Teachers gained insight into how field experiments are conducted. A collaboration was built among university, public agency, and the high school community. As a result of my time spent teaching at Big Sky High School, I am invited to take students on annual field trip to burned forests to teach about fire ecology.

One of the most encouraging lessons from this activity was how willing various community members were to help education-related projects. We received donations of time and equipment from Montana Department of Natural Resources, scientists at the fire lab, soil scientists at UM, as well as numerous scientists volunteering their time to help collect data. A field experiment does not need to be as large-scale as the one we conducted to be effective. Most schools can find a small area outside to conduct a field experiment. Most importantly, students need to actively participate in process, not just observe. Student participation is more important than data quality as the goal of the experiment was to teach science.

At the end of the academic year, we gave an anonymous survey to all students at the end of the year asked what they learned. These are a few of the comments from students about the program:

- "*I learned how to ask a question and break it down to learn how to answer it"*
- *"ECOS taught me more about the scientific process than any one thing"*

- *"It gave me a better insight into ecology and how scientists draw their conclusions"*
- *"That ecology is in our everyday life"*

These comments provide the best evidence of the success of our program.

Table B-1 The number of insects collected pre-treatment and post-treatment for the prescribed burn experiment. The class abbreviations are as follows: KP1: Kathleen Kennedy's period 1; OP1: Dave Oberbillig's period 1; Dave Oberbillig's period 2; P3: period 3; P4: period 4; P8: period 8.

PRE-TREATMENT

class	flies	moths	grasshoppers	beetles	caterpillar	ants	earwig	spider	worm	bee	planthopper	unknown	total
KP1	0	0	1	0	0	0	0	1	0	0	0	0	2
OP1	0	0	0	0	0	0	0	1	0	0	0	6	7
OP2	4	0	0	5	1	3	0	1	0	0	1	2	17
P3	3	0	2	4	0	1	0	1	0	1	0	0	12
P4	2	0	5	5	0	0	0	2	0	0	0	0	14
P8	0	0	0	2	1	0	0	1	0	0	0	0	4

POST-TREATMENT

class	flies	moths	grasshoppers	beetles	caterpillar	ants	earwig	spider	worm	bee	planthopper	unknown	total
KP1	0	2	17	0	4	0	0	1	0	0	0	0	24
OP1	3	0	1	6	0	1	0	0	0	0	0	0	11
OP2	15	0	0	2	1	91	0	2	0	0	30	0	141
P3	2	1	0	0	0	18	1	2	0	2	0	0	26
P4	5	5	1	1	1	2	2	0	0	0	0	0	17
P8	52	0	0	13	0	14	0	0	1	1	0	0	81

Figure B-1 A schematic (left) of the experimental design from the prescribed burn experiment. Two different fuel augmentation treatments were tested, the addition of leaves and the addition of weed free straw, as well as control plots. The photo shows the actual plots post-burn.

Figure B-2 University scientists assisting Big Sky High School students collect soil samples and estimating plant cover.

Figure B-3 Montana Department of Natural Resources firefighters conducting prescribed burn with Big Sky High School students observing in the background.

Figure B-4 Pre- and post-treatment results for the effects of fire on a) cheatgrass, b) number of plants, c) biomass, d) percent cover moss; solid black bars: pre-treatment, diagonal lines: post-treatment. The class abbreviations are as follows: KP1: Kathleen Kennedy's period 1; KP2: Kathleen Kennedy's period 2; OP1: Dave Oberbillig's period 1; Dave Oberbillig's period 2; P3: period 3; P4: period 4; P5: period 5; P8: period 8.

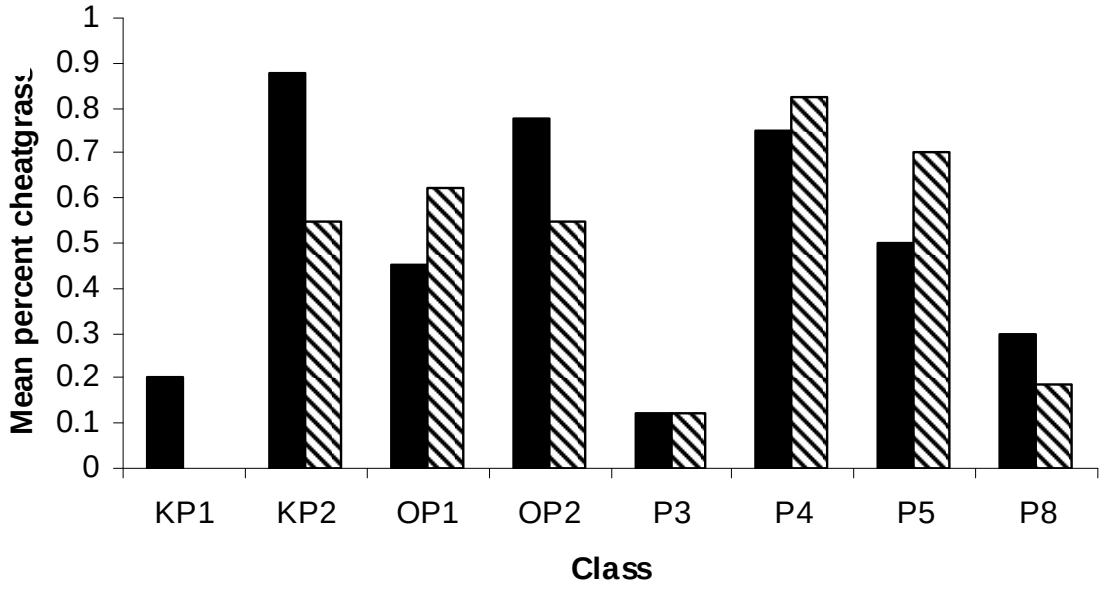

Experimental Design
20 m
20 m
Leaves added
Straw added
Control

Figure B-1

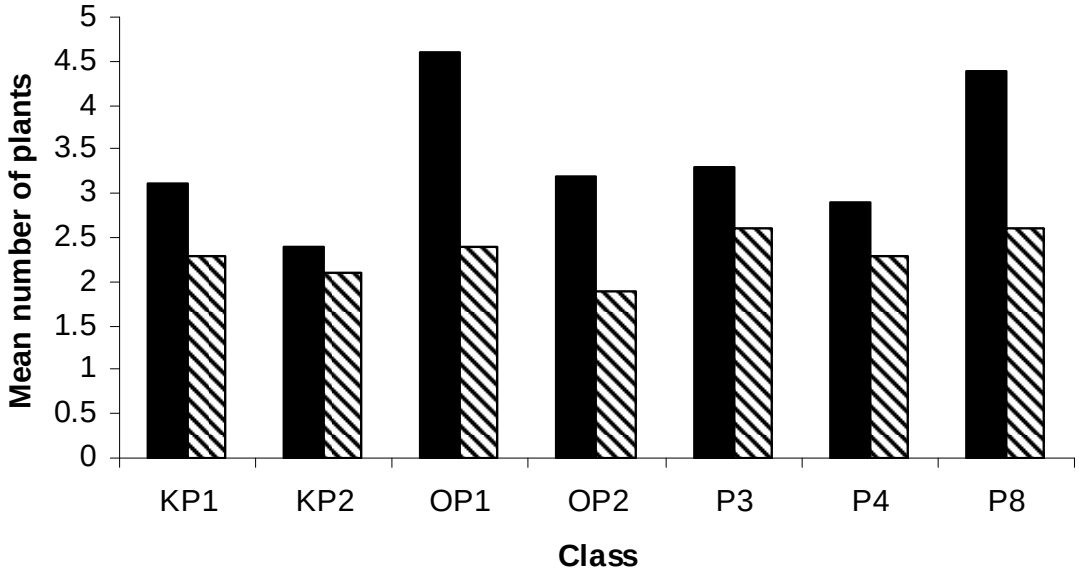
Figure B-2

Figure B-3

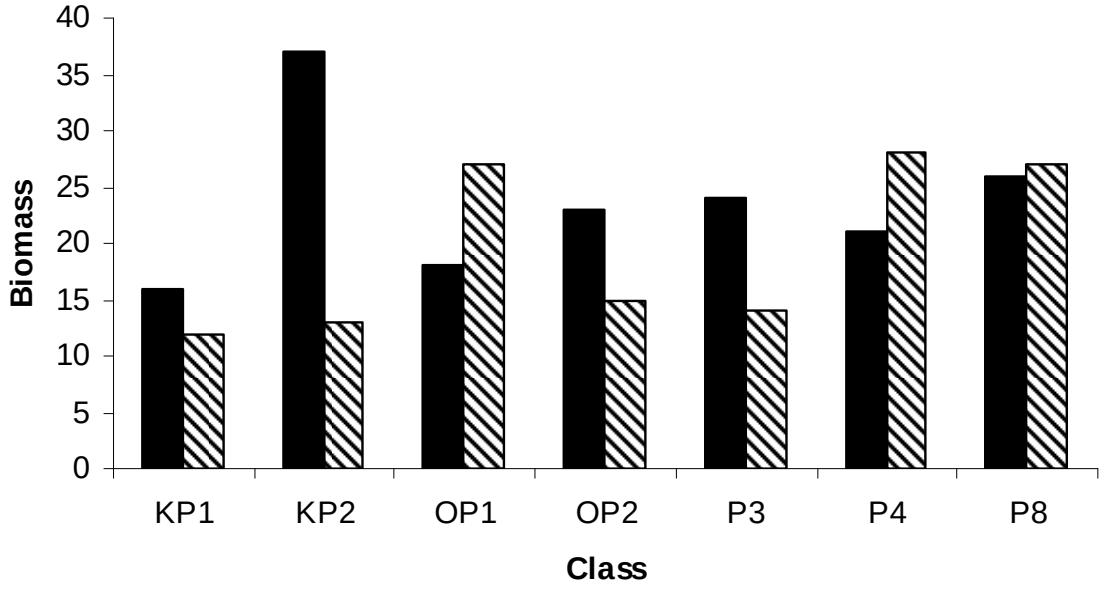
a)


Mean percent cheatgrass
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
KP1
KP2
OP1
OP2
P3
P4
P5
P8
Class

b)


Mean number of plants
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
KP1
KP2
OP1
OP2
P3
P4
P8
Class

c)


40
35
30
25
20
15
10
5
0
Biomass
KP1
KP2
OP1
OP2
P3
P4
P8
Class

d)

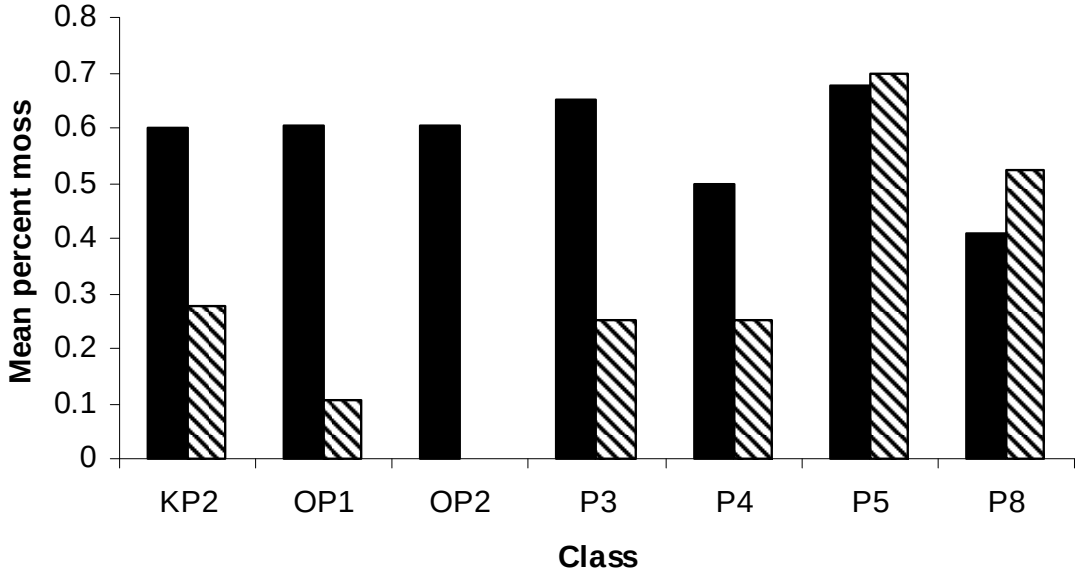

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Mean percent moss
KP2
OP1
OP2
P3
P4
P5
P8
Class

Figure B-4

Appendix C. ECOS Inquiry: Sampling Safari

1. CONTRIBUTOR'S NAME: Andrew Whiteley, Jennifer Woolf, and Frank Janes
2. NAME OF INQUIRY: Sampling Safari
3. GOALS AND OBJECTIVES:

a. Inquiry Questions: 1. How to biologists accurately count organisms?

2. How to estimate population size?

b. Ecological Theme(s): sampling design, population monitoring, population increase/decline, field biology in practice
c. General Goal: The goal of this exercise is teach students that sampling design can affect the accuracy of an estimate.
d. Specific Objectives:
Academic:

1) determine the sampling effort required for species distributed differently

across the landscape

2) learn about technical terms such as sample and distribution

3) understand concept of scaling

4) introduce concept of randomization in science

Procedural/technical:
1) graphing skills – labeling axis, identifying points from own data
2) math – determine scaling factor and calculate population estimate
3) data recording

Social:
1) work in a team to collect data
e. Grade Level: This exercise is aimed at sophomores – seniors in high school, but could easily be scaled down to 5th? Grade and up to lower level college courses
f. Duration/Time Required:
- Prep time: outside the classroom includes acquiring animals and setting up the sampling grid. The grid can be painted on with field paint, which will take ~ 2 hours or made with string, ~3 hour
- Implementing Exercise During Class: Introduction ~30min.
Activity ~1 hour
Review ~ 30min.
- Assessment ~15 min

4. ECOLOGICAL AND SCIENCE CONTEXT:

a. Background (for Teachers): Teachers: Estimating population size can be done in a number of ways. Often, a full census is impractical due to lack of resources to count every organism and the inability to detect all organisms even when trying to count a majority of animals. Biologists design ways to sample a portion of the population to estimate what they are really interested in: population size.
One way is to determine the density of animals in a small area and extrapolate that to your area of interest. Important considerations in this include

1) **Sampling design**: sampling designs can be very diverse. The simplest sampling design is simple random sampling, which is where every item in the sample has an equal probability of being included. When one wants to ensure the population is well covered by the sampling effort, stratified sampling can be implemented, where the sampling units are divided into non-overlapping strata and random samples are drawn from within the strata. Systematic sampling can be used when a population covers a well-defined area. The advantage of systematic sampling is it is often easier and can be more representative of a population, thereby giving a more precise estimate.

2) **Species distribution**: the way individuals are spread out across a landscape. For example, many species are found in clumps, such as herd animals like deer and elk. Other species are spread across the landscape in a random pattern, such as mountain lions or giraffes. Organisms are rarely uniformly distributed across landscape, such as common weeds. An organism's distribution will affect how you best sample. Clumped species will take more effort to sample effectively because you may count alot if you are in an area with a herd, or count none if you are not. Therefore you must count more areas to assess the real number on the landscape.

3) **Movement of organisms**: If organisms are able to move in and out of your sampling units while you are counting them it may cause you to over or under count them.

4) **Density**: If you are interested in the density of animals, it is important to define the area you are interested in before sampling. For example, you may be interested in the number of deer in western Montana or in Missoula County. One would design a sampling effort differently for each case based on the resources available.

5) **Resources available**: what is a realistic amount of effort that can be used in total, including hours of effort, gas, etc.

6) **Detectability**: some animals are easy or harder to count because they are hiding, etc.

7) **Time frame**: when estimating abundance or density, it is important that the population is considered "closed". A closed population does not have any

births, deaths, immigration or emigration. So you have to be able to sample your areas in a time frame that will follow these assumptions, such as one week.

8) **Random**: the concept of randomness is important to reduce bias, if one picks random samples through the use of a random number generator or a similar method, one can reduce bias in their estimate

9) **Accuracy vs. precision review**: The goal is to get an accurate sample, which means it is as close to the true value as possible. A precise estimate is one that has little variation, that is, if you conducted the same sampling procedure ten times, you would get a similar result. However a precise result can still be very biased (or wrong).

b. Background (to present to Students): Students: Often, field ecologists are interested in knowing the number of individuals present in a certain area. However, it is usually not possible to count every individual
So we count animals in smaller areas and estimate the population size from there.

1) sample – define

2) introduce idea of counting smaller areas and scaling up

3) scaling – define

4) introduce species distributions, examples

5) accuracy – define

6) why important to know population size

7) why ecologists have to sample vs. census

8) how ecologists sample in similar ways to exercise (i.e. flights for ungulates)

5. MOTIVATION AND INCENTIVE FOR LEARNING:
The motivation for this lesson can be in the introduction when explaining to kids how we actually count animals. Great example in areas where hunting is popular is talking about ungulate counts and how that relates to hunting tags. In more urban areas, great examples are trends in migratory bird counts such as the Breeding bird survey. Lesson is held outdoors, so atmosphere is relaxed. Safari animals can lead into great stories of the African safari

6. VOCABULARY:
Simple random sample: a sample that is drawn from a population in such a way that all items are given the same chance of selection.

Sample with replacement: sampling in a manner that the same "unit" can be chosen repeatedly
Sample without replacement: sampling in a manner that the same "unit" can be chosen only once and then is taken out of the possible choices.
Subsample: individual unit of measurement that is obtained to form a sample. A sample can consist of numerous subsamples
Species distribution: the way individuals are spread out across a landscape. For example, some species are found in clumps, some species are randomly found in the landscape and some are uniformly distributed.
Accuracy: The measure of the correctness of data, as given by the difference between the measured value and the true or standard value.
Precision: the quality of being reproducible
Effort: in this case, number of subsamples taken per estimate
7. SAFETY INFORMATION:

8. MATERIALS LIST (including any handouts or transparency masters):
50 animals for even distribution (zebra, giraffe)
50 animals for clumped distribution (elephant, hippo)
string or field paint to make a 10m x 10m grid
meter tape for measuring grid
pin flags helpful in marking out grid corners
clipboards or other tools for outdoor data collection
data sheets
bucket/hat
random numbers (squares of paper with numbers that correspond to grid cells; include figure)
Cost of materials: ~$40 for animals (could reduce cost on less expensive animals), field paint ~ $5, pin flags ~$5

9. METHODS/PROCEDURE FOR STUDENTS:
The goal of this activity is to demonstrate to students how to sample a small area and use the number of animals counted in the small area to estimate the number in the larger area. This idea is expanded upon by using animals with two different distributions, clumped and even (or random). We do this by designing a grid in the schoolyard and placing animals in the grid according to their distribution (clumped/even). We use the same number of animals in each case to demonstrate the difference in accuracy and precision for each distribution at different levels of sampling effort. This activity is conducted easily in any schoolyard area. Preparation for activity is setting up a 10 x 10 m grid and distributing animals in an even and clumped distribution. A short classroom introduction that explains the background concepts to the students is helpful, see the attached handout for what can be covered introduction. This activity works best for students in groups of 2 –3, one student recording the data and one/two student (s) collecting the data (counting animals). This activity is designed to have students estimate population size with two different levels of effort five times each. The students repeat the estimate five times to see how precise the estimate is. The students begin by choosing random sample units from a hat. It is easy to label your grid 1-10 rows and A – J columns and have 100 pieces of

paper in a hat labeled 1A – 10J. Each group will fill out the random number sheet (see attached) prior to collecting samples. If time is an issue, the teacher can save time by supplying the random numbers, but if time permits it is an important lesson to see what random means.

Then the students will go to the grid and count the number of animals in each subsampled unit and fill this in on the datasheet. First, they will use five subsamples to estimate population size. Next, they will use ten subsamples to estimate population size. Population size is estimated by summing the animals counted in all the subsamples and using the scaling factor to calculate population size. The scaling factor is based on the idea that the density of animals in the area of interest is stable. So you can use the number of animals you counted in a small area and scale it up to a larger area. Population estimates are obtained from samples by assuming the density of individuals in the sample is the same as in the entire landscape. That is:

$NsampleAsample=NtotalAtotal$

N = number of individuals

A = area

So, N_{sample} is the number of animals you count in your sample, A_{sample} is the area covered by your sample, N_{total} is the population estimate and A_{total} is the total area occupied by the population of interest.

To estimate population size, you solve the equation for $Ntotal=Nsample(AtotalAsample)$

For example, let's say you are trying to estimate the population size of frogs in a 10m x 10m area ($100m^2$). You take subsamples from 5 1x1m quadrats (squares) and find a total of 10 frogs.

$N_{sample} = 10$

$A_{total} = 100\ m^2$

$A_{sample} = 5\ m^2$

Therefore $N_{total} = 10\ (100/5) = 10\ (20) = 200$, our population estimate = 200

(*AtotalAsample*) is also referred to as the **scaling factor**, which in this case was 20.

This will be done for both levels of effort so students can compare both the accuracy and precision of estimates for two levels of effort with two different species' distribution. The students will graph the results which will help with interpretation. The students can draw a line across the true population size (i.e. 50) and accuracy can be assessed by looking at how close the estimate is to true population size. Precision can be assessed by looking at how variable the 5 different estimates are (do the estimates bounce around alot or are they pretty similar?)

A good class discussion can include the students coming to the board and drawing the results on the board and comparing among groups.

A helpful task is to have students make predictions about which species distribution would take more effort to accurately count. Predictions should be that evenly distributed animals will take less effort to accurately estimate the population size.

10. ASSESSMENT:

The students are asked to graph their results and interpret the graphs. This is a good exercise in transferring tabular data to a graphed format, understanding axes, and overall graph interpretation. Additional questions are:
This activity includes questions for the kids to take home and answer
1) Briefly summarize your findings. Examine your graphs for the two species comparing the two different sampling efforts. How are they different? Is there a point in which increasing the sampling area (number of subsamples) seems to make little difference in increasing accuracy. Does this point depend on the distribution of the species? (Adapted from Ecobeaker)

2) Pick a species that you are interested in and describe how you think it is distributed on the landscape. Describe the amount of sampling effort you would use to accurately estimate the population size.

Quiz/Test questions are a good follow up to this exercise to ensure the scaling factor is understood, as well as what high vs. low levels of variation implicate.

11. EXTENSION IDEAS:
Good ways to extend this exercise are to include other sampling designs
Such as stratification or cluster sampling and to consider other species distributions such as random. Other extensions could include an addition on species distributions and reality of even vs. clumped vs. random.
For more advanced classes, a great extension would be having the students present the graphed results in a presentation that expanded to include recommendations on sampling designs for a local species that was detailed in how the results from sampling safari would inform the specific sampling design.

12. SCALABILITY
This lesson can be scaled down by simplifying the lesson to counting one type of animal, not considering distribution. Focus on estimating numbers in a big area from a small area. This could be scaled up by incorporating more advanced topics in sampling design and having the students do more work with the scaling factor and design of own sampling scheme.

13. REFERENCES: Manly BFJ. 1992. The design and analysis of research studies. New York: Cambridge University Press.

14. LIST OF EXPERTS AND CONSULTANTS

15. EVALUATION/REFLECTION BY FELLOWS AND TEACHERS OF HOW IT WENT:
Overall, sampling safari was a very successful exercise. The introduction and conclusion discussions are a very important part of this exercise. Students need to have a clear picture of why they are outside counting animals in little squares to make it a meaningful exercise. This can be accomplished by a good introduction that has links to local population monitoring efforts and how they are conducted. A good review of the scaling

factor, amount of effort, and variation is helpful in clarifying the exercise as well. Additional exercises involving the scaling factor and graph interpretation may be helpful in really bringing the points home.

Appendix D. ECOS Inquiry: Scratching Your Head Over Itchy Weeds: A Population Activity

1. CONTRIBUTOR'S NAME: Jennifer Woolf, Andrew Whiteley, and Frank Janes

2. NAME OF INQUIRY: Scratching Your Head Over Itchy Weeds: A Population Activity

3. GOALS AND OBJECTIVES:
a. Inquiry Questions: What is a population of knapweed/cheatgrass? How can we estimate population size of a plant population?
b. Ecological Theme(s): population ecology, species interactions, and sampling
c. General Goal: To provide a semi-guided inquiry that builds on a previous sampling activity and has the students think how to define a population in a real ecological situation.
d. Specific Objectives:

1) To teach students about how to define population boundaries
2) To communicate the reasons for monitoring population size over time
3) To build on sampling knowledge to determine the appropriate sampling design to estimate population size based on population characteristics
4) To determine factors that affect population based on field observations.

e. Grade Level: This activity is currently geared toward high school, and is most appropriate for high school to lower division college students
f. Duration/Time Required:
 Prep time: the only preparation involved is finding an appropriate area in your schoolyard that has two different species with different characteristics for comparison. Species that have very different spatial patterns visually, such as obvious clumps versus ubiquitous or rare, are the best ones to choose for a good comparison. This can even be grass, which is often ubiquitous vs. dandelions, which are often clumped.

Implementing Exercise During Class: a 15-minute introductory lecture is followed by 45 minutes outside. This could easily be shortened to 30 minutes outside. The students just need enough time to make observations necessary to answer the questions. It is helpful to talk with the students individually while they are making observations and ask them leading questions to direct them towards understanding how individuals in the population are interacting. For example, one of the main questions to ask is: what are the population boundaries? If the students have grouped things into many small populations in close proximity, then ask: if the individuals can reproduce with each other, are they in a separate population? For plants, this often requires thinking about how the seeds disperse and how pollination is done. If a bee pollinates a plant, then plants connected by bee pollination could be in the same population. Also, thinking about resources the plants are using, such as soil moisture and sunlight, are good areas to direct students to think about.

Assessment: The students are asked 11 questions in this activity, the first 8 are best answered outside while doing the activity and the next 3 can be answered at the end of class or as a take-home exercise.

4. ECOLOGICAL AND SCIENCE CONTEXT:

a. Background (for Teachers):

Background Information:

We began this exercise with a short introduction on the definition of a population. This can be a difficult concept because many people mean different things when they say the word population. Therefore, it is helpful to begin by defining the term in a general manner. We define a population as a group of organisms of the same species occupying a particular space at a particular time, with the potential to breed with each other. We then introduced students to concepts relating to interactions among individuals within a population. Individuals may interact with each other directly through territorial and reproductive behaviors or indirectly through use of common resources or occupation of common habitat. The area within which individuals are interacting often defines spatial boundaries of populations. Spatial boundaries can be easily defined or may be vague. An example of a situation where it is easy to define boundaries is when you have island, or an isolated patch of habitat. Large areas of continuous habitat or areas that have been somewhat fragmented but well connected by corridors are much more difficult. In this case, biologists often assume arbitrary boundaries for investigations, which may be appropriate under many circumstances. An important parameter in population biology is population size (i.e. abundance) or density (# individuals/unit). Biologists are often interested in how and why a population size may change over time. For instance, we are interested in know if a population growing, shrinking or staying the same size. Population changes over time can be expressed in a way that incorporates gains and losses:

$N(t+1) = N(t) + B(t) + I(t) – D(t) – E(t)$

$N(t+1)$: population size at time t plus time step (month, season, year)

$N(t)$: current population size

$B(t)$: births

$I(t)$: immigrants

$D(t)$: deaths

$E(t)$: emigrants

Birth, death, immigration and emigration are the four primary processes that affect populations. Factors affecting populations *or* affecting the four primary processes can be classified as abiotic and biotic. Abiotic factors include the physical and characteristics of an organism's environment. For terrestrial organisms, these factors include: soil type, water availability, temperature, and fire frequency. For aquatic organisms, these factors include: water salinity, pH, currents, light penetration, and dissolved oxygen. Biotic factors include interactions among members of the same species (intraspecific) or interactions involving another species (interspecific). Examples of these factors include: predation, competition, parasitism, and disease. Biotic factors can be further classified as direct (behavioral interactions such as excluding other individuals from food resources) and indirect (depletion of common resources and occupation of common habitat). There is almost always interaction between biotic and abiotic factors which often influence more than one primary process at once.

Background (to present to students):

1) population definitions
2) spatial boundaries
3) interactions: direct and indirect
4) importance of population size: abundance vs. density, how biologists track trends
5) factors influencing populations: abiotic vs. biotic (direct and indirect within these categories. Abiotic and biotic usually interacting with each other

5. MOTIVATION AND INCENTIVE FOR LEARNING: Students get to go outside and use field observations to synthesize and implement classroom knowledge

6. VOCABULARY:

Population: group of organisms of the same species occupying a particular space at a particular
time, with the potential to breed with each other.
Spatial boundary: outer edge of population, often defined by a landscape characteristic such as
a mountain range or river. Can be easily identified such as island boundaries, or vaguely identified by subtle changes in habitat type.
Abundance: number
Density: number per unit area
Immigration: individuals entering population
Emigration: individuals leaving population
Interaction: mutual or reciprocal action or influence
Direct interaction: when individuals have direct contact through reproductive or territorial
behaviors
Indirect interaction: when individuals affect each other indirectly often behaviorally, such as
use of common resources
Abiotic: physical and characteristics of an organism's environment
Biotic: interactions among members of the same species (intraspecific) or interactions involving
another species (interspecific)

7. SAFETY INFORMATION: SAFETY CONCERNS ARE LIMITED TO ONES INHERENT TO AREA VISITING FOR OUTDOOR PORTION OF ACTIVITY (I.E. HOLES IN GROUND, ETC)

8. MATERIALS LIST :

handouts, including local area map (see attached sample)
clipboards for outdoor questions. We recommend the following paper on Yellowstone National Park to talk about interactions: Smith, D.W., Peterson, R.O. and Houston, D.B. 2003. Yellowstone after wolves. Bioscience 53(4): 330-340.

9. METHODS/PROCEDURE FOR STUDENTS:
a. Pre-investigation work: teachers need to identify area in schoolyard that has two species of plant with two different distribution; background lecture ~15minutes
b. Investigation work:

1) Students go to area of interest and use field observations to define the population of interest.
2) Students draw the population boundaries of each species on supplied area map
3) Students use field observations to determine biotic and abiotic factor influencing both populations
4) Students describe the distribution of species and use this information to determine a sampling scheme * (only relevant to classes with a background in sampling)
5) Students present evidence through short answer questions and drawing?
6) See sample data sheets

10. ASSESSMENT: Students answer 11 questions, the first 8 are best answered in the field and the final three are good classroom exercises or take-home exercises. It is important to conduct a wrap-up of the exercise reviewing the main points. The second section of the exercise is only relevant if there is a background in sampling

11. EXTENSION IDEAS: a great extension would be to take another class period and have the students actually design and implement a sampling design to estimate the population size. If several groups estimate the population size in the same area, then a graph could be made of the variance with different methods

12. SCALABILITY: this exercise could be scaled down to upper middle school by simplifying to just a population activity without talking about interactions and factors affecting populations. Scaled up by asking tougher questions about populations and sampling design.

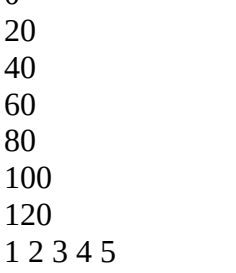
13. REFERENCES: Williams BK, Nichols JD, Conroy MJ. 2002. Analysis and management of animal populations. San Diego, California: Academic Press. 817 p.

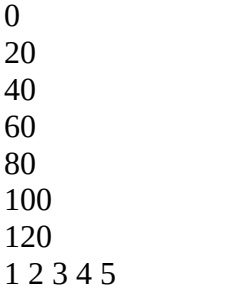
14. LIST OF EXPERTS AND CONSULTANTS:

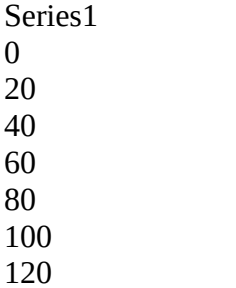
15. EVALUATION/REFLECTION BY FELLOWS AND TEACHERS OF HOW IT WENT:
This exercise went well overall, the questions could be re-phrased to be more clear. We could have spent more time developing the sampling schemes with the students out in the field. It was good to get the students outside and deciding in a real situation how to define population boundaries, however our map reflected the boundaries too well and could have provided a more “open-ended” way for the students to decide boundaries. Requires an interaction with each group while outside to encourage thinking in the right direction, so having 2-3 “teachers” in the field is helpful.

Field Lab Scratching Your Head Over Itchy Weeds Fall 2004
Name: Period:
Date:
In this activity we are interested in assessing the population size of two different plant species, knapweed (*Centaurea maculosa*) and cheatgrass (*Bromus tectorum*) at the DNRC field next to Big Sky. To do so, the first thing we need to do is determine what a population is for each type of plant.
1) What is a population in general?
The rest of these questions you will answer while we are at the DNRC field. Take a look at the field.
2) Describe a population of knapweed at DNRC. Is there one or more than one population?
3) Describe a population of cheatgrass at DNRC. Is there one or more than one population?
Usually we use a physical barrier like a river or a mountain divide to help us draw boundaries around a population. What boundaries could you use to help define populations of knapweed and cheatgrass in the DNRC field? Draw these boundaries in the box on the front of the next page, make sure you clearly label the boundaries.
4) How did you decide where the boundaries are?
5) What are three factors influencing populations of each species? Label each factor as either biotic or abiotic.
6) Name two ways the cheatgrass and knapweed plants within your population boundaries might be interacting with each other (keep in mind that now we are just talking about biotic factors).
7) For each of the biotic factors you just listed, say whether it is a direct interaction or an indirect interaction and why.
We would like to estimate the population size of knapweed and cheatgrass so we can determine if the population is changing over time. Remember from sampling safari that plants and animals can be distributed across the landscape in different ways. To figure out how to estimate the number of cheatgrass and knapweed plants, the first thing we need to think about is the distribution of each species. Then we can think about how much effort we need to use to get a good estimate of population size. Draw the distribution of each species within the population boundaries in the box on the front of the next sheet. Use different symbols for each species and draw how each species is distributed.
8) What is the distribution of each species (clumped, random, or even)?
9) Based on their distribution, how would you sample each species (according to the kind of sampling we used in sampling safari)? Be specific, in your answer talk about how you would take subsamples in an organized way for each species and how much effort you would use for each species.
a. knapweed
b. cheatgrass
10) When deciding how much effort we need to accurately estimate population size, our goal is to collect a certain number of subsamples and "scale up" to the correct population size. Remember in sampling safari we tested if 5 vs. 10 subsamples were enough to get an accurate population estimate. The graphs on this page are similar to the ones you made

during sampling safari. On these graphs, each point on the graph represents the population estimate from 10 subsamples. If 10 subsamples is enough to accurately estimate the actual number of animals, what would the graph look like?







11) We know that we need to estimate population size accurately to be able to track populations
over time. Give at least two reasons why we would want to track the population size of knapweed and cheatgrass over time.

a

b

c

Pop. size

Pop. size

Pop. size

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