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Genetic Diversity and Paternity Analysis of Endangered Canadian Greater Sage-Grouse (*Centrocercus urophasianus*)

by

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Abstract

Greater Sage-Grouse (*Centrocercus urophasianus*) are an endangered lekking species that has declined by 66%-92% during the last 35 years in Canada. Sage-Grouse have a lek mating system centered on communal breeding grounds where few males are thought to obtain most matings in a given year and females are believed to mate once. I used 13 microsatellites to genotype 2,519 adults 1,206 offspring sampled between 1998 – 2007 from 104 leks in Alberta, Saskatchewan, Montana, and Wyoming and 238 historic Canadian birds collected between 1895 and 1991. My goals were to determine the (1) genetic population structure, diversity, and dispersal ability of birds in the proposed northern Montana population, (2) diversity and relatedness of Sage-Grouse in Alberta, (3) paternity, polygamy (males and females mating with multiple individuals), and reproductive variance among individuals in Alberta, and (4) if genetic diversity, structure, and effective population size changed over time in Canada. I determined that northern Montana (northern Montana, Alberta, and Saskatchewan) formed a single genetic population with high diversity and no evidence that peripheral regions were genetically depauperate or highly structured. Both sexes disperse, but males disperse further and more frequently. Within Alberta, diversity was high and relatedness was close to zero for both sexes at the lek-level suggesting neither sex forms kin associations. I found that most clutches had a single father and mother, but there was evidence of multiple paternity and intraspecific nest parasitism. Annually, most males fathered single broods, the proportion of males in Alberta fathering offspring during their lifetime averaged 45.9%, and reproductive

variance was lower than expected if only a small proportion of males mated. For the historic analysis, I found high diversity during each time period with no decline through time. Genetic structure did not change and there was no evidence of a genetic bottleneck. Effective population size in Canada decreased with time and was estimated at 46.8 – 93.6 individuals for the most contemporary time period. Together, my findings suggest that more birds are breeding than expected for a lekking species and Sage-Grouse in Canada are part of a genetically diverse population that is maintaining genetic connectivity through dispersal.

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CHAPTER ONE

General Introduction

1. Sage-Grouse Biology

Greater Sage-Grouse (*Centrocercus urophasianus*; hereafter Sage-Grouse) are a polygynous galliform that inhabit the sage steppe of western North America. Historically Sage-Grouse inhabited three Canadian provinces (Alberta, Saskatchewan, and British Columbia) and 14 American states (Arizona, California, Colorado, Idaho, Montana, Nebraska, Nevada, New Mexico, North Dakota, Oregon, South Dakota, Utah, Washington, and Wyoming), but presently occur only in southeastern Alberta, southwestern Saskatchewan, and 11 U.S. states (Sage-Grouse have been extirpated from Arizona, Nebraska, New Mexico, and British Columbia; Schroeder et al. 2004). Rangewide, the amount of habitat has decreased by greater than 50% due to widespread distruction of sagebrush (Connelly et al. 2004; Schroeder et al. 2004). Sage-Grouse are entirely dependent on the sagebrush ecosystems of western North America, as they are sagebrush obligates (they are dependent on sagebrush as their primary food source and year-round habitat; Patterson 1952; Braun et al. 1977; Connelly et al. 2000; Connelly et al. 2004). Sage-Grouse adults primarily eat sagebrush throughout the year (Wallestad et al. 1975), but they also consume forbs and insects when available seasonally (Knowlton and Thornley 1942; Pyle 1993; Drut et al. 1994). Throughout most of the range, Sage-Grouse are associated with big sagebrush (*Artemisia tridenta*), but in Canada at the northern periphery of the species' range, Sage-Grouse are limited to the distribution of silver sagebrush (*A. cana*; Aldridge 1998; Connelly et al. 2004; Alberta Sage-Grouse Recovery Action Group 2005). The distribution of silver sagebrush is naturally patchy, so birds have adapted to move large distances to find suitable habitat. Based on population and habitat type, Sage-Grouse can be migratory, moving up to 161 km, or resident (Patterson 1952; Dalke et al. 1960; Berry and Eng 1985; Connelly et al. 1988; Bradbury et al. 1989; Connelly et al. 2004). Resident populations exhibit little movement year-

round, while birds from migratory populations can travel between winter/breeding and summer areas (two-stage migration), winter and breeding/summer areas (two-stage migration), or winter, breeding, and summer areas (three-stage migration; Connelly et al. 1988).

There are two species of Sage-Grouse, the Greater Sage-Grouse and Gunnison Sage-Grouse (*Centrocercus minimus*; Fig. 1-1; Young et al. 2000; Connelly et al 2004). Gunnison's Sage-Grouse were recently recognized as a distinct species using molecular, morphological, and behavioral data (Kahn et al. 1999, Oyler-McCance et al. 1999; Young et al. 2000) and occur in southwestern Colorado and southeastern Utah (Young et al. 2000). Greater Sage-Grouse were historically divided into two subspecies: the eastern subspecies (*Centrocercus urophasianus urophasianus*), which was believed to occur in Alberta, Saskatchewan, Colorado, Idaho, Montana, Nebraska, Nevada, New Mexico, North Dakota, South Dakota, Utah, and Wyoming and the western subspecies (*Centrocercus urophasianus phaios*) in British Columbia, California, Oregon, and Washington (Aldrich 1946; Benedict et al. 2003; Connelly et al. 2004). Recently, molecular analyses have shown that there is no genetic evidence for a subspecies division (Benedict et al. 2003), but there is evidence for distinct populations within the species (Benedict et al. 2003; Oyler-McCance et al. 2005).

Connelly et al. (2004) divided all Greater Sage-Grouse into 41 discrete populations with 24 subpopulations. These divisions were based on spatial isolation, although many populations were connected via narrow corridors of habitat (Connelly et al. 2004). Northern Montana was recognized as a discrete population separated from other populations by approximately 20 km and the Missouri River (Figs. 1-1 and 1-2). It was divided into three subpopulations: (1) Alberta, southwestern Saskatchewan, and the western part of northeastern Montana (Fig. 1-3), (2) north central Montana (Fig. 1-4), and (3) south central Saskatchewan and the eastern part of northeastern Montana (Fig. 1-5; Connelly et al. 2004). Subpopulation 1 was separated from other populations by approximately 20 km and the central Saskatchewan subpopulation by approximately 50 km. Subpopulation 2 was approximately 20 km from the nearest

adjacent population, separated from that population by the Missouri River, and loosely connected to subpopulations 1 and 3 in the north. Subpopulation 3 was highly fragmented and isolated from the rest of the northern Montana population by approximately 20 to 40 km (Connelly et al. 2004).

2. Lekking Behavior in Sage-Grouse

Sage-Grouse are a lekking species of galliform where males congregate on communal display grounds (leks) in the spring and females make repeated, lengthy visits to assess males before they mate and raise young on their own (Wiley 1973; Johnsgard 1983; Gibson 1992; Gibson 1996). Sage-Grouse are the largest North American grouse and are highly sexually dimorphic with males being approximately twice the size of females (Dalke et al. 1963; Eng 1963; Beck and Braun 1978; Hupp and Braun 1991). Females are cryptically coloured, allowing them to blend into their habitat, while males are more conspicuous with long pointed tails, elaborate filoplumes, white breasts, and two large yellowish air sacs that are visible on the lower neck/upper breast during display (Connelly et al. 2004). The noise produced by these air sacs is an acoustic signal that attracts females. Leks are generally in open habitat (e.g., windswept ridges, exposed knolls, flat sagebrush areas, or bare openings) with limited vegetation so that displaying males are highly visible to females (Patterson 1952; Giezentanner and Clark 1974; Connelly et al. 1981; Johnsgard 1983; Aldridge 1998). Leks vary in size from 0.04 to 16 hectares and can be used for up to 100 years (Scott 1942; Patterson 1952; Aldridge 1998). Male Sage-Grouse attend leks for up to three months each spring (Vehrencamp et al. 1989), generally arrive on leks prior to sunrise, and display for up to four hours each morning (Scott 1942; Patterson 1952; Hjorth 1970; Jenni and Hartzler 1978). Depending on the region, males begin displaying around the end of February to early April and end displaying in late May or early June (Eng 1963; Schroeder et al. 1999; Aldridge 2000a; Hausleitner 2003). In Canada, males return to leks at the end of winter and start displaying in March before females arrive in early April (Aldridge 1998). Once most of the females have visited the leks and mated, yearling males arrive in late

April to early May and some obtain territories at the periphery of the lek (Aldridge 1998). Displaying is believed to have dual purposes. Agonistic displays are used to defend lek territoies from other males (Scott 1942; Patterson 1952; Dalke et al. 1960; Wiley 1973; Gibson and Bradbury 1987; Gibson 1992; Gibson and Bradbury 1986; Bradbury et al. 1989) and strutting displays attract females (Johnsgard 1983; Aldridge 1998). Displays occur at both dusk and dawn, but increase in intensity at sunrise (Johnsgard 1983). The display is comprised of strutting, tail fanning, and chest puffing (Lumsden 1968; Wiley 1973; Johnsgard 1983). The male inflates his yellowish air sacs and pops them twice as he flaps his wings (Lumsden 1968; Wiley 1973; Johnsgard 1983; Young et al. 2000). Male Sage-Grouse have evolved this complex series of mating behaviour to attract females to leks to breed.

While males spend months on leks, females spend a much shorter period of time at the actual lek location, but many nest in close proximity to leks. Females are thought to visit a single lek over the period of two to three days and only mate once, presumably with dominant males (Wiley 1973). After breeding, nests are placed on average 2.7 to 7.8 km from the lek (Wallestad and Pyrah 1974; Wakkinen et al. 1992; Fischer 1994; Schroeder et al. 1999; Hausleitner 2003). Females lay an average of 7.3 eggs (Connelly et al. 2004) in a nest bowl on the ground that is sparsely lined with vegetation and feathers from the female's brood patch (Schroeder et al. 1999). The incubation period is 27 days (Aldridge and Brigham 2001). The likelihood of a female nesting in a given year ranges from 63% to 100%, with nest success being 14.5% to 86.1% (Gregg 1991; Gregg et al. 1994; Schroeder 1997; Chi 2004; see Connelly et al. 2004 for a review). Chicks are precocial, leave the nest soon after hatching, and are capable of weak flight at 10 days (Schroeder et al. 1999). Despite the short time period that leks are used by both sexes, they are a focal point for both breeding and nesting and have led to the evolution of unique mating behaviours.

Like most other grouse species, Sage-Grouse are polygynous and specifically exhibit a form of mating system called "lek polygyny" where multiple males display for females on the same arena or lek (Bergerud 1988). Lek systems

can be defined by four criteria: (1) males exhibit no parental care, (2) leks occur away from nesting areas, (3) displaying males occur in groups, and (4) females can choose any male as a mate (Gibson and Bradbury 1986). Only a few males are thought to obtain the majority of matings on any given lek in a given breeding season (Wiley 1973; Gibson et al. 1991). Based on behavioral studies, it is believed that intense competition between males results in the most dominant male fathering most of the young (Hernandez et al. 1999). A few males are thought to obtain 80% to 90% of all matings and several subordinate males obtain the remainder (Scott 1942; Wiley 1978). Dominance is likely determined by age, experience, ability to display and hold a territory, and potentially relatedness to other males on the lek. Both experience and ability were found to be associated with a male's display performance and location on the lek (Gibson et al. 1991). However, male mating behaviour is only one component of what makes the lekking system unique.

Lekking and active sampling of prospective mates is usually considered costly for females because they have to visit leks repeatedly to spend time with several different males before mating (Gibson and Bachman 1992). This results in additional movement requirements that may increase a female's energetic expenditure or expose her to an increased predation risk (Gibson and Bachman 1992). However, spending extra time assessing potential mates likely allows females to select high quality, healthy males that will contribute superior genes to their offspring. As such, females have been observed exhibiting relatively unanimous choice for individual males as mates (Gibson et al. 1991). Females are thought to assess male morphological and behavioural traits based on courtship ability when selecting a mate, but also employ secondary tactics such as copying other females' choice in mates and site fidelity (i.e., selecting a male based on the particular territory he holds; Gibson et al. 1991). Site fidelity is usually thought of as loyalty of a male to a particular territory on a lek and its effect on his mating success (Gibson et al. 1991). However, males can change territory locations annually so it is likely not a good predictor of a male's "attractiveness" across years. Copying is when a female copies the choices of other females because if a

male is popular, it may signal his quality as a mate (Gibson et al. 1991). It is unlikely that mate choice is maintained by selection unless offsetting benefits are sustained (Gibson 1992). These benefits could be obtained indirectly through production of offspring of the sex that most increases the fitness of the mother (Fisher 1930), increased attractiveness of sons ("sexy son hypothesis"; Weatherhead and Robertson 1979), or increased viability of both male and female offspring (Maynard Smith 1991; Gibson and Bachman 1992). Mate choice could also provide direct benefits, such as reduced disease transmission, decreased social interference, or increased fertility (Avery 1984; Gibson and Bachman 1992), which could maintain mate choice relative to the significant sampling costs.

Lekking is also considered costly for males because aggregations of males may attract predators and the majority of males are believed not to mate. Many hypotheses have been proposed to explain why males participate in leks when the majority of males apparently fail to mate. Explanations range from anticipating future breeding opportunities (Wiley 1973), parasite-host co-evolution (Boyce 1990), increased mating opportunity (Höglund and Alatalo 1995), unpredictable female copying behavior (Kokko 1997), reduced predation risk (Boyko et al. 2004) to kin selection (Kokko and Lindström 1996; Sherman 1999; Sœther 2002), but the paradox remains unsolved.

3. Sage-Grouse in Canada

Sage-Grouse are endangered at both the provincial and national levels in Canada (Alberta Sage-Grouse Recovery Action Group 2005; Lungle and Pruss 2008) and sutiable sagebrush habitat has declined from 100,000 km^2 - 6,000 km^2 (Aldridge & Brigham 2003). Only 6% of historical habitat remains and is split into two disjunct regions (Alberta/western Saskatchewan and central Saskatchewan) separated by more than 100 km. Sage-Grouse in Canada are located at the northern periphery of the range and inhabit the sagebrush-grasslands of the semi-arid mixed-grass prairie (Aldridge 1998; Braun 1998; Connelly et al. 2000, Connelly et al. 2004).

Sage-Grouse have been enumerated on their lekking grounds in Alberta biannually from 1968 to 1991 (Aldridge 2000b). After a population crash in 1994, surveys occurred annually and increased in intensity and search effort (Alberta Fish and Wildlife, unpubl. data; Table 1-1). In the late 1960s, counts revealed an average of approximately 600 males in the province (Harris et al. 2001). The number of active leks peaked at 16 with 524 males in 1981 and has since continued to decline (Harris et al. 2001; Connelly et al. 2004; Table 1-1). The majority of leks in Alberta are small (less than 20 males) and there has not been a lek with more than 40 enumerated males since 1991 (Connelly et al. 2004). In spring 2009, only 66 males were counted on 10 active leks (Alberta Fish and Wildlife, unpubl. data; Table 1-1).

Saskatchewan has exhibited similar declines. Prior to 1994, lek counts were sporadic and not well documented. In 1987, there were 30 active leks with a total of 515 males (Harris et al. 2001), but because most of these "leks" were observed only once and were in very close proximity to one another, it is likely that some were actually off-lek foraging or loafing sites and the number of birds in Saskatchewan was considerably lower. In 1994, 93 males were counted on 15 active leks (Parks Canada and Saskatchewan Environment, unpubl. data; Table 1-1). This declined to 10 active leks in 2003 with 81 males. The decline has been greater in Saskatchewan than Alberta; in 2009, there were only four active leks with 45 males (Parks Canada and Saskatchewan Environment, unpubl. data; Table 1-1).

Based on the 1987 lek counts, the Canadian spring population size was estimated to be 2,745 to 4,067 individuals (Aldridge 1998). These estimates were based on the assumption that there is an average spring sex ratio of two females to one male, that counts represent as few as 75% of all males associated with the lek due to the inability of yearling males to obtain territories, and that 90% of all active leks are located and surveyed (Aldridge 1998). The 1997 spring Canadian population was estimated to be between 549 and 813 individuals, which represented an 80% decline since 1987 (Aldridge 1998) and a 66% to 92% decline since the 1970s (Aldridge and Brigham 2001). The population slightly

rebounded in subsequent years (an estimate of 873 to 1293 individuals in 1998 and 1999; Aldridge 1998; Harris et al. 2001), but has since suffered another substantial decline. The current estimated population size is approximately 400 birds based on 2006 – 2009 lek counts and assuming the 1:1 sex ratio observed for Sage-Grouse chicks in Alberta (K. L. Bush, unpubl. data).

Saskatchewan listed Sage-Grouse as threatened in 1987 (Harris et al. 2001). In Alberta they were given a "Blue" listing in 1996 and were considered to be a species at risk (Aldridge 1998). Sage-Grouse were listed as threatened by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 1997 and were upgraded to endangered in 1998 (Connelly et al. 2004). In 1999, Sage-Grouse were listed as endangered in Saskatchewan (Harris et al. 2001) and in 2000, the Alberta Endangered Species Conservation Committee listed them as endangered under the Alberta Wildlife Act (Connelly et al. 2004). Several management actions were implemented after the listing of Sage-Grouse in Alberta (Aldridge 2000b). The hunting season was closed in Alberta in 1996 (hunting was allowed from 1967 to 1995) and the endangered listing protected Sage-Grouse from capture, killing, or harming of individuals and their nests (Aldridge 2000b; Lungle and Pruss 2008). Prior to the closure of the hunting season, it was estimated that at least 272 Sage-Grouse were taken annually from Alberta (Johnsgard 1973) or less than 10% of the population (Harris et al. 2001). Hunting was allowed in Saskatchewan until 1938, when the species received protection under the Wildlife Act (Harris et al. 2001; Lungle and Pruss 2008). In 1997, a recovery team was put together to develop a recovery plan for Sage-Grouse in Canada and assess threats to the species' persistence (Aldridge 2000b).

There are multiple anthropogenic and natural threats to Sage-Grouse in Canada which have contributed to the species' decline. These include oil and gas development (Braun et al. 2002), poor grazing practices (Aldridge et al. 2004; Lungle and Pruss 2008), hydrological impacts (i.e., water impoundments preventing sporadic flooding necessary for sagebrush and forb growth; McNeil and Sawyer 2003; McNeil 2009), conversion of land to agriculture (McAdam 2003; Thorpe et al. 2005), wildlife viewing (Alberta Sage-Grouse Recovery

Action Group 2005; Lungle and Pruss 2008), fragmentation by roads (Aldridge 1998; Braun 1998; Connelly et al. 2000; Holloran 2005), changes to the predator community (Alberta Sage-Grouse Recovery Action Group 2005; Lungle and Pruss 2008), climate change (McNeil et al. 2007), West Nile virus (Naugle et al. 2004; Carpenter 2007), and widespread destruction of sagebrush habitat in neighboring Montana (Alberta Sage-Grouse Recovery Action Group 2005).

In Alberta, anthropogenic disturbance has dramatically increased over the past 30 years (Harris et al. 2001; Braun et al. 2003). The oil and gas industry has removed localized patches of vegetation for well sites, pumping stations, pipelines, and roads (Aldridge 1998, Harris et al. 2001). There has been increased vehicular traffic, mechanical noise, and fragmentation of habitat (Harris et al. 2001; Braun et al. 2003). Over 1800 wells have been drilled across the current Sage-Grouse range in Alberta and approximately 514 well sites were still active in 2009 (Alberta Fish and Wildlife, unpubl. data). This has resulted in the highest concentration of roads and wells in Alberta and has caused the abandonment of four of six leks in this area (Aldridge 1998; Braun et al. 2003). Grazing and agricultural conversion may also have negative impacts on Sage-Grouse. Grazing over extended periods is known to alter the structure of habitat and the composition of the plant community in upland and riparian areas (Aldridge 1998; Hays et al. 1998; Harris et al. 2001). To make the land suitable for grazing and crops, shrub steppe is plowed, sprayed, burned, mechanically treated, flattened, and cut (Hays et al. 1998). Sage-Grouse habitat in Canada is limited by the distribution of silver sagebrush, which is normally confined to riparian corridors (Aldridge 1998; Harris et al. 2001). Degradation of this habitat will be detrimental to Sage-Grouse since it is the primary food source for adults throughout the year and it provides shelter and protection from predators (Aldridge 1998; Harris et al. 2001). Similarly, water impoundments alter the health of sagebrush communities and reduce the availability of mesic meadows required for successful chick rearing (McNeil and Sawyer 2003). Impoundments reduce the frequency of flood events, which maintain sagebrush habitat (McNeil and Sawyer 2003; McNeil 2009). In the last 50 years, the number of impoundments in Sage-Grouse habitat

has quadrupled (McNeil and Sawyer 2003) and increased by 20% to 200% (Watters et al. 2004) in Alberta and Saskatchewan, respectively. However, anthropogenic disturbance is not the only threat to Sage-Grouse in Canada.

Natural factors, such as predation, disease, and environmental conditions, may be affecting Sage-Grouse as well. Population growth is potentially limited by increased nest predation and nest destruction by coyotes (*Canis latrans*), ground squirrels (*Spermophilus* spp.), striped skunks (*Mephitis mephitis*), American Crows (*Corvus brachyrhynchos*), and Black-billed Magpies (*Pica pica*; Johnsgard 1975; Aldridge 1998). Predation on adults and juveniles by coyotes, hawks, eagles, and badgers (*Taxidea taxus*) has the potential to increase due to the construction of infrastructure for industry, which acts as perching sites, and roads, pipelines, and fence lines, which serve as dispersal corridors (Aldridge 1998). West Nile Virus has shown to decrease survival in Alberta (Naugle et al. 2004; Carpenter 2007). Global warming may also affect the species by altering and decreasing the amount of habitat available through drought or extreme weather events (Harris et al. 2001; McNeil and Sawyer 2003; McNeil et al. 2007; Lungle and Pruss 2008).

4. Genetics and Sage-Grouse

To date, there have been five major genetic studies on Greater Sage-Grouse that examine species and subspecies delineation, population structure, male relatedness, and paternity. Kahn et al. (1999) used part of the control region of mitochondrial DNA to identify unique haplotypes to differentiate the large and small-bodied forms of Sage-Grouse into two separate species. Kahn et al. (1999) found that the small-bodied form was comprised almost entirely of one haplotype while the large-bodied form was a mixture of four common haplotypes with several unique haplotypes in each population. The haplotypes formed two distinct clades that separated the two forms, suggesting an absence of gene flow between them. Oyler-McCance et al. (1999) combined the mitochondrial sequence data with microsatellite loci to further examine differences. The small-bodied birds were much less polymorphic at all microsatellite loci and had fewer alleles per

locus (Oyler-McCance et al. 1999). There was also a significant population subdivision between the two forms, but little population subdivision within the large-bodied form (Oyler-McCance et al. 1999). This data was used in conjunction with morphology and behaviour to list the small-bodied form as a new species, Gunnison Sage-Grouse (Young et al. 2000).

Benedict et al. (2003) examined the relationship between the eastern and western subspecies of Greater Sage-Grouse by sequencing part of the mitochondrial control region in birds spanning the boundaries of the two predicted subspecies. They found no genetic evidence for the subspecies designations, but did find two distinct populations. The Lyon/Mono population in southwestern Nevada and eastern California contained a high number of unique haplotypes indicating that the population had been isolated from neighboring populations for a considerable amount of time (Benedict et al. 2003). The birds in Washington were also shown to possess very low haplotype diversity compared to all other populations in the range (Benedict et al. 2003). Oyler-McCance et al. (2005) used microsatellites and mitochondrial DNA to determine the population structure of Sage-Grouse across the species' range. They identified 10 populations that followed a pattern of isolation-by-distance indicating that Sage-Grouse primarily move among neighboring populations. As with Benedict et al. (2003), Washington and Lyon/Mono were identified as unique with low genetic diversity. The remainder of Sage-Grouse had high genetic diversity.

The final two studies used microsatellites to examine paternity and relatedness within Sage-Grouse. Semple et al. (2001) examined mating behaviour and paternity in Sage-Grouse. They studied 10 broods from California and found that 40% of broods were sired by territorial males, 40% were sired by non-territorial males, males from unstudied leks, or males off-lek, and 20% were sired by multiple males. Gibson et al. (2005) investigated whether kin selection occurs on Sage-Grouse leks. They found that relatedness of males within individual leks was indistinguishable from zero and that male relatives did not cluster on leks while displaying. However, they did find that male relatives were observed off-lek together. The Semple et al. (2001) and Gibson et al. (2005) studies were the

first evidence that Sage-Grouse leks are not composed of highly related males where only a few dominant males breed in a given year.

Conservation genetics has become an essential part of management because it bridges the worlds of genetics and ecology and answers questions that could not be examined with solely ecological methods. Genetics is becoming increasingly important as the distributions of many species become fragmented by human activities, leading to small populations with expected reductions in genetic diversity and reduced ability to adapt to biological stressors and changing environments (Frankham et al. 2002). Conservation genetics investigates the relationships among species, populations, family groups, and individuals, providing concrete information on populations and behavioural processes that were only speculative before. At the population level, genetics can be used to estimate levels of gene flow, population differentiation, and population structure, which can identify factors influencing population persistence, such as barriers to gene flow (Slatkin 1987; Piertney et al. 1998; Fedy et al. 2008). Genetic methods can also investigate the relationship between the landscape and genetic structure (Giles and Goudet 1995) because the extent of spatial separation between populations influences the replenishment of genetic diversity via gene flow (Eckert et al. 2008). Identifying population boundaries is also a critical first step for assessing evolutionary processes and identifying management units for the preservation of biodiversity (Petit et al. 1998; Taylor and Dizon 1999; Ji and Leberg 2002; Manel et al. 2007). Similarly, understanding the dynamics of smaller groups, such as leks, or even the relationship between individuals, provides a greater understanding of a species' biology, dispersal, and behaviour. Therefore, combining genetic and ecological methods to study Sage-Grouse allows populations, leks, and individual relationships and processes to be fully examined and used to make more informed conservation decisions.

5. Goals of Thesis

The primary goals of this thesis were to identify the genetic diversity, structure, gene flow, and relatedness of endangered Sage-Grouse in Canada, to

determine if diversity declined over time, and to provide managers with genetic information to help guide conservation decisions. In chapter 2, I determined the genetic structure of birds from Alberta, Saskatchewan, and northern Montana using 13 polymorphic microsatellites to identify population and subpopulation boundaries. I then assessed genetic diversity at the large scale (population, subpopulations, and periphery versus core) to identify if any regions were genetically depauperate. I examined dispersal by both sexes using isolation-by-distance and the assignment test (Cornuet et al. 1999; Pritchard et al. 2000; Bergl Vigilant 2007). I also investigated relatedness within leks to determine if leks were composed of male kin (Kokko and Lindström 1996; Sherman 1999; Sœther 2002) or if any leks contained highly related birds and had a potential for inbreeding. One purpose of this chapter was to determine at what spatial scale management should occur at, as each separate jurisdiction (Alberta, Saskatchewan, and three counties in Montana) had been managed separately and with minimum coordination in the past. Another goal was to provide information on diversity, relatedness, and gene flow to managers so that areas requiring special attention (i.e., areas with low diversity, evidence of inbreeding, or signs of isolation) could be managed to maintain optimal genetic diversity and connectivity.

In chapter 3, I assessed the relatedness of birds in Alberta to determine the degree of sex-specific relatedness within and between leks and if Sage-Grouse exhibited kin association (Kokko and Lindström 1996; Sherman 1999; Sœther 2002). I determined the genetic diversity of each active Alberta lek. I also examined isolation-by-distance within Alberta for both sexes to identify sex-specific patterns of philopatry and dispersal. Goals of this research were to provide Alberta managers with basic genetic information to assess the diversity of Sage-Grouse in Alberta and in each active lek and to determine if there is currently gene flow between leks. The data will also be used to help determine if kin selection operates on Sage-Grouse leks and identify any leks that are composed of highly related individuals, which may be the result of lek isolation.

In chapter 4, I determined the paternity, polygamy (males and females mating with multiple mates), and reproductive variance among individuals for Sage-Grouse in Alberta. I used paternity analyses (Marshall et al. 1998; Kalinowski et al. 2007; Wang 2008) to identify the number of fathers in each clutch, how many broods each male fathered annually and over his sampled lifetime, and the proportion of the sampled male population that fathered offspring. I used genetic methods to identify the rate of intraspecific nest parasitism. I also measured reproductive variance, defined as opportunity for selection (Wade and Arnold 1980), for both sexes and compared these values to other studies of Sage-Grouse and lekking bird species. One purpose of this chapter was to assess mechanisms that might affect genetic diversity of Sage-Grouse in Alberta. If Sage-Grouse leks in Alberta function as previously thought, with only a few males obtaining most of the copulations (Wiley 1973), genetic drift would be accelerated due to a smaller effective population size (Wright 1938; Nunney 1993). However, if more males breed, this reduces variance in reproductive success and increases effective population size (Nunney 1993).

In chapter 5, I evaluated the genetic diversity and structure of Canadian birds from 1895 – 2007 using both historic (museum and private) and contemporary samples. This was done to determine if genetic diversity declined through time as expected for populations suffering severe demographic reductions (Frankham et al. 2002). I determined if birds in Canada had become more genetically structured with increasing habitat fragmentation. I assessed if there was evidence that a genetic bottleneck occurred in concert with the demographic bottleneck in 1994. I also estimated current and past effective population size to determine if it had declined over time. The goal of this chapter was to use historic specimens to provide a genetic baseline against which to evaluate the current genetic state of the population and to determine if genetic diversity had declined with the demographic decline. In chapter 6, I summarize all of my findings by putting them into a management framework and give recommendations for future conservation efforts in Canada and Montana.

6. Literature Cited

Alberta Sage-Grouse Recovery Action Group (2005) Alberta Greater Sage-Grouse Recovery Plan. Alberta Sustainable Resource Development, Fish and Wildlife Division, Alberta Species at Risk Recovery Plan No. 8. Edmonton, Alberta.

Aldrich JW (1946) New subspecies of birds from western North America. Proceedings of the Biological Society of Washington 59: 129 – 136.

Aldridge CL, Boyce MS, Baydack RK (2004) Adaptive management of prairie grouse: how do we get there? Wildlife Society Bulletin 32: 92 - 103.

Aldridge CL, Brigham RM (2003) Distribution, abundance, and status of the greater sage-grouse, *Centrocercus urophasianus*, in Canada. Canadian Field Naturalist 117: 25-34.

Aldridge CL (2001) Do Sage-Grouse have a future in Canada? Population dynamics and management suggestions. Proceedings of the 6th Prairie Conservation and Endangered Species Conference, Winnipeg, Manitoba, February 22 - 25, 2001. 11pp.

Aldridge CL, Brigham RM (2001) Nesting and reproductive activities of greater sage-grouse in a declining northern fringe population. Condor 103: 537 - 543.

Aldridge CL (2000a) Reproduction and habitat use by sage grouse (*Centrocercus urophasianus*) in a northern fringe population. Msc. Thesis, University of Regina, Regina, Saskatchewan.

Aldridge CL (2000b) Assessing chick survival of Sage Grouse in Canada. Alberta Sustainable Resource Development, Fish and Wildlife Service, Alberta Species at Risk Report No. 19. Edmonton, Alberta. 25pp.

Aldridge CL (1998) Status of the Sage Grouse (*Centrocercus urophasianus urophasianus*) in Alberta. Alberta Environmental Protection, Wildlife Management Division, and Alberta Conservation Association, Wildlife Status Report No. 13, Edmonton, AB. 23pp.

Avery MI (1984) Lekking in birds: choice, competition, and reproductive constraints. Ibis 126: 177 - 187.

Beck TDI and Braun CE (1978) Weights of Colorado sage grouse. Condor 80: 241 – 243.

Benedict NG, Oyler-McCance SJ, Taylor SE, Braun CE, Quinn TW (2003) Evaluation of the eastern (*Centrocercus urophasianus urophasianus*) and western (*Centrocercus urophasianus phaios*) subspecies of Sage-grouse using mitochondrial control-region sequence data. Conservation Genetics 4: 301 - 310.

Berry JD, Eng RL (1985) Interseasonal movements and fidelity to seasonal use areas by female sage grouse. Journal of Wildlife Management 49: 237 – 240.

Bergerud AT (1988) Mating systems in grouse. *In* Adaptive strategies and population ecology of northern grouse (Bergerud AT, Gratson MW eds). University of Minnesota Press, Minneapolis, Minnesota.

Bergl RA, Vigilant L (2007) Genetic analysis reveals population structure and recent migration within the highly fragmented range of the Cross River gorilla (*Gorilla gorilla diehli*). Molecular Ecology 16: 501 - 516.

Boyce MS (1990) The Red Queen visits sage grouse leks. American Zoologist 30: 263 - 270.

Boyko AR, Gibson RM, Lucas JR (2004) How predation risk affects the temporal dynamics of leks: Greater sage grouse versus golden eagles. American Naturalist 163: 154 – 165.

Bradbury JW, Vehrencamp SL, Gibson RM (1989) Dispersion of displaying male sage grouse. I. Patterns of temporal variation. Behavioral Ecology and Sociobiology 24: 1 – 24.

Braun CE, Oedekoven OO, Aldridge CL (2003) Oil and gas development in western North America: effects on sagebrush steppe avifauna with particular emphasis on Sage Grouse. Transactions of the 67th North American Wildlife and Natural Resources Conference. 337-349.

Braun CE (1998) Sage-Grouse declines in western North America: What are the problems? Proceedings of the Western Association of State Fish and Wildlife Agencies 78: 139 - 156.

Braun CE, Britt TE, Wallestad RO (1977) Guidelines for maintenance of sage grouse habitats. Wildlife Society Bulletin 5: 99 – 106.

Carpenter JE (2007) West Nile virus and parasites in greater sage-grouse (*Centrocercus urophasianus*) populations. Msc. Thesis, University of Alberta, Edmonton, Alberta.

Chi RY (2004) Greater sage-grouse on Parker Mountain, Utah. MSc. Thesis, Utah State University, Logan, Utah.

Connelly JW, Knick ST, Schroeder MA, Stiver SJ (2004) Conservation Assessment of Greater Sage-Grouse and Sagebrush habitats. Western Association of Fish and Wildlife Agencies. Unpublished Report. Cheyenne, Wyoming.

Connelly JW, Schroeder MA, Sands AR, Braun CE (2000) Guidelines to manage sage grouse populations and their habitats. Wildlife Society Bulletin 28: 967 – 985.

Connelly JW, Browers HW, Gates RJ (1988) Seasonal movements of sage grouse in southeastern Idaho. Journal of Wildlife Management 52: 116 – 122.

Connelly JW, Arthur WJ, Markham OD (1981) Sage grouse leks on recently disturbed sites. Journal of Range Management 52: 153 – 154.

Cornuet J-M, Piry S, Luikart G, Estoup A, Solignac M (1999) New methods employing multilocus genotypes to select of exclude populations as origins of individuals. Genetics 153: 1989 – 2000.

Dalke PD, Pyrah DB, Stanton DC, Crawford JE, Schlatterer EF (1963) Ecology, productivity, and management of sage grouse in Idaho. Journal of Wildlife Management 27: 811 – 841.

Dalke PD, Pyrah DB, Stanton DC, Crawford JE, Schlatterer EF (1960) Seasonal movements and breeding behavior of sage grouse in Idaho. Transactions of the North American Wildlife Conference 25: 396 – 407.

Drut MS, Crawford JA, Gregg MA (1994) Brood habitat use by sage grouse in Oregon. Great Basin Naturalist 54: 170 – 176.

Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. Molecular Ecology 17: 1170 – 1188.

Eng RL (1963) Observations of the breeding biology of male sage grouse. Journal of Wildlife Management 27: 841 – 846.

Fedy BC, Martin K, Ritland C, Young J (2008) Genetic and ecological data provide incongruent interpretations of population structure and dispersal in naturally subdivided populations of white-tailed ptarmigan (*Lagopus leucura*). Molecular Ecology 17: 1905 – 1917.

Fischer RA (1994) The effects of prescribed fire on the ecology of migratory sage grouse in southeastern Idaho. Ph.D. Dissertation, University of Idaho, Moscow, Idaho.

Fisher RA (1930) The genetical theory of natural selection. Clarendon Press, Oxford, UK.

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to Conservation Genetics. Cambridge University Press, Cambridge, UK.

Gibson RM, Pires D, Delaney KS, Wayne RK (2005) Microsatellite DNA analysis shows that greater sage grouse leks are not kin groups. Molecular Ecology 14: 4453 – 4459.

Gibson RM (1996) Female choice in sage grouse: the roles of attraction and active comparison. Behavioural Ecology and Sociobiology 39: 55-59.

Gibson RM (1992) Lek formation in sage grouse: the effect of female choice on male territorial settlement. Animal Behaviour 43: 443 – 450.

Gibson RM Bachman,GC (1992) The costs of female choice in a lekking bird. Behavioral Ecology 3: 300 - 309.

Gibson RM, Bradbury JW, Vehrencamp SL (1991) Mate choice in lekking sage grouse revisited: the roles of vocal display, female site fidelity, and copying. Behavioral Ecology 2: 165 – 180.

Gibson RM, Bradbury JW (1987) Lek organization in sage grouse: variations on a territorial theme. Auk 104: 77 – 84.

Gibson RM, Bradbury JW (1986) Male and female mating strategies on sage grouse leks. *In* Ecological aspects of social evolution: birds and mammals (Rubenstein DI, Wrangham RW eds). Princeton University Press, Princeton, New Jersey.

Giezentanner KI, Clark WH (1974) The use of western harvester ant mounds as strutting locations by sage grouse. Condor 76: 218 – 219.

Giles BE, Goudet J (1995) A case study of genetic structure in a plant metapopulation. *In*: Metapopulation Biology: Ecology, Genetics, and Evolution (eds Hanksi IA, Gilpin ME), pp. 429–454. Academic Press, San Diego, California.

Gregg MA, Crawford JA, Drut MA, DeLong AK (1994) Vegetational cover and predation of sage grouse nests in Oregon. Journal of Wildlife Management 58: 162 – 166.

Gregg MA (1991) Use and selection of nesting habitat by sage grouse in Oregon. MSc. Thesis, Oregon State University, Corvallis, Oregon.

Harris W, Lungle K, Bristol B, Dickinson D, Eslinger D, Fargey P, Kroshus J, Livingston T. Lunn S, McAdam S, Michaud I, Milner D, Poirier T, Scobie D, Veitch L (2001) Canadian Sage Grouse Recovery Strategy. The Canadian Sage Grouse Recovery Team. 55pp.

Hausleitner D (2003) Population dynamics, habitat use and movements of greater sage-grouse in Moffat County, Colorado. MSc. Thesis, University of Idaho, Moscow, Idaho.

Hays DW, Tirhi MJ, Stinson DW (1998) Washington State status report for the sage grouse. Washington Department of Fish and Wildlife, Olympia. 62 pp.

Hernandez ML, Houston AI, McNamara JM (1999) Male rank and optimal lek size. Behavioral Ecology 10: 73 - 79.

Hjorth I (1970) Reproductive behavior in Tetraonidae with special reference to males. Viltrevy 7: 351 – 357.

Höglund J, Alatalo RV (1995) Leks. Princeton University Press, Princeton, New Jersey.

Holloran MJ (2005) Greater Sage-Grouse (*Centrocercus urophasianus*) population response to natural gas field development in western Wyoming. Ph.D. Thesis. University of Wyoming. Larmie, Wyoming. 209 pp.

Hupp JW, Braun CE (1991) Geographic variation among sage grouse in Colorado. Wilson Bulletin 103: 255 – 261.

Jenni SA, Hartzler JE (1978) Attendance at a sage grouse lek: implications for spring censuses. Journal of Wildlife Management 54: 89 – 91.

Ji W, Leberg P (2002) A GIS-based approach for assessing the regional conservation status of genetic diversity: an example from the Southern Appalachians. Environmental Management 29: 531 – 544.

Johnsgard PA (1983) The Grouse of the World. University of Nebraska Press, Lincoln, Nebraska.

Johnsgard PA (1973) Grouse and Quails of North America. University of Nebraska Press, Lincoln, Nebraska.

Kahn NW, Braun CE, Young JR, Wood S, Mata DR, Quinn TW (1999) Molecular analysis of genetic variation among large- and small-bodied s age grouse using mitochondrial control-region sequences. Auk 116: 819 - 824.

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16: 1099 - 1106

Knowlton GF, Thornley HF (1942) Insect food of the sage grouse. Journal of Economic Entomology 35: 107 – 108.

Kokko H (1997) The lekking game: can female choice explain aggregated male displays? Journal of Theoretical Biology 187: 57 – 64.

Kokko H, Lindström J (1996) Kin selection and the evolution of leks: whose success do young males maximize? Proceedings of the Royal Society of London Series B 263: 919 - 923.

Lumsden HG (1968) The displays of the sage grouse. Ontario Department of Lands and Forests Research Report (Wildlife) no. 83.

Lungle K, Pruss S (2008) Recovery strategy for the greater sage-grouse (*Centrocercus urophasianus urophasianus*) in Canada. *In* Species at Risk Act Recovery Strategy Series. Parks Canada Agency. Ottawa. vii + 43 pp.

Manel S, Berthoud F, Bellemain E, Gaudeul M, Luikart G, Swenson JE, Waits LP, Taberlet P, Intrabiodiv Consortium (2007) A new individual-based spatial approach for identifying genetic discontinuities in natural populations. Molecular Ecology 16: 2031 – 2043.

Marshall TC, Slate J, Kruuk EB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology 7: 639 - 655

Maynard Smith J (1991) Theories of sexual selection. Trends in Ecology and Evolution 6: 146 - 150.

McAdam S (2003) Lek occupancy by greater Sage-Grouse in relation to habitat in southwestern Saskatchewan. M.Sc. Thesis. Royal Roads University, Victoria, British Columbia.

McNeil R (2009) Strategies to maintain or enhance silver sagebrush habitats for greater sage grouse in southwestern Saskatchewan. Prepared by LandWise Inc. for Interdepartmental Recovery Fund (IRF), Prairie Farm Rehabilitation Administration, Agriculture and Agri-Food Canada (PFRA), Grasslands National Park, Parks Canada (GNP), and Saskatchewan Watershed Authority (SWA) 182 pp.

McNeil J, Yin H, McNeil R (2007) Analysis of the climate in the Greater Sage Grouse range of southwestern Saskatchewan and southeastern Alberta. Prepared for PFRA, Grasslands National Park, Sask. Watershed Authority and Interdepartmental Recovery Fund (IRF), by LandWise Inc., Lethbridge, Alberta. 43 pp.

McNeil RL, Sawyer BJ (2003) Effects of water management practices and precipitation events on sagebrush habitat in southeastern Alberta. Prepared for Alberta Conservation Association and Alberta Sustainable Resource Development, by LandWise Inc., Lethbridge, Alberta. 36 pp.

Naugle DE, Aldridge CL, Walker BL, Cornish TE, Moynahan BJ, Holloran MJ, Brown K, Johnson GD, Schmidtmann ET, Mayer RT, Kato CY, Matchett MR, Christiansen TJ, Cook WE, Creekmore T, Falise RD, Rinkes ET, Boyce MS (2004) West Nile virus: pending crisis for greater sage-grouse. Ecology Letters 7: 704 – 713.

Nunney L (1993) The influence of mating system and overlapping generations on effective population size. Evolution 47: 1329 – 1341.

Oyler-McCance SJ, Taylor SE, Quinn TW (2005) A multilocus population genetic survey of the greater sage-grouse across their range. Molecular Ecology 14: 1293 – 1310.

Oyler-McCance SJ, Kahn NW, Burnham KP, Braun CE, Quinn TW (1999) A population genetic comparison of large- and small-bodied sage grouse in Colorado using microsatellite and mitochondrial DNA markers. Molecular Ecology 8: 1457 - 1465.

Patterson RL (1952) The sage grouse in Wyoming. Sage Books, Inc. Denver, Colorado.

Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. Conservation Biology 12: 844–855.

Piertney SB, MacColl ADC, Bacon PJ, Dallas JF (1998) Local genetic structure in red grouse (*Lagopus lagopus scoticus*): evidence from microsatellite DNA markers. Molecular Ecology 7: 1645 – 1654.

Pritchard JK, Stephens M, Donelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945 - 959

Pyle WH (1993) Response of brood-rearing habitat of sage grouse to prescribed burning in Oregon. Msc. Thesis, Oregon State University, Corvallis, Oregon.

Sœther SA (2002) Kin selection, female preference and the evolution of leks: direct benefits may explain kin structuring. Animal Behaviour 63: 1017 - 1019.

Scott JW (1942) Mating behavior of the sage grouse. Auk 59: 477 – 498.

Schroeder MA, Aldridge CL, Apa AD, Bohne JR, Braun CE, Bunnell SD, Connelly JW, Diebert PA, Gardner SC, Hilliard MA, Kobriger GD, McAdam SM, McCarthy CW, McCarthy JJ, Mitchell DL, Rickerson EV, Stiver SJ. (2004) Distribution of sage-grouse in North America. Condor 106: 363 - 376.

Schroeder MA, Young JR, Braun CE (1999) Sage grouse (*Centrocercus urophasianus*) in The Birds of North American, No. 425 (Poole A, Gill F eds). The Birds of North American, Inc. Philadelphia. 28pp.

Schroeder MA (1997) Unusually high reproductive effort by sage grouse ina fragmented habitat in north-central Washington. Condor 99: 933 – 941.

Semple K, Wayne RK, Gibson RM (2001) Microsatellite analysis of female mating behaviour in lek-breeding sage grouse. Molecular Ecology 10: 2043 – 2048.

Sherman PW (1999) Birds of a feather lek together. Nature 401: 119 – 120.

Slatkin M (1987) Gene flow and geographic structure of natural populations. Science 236: 787 – 792.

Taylor BL, Dizon AE (1999) First policy then science: why a management unit based solely on genetic criteria cannot work. Molecular Ecology 8: S11 – S16.

Thorpe J, Godwin B, McAdam S (2005) Sage-Grouse habitat in southwestern Saskatchewan: Differences between active and abandoned leks. SRC Publication No. 11837-1E05. Saskatchewan Research Council, Saskatoon, Saskatchewan. 39 pp.

Vehrencamp SL, Bradbury JW, Gibson RM (1989) The energetic cost of display in male sage grouse. Animal Behavior 38: 885 – 896.

Wade M J, Arnold SJ (1980) The intensity of sexual selection in relation to male sexual behaviour, female choice, and sperm precedence. Animal Behavior 28: 446 – 461.

Wakkinen WL (1990) Nest site characteristics and spring-summer movements of migratory sage grouse in southeastern Idaho. Msc. Thesis, University of Idaho, Moscow, Idaho.

Wallestad RO, Peterson JG, Eng RL (1975) Foods of adult sage grouse in central Montana. Journal of Wildlife Management 39: 628 – 630.

Wallestad RO, Pyrah DB (1974) Movement and nesting of sage grouse hens in Central Montana. Journal of Wildlife Management 38: 630 – 633.

Wang J (2008) COLONY 2.0 Manual. Downloadable from http://www.zoo.cam.ac.uk/ioz/index.htm

Watters M, McMaster G, Springer G (2004) Site plans for Sage-Grouse (*Centrocercus urophasianus*) in southern Saskatchewan. Unpublished Report, Saskatchewan Watershed Authority, Regina, Saskatchewan.

Weatherhead PJ, Robertson RJ (1979) Offspring quality and the polygyny threshold: 'the sexy son hypothesis'. American Naturalist 113: 201 - 208.

Wiley RH (1978) The lek mating system of the sage grouse. Scientific American 238: 114 – 125.

Wiley RH (1973) Territoriality and non-random mating in the sage grouse, *Centrocercus urophasianus*. Animal Behaviour Monographs 6: 87 - 169.

Wright S (1938) Size of population and breeding structure in relation to evolution. Science 87: 430 – 431

Young JR, Braun CE, Oyler-McCance SJ, Hupp JW, Quinn TW (2000) A new species of Sage-Grouse (Phasianidae: *Centrocercus*) from southwestern Colorado. Wilson Bulletin 112: 445 - 453.

Table 1-1. Lek counts and number of active leks in Alberta and Saskatchewan in years in which lek counts were conducted. For these purposes "active leks" are considered leks with at least one male counted in a given year. Years in which lek counts were not performed are blank. Data is from Alberta Fish and Wildlife, Parks Canada, and Saskatchewan Environment.

Year	Number of active leks in Alberta	Number of males counted in Alberta	Number of active leks in Saskatchewan	Number of males counted in Saskatchewan
1968	21	613		
1969	19	554		
1975	19	212		
1976	19	347		
1977	13	286		
1978	13	235		
1979	11	198		
1980	16	482		
1981	16	524		
1983	18	358		
1985	14	208		
1987	13	400		
1988			61*	934*
1989	12	344		
1991	11	241		
1994	8	70	15	93
1995	12	110	16	105
1996	10	136	19	123
1997	8	122	10	61
1998	8	124	11	122
1999	9	117	8	101
2000	8	126	10	126
2001	9	114	10	106
2002	10	91	10	84
2003	9	96	10	81
2004	9	94	8	60
2005	9	95	8	62
2006	9	90	6	60
2007	10	90	6	55
2008	9	78	5	51
2009	10	66	4	45

*Unlikely to be accurate based on the 1994 – 2009 Saskatchewan counts and the 1987 Alberta counts. It is likely that the counts were similar to Alberta for that time period.

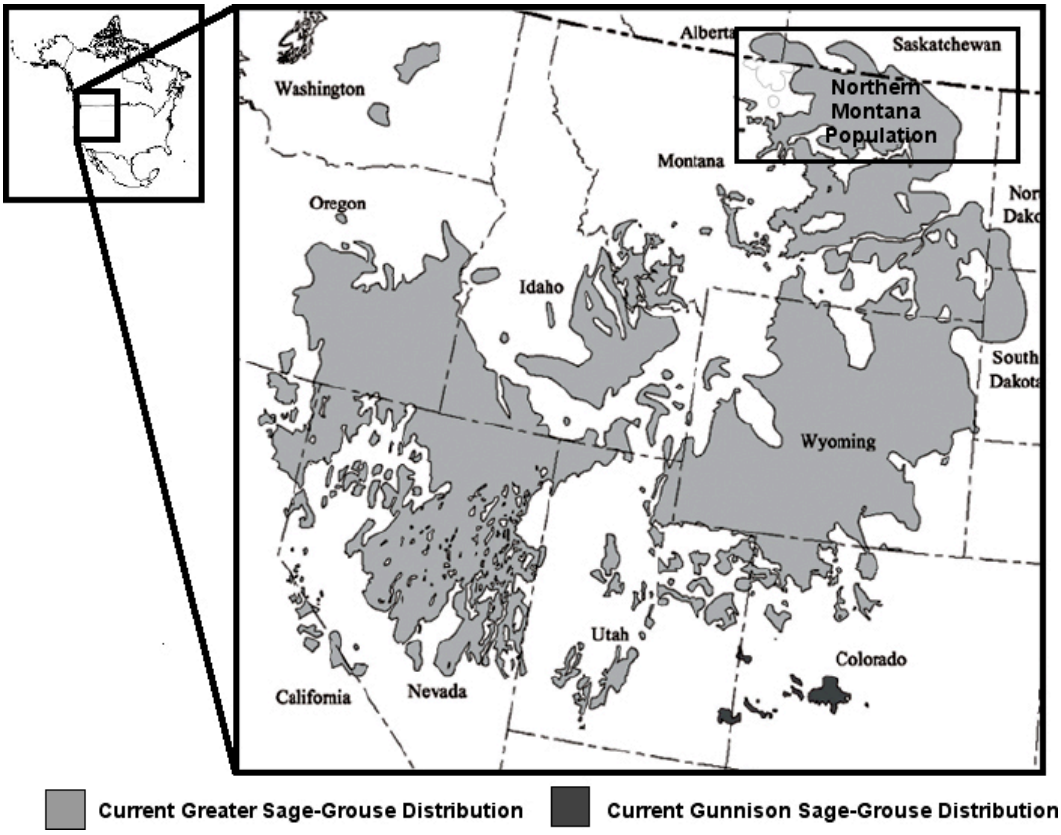

Figure 1-1. Current Greater and Gunnison Sage-Grouse range with the northern Montana population highlighted. Adapted from Schroeder et al. (2004).

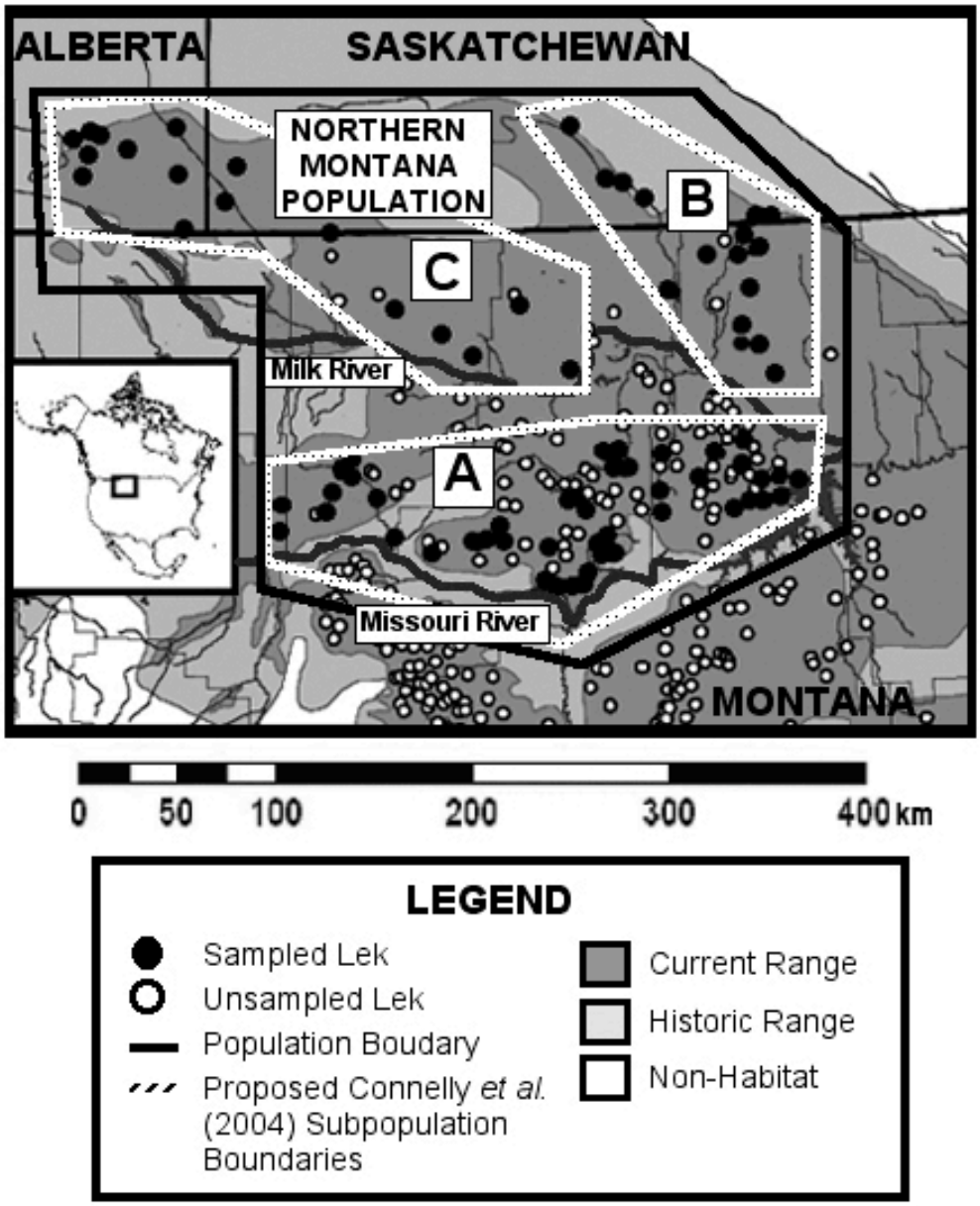

Figure 1-2. The northern Montana population with the three subpopulations (A, B, and C) suggested by Connelly *et al*. (2004). Milk and Missouri Rivers are indicated by wide black lines in the middle and bottom of the northern Montana population, respectively. Adapted from Schroeder et al. (2004).

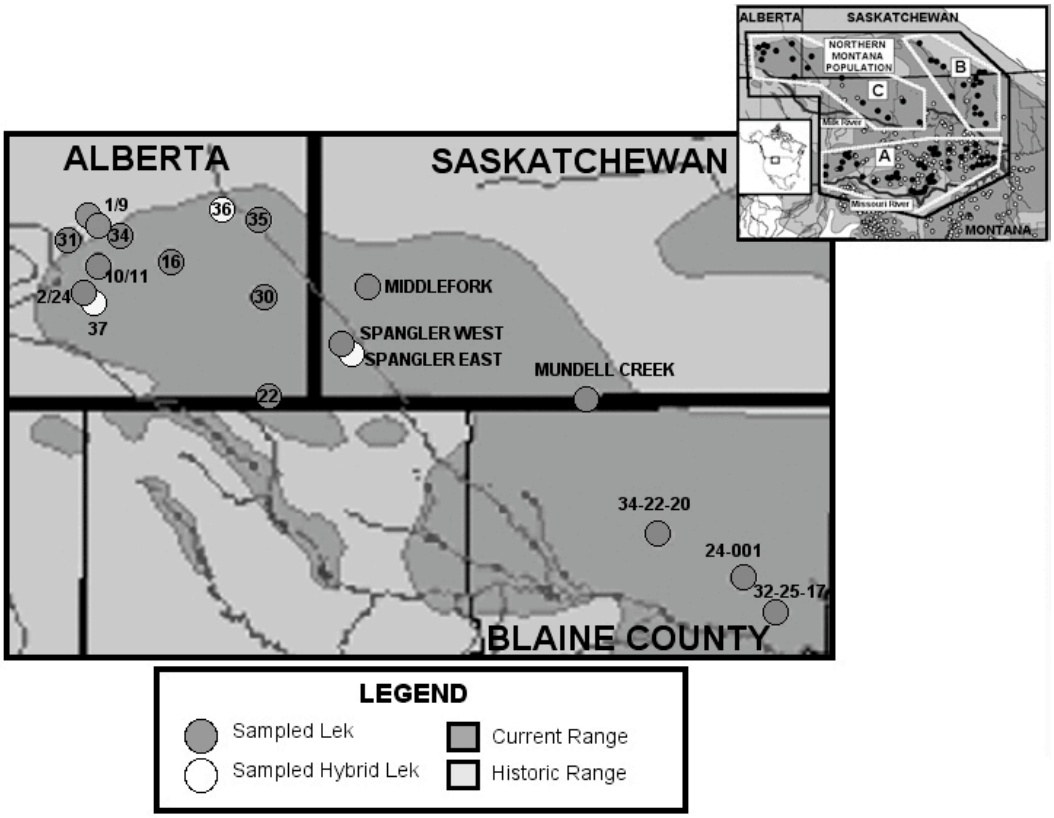

Figure 1-3. Subpopulation 1 ("C" in Figure 1-2; sage creek) of the northern Montana population suggested by Connelly *et al*. (2004). Sage creek includes Alberta, southwestern Saskatchewan, and north Blaine County, Montana**.** Adapted from Schroeder et al. (2004).

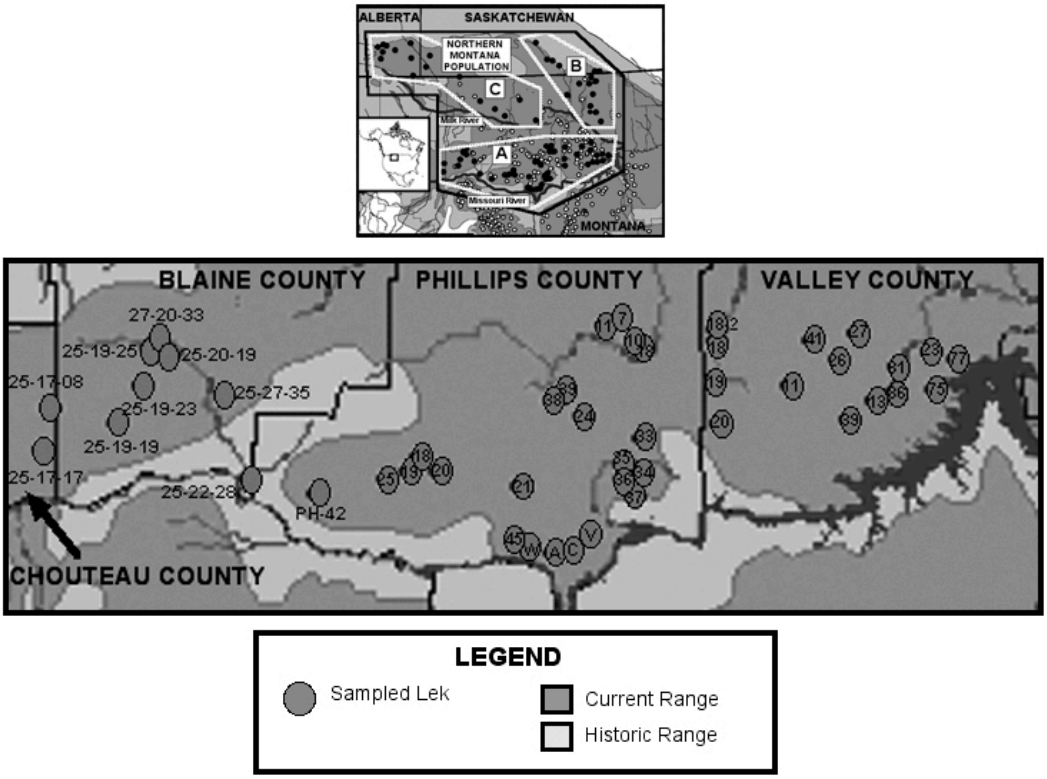

Figure 1-4. Subpopulation 2 ("A" in Figure 1-2; Milk/Missouri transition zone) of the northern Montana population suggested by Connelly *et al*. (2004). The Milk/Missouri transition zone involves north central Montana (Chouteau County and south Blaine, Phillips, and Valley Counties). Adapted from Schroeder et al. (2004).

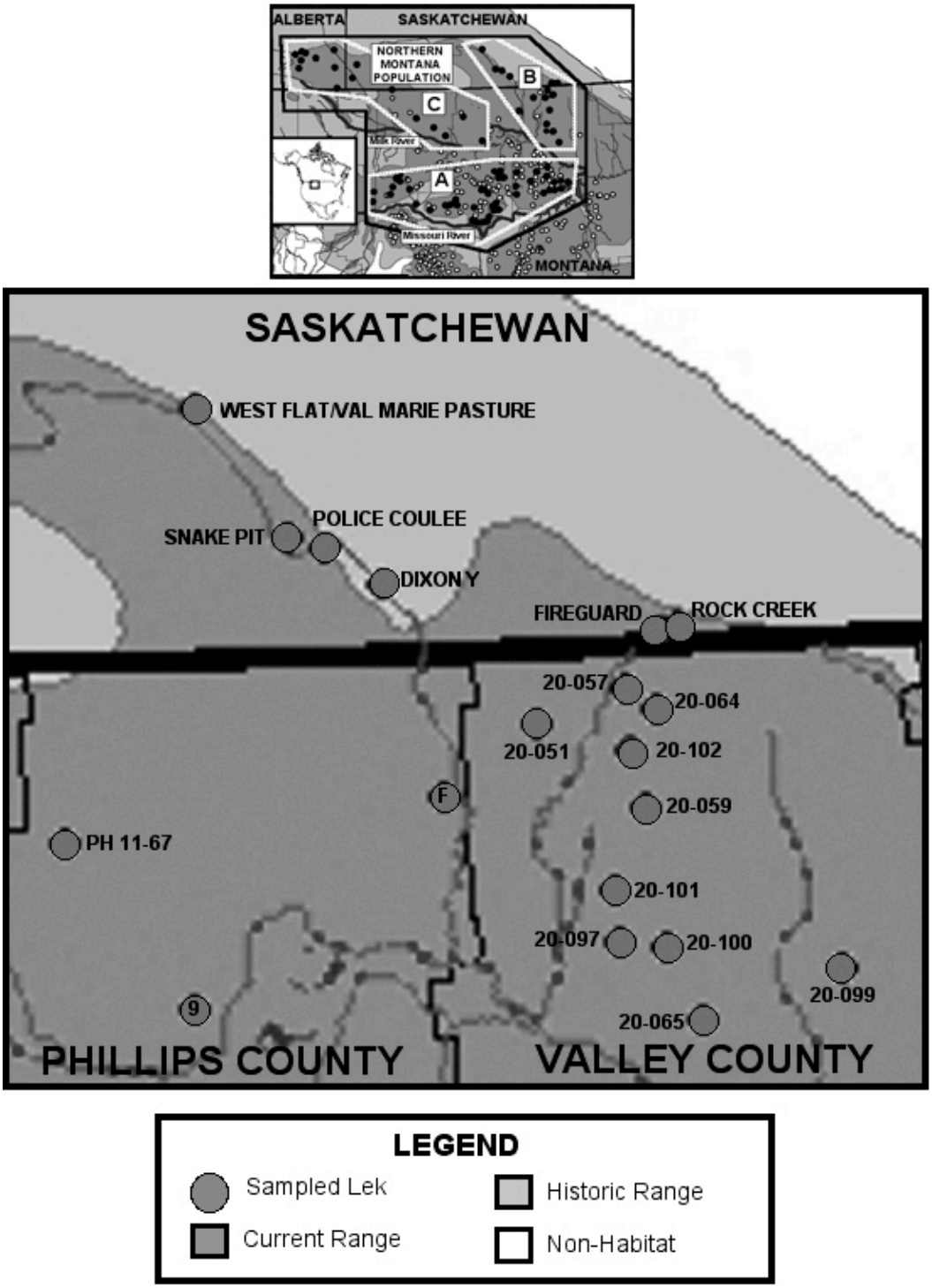

Figure 1-5. Subpopulation 3 ("B" in Figure 1-2; Frenchman River) of the northern Montana population suggested by Connelly *et al*. (2004). The Frenchman River region includes south central Saskatchewan and north Phillips and Valley Counties, Montana. Adapted from Schroeder et al. (2004).

CHAPTER TWO

Population Structure and Genetic Diversity of Greater Sage-Grouse (*Centrocercus urophasianus*) in Fragmented Landscapes at the Northern Edge of Their Range[1]

1. Introduction

The effects of habitat fragmentation and peripheral habitat on genetic diversity are important topics in conservation genetics. Fragmentation impacts gene flow by decreasing dispersal and population size, and increasing genetic drift in isolated pockets (Frankel & Soulé 1981). Declining populations experience greater loss of genetic diversity, inbreeding, and fixation of deleterious alleles, all of which may increase probability of extinction and reduce adaptive potential of populations (Frankel & Soulé 1981). Species with the ability to fly such as birds should be more resilient to fragmentation (Galbusera et al. 2004; Veit et al 2005; Martínez-Cruz et al. 2007), but more sedentary species, particularily galliforms, display significant genetic structure and differentiation from fragmentation at varying spatial scales (Johnson et al. 2003, greater prairie-chicken [*Tympanuchus cupido pinnatus*]; Caizergues et al. 2003a, black grouse [*Tetrao tetrix*]; Segelbacher et al. 2003, capercaillie [*Tetrao urogallus*]; Caizergues et al. 2003b, rock ptarmigan [*Lagopus mutus*]; Bouzat & Johnson 2004, lesser prairie-chicken [*Tympanuchus pallidicinctus*]).

Peripheral populations are often touted as sources of unique genetic variation, which may allow adaptation to future climate change, habitat alteration, range expansion, or speciation events, but they can also be viewed as genetically depauperate, doomed to extinction, and not worth conservation effort (Eckert et al. 2008). Populations at range peripheries are considered more susceptible to declines because they occupy marginal habitat and are isolated from larger central

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[1] This chapter is formatted for Conservation Genetics with the following authors: KL Bush, CK Dyte, BJ Moynahan, CL Aldridge, HS Sauls, AM Battazzo, BL Walker, KE Doherty, J Tack, J Carlson, D Eslinger, J Nicholson, MS Boyce, DE Naugle, CA Paszkowski, and DW Coltman

populations (Lesica & Allendorf 1995; Sargarin & Gaines 2002). Peripheral populations are usually smaller in census and effective population sizes, are more genetically isolated, exhibit founder effects or genetic drift, and are prone to extinction from stochastic or catastrophic events (Lammi et al. 1999; Vucetich & Waite 2003). Some studies have found peripheral populations to be less genetically diverse than central populations (Lammi et al. 1999; Vucetich & Waite 2003; Bouzat & Johnson 2004), while others have not (Kirkpatrick & Ravigne 2002; Eckert et al. 2008).

In this study I assess how range periphery and fragmentation impact genetic diversity and structure in greater sage-grouse (hereafter sage-grouse; *Centrocercus urophasianus*). Sage-grouse are a good model system because large sample sizes are obtainable, they are well studied, and basic biological and habitat parameters are known. Microsatellite markers and baseline genetic data are available for the species (Oyler-McCance et al. 2005). They are also a species of concern in North America due to rapid population declines and habitat destruction (Connelly et al. 2004) and my study population is on the northern range edge, which has experienced substantial anthropogenic fragmentation.

Sage-grouse are polygamous galliforms where males congregate and strut on communal display grounds (leks) in the spring and females select a mate, breed, and incubate and raise the young on their own (Gibson 1996). Grouse leks are thought to contain philopatric, related males (Kokko & Lindström 1996) and mating success is highly skewed (Wiley 1973) so there should be reduced effective population size, increased genetic structuring, and inbreeding potential, especially in fragmented landscapes. Historically, sage-grouse inhabited three Canadian provinces (Alberta, Saskatchewan, and British Columbia) and 14 U.S. states, but presently occur only in southeastern Alberta, southwestern Saskatchewan, and 11 U.S. states (Schroeder et al. 2004). In Canada, sage-grouse numbers have declined by 66-92% since the 1970s (Aldridge & Brigham 2003) with an estimated 2007 population size of approximately 450 birds. Populations in the United States have declined at a slower rate, ranging from 45-80% across the species' range, with the central Montana and central/southern Wyoming regions

remaining relatively stable (Connelly et al. 2004). Rangewide, the amount of habitat has decreased by over 50% (Schroeder et al. 2004) from conversion of native sage steppe to agriculture, municipal infrastructure, and energy development (Connelly et al. 2004).

Determining genetic population structure is essential for managing declining, peripheral, and fragmented populations. Connelly et al. (2004) classified sage-grouse into 41 populations across North America based on spatial isolation from other populations by at least 10 km. The Northern Montana population (NMP; Canada and Montana north of the Missouri River) was split into three subpopulations based on potential habitat barriers (Fig. 2-1). The Milk River separates subpopulation "A" to the south and a 100 km strip of agriculture separates subpopulations "B" and "C" in the north (Fig. 2-2; Connelly et al. 2004). Genetic work on sage-grouse showed isolation-by-distance (IBD) with restricted gene flow across the range and identified only 10 populations (Oyler-McCance et al. 2005). One genetic population included Alberta and all of Montana, but likely overestimated population size because of small sample sizes and sparsely distributed sampling locations.

I used polymorphic microsatellites to examine genetic structure and diversity in a fragmented and peripheral sage-grouse population. I examined three topics:

(1) Genetic structure and diversity - do birds north of the Missouri River (the proposed NMP) form one or more populations?
(2) Lek genetic structure – Are leks composed of unrelated males?
(3) Periphery and fragmentation - Are there genetic consequences of being situated near the range periphery and in areas impacted by fragmentation?

I expected to find population structure within the NMP due to substantial declines in lek counts, extensive natural and anthropogenic habitat fragmentation, and isolation at the northern periphery of the species' range. I predicted that leks were not composed of related males based on the Gibson et al (2005) study, where they found sage-grouse males within leks displayed low levels of relatedness and because recruitment within parts of the study area was low (Aldridge & Boyce 2007). The latter suggests few offspring survive to potentially

lek with their relatives. I anticipated lower genetic diversity in fragmented and isolated regions and predicted that the northern periphery would be genetically depauperate compared to high-density regions near the Missouri River.

2. Methods

2.1. Study location and sample collection

This study was conducted on sage-grouse from the NMP (14.2% of the total sage-grouse range) and part of the Powder River Basin (PRB) populations (Fig. 2-1). Only the northern part of the PRB was sampled and was included as an outgroup to delineate structure of the NMP. Birds were captured using walk-in funnel traps (Schroeder & Braun 1991), night-lighting (Giesen et al. 1982), rocket nets (Giesen et al. 1982), and drop-nets (Bush 2008). Blood (n = 290), plucked feather (n = 974), mouth swab (n = 104), and shed feather (n = 2,441) samples were collected from adult sage-grouse as part of research projects in the NMP (Alberta [1998-2006] and Montana: Phillips [2001-2005] and Valley [2006] counties) and northern PRB (Montana: Bighorn county [2003-2006] and Wyoming: Sheridan [2003-2006], Campbell [2003-2004], and Johnson [2004-2006] counties). The NMP was sampled using molted feathers collected from leks in Alberta and Saskatchewan (2003-2006), Valley (2005), Blaine (2005 and 2006), Phillips (2006), and Choteau (2006) counties, Montana. Not all active leks were sampled in both populations (Fig. 2-1). I only sampled leks that were being surveyed and/or studied in the NMP and the PRB. To increase the sample size for birds in Canada, I opportunistically sampled birds off-lek. Off-lek birds consisted of females captured in the company of radio-collared females, carcasses of unmarked vehicular or predator mortalities, and molted feathers found at roost sites. All birds sampled off lek were assigned an unknown lek status and were not used in any lek-specific analyses. Overall, I collected 3,824 samples (3,616 from 104 leks [83 NMP, 21 PRB] and 208 off-lek). All samples were used for all population and subpopulation level analysis, while only leks over 10 (lek-level) and five (sex-specific) sampled birds were used for finer scale analyses.

2.2. Microsatellite genotyping

DNA was extracted using Qiagen DNeasy® Tissue and QIAamp® DNA Micro kits using modifications from Bush et al. (2005). All samples were DNA-sexed using the Bush et al. (2005) procedure. Thirteen microsatellite loci developed from sage-grouse (SGCA9-2 [redesigned primer set; S. Taylor, pers. comm.] and SGCA5; Taylor et al. 2003), capercaillie (TUT3, TUT4, TUD1, and TUD3; Segelbacher et al. 2000), black grouse (BG6 and BG15; Piertney & Höglund 2001; TTD6 and TTT1; Caizergues et al. 2001; TTT3; Caizergues et al. 2003a), red grouse (*Lagopus lagopus*; LLSD8; Piertney & Dallas 1997), and domestic chicken (*Gallus gallus*; ADL230; Cheng et al. 1994) were used. I identified null alleles by examining 20 sage-grouse females and their known offspring. I did not detect null alleles, therefore the 13 loci were used for all analyses. Microsatellite PCRs (15μl total volume) were carried out as described in Bush et al. (2005). Forward primers were fluorescently labeled with 6-FAM, TET, and HEX (Applied Biosystems). I followed the PCR cycling conditions outlined for each microsatellite in the original publications using Perkin Elmer Cetus GeneAmp PCR System 9600® and Eppendorf Mastercycler® ep machines. All non-invasive samples were run in triplicate as outlined in Bush et al. (2005). The PCR products were visualized using an ABI 377® automated sequencer with GENESCAN ANALYSIS3.1® software (Applied Biosystems). Alleles were scored using GENOTYPER®2.0 software (Applied Biosystems).

2.3. Duplicate samples

Shed feathers are normally considered non-invasive samples, but on leks, most result from fighting and are equivalent in quality to plucked feathers. I quantified the DNA quality of each feather by amplifying the five strongest microsatellites (TUT3, TUT4, SGCA5, SGCA9-2, and TTD6) once and assessing peak height and quality. Then triplicate PCR replicates were performed with 3 - 5μl DNA. For shed feathers with lower quality DNA, a maximum of 7 - 11 microsatellites were successfully amplified in triplicate for each sample. For all other samples, all 13 loci were amplified. In low quality feather samples, low

rates of drop out and no false alleles were detected. For all samples that failed to produce the same genotype in three of three replicates for any locus, the genotype for that locus was excluded and only consistent genotypes (three of three replicates) were kept for that sample (i.e. if a sample produced the same genotype at one locus in two of three runs, the genotype for that locus was excluded from the composite genotype, which was composed of all 13 loci). Duplicate samples were identified using GENALEX version 5.1 (Peakall & Smouse 2001). Two samples were considered duplicates if they were identical or differed by no more than one allele at up to two loci in a manner consistent with allelic drop out. Missing data was ignored to allow for matches between fully genotyped samples and samples with one or more missing loci. Probability of identity (PI) was calculated in GENALEX.

2.4. Population structure

I investigated spatial genetic structure using the Bayesian program STRUCTURE (Pritchard et al. 2000), which puts individuals into clusters (*K*) based on multilocus genotypic data, independent of sample location. Highly related individuals (parent-offspring and full-siblings) were identified with COLONY, version 1.2 (Wang 2004) and all but one relative was removed prior to STRUCTURE analysis to minimize lower-level structure caused by first-order relatives. I examined three levels of population structure to delineate the boundaries for sage-grouse populations in the region. First, all birds from the NMP and the PRB were included to identify the number of populations. Second, the NMP birds were used to identify the number of subpopulations within the population. Third, I determined lower level structure (genetically distinctive leks and lek clusters [groups of related neighboring leks]) by breaking the NMP into geographic regions containing < 20 leks (i.e. Alberta and western Saskatchewan; Fig. 2-1). I determined *K* for the number of (1) populations, (2) subpopulations, and (3) lek clusters/leks by running 20 independent simulations for each *K* (1 - 20) with 100,000 burn-in iterations and 1,000,000 data repetitions assuming an admixture model, correlated allele frequencies (within the NMP; 0.01), and no

prior population information. I used the method of Evanno et al. (2005), which calculates ΔK, a measure of the second order rate of chance in the likelihood of K, to estimate the true K. I used this method because both Evanno et al. (2005) and the software documentation note that it is computationally difficult to obtain accurate estimate of K using $\Pr(X|K)$ values and its biological interpretation may not be straightforward.

I examined genetic population structure within the NMP with hierarchical analysis of molecular variance (AMOVA) in ARLEQUIN, version 3.1 (Excoffier et al. 2006) using F_{ST} as the genetic distance measure.

2.5. Genetic diversity and differentiation

I calculated expected (H_E) and observed (H_O) heterozygosity for each locus and tested for deviations from Hardy-Weinberg and linkage equilibrium using GENEPOP, version 3.4 (Raymond & Rousset 1995). Number of alleles per locus (A) was calculated in GENALEX and allelic richness (number of alleles corrected for the smallest sample size; AR) in FSTAT, version 2.9.3 (Goudet 2001). Average relatedness (R) within and between-leks was computed in RELATEDNESS 5.0 (Queller & Goodnight 1989). Pairwise-F_{ST} was calculated in GENEPOP and significance tests were performed in FSTAT using 1,000 permutations. The preceding diversity indices were calculated for the NMP, both subpopulations, and all leks. Levels of significance were adjusted using the Dunn-Sidák method of Bonferroni correction (Sokal & Rohlf 1995) when multiple statistical tests were conducted simultaneously. Tests for differences among groups for AR, H_O, R, and F_{ST} were performed in FSTAT using 1,000 permutations and two-sided tests.

I characterized population differentiation by calculating pairwise-F_{ST} between leks for the population (49 leks), each sex within the population (males = 57 leks, females = 23 leks), each subpopulation (north of the Milk River subpopulation [NMRS] = 22, south of the Milk River subpopulation [SMRS] = 27), and each sex within each subpopulation (NMRS males = 24, NMRS females = 11, SMRS males = 33, SMRS females = 12; see results for subpopulation

descriptions). For the analyses at the population and subpopulation levels, all birds and leks were retained. For analyses at the lek level and for each sex, I used leks with a minimum sample size of 10 and five, respectively. I regressed F_{ST} against geographic distance to test for IBD and tested for significance using a Mantel test (Mantel 1967) in R-PACKAGE, version 4.0 (Casgrain & Legendre 2001). I estimated contemporary dispersal using assignment tests in STRUCTURE, which places individuals into their most likely unit (lek or subpopulation) based on the method from Bergl & Vigilant (2007).

2.6. Lek structure

I computed mean coefficients of relatedness (*R*) for males and females within leks using RELATEDNESS and compared sample means to a null expectation of zero using a t-test to determine whether males and females attending the same lek were more related than expected by chance (Gibson et al. 2005). Population allele frequencies did not differ significantly between years, sexes, or leks, excluding lek 1/9 (K. L. Bush, unpubl. data) therefore, I used the NMP frequencies for all analyses. Relatedness among males and females within leks was estimated and standard errors were calculated using the jackknife re-sampling procedure in RELATEDNESS. Within-lek *R* was calculated for each sex in each lek along with jackknifed standard errors. To calibrate my estimates of relatedness, I calculated estimates of relatedness within families, specifically known mother-offspring, full-siblings, and half-siblings, in RELATEDNESS and compared the means to a null expectation of 0.5 (mother-offspring and full-siblings) and 0.25 (half-siblings) using a one sample *t*-test.

2.7. Range periphery & fragmentation

To determine whether part (or all) of the NMP fit the assumptions of a peripheral population, I regressed density (males/km^2; range of 0.05 – 0.40; based on Fig. 13.1 in Connelly et al. [2004]), distance to the nearest active neighbor lek, and lek counts (number of males counted on a given lek in a given morning each spring) against geographic distance to the range edge. To investigate whether

genetic diversity was significantly lower in (a) low density and (b) peripheral regions, I calculated AR, H_O, R, and F_{ST} and tested for differences among groups (low density [0.05 males/km^2] vs. high density [> 0.15 males/km^2] and periphery vs. core) in FSTAT using 1,000 permutations and two-sided tests. Low and high-density regions were categorized using Connelly et al. (2004). Leks situated on the northern range periphery were identified by measuring the geographic distance of each lek to the closest point on the current northern range edge (white line in Fig. 2-2). All leks within 50 km of the range edge were considered peripheral and the rest were classified as core. This is an arbitrary distance, but was chosen because leks were either < 50 km or > 100 km from the range edge and it provided a natural break for classification purposes. I did not use the species' historic range edge because it was based on several unsubstantiated observations and erroneous locations for historic specimens resulting in an inflated range (chapter 5). To determine whether proximity to range periphery impacted genetic diversity, I regressed all four measures against geographic distance to the range edge and tested for significance using a Mantel test in R-PACKAGE. This test was also performed independently for both sexes.

I tested whether habitat features (i.e. Milk and Missouri Rivers and a 100 km strip of agriculture in Saskatchewan; Fig. 2-2) acted as dispersal barriers to sage-grouse using partial Mantel tests in R-PACKAGE. Partial Mantel tests were performed using lek-to-lek F_{ST} (matrix A), lek-to-lek geographic distance (matrix B), and a barrier matrix (0 = leks on the same side of the barrier, 1 = leks on the opposite side of the barrier; matrix C) to assess whether potential barriers impeded gene flow. For the population and subpopulation-level analyses, I used the Missouri and Milk Rivers (and surrounding areas of non-habitat) as barriers. Tests were performed with sexes combined and separate to detect differences in sex-specific dispersal. I also regressed the diversity indices (AR, H_O, R, and F_{IS}) on distance to the nearest active lek to examine the effects of isolation. Fragmentation levels and type differed greatly between the north and south halves of the NMP. Primary causes of fragmentation north of the Milk River included oil and gas development (Alberta; Lungle & Pruss 2008) and agriculture

(Saskatchewan [Lungle & Pruss 2008]. Habitat was much less fragmented south of the Milk River (J. Carlson, pers. comm.), but I could not quantify or test for differences between regions because high resolution mapping of land cover types was not available for the entire study area.

3. Results

3.1. Identification of unique individuals

A total of 3,810 of 3,824 (99.6%) samples contained enough DNA to amplify seven or more loci in triplicate. Of the 3,824 samples, 2,519 (65.4%) were unique. Because most shed feathers were replicates of another sample (range of replicates = 1 - 43), most individual samples with one or more loci that failed to amplify could be fully characterized because a duplicate sample filled in the missing gap(s). PI and PI for siblings were set to 0.001 and achieved at four and seven loci respectively. Of the 2,519 samples, 1,075 were from the NMRS, 1062 from the SMRS, and 382 from the PRB; 969 (286 NMRS, 380 SMRS, 303 PRB) were female and 1,550 (789 NMRS, 682 SMRS, 79 PRB) male.

3.2. Population structure

At the population level, the most likely K produced by STRUCTURE was two (ΔK = 174.1 for K = 2 vs. the next highest ΔK = 96.1 for K = 3), with the PRB separate from the NMP. The Pr(X/K) method selected K= 3 as the most likely (-lnP(D) = 83,052.6) compared to K = 2 (-lnP(D) = 83,797.3), but two of the three suggested populations contained birds from all parts of both populations so K = 2 was retained. No further data are presented from the PRB as it was only included to define the NMP boundary. Within the NMP, the most likely number of subpopulations was two (ΔK = 142.8 for K = 2 vs. the next highest ΔK = 7.2 for K = 5; -lnP(D) for K of 2 = 14,098.9). Using the Pr(X/K) method, the most likely number of subpopulations was seven (-lnP(D) = 13,660.5), but all seven groups contained birds from all parts of the NMP, therefore I recognized two subpopulations. When leks were plotted for the percentage of birds assigning to subpopulation one, subpopulation two, or assigning to both subpopulations, the

most likely subpopulation boundary was the Milk River. The two subpopulations identified were north (NMRS) and south (SMRS) of the Milk River. I identified no lek clusters and only one genetically unique lek, 1/9 in Alberta.

Almost all genetic variation (96.8%, $P < 0.001$) detected within the NMP was within leks and due to inter-individual differences (Table 2-1a). Very little genetic variation was found among subpopulations (0.5%, $P < 0.001$) or among leks within subpopulations (2.7%, $P < 0.001$; Table 2-1a). Independent analysis of subpopulations confirmed that the majority of variation was found within individual leks (NMRS, 96.9%, $P < 0.001$; Table 2-1b; SMRS, 97.4%, $P < 0.001$; Table 2-1c).

3.3. Genetic diversity and differentiation

I examined departures from Hardy-Weinberg and linkage equilibrium within the NMP and its subpopulations. There was disequilibrium after loci were corrected for multiple comparisons at both the population and subpopulation levels. However, at the lek level, all loci were in equilibrium. When linkage disequilibrium was examined at the population level, 22 of 78 comparisons were in disequilibrium. At the subpopulation level, seven (SMRS) and 43 (NMRS) comparisons were in disequilibrium, but at the lek level, no two pairs of loci were in disequilibrium. Because the lek was the major level of population structure and there was no disequilibrium at this level, I did not exclude any loci.

All loci were polymorphic (6 - 31 alleles per locus) with high A, AR, H_O, and H_E for the NMP and both subpopulations (Table 2-2). There was no statistical difference in any genetic diversity or relatedness measure between subpopulations (AR [$P = 0.83$], F_{ST} [$P = 1.00$], R [$P = 1.00$], H_O [$P = 1.00$]).

I observed significant isolation-by-distance relationships between leks for the NMP (Mantel r = 0.21, $P = 0.001$, Fig. 2-4a), northern Montana females (Mantel r = 0.27, $P = 0.001$, Fig. 2-4b), northern Montana males (Mantel r = 0.18, $P = 0.001$, Fig. 2-4c), the NMRS (Mantel r = 0.17, $P = 0.005$, Fig. 2-4d), NMRS females (Mantel r = 0.30, $P = 0.05$, Fig. 2-4e), NMRS males (Mantel r = 0.17, $P = 0.001$, Fig. 2-4f), the SMRS (Mantel r = 0.37, $P = 0.006$, Fig. 2-4g), and SMRS

males (Mantel r = 0.21, $P = 0.01$, Fig. 2-4i), but not SMRS females (Mantel r = 0.05, $P = 0.28$, Fig. 2-4h).

Genetic differentiation was too low between the NMRS and the SMRS to measure levels of contemporary dispersal between subpopulations accurately (433 NMRS and 197 SMRS first-generation dispersers). I conservatively defined first-generation dispersers as individuals assigning > 80% to the other subpopulation, lek cluster, or lek. Leks were not sufficiently differentiated to measure lek-to-lek dispersal, with the exception of lek 1/9 in Alberta, which was genetically divergent. I identified 61 first-generation dispersers of both sexes (44 males, 17 females) that moved 3 - 316 km to 14 leks across the NMP (Table 2-3; Fig. 2-2). No lek 1/9 resident assigned to other leks. Maximum (lek 1/9) and minimum (lek 22) dispersal distances are presented because most Alberta leks contained multiple birds assigning to lek 1/9 making it possible that dispersers were produced on these other leks (Table 2-3). Lek 22 was chosen for the minimum distance because no location outside Alberta produced a cluster of birds with the unique 1/9 genetic signature, lek 22 was the southern-most Alberta lek, and closest to the remainder of the population.

3.4. Lek structure

Mean estimates of R did not differ statistically from the expected value of 0.5 for mother-offspring (mean ± SD = 0.49 ± 0.07, $P = 0.22$) and full-siblings (0.53 ± 0.09, $P = 0.14$) and 0.25 for half-siblings (0.27 ± 0.04, $P = 0.75$). Both average male (mean ± SE = 0.01 ± 0.09, $t = 1.2$, df = 56, $P = 0.24$) and female (mean ± SE = 0.001 ± 0.06, $t = -0.5$, df = 24, $P = 0.65$) R did not differ from zero for all leks combined. When individual leks were examined independently for each sex, only R from males on three of 54 leks; leks 1/9 (Alberta; $R = 0.57 \pm 0.13$, $P = < 0.001$), 35 (Alberta; $R = -0.03 \pm 0.02$, $P = 0.003$) and BL27-19-25 (Montana; $R = -0.23 \pm 0.08$, $P = 0.04$) was significantly different from zero. Female R differed significantly from zero for six of 23 leks; leks 10/11 (Alberta; $R = 0.24 \pm 0.01$, $P = 0.001$), 30 (Alberta; $R = 0.17 \pm 0.06$, $P < 0.001$), Mundell Creek (MC; Saskatchewan; $R = -0.09 \pm 0.06$, $P = 0.01$), Dixon Y (DY;

Saskatchewan; $R = -0.10 \pm 0.07$, $P = 0.04$), PH-19 (Montana; $R = -0.08 \pm 0.05$, $P = 0.03$), and PH-33 (Montana; $R = -0.04 \pm 0.08$, $P < 0.001$).

3.5 Range periphery & fragmentation

Regressions of density ($R^2 = 0.61$, $t = 8.62$, $P < 0.001$) and lek counts ($R^2 = 0.51$, $t = 6.96$, $P < 0.001$) against geographic distance to the range edge increased significantly with increasing distance from the edge, while distance to nearest neighbor lek ($R^2 = 0.13$, $t = -2.70$, $P = 0.009$) decreased, therefore northern leks fit non-genetic assumptions of peripherality. High and low density leks did not differ in *AR* ($P = 0.83$), H_O ($P = 1.00$), F_{IS} ($P = 1.00$), or *R* ($P = 1.00$), nor did peripheral leks ($P = 0.92$, 1.00, 1.00, and 0.89, respectively). For my population, which is situated at the species' northern range edge, none of the four measures were related to geographic distance from the northern range edge for all birds combined, females only, or males only (Table 2-4; Fig. 2-5).

Both the Missouri (partial Mantel r = 0.19, $P = 0.001$) and Milk (partial Mantel r = 0.22, $P = 0.001$) Rivers (and associated non-habitat) and the 100 km strip of agriculture in Saskatchewan (partial Mantel r = 0.19, $P = 0.001$) were significant barriers to dispersal. When the sexes were examined independently, the Milk River and surrounding disturbance was a barrier to both males (partial Mantel r = 0.18, $P = 0.001$) and females (partial Mantel r = 0.20, $P = 0.03$), as was the Saskatchewan cropland (male partial Mantel r = 0.15, $P = 0.001$; female partial Mantel r = 0.27, $P = 0.001$). Distance to the nearest active lek did not explain variation in any of the diversity indices for either both sexes combined (*P*-values = 0.83, 0.45, 0.07, 0.66) or males alone (*P*-values = 0.95, 0.31, 0.89, 0.42), but allelic richness vs. distance to the nearest active lek was significant for females (*P*-values = 0.0002, 0.06, 0.85, 0.40).

4. Discussion

I found that all sage-grouse in the NMP formed a single population despite fragmentation and proximity to the range periphery. There was substructure within the NMP north and south of the Milk River, but genetic diversity was high

and equivalent in both subpopulations. Male sage-grouse did not form kin groups and appeared to disperse greater distances that females. No diversity value was impacted by distance to the range edge, but rivers with associated anthropogenic disturbance and cropland represented significant barriers to dispersal.

4.1. Population structure

I identified two genetically distinct sage-grouse populations, the NMP and the PRB, and two subpopulations within the NMP (NMRS and SMRS). Connelly et al. (2004) predicted both populations and the SMRS using habitat breaks (rivers and areas containing unsuitable habitat; Fig. 2-1), which suggests gene flow in sage-grouse is impeded by these geographic features. However, the NMRS was not genetically subdivided by agriculture in Saskatchewan. This is likely because birds circumvent the agricultural disturbance by traveling east-west via corridors of suitable habitat south of the patch of agriculture (Fig. 2-2). I also found that while the NMRS and the SMRS are separate subpopulations, there is little genetic difference between them.

Potential subpopulation barriers included the Milk River itself, extensive agricultural conversion in the Milk River valley, and change in dominant sagebrush species on either side of the river. No radio-marked birds flew across the Missouri River during a three-year study (B. Moynahan, pers. comm.), but the Missouri River is substantially larger than the Milk River. The Milk River itself likely does not pose a barrier because it is narrow, it lacks rugged or steep banks in Montana, and sage-grouse commonly fly over non-suitable habitat (crops, roads, etc.). The change in sagebrush species constitutes another potential barrier because sagebrush is the primary habitat and food source for sage-grouse. Silver sagebrush (*Artemisia cana*) is the only woody sagebrush species present north of the Milk River (Aldridge and Brigham 2003), whereas both silver and big (*A. tridentata*) sagebrush are present south of the river where big sagebrush is the primary food (Sauls 2006). While birds on both sides of the river assign to opposite subpopulations, lek 1/9 birds disperse across the river, and some silver sagebrush birds winter in big sagebrush south of the Milk River (J. Tack, pers.

comm.) suggesting that sagebrush species is not a barrier. Agricultural conversion along the Milk River over the past 30 - 100 years is likely the largest barrier because most sagebrush within the valley has been destroyed and historic leks are inactive (Fig. 2-1). However, there are a few locations where sagebrush still extends up to the river's edge, which may allow for dispersal between subpopulations.

All leks were genetically undifferentiated from one another, except for one highly differentiated lek (lek 1/9) near the range edge in Alberta. Lek 1/9 was genetically unique and behaviourally unusual. This lek was extirpated and refounded 25 years later (Alberta Fish and Wildlife; unpubl. data) by a single banded male whose offspring produced the males sampled on the lek (K.L. Bush; unpubl. data). The lek is also unusual because it changes location throughout the year and even during a single day, which is rare in lekking species and typically occurs because of temporary (e.g. flooding) or permanent (e.g. tilling) habitat alterations. However, the lek site and surrounding pasture remains relatively undisturbed (Alberta Fish and Wildlife; unpubl. data). This, coupled with the discovery of another lek that changes location (Alberta Fish and Wildlife; unpubl. data), suggests that birds have adopted this behaviour for another reason. Males most likely change the location of the lek in response to the current location of females, as suggested by Gibson (1996) for migratory sage-grouse. If females forage in the vicinity, but do not attend the lek, it would be beneficial for males to shift where they display to where females currently are to increase their mating opportunities. Lek 1/9 was also unusual because within-lek relatedness was higher than for any other lek in the NMP. This result, coupled with the unique genetic signature, suggests that primarily related birds mate on this lek and some form of behavioural isolation prevents unrelated birds from recruiting. However, dispersal to other leks is high (Table 2-3) indicating that the isolation is one-way. It is possible that lek 1/9 birds are immigrants from other leks containing this unique genetic signature, but this seems unlikely because lek 1/9 is the only lek solely composed of these distinctive birds (Table 2-3) and the probability of birds from a unique genetic lineage dispersing from multiple leks to a single location is low.

4.2. Genetic diversity and differentiation

Most genetic studies on grouse have focused on highly fragmented and isolated populations experiencing extreme population declines (Segelbacher & Storch 2002; Caizergues et al. 2003a; Johnson et al. 2003; Van Den Bussche et al. 2003; Bouzat & Johnson 2004). These studies have found low genetic diversity with extensive population structure and differentiation. Sage-grouse in Canada have undergone dramatic declines, but the NMP exhibited high diversity, little population structure, and low levels of differentiation. The NMP and both subpopulations had H_E in the range of core sage-grouse populations and higher H_E and H_O than fragmented and/or isolated greater and Gunnison sage-grouse populations (Table 2-5). At shared loci, sage-grouse in the NMP had similar levels of H_O to fragmented populations of black grouse and lower levels than contiguous populations (Table 2-5). Overall, H_O was higher than peripheral populations of lesser prairie-chicken and fragmented populations of greater prairie chicken, and in the range of values for both fragmented and contiguous populations of European grouse (Table 2-5). Similar to the range-wide analysis on sage-grouse, the NMP exhibited IBD, but lek-to-lek F_{ST} values were considerably lower than for Gunnison sage-grouse (NMP average = 0.05 [range 0 – 0.40]; Gunnison average = 0.26; Oyler-McCance et al. 2005b). My average F_{ST} of 0.05 was consistent with regional values for capercaillie (0.05 [range 0 – 0.15]; Segelbacher & Storch 2002) and lesser prairie-chicken (0.008 – 0.1; Johnson et al. 2003). Sage-grouse inhabit both naturally and anthropogenically fragmented landscapes, but my results suggest that fragmentation either does not greatly impede the ability of northern Montana sage-grouse to disperse or fragmentation has occurred too recently to have had a measurable effect.

Despite various forms of fragmentation, most leks in the NMP were connected by contiguous habitat to at least one other lek, which facilitated gene flow and prevented isolation of any given region. However, the NMP, NMRS, and SMRS exhibited significant IBD (Fig. 2-4) suggesting that distance limits gene flow. Similarly, both males and females in the NMP and the NMRS and males in the SMRS displayed IBD. Females within the SMRS were the only class

not exhibiting IBD, but because I sampled approximately one-third fewer leks in the SMRS containing females, I may have failed to detect an existing pattern. Evidence for both sexes dispersing is contrary to the "dogma" that sage-grouse females disperse and males are philopatric (Dunn and Braun 1985). While males displayed a stronger IBD pattern for both subpopulations (Fig. 2-4), they also dispersed farther than females. All putative lek 1/9 dispersers sampled on leks > 100 km from their natal lek were male (Table 2-3). Females from lek 1/9 dispersed only 8 - 19 km and constituted 28% of dispersers. While it is difficult to generalize trends in dispersal from one lek, this pattern was also apparent in dispersal between subpopulations. Sage-grouse males may be more prone to long-distance dispersal because they are larger than females and may have greater energy reserves to enable extended searches for suitable habitat. Traditional radio-transmitter studies have documented individual sage-grouse dispersal of only 5 - 20 km (e.g. Beck et al. 2006), but have not captured maximum dispersal distances due to infrequency of long distance dispersal events, lack of monitoring juvenile birds, and logistical difficulty of tracking individuals over long distances. These studies have not accurately documented sex-biases because most sage-grouse telemetry studies have been conducted on females.

While dispersal is important for bolstering lek size, gene flow is ultimately the most important factor because its presence reveals successful reproduction by dispersers. IBD suggests that gene flow is constrained by geographic distance, but the presence of possible second-generation dispersers from lek 1/9 (assignment probabilities of 0.60 – 0.79; Fig. 2-3) across the NMP suggests that some dispersers contribute to the gene pool and the low levels of differentiation across the NMP indicate that this has always been the case. However, the success of dispersers is unknown because I do not know the proportion of birds that successfully reproduce on each lek across the NMP or if dispersers move between leks until they find success. These questions need to be addressed to understand how dispersal and gene flow are correlated in sage-grouse. Gene flow is also expected to be more sensitive to fragmentation because few dispersers are expected to reproduce and fragmentation likely reduces dispersal resulting in very

few dispersers passing on their genes. Determining dispersal ability and levels of gene flow are important directives for devising management strategies for sage-grouse because if disturbance exceeds movement capabilities, regions can become permanently isolated.

4.3. Lek structure

Sage-grouse leks were congregations of primarily unrelated males and females exhibiting little kin association. This is contrary to expectations if male kin selection is responsible for the formation and maintenance of leks (Kokko and Lindström 1996; Sherman 1999). Only one lek in the NMP contained males that were significantly more related to each other than random (a mean expectation of zero) and that was the unusual lek 1/9. My finding of low within-lek male relatedness is consistent with patterns observed in sage-grouse in California (Gibson et al. 2005) suggesting that the species does not exhibit kin association on leks. However, ruling this possibility out still leaves many alternative explanations for lek formation in sage-grouse and other grouse species ranging from decreased predation risk (Boyko et al. 2004), increased mating opportunity (Höglund and Alatalo 1995), and queuing for future breeding status (Wiley 1973).

4.4. Range periphery & fragmentation

The birds I studied at the northern edge of the sage-grouse range fit the non-genetic assumptions of peripherality. However, the northern edge was no more structured than areas farther from the periphery, was not genetically depauperate compared to the core, and diversity indices did not vary with distance from the periphery. Although my results are consistent with findings for peripheral populations of capercaillie (Seglbacher & Storch 2002), they do not fit the expectations that peripheral regions are more isolated, more differentiated, and have lower diversity than areas closer to the range centre. Based on lek 1/9, it appears that peripheral birds disperse towards the core suggesting that the northern periphery may not be a typical“sink”. This coupled with IBD and the presence of dispersers across the population, indicate that birds from across the

NMP successfully disperse. I did not detect any association between periphery and diversity and only rivers and their associated anthropogenic disturbance, and a century old patch of agriculture were identified as somewhat permeable barriers to dispersal. Sage-grouse may violate the genetic assumptions of peripherality for historical reasons. Sage-grouse likely underwent a range expansion and subsequent contraction in the recent past (Fig. 2-1; Oyler-McCance et al. 2005a) resulting in a system that has not reached equilibrium between mutation, migration, and drift. However, if range contraction and expansion occur in a species, they occur at the periphery, making all peripheral habitat regions inherently unstable and panmictic.

If either periphery or fragmentation were currently impacting sage-grouse, the NMRS should exhibit more differentiation than I am currently detecting between leks, lower diversity, and evidence for genetic isolation of leks or regions. Sage-grouse may not be sensitive to the separate or combined effects of peripherality and fragmentation for several reasons. First, sage-grouse are capable of long distance dispersal, which homogenizes genetic diversity regardless of location in relation to the range periphery. Theoretically, only one effective migrant per generation is required to prevent population differentiation (Wright 1964), therefore, rare long-distance dispersers and moderate numbers of shorter distance dispersers may maintain high diversity in peripheral and fragmented regions. The presence of significant IBD and gene flow suggests that leks form a stepping stone network across the landscape that allows dispersal through contiguous habitat strips of habitat even in the presence of substantial fragmentation. Second, disturbance is mainly occurring in the NMRS, but silver sagebrush habitat is naturally patchy (Aldridge & Brigham 2003). Peacock & Ray (2001) and Aars et al. (2006) found mammal populations inhabiting patchy habitat retain higher levels of genetic variability compared to high-density continuous populations due to efficient and frequent long-distance dispersal. Both sage-grouse subpopulations exhibit IBD and equivalent diversity, but I have evidence of long-distance dispersal within the NMP (Table 2-3). Therefore, patchy habitats may result in an increased propensity for some individuals to

move farther in search of new or better quality habitat. This, coupled with high dispersal between subpopulations, has led to high diversity and connectivity across the NMP, including areas at the very periphery of the species' range. Finally, despite adaptations to natural disturbance by sage-grouse at the northern periphery, anthropogenic disturbance may have occurred too recently to affect genetic diversity due to the species' high dispersal capabilities. The single area of disturbance that I could evaluate (patch of agriculture in Saskatchewan) exhibited a significant genetic impact, but it is also the oldest conversion of land affecting the population (> 100 years; Lungle & Pruss 2008). Most other disturbances occurred in the last 30 years (J. Carlson, pers. comm.; Lungle & Pruss 2008), so there may have not been time for detectable genetic change.

5. Conservation Implications

My findings reject the idea that sage-grouse inhabiting fringe and fragmented habitats in Alberta, Saskatchewan, and Montana are genetically impoverished or isolated. Both subpopulations have comparable levels of genetic diversity and dispersal between them, suggesting gene flow maintains genetic diversity. Nevertheless, both subpopulations are differentiated from each other despite gene flow across the Milk River. Sage-grouse in the NMRS have undergone massive demographic declines in the last half century, possibly from increasing fragmentation and destruction of sagebrush along the Milk River. With increasing habitat alteration, fewer dispersers from the SMRS likely disperse across the river, leading to fewer birds supplementing the less productive (Aldridge & Boyce 2007) and declining NMRS. While there are still enough birds dispersing to maintain genetic diversity, increased fragmentation will likely only exacerbate demographic declines. Management efforts need to focus on maintaining current sage-grouse habitat to allow for dispersal and gene flow. In areas that continue to suffer declines, population augmentation from high-density areas in the SMRS may be necessary to maintain a viable breeding population and genetic diversity. Studies need to be conducted to identify juvenile dispersal corridors to better understand when and where birds are dispersing and what

forms of fragmentation they can cross. Finally, sage-grouse conservation has to switch from a lek-based to population model because without habitat connecting segments of the population, leks will likely become isolated and suffer population declines and genetic problems (i.e. inbreeding, lek differentiation, etc.; Keyghobadi 2007), making management and/or population rescue a much more daunting task.

6. Literature Cited

Aars J, Dallas JF, Piertney SB, et al. (2006) Widespread gene flow and high genetic variability in populations of water voles *Arvicola terrestris* in patchy habitats. Mol Ecol 15: 1455-1466.

Alberta Sage Grouse Recovery Action Group (ASGRAG) (2005) Alberta Greater Sage-Grouse Recovery Plan. Alberta Sustainable Resource Development, Fish and Wildlife Division, Alberta Species at Risk Recovery Plan No. 8. Unpublished Report. Edmonton, Alberta.

Aldridge CL, Boyce MS (2007) Linking occurrence and fitness to persistence: habitat-based approach for endangered greater sage-grouse. Ecol App. 117: 508-526.

Aldridge CL, Brigham RM (2003) Distribution, abundance, and status of the greater sage-grouse, *Centrocercus urophasianus*, in Canada. Can Field-Nat 117: 25-34.

Beck JL, Reese KP, Connelly JW, Lucia MB (2006) Movements and survival of juvenile greater sage-grouse in southeastern Idaho. Wildlife Soc B 34: 1070-1078.

Bergl RA, Vigilant L (2007) Genetic analysis reveals population structure and recent migration within the highly fragmented range of the Cross River gorilla (*Gorilla gorilla diehli*). Mol Ecol 16: 501-516.

Bouzat JL, Johnson K (2004) Genetic structure among closely spaced leks in a peripheral population of lesser prairie-chickens. Mol Ecol 13: 499-505.

Bouzat JL, Cheng HH, Lewin HA, *et al*. (1998) Genetic evaluation of a demographic bottleneck in the greater prairie chicken. Conserv Biol 12: 836-843.

Boyko AR, Gibson RM, Lucas JR (2004) How predation risk affects the temporal dynamics of avian leks: greater sage grouse versus golden eagles. Am Nat 163: 154-165.

Bush KL (2008) A pressure-operated drop net for capturing Greater Sage-Grouse. J Field Ornithol 79: 64-70.

Bush KL, Vinsky MD, Aldridge CL, Paszkowski CA (2005) A comparison of sample types varying in invasiveness for use in DNA sex determination in an endangered population of greater sage-grouse (*Centrocercus urophasianus*). Conserv Genet 6: 867-870.

Caizergues A, Rätti O, Helle P, et al. (2003a) Population genetic structure of male black grouse (*Tetrao tetrix* L.) in fragmented vs. continuous landscapes. Mol Ecol 12: 2297-2305.

Caizergues A, Bernard-Laurent A, Brenot JF, *et al*. (2003b) Population genetic structure of rock ptarmigan *Lagopus mutus* in Northern and Western Europe. Mol Ecol 12: 2267-2274

Caizergues A, Dubois S, Mondor G, Rasplus J-Y (2001) Isolation and characterisation of microsatellite loci in black grouse (*Tetrao tetrix*). Mol Ecol Notes 1: 36-38.

Casgrain P, Legendre P (2001) The R Package for Multivariate and Spatial Analysis, Version 4.0 User's Manual. http://www.fas.umontreal.ca/BIOL/legendre. Department des Sciences biologiques, Université de Montreal.

Cheng HH, Levin I, Vallejo RL, *et al*. (1995) Development of a genetic map of the chicken with high-utility markers. Poultry Sci 74: 1855-1874.

Connelly JW, Knick ST, Schroeder MA, Stiver SJ (2004) Conservation Assessment of Greater Sage-grouse and Sagebrush Habitats. Western Association of Fish and Wildlife Agencies. Unpublished Report. Cheyenne, Wyoming.

Dunn PO, Braun CE (1985) Natal dispersal and lek fidelity of sage-grouse. Auk 102: 621-627.

Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. Mol Ecol 17: 1170-1188.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14: 2611-2620.

Excoffier L, Laval G, Schneider S (2006) ARLEQUIN ver 3.1: An integrated software package for population genetic analysis. Computational and Molecular Population Genetics Lab, University of Berne, Switzerland.

Frankel OH, Soulé ME (1981) Conservation and Evolution. Cambridge University Press, Cambridge, UK.

Galbusera P, Githiru M, Lens L, Matthysen E (2004) Genetic equilibrium despite habitat fragmentation in an Afrotropical bird. Mol Ecol 13: 1409-1421.

Gibson RM, Pires D, Delaney KS, Wayne RK (2005) Microsatellite DNA analysis shows that greater sage grouse leks are not kin groups. Mol Ecol 14: 4453-4459.

Gibson RM (1996) Female choice in sage grouse: the roles of attraction and active comparison. Behav Ecol Sociobiol 39: 55-59.

Giesen KM, Schoenberg TJ, Braun CE (1982) Methods for trapping sage grouse in Colorado. Wildlife Soc B 10: 223-231.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). http://www.unil.ch/izea/softwares/fstat.html. Institute of Ecology, Lausanne

Höglund J, Larsson JK, Jansman HAH, *et al*. (2007) Genetic variability in European black grouse (*Tetrao tetrix*). Conserv Genet 8: 239-243.

Höglund J, Alatalo RV (1995) Leks. Princeton University Press, Princeton, New Jersey.

Johnson JA, Toepfer JE, Dunn PO (2003) Contrasting patterns of mitochondrial and microsatellite population structure in fragmented populations of greater prairie chickens. Mol Ecol 12: 3335-3347.

Keyghobadi N (2007) The genetic implications of habitat fragmentation for animals. Can J Zool 85: 1049-1064.

Kirkpatrick M, Ravigne V (2002) Speciation by natural and sexual selection: models and experiments. Am Nat 159: S22-S35.

Kokko H, Lindström J (1996) Kin selection and the evolution of leks: whose success do young males maximize? Proc R Soc London, Sect B 263: 919-923.

Lammi A, Siikamäki P, Mustajärvi K (1999) Genetic diversity, population size, and fitness in central and peripheral populations of a rare plant *Lychnis viscaria*. Conserv Biol 13: 1069-1078.

Lebigre C, Alatalo RV, Siitari H, Parri S (2007) Restrictive mating by females on black grouse leks. Mol Ecol 16: 4380-4389.

Lesica P, Allendorf FW (1995) When are peripheral populations valuable for conservation? Conserv Biol 9: 753-760.

Lungle K, Pruss S (2008) Recovery strategy for greater sage-grouse (*Centrocercus urophasianus urophasianus*) in Canada. Species at Risk Act recovery strategy series. Parks Canada Agency. Unpublished report. Ottawa, Ontario.

Mantel N (1967) The detection of disease clustering and generalized regression approach. Cancer Res 27: 209-220.

Martínez-Cruz B, Godoy JA, Negro JJ (2007) Population fragmentation leads to spatial and temporal genetic structure in the endangered Spanish imperial eagle. Mol Ecol 16: 477-486.

Oyler-McCance SJ, Taylor SE, Quinn TW (2005a) A multilocus population genetic survey of the greater sage-grouse across their range. Mol Ecol 14: 1293-1310.

Oyler-McCance SJ, St. John J, Taylor SE, *et al*. (2005b) Population genetics of Gunnison sage-grouse: implications for management. J Wildl Manage 69: 630-637.

Peacock MM, Ray C (2001) Dispersal in pikas (*Ochotona princes*): combining genetic and demographic approaches to reveal spatial and temporal patterns. In: Clobert J, Danchin E, Dhondt A, Nichols JD (eds) Dispersal, Oxford University Press, Oxford, pp. 44-56

Peakall R, Smouse PE (2001) GENALEX version 5.1. Genetic analysis in Excel. Population genetic software for teaching and research. http://www.anu.edu.au/BoZo/GenAlEx. Australian National University, Canberra, Australia.

Piertney SB, Höglund J (2001) Polymorphic microsatellite DNA markers in black grouse (*Tetrao tetrix*). Mol Ecol Notes 1: 303-304

Piertney SB, Dallas JF (1997) Isolation and characterization of hypervariable microsatellites in the red grouse. *Lagopus lagopus scoticus*. Mol Ecol 6: 93-95

Pritchard JK, Stephens M, Donelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945-959

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43: 258-275

Raymond M, Rousset F (1995) Genepop version 3.1d: population genetics software for exact test and ecumenism. J Hered 86: 248-249

Sargarin RD, Gaines SD (2002) The 'abundant centre' distribution: to what extent is it a biogeographical rule? Ecol Lett 5: 137-147

Sauls, H (2006) The role of selective foraging and cecal microflora in sage-grouse nutritional ecology. MSc. Thesis, University of Montana

Schroeder MA, Aldridge CL, Apa AD, *et al*. (2004) Distribution of sage-grouse in North America. Condor 106: 363-376

Schroeder MA, Braun CE (1991) Walk-in traps for capturing greater prairie-chickens on leks. J Field Ornithol 62: 378-385

Segelbacher G, Höglund J, Storch I (2003) From connectivity to isolation: genetic consequences of population fragmentation in capercaillie across Europe. Mol Ecol 12: 1773-1780

Segelbacher G, Storch I (2002) Capercaillie in the Alps: genetic evidence of metapopulation structure and population decline. Mol Ecol 11: 1669-1677

Segelbacher G, Paxton RJ, Steinbrueck G, Trontelj P, Storch I (2000) Characterisation of microsatellites in capercaillie (*Tetrao urogallus*) (AVES). Mol Ecol 9: 1934-1935

Semple K, Wayne RK, Gibson RM (2001) Microsatellite analysis of female mating behaviour in lek-breeding sage grouse. Mol Ecol 10: 2043-2048

Sherman PW (1999) Birds of a feather lek together. Nature 401: 119-120.

Sokal RR, Rohlf FJ (1995) Biometry, the principles and practice of statistics in biological research, 3rd edition. W.H. Freeman and Company, New York

Taylor SE, Oyler-McCance SJ, Quinn TW (2003) Isolation and characterization of microsatellite loci in greater sage-grouse (*Centrocercus urophasianus*). Mol Ecol Notes 3: 262-264

Van Den Bussche RA, Hoofer SR, Wiedenfeld DA *et al*. (2003) Genetic variation within and among fragmented populations of lesser prairie-chickens (*Tympanuchus pallidicinctus*). Mol Ecol 12: 675-683

Veit ML, Robertson RJ, Hamel PB, Friesen VL (2005) Population genetic structure and dispersal across a fragmented landscape in cerulean warblers. Conserv Genet 6: 159-174

Vucetich JA, Waite TA (2003) Spatial patterns of demography and genetic processes across the species' range: Null hypotheses for landscape conservation genetics. Conserv Genet 4: 639-645.

Wang J (2004) Sibship reconstruction for genetic data with typing errors. Genetics 166: 1963-1979

Wiley RH (1973) Territoriality and non-random mating in the sage grouse, *Centrocercus urophasianus*. Anim Behav Mono 6: 87-169.

Wright S (1964) Stochastic processes in evolution. In: Garland J (ed) Stochastic Models in Medicine and Biology, University of Wisconsin Press, Madison, pp. 199-241.

Table 2-1. AMOVA comparing genetic variation in microsatellite data for the (a) the northern Montana population, (b) north of the Milk River subpopulation, and (c) south of the Milk River subpopulation

Source of variation	d.f.	Sum of Squares	Variance Components	Fixation indices	*P* value	Percentage of variation
(a) Among subpopulations (n = 2114)	1	13.1	0.003	0.03	<0.001	0.5
Among leks within subpopulations	47	95.4	0.02	0.03	<0.001	2.7
Within leks	3641	2505.2	0.7	0.005	0.001	96.8
Total	3689	2613.7	0.7			
(b) Among NMRS leks (n = 1075)	37	66.9	0.02	0.03	<0.001	3.1
Within NMRS leks	2007	1399.5	0.7	0.03	<0.001	96.9
Total	2114	1466.4	0.7			
(c) Among SMRS leks (n = 1039)	46	103.4	0.03	0.03	<0.001	2.6
Within SMRS leks	2031	2141.2	1.05	0.03	<0.001	97.4
Total	2077	2244.6	1.08			

Table 2-2. Estimated genetic variation for the northern Montana population and its subpopulations: NMRS and SMRS.

Population	Subpopulation	n	A	AR	H_O	H_E	R	F_{ST}
Northern Montana	Total Population	2137	14.8	12.36	0.66	0.71	0.016	0.009
	NMRS	1075	12.0	11.89	0.66	0.71	0.015	0.008
	SMRS	1062	12.0	11.78	0.66	0.70	0.008	0.004

Table 2-3. Number of birds assigning to lek 1/9 from the leks with first generation dispersers detected, and the minimum and maximum distance potentially dispersed. Most birds that assign to lek 1/9 are located in Alberta (57 of 67) and most leks within Alberta contain at least one lek 1/9 disperser, therefore it is possible that dispersers originated from any Alberta lek listed below. Distances are listed from two locations to give an estimate of maximum and minimum dispersal distances into Montana. These leks are lek 1/9 (the most likely source of dispersers) or lek 22 (the southern most lek in Alberta containing birds with the lek 1/9 signature). See figure 2-3.

Lek name	Region (province or portion of Montana county)	Number of first generation dispersers assigning to lek 1/9 and sex	Assignment probability to lek 1/9 cluster*	Distance dispersed (km) measured from lek 1/9	Distance dispersed (km) measured from lek 22 (southern most lek of Alberta cluster)
1/9	Alberta	All birds (6M)	0.93-1.00	N/A	N/A
34	Alberta	4 (4M)	0.81-0.93	5	54
31C	Alberta	14 (9M, 5F)	0.82-0.95	8	61
10/11	Alberta	15 (8M, 7F)	0.81-0.91	10	53
16	Alberta	13 (8M, 5F)	0.81-0.92	19	42
2/24	Alberta	3 (3M)	0.85-0.93	19	49
35	Alberta	1 (1M)	0.86	37	44
22	Alberta	1 (1M)	0.92	59	N/A
BL27-19-25	South Blaine	1 (1M)	0.82	184	126
PH-20	South Phillips	1 (1M)	0.82	253	194
PH-45	South Phillips	2 (2M)	0.81-0.86	279	220
Airport	South Phillips	1 (1M)	0.89	287	228
V20-102	North Valley	1 (1M)	0.86	291	245
V20-086	South Valley	1 (1M)	0.86	306	256
V20-065	North Valley	3 (3M)	0.87-0.90	316	265

* No bird assigning to lek 1/9 assigned to another lek in the NMP at > 7.5% probability

Table 2-4. Summary statistics for regression analyses of allelic richness, observed heterozygosity, the inbreeding coefficient (F_{IS}), and average relatedness against geographic distance from the northern range edge within the northern Montana sage-grouse population for all birds, males, and females. *P* is the probability of obtaining a greater correlation than that observed under the null hypothesis (one tailed).

Diversity Index	All Birds				Males				Females			
	R^2	Slope (SE)	*t*	*P*	R^2	Slope (SE)	*t*	*P*	R^2	Slope (SE)	*t*	*P*
Allelic Richness	0.03	0.0005 (0.0005)	1.15	0.26	0.02	0.0005 (0.0004)	1.18	0.24	0.10	0.0007 (0.0005)	1.57	0.13
H_O	0.03	-0.0002 (0.0002)	-1.27	0.21	0.05	-0.0001 (0.0001)	-1.69	0.10	0.04	0.0009 (0.0001)	-0.93	0.36
F_{IS}	0.06	0.00003 (0.0002)	1.72	0.09	0.05	0.0004 (0.0002)	1.72	0.09	0.14	0.0003 (0.0002)	1.87	0.08
Relatedness	0.05	-0.0003 (0.0002)	-1.50	0.14	0.02	-0.0003 (0.0003)	-1.09	0.28	0.007	0.0007 (0.0002)	-0.37	0.71

Table 2-5. Comparison of genetic diversity values for the same microsatellite loci across different grouse studies. Values from this study are in bold.

Species/study/ population subset	Microsatellites											Average for all microsatellites used in study
	SGCA5	SGCA9-2	LLSD8	ADL230	TUT3	TUT4	TTD6	TTT1	TTT3	BG6	BG15	
H_E												
• Sage-Grouse												
(a) NMP	**0.75**	**0.89**	**0.74**	**0.70**	**0.64**	**0.84**	**0.76**	**0.66**	**0.55**	**0.85**	**0.66**	**0.71**
(a) NMRS	**0.73**	**0.88**	**0.74**	**0.70**	**0.64**	**0.84**	**0.73**	**0.67**	**0.55**	**0.82**	**0.71**	**0.71**
(a) SMRS	**0.77**	**0.89**	**0.73**	**0.71**	**0.64**	**0.82**	**0.79**	**0.65**	**0.56**	**0.86**	**0.60**	**0.70**
(b) Core/ Contiguous	0.66 – 0.88	0.61 – 0.93	0.64 – 0.78	0.70 – 0.83								0.62 – 0.75
(b) Fragmented Periphery	0.07 – 0.87	0.42 – 0.91	0.09 – 0.79	0.58 – 0.80								0.45 – 0.71
(c) Gunnison												0.37 – 0.57
(d) California			0.59									0.64
H_O												
• Sage-Grouse												
(a) NMP	**0.74**	**0.62**	**0.73**	**0.72**	**0.69**	**0.68**	**0.76**	**0.62**	**0.52**	**0.79**	**0.66**	**0.66**
(a) NMRS	**0.70**	**0.60**	**0.74**	**0.70**	**0.71**	**0.68**	**0.72**	**0.62**	**0.51**	**0.80**	**0.72**	**0.66**
(a) SMRS	**0.78**	**0.64**	**0.72**	**0.74**	**0.67**	**0.68**	**0.80**	**0.62**	**0.53**	**0.79**	**0.60**	**0.66**
(c) Gunnison												0.36 – 0.51
(d) California			0.62									0.64
(e) California			0.53 – 0.60		0.64 – 0.67							0.49 – 0.53
• Black Grouse												
(f) Contiguous					0.73	0.79	0.82	0.86	0.74			0.76
(f) Fragmented					0.67	0.72	0.77	0.78	0.68			0.74
(g) Contiguous					0.75 – 0.88	0.70 – 0.81		0.79 – 0.99		0.72 – 0.88	0.65 – 0.78	0.72 – 0.75

(h) Continuous				0.66 – 0.67
(h) Contiguous				0.64 – 0.71
(h) Isolated				0.44 – 0.57
• Capercaillie				
(i) Alps Core				0.56 – 0.78
(i) Alps Edge				0.63 – 0.86
(j) Contiguous				0.56 – 0.72
(j) Fragmented				0.44 – 0.71
• Rock Ptarmigan		0.92	0.80	0.81
(k) Contiguous				
(k) Fragmented		0.49 – 0.94	0.66 – 0.92	0.64 – 0.88
• Lesser Prairie Chicken	0.60 – 0.89			0.53 – 0.55
(l) New Mexico leks				
(m) Oklahoma leks				0.22 – 0.75
(m) New Mexico leks				0.40 – 0.58
• Greater Prairie Chicken	0.75 – 0.89			0.57 – 0.65
(n) Fragmented & Contiguous				
(o) Fragmented & Contiguous				0.59 – 0.75

(a) This study, (b) Oyler-McCance et al. (2005a), (c) Oyler-McCance et al. (2005b), (d) Semple et al. (2001), (e) Gibson et al. (2005), (f) Caizergues et al. (2003a), (g) Lebigre et al. (2007), (h) Höglund et al. (2007), (i) Segelbacher & Storch (2002), (j) Segelbacher et al. (2003), (k) Caizergues et al. (2003b), (l) Bouzat & Johnson (2004), (m) Van Den Bussche et al. (2003), (n) Bouzat et al. (1998), (o) Johnson et al. (2003)

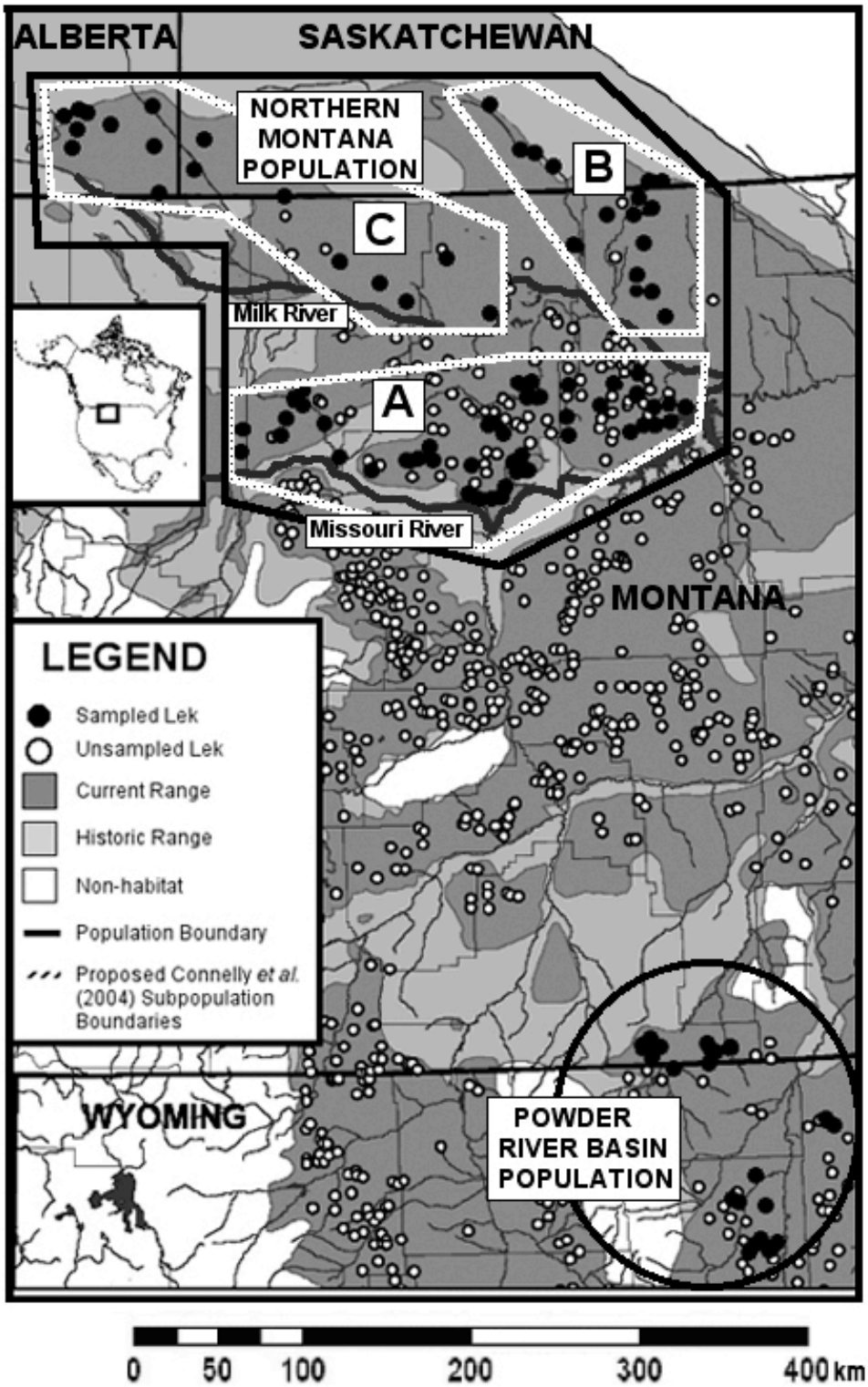

Figure 2-1. Study area map with the northern Montana and Powder River Basin populations highlighted. Dashed lines represent the three NMP subpopulations (A, B, and C) suggested by Connelly *et al*. (2004). Milk and Missouri Rivers are indicated by wide grey lines in the middle and bottom of the northern Montana population, respectively. Map modified from Schroeder *et al*. (2004).

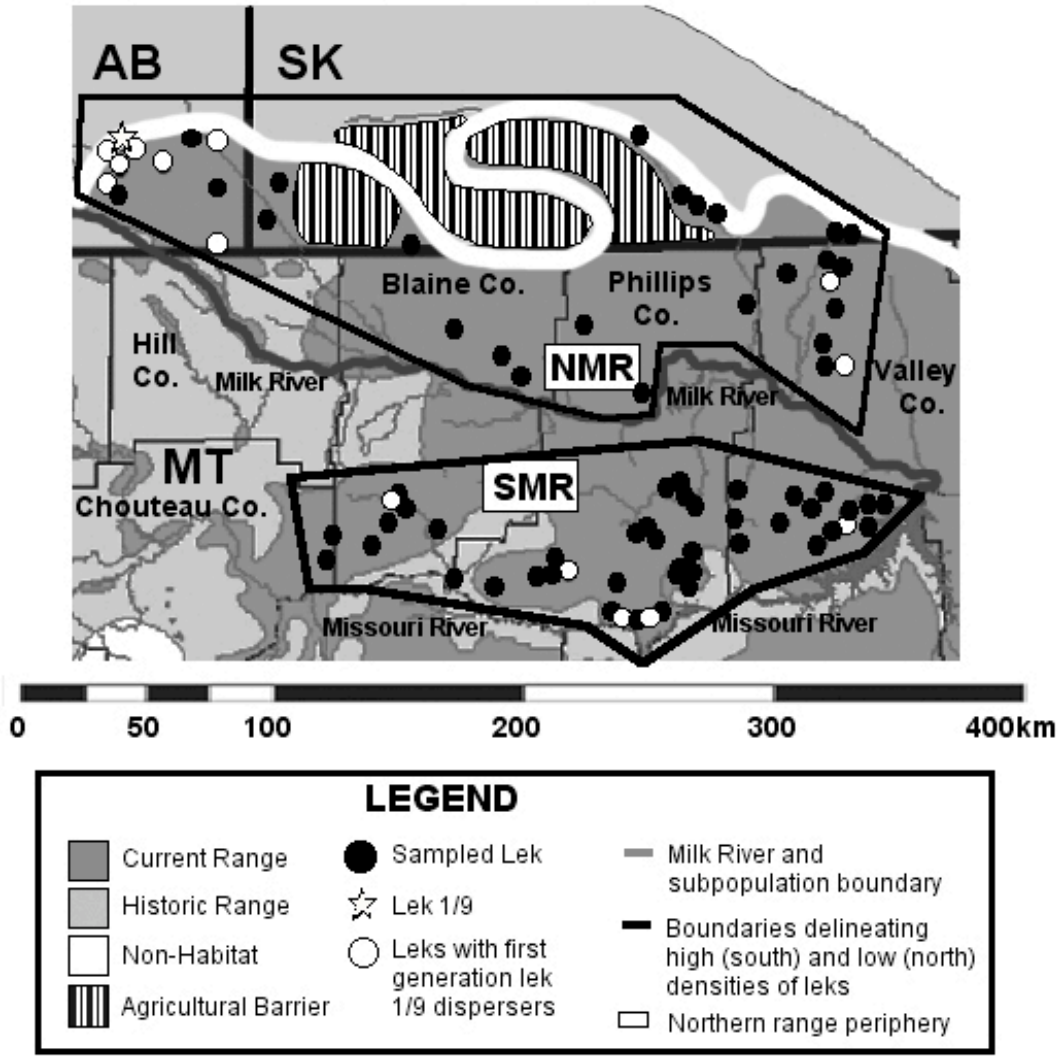

Figure 2-2. Map depicting the two subpopulations identified within the northern Montana population by STRUCTURE and partial Mantel analyses: north of the Milk River (NMRS) and south of the Milk River (SMRS). The white star represents the only genetically unique lek identified by the assignment test within STRUCTURE and open circles depict leks with birds assigning to lek 1/9 with greater than 80%. Dark lines represent boundaries delineating high (south of the Milk River) and low (north of the Milk River) densities of sage-grouse and leks. The white line represents the northern range periphery. Map modified from Schroeder *et al*. (2004). To see enlarged maps with all of the sampled leks labeled, go to: http://www.aviangenetics.com/northern_montana_maps/

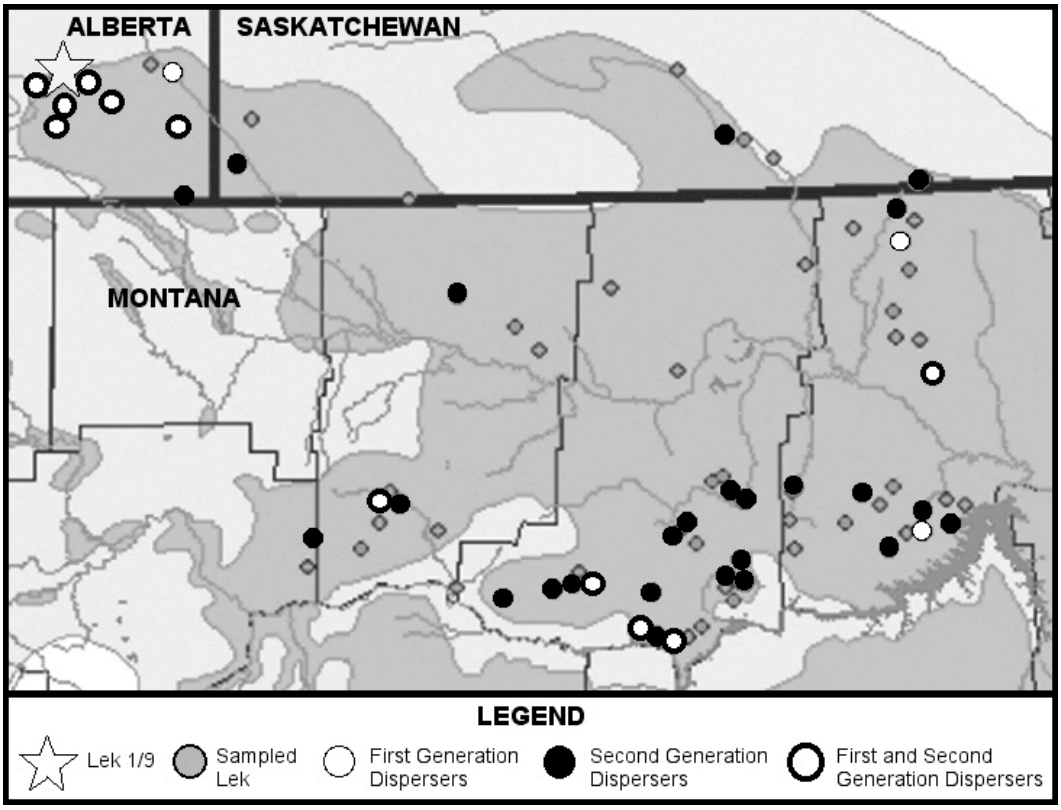

Figure 2-3. First and second-generation dispersers originating from lek 1/9. See table 2-3 for dispersal distances. Map modified from Schroeder *et al*. (2004).

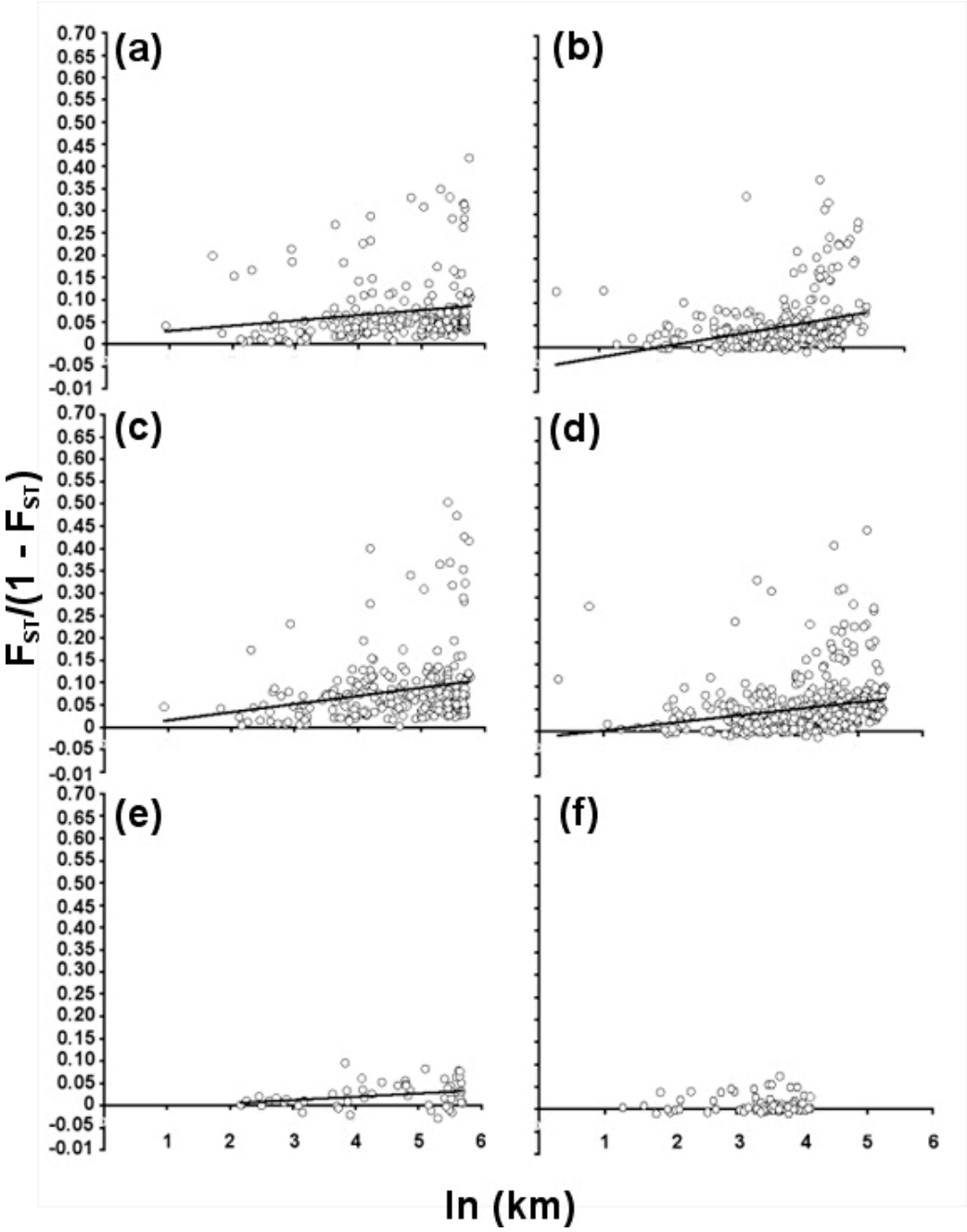

Figure 2-4. Plots illustrating spatial genetic structure as an isolation-by-distance correlation between genetic distance ($F_{ST}/(1-F_{ST})$) and geographical distance (ln[km]) for (a) the north of the Milk River subpopulation, (b) south of the Milk River subpopulation, males in the (c) north and (d) south of the Milk River subpopulation, females in the (e) north and (f) south of the Milk River subpopulation.

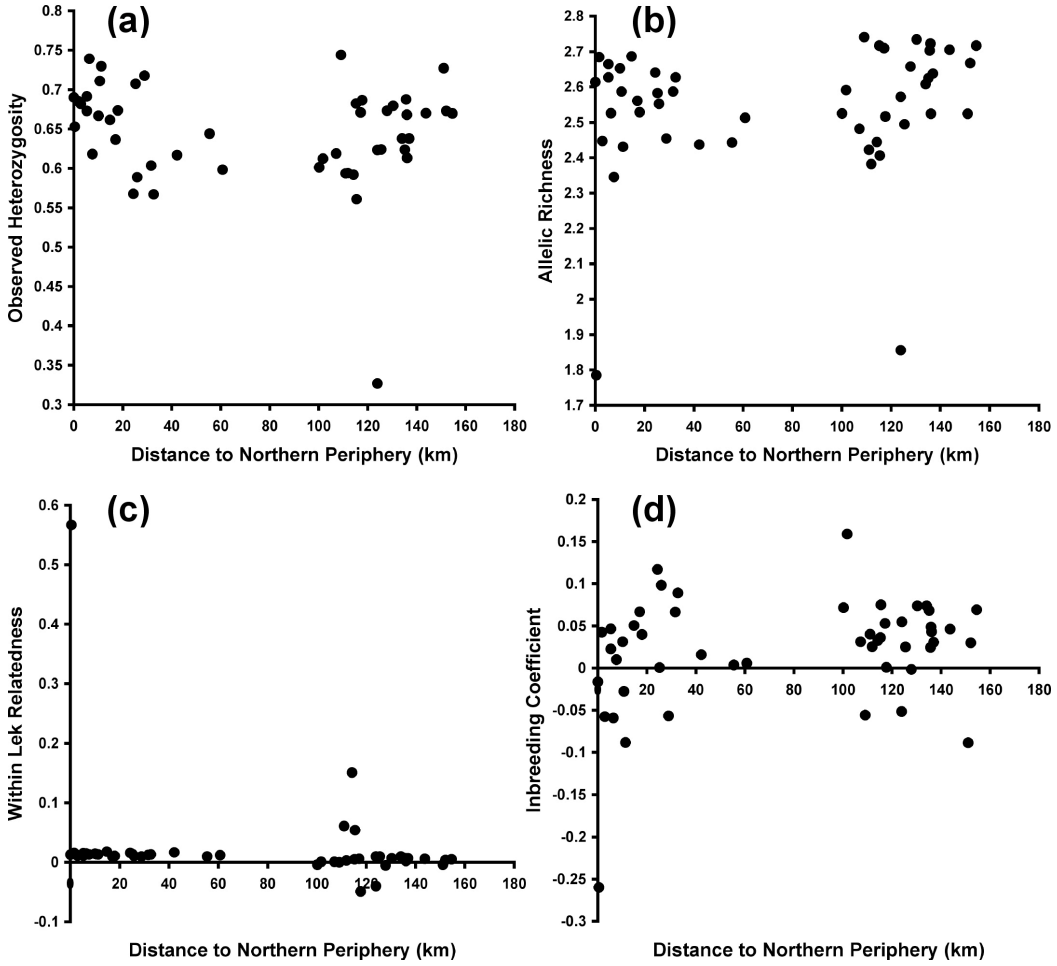

Figure 2-5. Regressions of (a) observed heterozygosity, (b) allelic richness, (c) within lek relatedness, and (d) inbreeding coefficient (F_{IS}) against geographic distance to the current northern peripheryof the species' range for all leks containing more than 10 sampled birds in the northern Montana sage-grouse population.

CHAPTER 3

Birds of a Feather Do Not Always Lek Together: Genetic Diversity and Kinship Structure of Greater Sage-Grouse in Alberta[2]

1. Introduction

Understanding the genetic structure and diversity of threatened and endangered populations, especially those that occur in fragmented or disturbed habitats, is necessary for devising effective management strategies to preserve these populations, determine their risk of extirpation, and aid in their recovery (Crozier 1997; Kraaijeveld-Smit et al. 2005). Anthropogenic fragmentation is a major threat that limits gene flow by reducing dispersal, decreasing population size, and increasing genetic drift in remnant habitat patches (Sherwin and Moritz 2000; Frankham 2003; Coulon et al. 2004). Most threatened species, regardless of habitat disturbance, exhibit decreased genetic diversity compared to their non-threatened taxonomic relatives (Spielman et al. 2004) because they are at higher risk of genetic diversity erosion, fixation of deleterious alleles, and inbreeding (Crozier 1997; Kraaijeveld-Smit et al. 2005). Because birds are mobile, they are expected to withstand the effects of fragmentation better than more sedentary species (Veit et al. 2005), but galliforms have been found to be particularly susceptible to the genetic effects of disturbance (Johnson et al. 2003; Caizergues et al. 2003a, b; Segelbacher et al. 2003; Bouzat and Johnson 2004).

Greater Sage-Grouse (*Centrocercus urophasianus*; hereafter Sage-Grouse) are endangered at the provincial (Alberta Sage-Grouse Recovery Action Group 2005) and federal (Lungle and Pruss 2008) levels in Canada where they are located at the northern periphery of the species' range. Sage-Grouse in Alberta have declined by 66-92% over the last 35 years (Aldridge and Brigham 2003) with an estimated population size of approximately 400 birds in the spring of 2007. Suggested causes for the decline include oil and gas development (Braun et

[2] This chapter is formatted for the Auk with the following authors: KL Bush, CL Aldridge, JE Carpenter, MS Boyce, CA Paszkowski, and DW Coltman

al. 2002), intensive grazing practices (Aldridge et al. 2004), wildlife viewing, changes in the predator community, climate change, and widespread destruction of sagebrush habitat in neighboring Montana (Alberta Sage-Grouse Recovery Action Group 2005). Sage-Grouse occur in the mixed-grass ecoregion of southeastern Alberta, but are primarily limited to the distribution of silver sagebrush (*Artemisia cana*), which keeps its leaves year round and is the main food and source of cover for Sage-Grouse (Alberta Sage-Grouse Recovery Action Group 2005). The distribution of silver sagebrush is naturally patchy, so birds have adapted to move large distances to find suitable habitat.

Sage-Grouse exhibit lekking behavior where males congregate on communal display grounds (leks) and females select a mate, breed, and then incubate eggs and raise young on their own (Wiley 1973). Many hypotheses have been proposed to explain why males participate in leks when the majority of males apparently fail to mate. Explanations range from anticipating future breeding opportunities (Wiley 1973), unpredictable female copying behavior (Kokko 1997), reduced predation risk (Boyko et al. 2004), parasite-host co-evolution (Boyce 1990), increased mating opportunity (Höglund and Alatalo 1995) to kin selection (Kokko and Lindström 1996), with the latter hypothesis having been tested on lekking grouse species using genetic data (Höglund et al. 1999; Bouzat and Johnson 2004; Gibson et al. 2005; Lebigre et al. 2007; Segelbacher et al. 2007). Kin selection is thought to drive the participation of low-ranking males in leks because they may indirectly and directly increase their own fitness by joining male relatives (Kokko and Lindström 1996; Sherman 1999). Subordinate males may benefit indirectly if their presence at the lek increases the reproductive success of related males. Direct benefits to subordinate males include increased mating opportunities with increased lek size, increased number of females attending the lek, or attraction of females to the lek that might be interested in males other than dominant individuals (Kokko and Lindström 1996; Sherman 1999; Sœther 2002). Several genetic studies have found evidence for kin association on leks (Höglund et al. 1999; Petrie et al. 1999; Bouzat and Johnson 2004), but others have not (McDonald and Potts 1994, Martin et al. 2002; Madden

et al. 2004; Höglund and Shorey 2004; DuVal 2007; Loiselle et al. 2007; Segelbacher et al. 2007; Knopp et al. 2008) including the only study on Sage-Grouse (Gibson et al. 2005).

I used polymorphic microsatellites to answer two main questions. First, what is the genetic diversity and connectivity of Sage-Grouse in Alberta? Second, are leks composed of related males? I expected to find low diversity and high differentiation between leks because of low estimated population size and extensive habitat fragmentation across the species' range in Alberta. For within-lek relatedness, I predicted low levels of male kinship within leks because a study of Sage-Grouse in California found males were typically unrelated (Gibson et al. 2005) and there is no evidence to suggest Sage-Grouse in Alberta would show different patterns of lek organization.

2. Methods

2.1. Study location and sample collection. - This study was conducted on Sage-Grouse from the extreme southeastern corner (4,000 km^2; Aldridge and Brigham 2003) of Alberta, Canada near Manyberries (Fig. 3-1). Birds were captured using walk-in funnel traps (Schroeder and Braun 1991), night lighting (Giesen et al. 1982), and drop-nets (Bush 2008). Blood, feather, and mouth swab samples were collected from captured Sage-Grouse between 1998-2006. All captured birds were aged following Eng (1955). "Yearlings" were birds entering their first breeding season and "adults" were birds entering their second (or subsequent) breeding season (Dalke et al. 1963). Vehicular and predator mortalities were opportunistically sampled and molted feathers were collected on leks from 2003 - 2007. All samples were collected during the lekking season (mid-March to mid-May) after dispersal had taken place and included both adults and yearlings. I did not attempt to separate birds into age categories for analysis because most of my samples were molted feathers that could not be aged. Survival of chicks was low (12%; Aldridge and Brigham 2008) so I had few samples from yearlings. In total, I collected 1,422 samples (327 blood, plucked feather, mouth swab, and road kill and 1,095 molted feathers); 1,391 were from the 11 known active leks in Alberta

and 31 samples were collected off lek. Off-lek birds consisted of females captured in the company of radio-collared females, carcasses of unmarked vehicular or predator mortalities, and molted feathers found at roost sites. All 31 birds sampled off-lek were assigned an unknown lek status and were not used in any lek-specific analyses. Nine leks were retained for analyses because only one male was sampled on lek 28, and leks 1 and 9 were combined into lek 1/9 as the single bird from lek 9 relocated to lek 1's site.

2.2. Microsatellite genotyping. - DNA was extracted using Qiagen DNeasy® Tissue and QIAamp® DNA Micro kits using modifications from Bush et al. (2005). All samples were sexed using DNA methods following Bush et al. (2005). Thirteen microsatellite loci developed from Sage-Grouse (SGCA9-2 [redesigned primer set; S. Taylor, personal communication] and SGCA5; Taylor et al. 2003), Capercaillie (*Tetrao urogallus*; TUT3, TUT4, TUD1, and TUD3; Segelbacher et al. 2000), Black Grouse (*Tetrao tetrix*; BG6 and BG15; Piertney and Höglund 2001; TTD6 and TTT1; Caizergues et al. 2001; TTT3; Caizergues et al. 2003a), Red Grouse (*Lagopus lagopus*; LLSD8; Piertney and Dallas 1997), and domestic chicken (*Gallus gallus*; ADL230; Cheng et al. 1994) were used. I assessed the presence of null alleles by examining 20 Sage-Grouse females and their known offspring (full nests; offspring were not included in the general analyses). I detected no null alleles, therefore the 13 loci were used for all analyses. Microsatellite PCRs (15μl total volume with 3, 4, or 5μl extracted DNA) were carried out as described by Bush et al. (2005). Forward primers were fluorescently labeled with 6-FAM, TET, and HEX (Applied Biosystems). I followed the PCR cycling conditions outlined for each microsatellite in the original publications using Perkin Elmer Cetus GeneAmp PCR System 9600® and Eppendorf Mastercycler® ep machines. All non-invasive samples were run in triplicate using the modified multiple-tubes approach (Segelbacher and Steinbrück 2001) as outlined in Bush et al. (2005). The PCR products were visualized using an ABI 377® automated sequencer with GENESCAN ANALYSIS3.1® software (Applied

Biosystems). Alleles were scored using GENOTYPER®2.0 software (Applied Biosystems).

2.3 Duplicate samples. - Molted feathers are normally considered a non-invasive source of genetic material because their collection does not involve handling birds. On leks, I observed that most molted feathers were pulled out during fights between males, which resulted in DNA equivalent in quality to hand-plucked feathers. Duplicate samples were identified using Microsoft Excel Microsatellite toolkit (Park 2001). For all non-invasive samples, the triplicate runs were first compared to one another. If the genotype for a given microsatellite was the same in all three runs, that genotype was retained. If inconsistent genotypes were found (different alleles in different runs) for a locus, no genotype was assigned and the locus was considered missing in all analyses. This approach decreased likelihood of allelic dropout and limited error. Two samples were considered duplicates if they were identical or differed by no more than one allele at up to two loci in a manner consistent with allelic drop out.

I determined DNA quality of each feather by amplifying five microsatellites (TUT3, TUT4, SGCA5, SGCA9-2, and TTD6) once and assessing peak height (amplification strength) and peak quality (presence/absence and amplitude of stutter peaks) on GENESCAN ANALYSIS electropherograms. Each feather was then assigned as high (high peaks with no stutter), medium (medium height peaks with little to no stutter), or low quality (short peaks and/or those exhibiting stutter) and triplicate PCR replicates were performed with 3, 4, and 5μl DNA respectively. Identification of genotyping errors was performed in MICRO-CHECKER (Van Oosterhout et al. 2004). Probability of identity (PI), the probability that two unrelated individuals drawn from a single population have the same multilocus genotype was calculated in GENALEX version 5.1 (Peakall and Smouse 2001) using the Paetkau and Strobeck (1994; random mating) and Taberlet and Luikart (1999; siblings) methods.

2.4. Genetic diversity, differentiation, and gene flow. – I used the Bayesian program STRUCTURE (Pritchard et al. 2000) to investigate spatial genetic substructure within Alberta. Previous research using STRUCTURE showed that Alberta birds are part of the northern Montana Sage-Grouse population (Alberta, Saskatchewan, and Blaine, Choteau, Phillips, and Valley counties in Montana) and belong to a subpopulation that occurs north of the Milk River (Alberta, Saskatchewan, and north Blaine, Phillips, and Valley counties; chapter 2). I ran 20 independent simulations for each K (1-19) with 100,000 burn-in iterations and 1,000,000 data repetitions assuming an admixture model and no prior population information. I used the method of Evanno et al. (2005), which calculates ΔK, a measure of the second order rate of chance in the likelihood of K, to estimate the true K, or number of clusters.

I calculated all genetic diversity measures at the Alberta (all birds combined), lek, and year levels. I calculated expected (H_E) and observed (H_O) heterozygosity for each locus and tested for deviations from Hardy-Weinberg and linkage equilibrium in GENEPOP, version 3.4 (Raymond and Rousset 1995). Number of alleles per locus (A) was calculated in Microsatellite toolkit. Allelic richness (AR; number of alleles corrected to the smallest sample size) and the inbreeding coefficient F_{IS} were calculated using FSTAT, version 2.9.3 (Goudet 2001); F_{IS} was calculated using Weir and Cockerham's (1984) estimator. Average relatedness (R) within leks and pairwise-R between leks and individuals were calculated in SPAGEDI version 1.1 (Hardy and Vekemans 2002) using the relationship coefficient of Queller & Goodnight (1989). This is a widely used method that gives results similar to other estimators. I used Wald statistics to test if diversity changed over time using linear mixed models, fitting year as a covariate.

To evaluate lek differentiation and dispersal within Alberta, I calculated average lek-to-lek R for leks with greater than 5 birds sampled both annually and overall (1998 – 2007) for both sexes combined, males, and females. I regressed average lek-to-lek R onto lek-to-lek geographic distance (5.4 – 61.3 km; Fig. 3-1) to test for isolation-by-distance (IBD) and determined significance using a Mantel

test (Mantel 1967) in R-PACKAGE, version 4.0 (Casgrain and Legendre 2001). I assessed IBD of males and females separately to identify sex-specific differences in dispersal.

2.5. Lek genetic structure. – I computed mean coefficients of relatedness for males and females within-lek years using SPAGEDI. All birds belong to a single population (Alberta; see STRUCTURE results), therefore I used allelic frequencies from the entire population across years for all analyses. *R* among males, females, and overall (males and females combined) within Alberta, leks, years, and lek-years was estimated and standard errors were calculated using the jackknife re-sampling procedure in SPAGEDI. To determine whether males and females attending the same lek in a given year were more related than expected by chance, I compared sample means to a null expectation of zero using a one-sample *t*-test (Gibson et al. 2005).

3. Results

3.1. Duplicate samples. - Of the 1,095 molted feather samples, 1,093 (99.8%) contained enough DNA to successfully amplify 7 – 13 loci in triplicate. For low and medium quality molted feathers and several plucked feathers with limited DNA quantities, a maximum of 11 microsatellites were successfully amplified for each sample. Amplification rates were consistent across lek (mean number of complete genotypes per sample ± SE; 12.0 ± 0.3), year (12.1 ± 0.4), sex (12.1 ± 0.2), and sample type (12.1 ± 0.6) and individual loci did not fail to amplify for an entire year, lek, sex, or in a single sample type. Therefore, it is unlikely that my estimations of genetic diversity or relatedness were biased due to missing data. For all samples that failed to produce the same genotype in two of three replicates (due to drop out), the genotype for that locus was excluded and only consistently accurate genotypes (three of three replicates) were included in the duplicate analysis to minimize error. Of the 1,422 samples, 604 were unique and 82% of these samples were genotyped at all 13 loci. Some birds were sampled up to 43

times by molted feathers. Probability of identity (PI) and PI for siblings were set at 0.001 and achieved at four and seven loci, respectively.

Of the birds genetically sampled more than once on a single lek, 98 (59.0%) males and 28 (80.0%) females were identified in only 1 year, whereas 68 (41.0%) males and seven (20.0%) females were sampled over multiple years at the same lek. Three leg-banded males were genetically sampled on more than one lek, but not in the same year and once a male relocated to a new lek, he stayed on that lek for the remainder of his life. One male moved from a lek that disbanded to the next closest lek (8.7 km), one male relocated to a slightly larger lek 8.8 km away, and one male relocated to a lek of approximately the same size 8.8 km away. No female was genetically detected on a lek other than the one where she was first captured/sampled, but 12 females were either physically recaptured or were detected via radio-telemetry on or near different leks during counts. Females attended different leks, both in the same year and across years, separated by 8.7 km (n = 5), 8.8 km (n = 1), 11.7 km (n = 2), 13.7 km (n = 2), 17.5 km (n = 1), and 24.1 km (n = 1).

3.2. Genetic diversity, differentiation, and gene flow. – Twelve of 13 loci were in Hardy-Weinberg disequilibrium at the Alberta level after corrections for multiple comparisons. At the lek level, all loci were in equilibrium. Nine of 78 comparisons were in linkage disequilibrium in Alberta, but since no loci were in disequilibrium at the lek level, all loci were considered unlinked and retained for analysis.

The most likely number of genetic clusters within Alberta was one (ΔK = 12.3 for K = 1 vs. the next highest ΔK = 4.8 for K = 3). All microsatellite loci were polymorphic with 5 - 23 alleles per locus at the Alberta level and 1 - 19 alleles at the lek level (Table 3-1). Global (across years) genetic diversity measures and relatedness were consistent with annual values within leks and across most leks (Table 3-1). Allelic richness (*AR)* was highest in the larger leks (average lek counts of ≥ 8 males; 10/11, 16, 30, 31, and 34) and was lowest in lek 1/9. Observed heterozygosity (H_O) was consistent across all leks. Relatedness (R)

was very high (0.63) and F_{IS} very low (-0.33) for lek 1/9. Diversity did not vary across years (H_O, Wald = 1.53, P = 0.13; AR, Wald = 1.39, P = 0.16; F_{IS}, Wald = 1.37, P = 0.17).

There was a weak negative relationship between lek relatedness and geographic distance for all Alberta birds combined, males, and females (Table 3-2). When individual years were examined, there were stronger negative relationships for both sexes combined in 2002 (Mantel r = -0.46, P = 0.004), 2003 (Mantel r = -0.45, P = 0.02), and 2004 (Mantel r = -0.36, P = 0.03); for males in 2003 (Mantel r = -0.37, P = 0.03) and 2004 (Mantel r = -0.34, P = 0.02); for females in 1998 (Mantel r = -0.68, P = 0.04) and 2002 (Mantel r = -0.58, P = 0.03; Table 3-2, Fig. 3-2).

3.3. Lek genetic structure. – Global Alberta average R for males across years was near zero, whereas overall and female R were slightly, but significantly different from zero (Table 3). Birds on several individual leks were positively related (three leks for both sexes combined, six leks for males only, and three leks for females only; Table 3-3). Most of these cases involved the three most eastern or isolated leks (Fig. 3-1). Lek 22 was the most geographically isolated lek and exhibited the most positive within-lek R for all three categories (combined, males, and females; Table 3). When means were taken across leks, R was close to zero for females, but greater than zero for males and both sexes combined.

For all years, birds were consistently more related within-leks than between leks (Table 3-4). Males and females displayed similar relatedness within leks (Table 3-4). Within-lek R varied among years and was highest in 2005 and 2006 for all three categories (Table 3-4). Averages based on all years were close to zero for all three categories for most leks, with the exception of lek 22 males and females, lek 35 overall and males, and lek 30 females (Fig. 3-3). Within individual leks, variation in R could be attributed to lek-years when fewer than 5 birds were sampled, lek location (22, 30, and 35 were peripheral leks), and lek size (2/24 was small with lek counts ranging from 1-11 males; Fig. 3-3).

Over the study period, annual within-lek *R* increased for some leks (lek 2/24 females and overall; lek 10/11 and 16 males, females, and overall; lek 22 males and overall); *R* decreased for others (lek 2/24 males; lek 30, 31, and 35 females; lek 34 males, females, and overall); or remained relatively constant (lek 30, 31, and 35 males and overall; data not shown). Annual mean *R* for each lek and sex was generally within a standard error of each previous and following year, with a few exceptions. Most cases involved peripheral (22, 30, and 35) and small (2/24) leks, but lek 10/11 females in 2006, lek 16 males in 2007, lek 31 males in 2007, and lek 34 males in 2000 and 2007 had elevated *R*-values (Fig. 3-3).

4. Discussion

Endangered Sage-Grouse in Alberta exhibited high genetic diversity and connectivity and leks were not primarily composed of kin. Leks in Alberta were not highly differentiated from one another despite population declines and habitat fragmentation. Isolation-by-distance was not detected for all birds combined or for either sex separately indicating that both sexes disperse. Overall within-lek relatedness for males and females was consistently close to zero in all years and for most leks, with the exception of lek 1/9. However, some lek-years had positive or negative relatedness for both sexes suggesting that while the overall pattern of kin association within leks was generally weak, there was considerable variation in the degree of relatedness detected for both sexes among lek-years. Although kin structure does not maintain leks in Alberta, it may be an indicator of lek health or recruitment in specific years.

4.1. Genetic diversity, differentiation, and gene flow. - I observed no population structure at the Alberta scale, which is consistent with my other analyses that have shown that birds north of the Missouri River (Alberta, Saskatchewan, and northern Montana) formed a single genetic population with two subpopulations (north and south of the Milk River; chapter 2). The lack of genetically

differentiated lek clusters can be attributed to the close geographic proximity of leks (Fig. 3-1) and high gene flow across the study area (Fig. 3-2; Table 3-2).

Genetic diversity was high across Alberta despite the endangered status of Sage-Grouse and habitat fragmentation. Genetic diversity (H_O, AR, and F_{IS}) did not change over the 10 years of the study, likely because the study leks are part of a larger, demographically stable population. The exception to the high diversity was lek 1/9, which had low allelic richness and high relatedness (Table 3-1) suggesting it was composed of highly related males (the average within-lek R of the five males sampled in 2004 was 0.79). I created a partial lek pedigree based on sampled birds, which revealed that all males present on lek 1/9 in 2004 were descendants of a single male sampled in 1999 on lek 9. Lek 1/9 was re-established in 2001 after 25 years of inactivity (Alberta Fish and Wildlife, unpubl. data). However, within-lek R of two other recently formed leks sampled in Montana and Wyoming did not differ from zero (K. L. Bush, unpubl. data), suggesting that male relatives are not always the founders of new leks. Sage-Grouse in Alberta maintained stable genetic diversity despite habitat fragmentation and population declines, with the exception of one unusual and highly related lek.

I compared my estimates of heterozygosity to published studies to assess the relative diversity of Sage-Grouse in Alberta. A common trend across all published grouse studies was that contiguous regions had the highest diversity and fragmented and/or peripheral regions had the lowest diversity (Table 3-5). Expected heterozygosity in Alberta was in the range detected for Sage-Grouse populations at the core of the species' current distribution (Montana, Wyoming, Nevada, Oregon, and Idaho) and was at the high end for peripheral and/or fragmented populations (California, Canada, Colorado, North Dakota, South Dakota, Utah, and Washington; Oyler-McCance et al. 2005; Table 3-5). Compared to a peripheral and isolated Sage-Grouse population in California, H_O was higher in Alberta (Table 3-5), suggesting that Sage-Grouse in Alberta are not isolated. Sage-Grouse in Alberta had lower levels of H_O than both fragmented (Alps) and contiguous (Finland) populations of Black Grouse and Rock Ptarmigan (*Lagopus mutus*; Table 3-5). Diversity was likely higher in the European grouse

because many of the microsatellite loci were developed on these species. H_O was comparable to a peripheral population of Lesser Prairie-Chicken and isolated Rock Ptarmigan (Pyrenees; Table 3-5), but slightly lower than all populations of Greater Prairie-Chicken. Sage-Grouse in Alberta had similar heterozygosity to fragmented populations of North American grouse and isolated populations of European Rock Ptarmigan in the Pyrenees (Table 3-5) suggesting that while diversity has not declined in Alberta, it may be lower by virtue of the birds' peripheral location in the species' range and smaller population size.

The absence of isolation-by-distance patterns and low relatedness within and among leks suggests extensive gene flow and little differentiation between leks in Alberta. Neither males nor females exhibited a correlation between genetic and geographic distances. Both sexes exhibited low average relatedness within-leks, but lower relatedness for females suggests that they may have a greater predisposition to disperse. When analyzed separately, three of 10 years displayed significant IBD, suggesting a weak pattern of IBD varying amongst years, perhaps driven by population density, weather, or chance. Work on the entire northern Montana population revealed significant IBD for both sexes combined and separately, but IBD was not significant at the smaller regional scale (contiguous habitat less than 100 km across; chapter 2). However, data from the entire northern Montana population was not analyzed on a year-by-year basis and lumping data across years may have masked a similar, weak pattern of IBD, such as that documented for Alberta. Dispersal of Sage-Grouse in Alberta deviates from the typical avian pattern of male philopatry and dispersal by females that was observed for Sage-Grouse in Colorado (Dunn and Braun 1985) because both sexes appeared to disperse at the regional scale.

4.2. Lek genetic structure. - Sage-Grouse leks in Alberta were congregations of primarily unrelated males and females with both sexes exhibiting limited kin association across years, but a greater potential for kin association within-years. An absence of strong male-biased kin structure in most leks suggests that males were not highly philopatric (remaining at their natal lek). Birds from all years and

leks displayed higher relatedness within each of the nine study leks than between leks (Table 3-4) or for all leks combined (Table 3-3), indicating weak familial associations within leks for both sexes. This variable pattern indicated that kin association by both sexes might play a role in the organization of some leks in some years. In Red Grouse (*Lagopus lagopus scoticus*) temporal variation in male kin structure was caused by delayed density-dependent changes in aggressiveness between males, which influenced recruitment to leks and regulated density (Piertney et al. 2008). It is possible that a similar mechanism operates in Sage-Grouse, but the cycle is obscured in Alberta due to the demographic decline. As for females, if productivity is high in the previous year, recruitment of siblings to individual leks, either via kin association or by chance, will be higher than in years following poor productivity. It is also possible that in a population as small as Alberta, relatedness varies from year to year strictly by chance as I documented no clear pattern of increasing, decreasing, or stable relatedness within leks or sexes over time. Most leks that exhibited elevated or more variable relatedness were either peripheral (22, 30, and 35) or small (1/9 and 2/24) suggesting that lek location and size may influence kin association. Small leks, just by virtue of their size, will have elevated relatedness even if they contain only a few relatives. In contrast, peripheral leks may have increased relatedness by necessity. Birds in more isolated leks may be more philopatric because the costs of dispersing through inhospitable habitat or over long distances outweigh the benefits for most individuals.

Low overall within-lek male relatedness in Alberta Sage-Grouse resembles patterns seen in California leks (Gibson et al. 2005). With the exception of lek 1/9 (R = 0.64), within-lek male relatedness (-0.01 to 0.09) were considerably lower than values reported for grouse populations studied over multiple years where leks were seen as a product of male kin selection (R = 0.17 to 0.36 for Lesser Prairie-Chicken leks; Bouzat and Johnson 2004; R = 0.11 to 0.21 for kin clusters within Capercaillie leks, Segelbacher et al. 2007). However, my relatedness values were similar to those reported for other grouse leks not exhibiting kin association (R = -0.05 to -0.11 for Lesser Prairie-Chicken, Bouzat

and Johnson 2004; R = 0.003 for California Sage-Grouse, Gibson et al. 2005; R = -0.02 to -0.05 for Capercaille, Segelbacher et al. 2007). None of these studies examined inter-annual variation in relatedness among males on individual leks, so it is difficult to determine whether kin association in other species fluctuates across multiple years or if there is a consistent lack of kin association over years. Also, none of these studies considered females, which I found to display similar degrees of kin association as males. My results suggest that some members of both sexes are philopatric in Alberta (Table 3-3, Fig. 3-3), while others disperse (Table 3-2, Fig 3-2) and kin association does not play a major role in maintaining Sage-Grouse leks in the long term. Therefore, alternative mechanisms for the evolution or maintenance of leks deserve examination.

5. Conservation implications

Sage-Grouse in Alberta have maintained high genetic diversity over recent years. The lek system of Sage-Grouse should lead to reduced effective population size, increased genetic structuring, and increased inbreeding potential if only one or a few males mate on a lek in a given year. However, I observed high diversity and low relatedness for both sexes. A relatively large effective population size and high levels of diversity may be maintained in Alberta via gene flow from other parts of the northern Montana population despite the recent demographic decline. This connection is positive for the conservation of endangered Alberta birds, but habitat destruction in adjacent northern Montana and Saskatchewan is a continuing process. Sage-Grouse exhibit evidence of gene flow (this study) and movement (Aldridge 2005) between Alberta leks, despite locally fragmented habitat, indicating that these birds can traverse or circumvent unsuitable habitat. However, some leks appear to be more genetically isolated (leks 1/9 and 22), based on elevated relatedness and the population continues to decline even though there is genetic connectivity with Montana and within Alberta. It would be useful to identify limiting factors (e.g., habitat availability) and to better understand the connectivity of leks in Alberta to the rest of the population so that corridors or

areas of critical habitat can be protected to minimize the impact of future fragmentation and isolation.

6. Literature Cited

Alberta Sage-Grouse Recovery Action Group. 2005. Alberta Greater Sage-Grouse Recovery Plan. Alberta Sustainable Resource Development, Fish and Wildlife Division, Alberta Species at Risk Recovery Plan No. 8. Edmonton, Alberta.

Aldridge, C. L., and R. M. Brigham. 2003. Distribution, abundance, and status of the Greater Sage-Grouse, *Centrocercus urophasianus*, in Canada. Canadian Field-Naturalist 117:25 - 34.

Aldridge, C. L., M. S. Boyce, and R. K. Baydack. 2004. Adaptive management of prairie grouse: how do we get there? Wildlife Society Bulletin 32:92 - 103.

Aldridge, C.L. 2005. Identifying habitats for persistence of Greater Sage-Grouse (*Centrocercus urophasianus*) in Alberta, Canada. Ph.D. dissertation, University of Alberta, Edmonton.

Aldridge, C. L. and M. S. Boyce. 2008. Accounting for fitness: combining survival and selection when assessing wildlife-habitat relationships. Israel Journal of Ecology and Evolution 54:389-419.

Bouzat, J. L., H. H. Cheng, H. A. Lewin, R. L. Westemeier, J. D. Brawn, and K. N. Paige. 1998. Genetic evaluation of a demographic bottleneck in the Greater Prairie Chicken. Conservation Biology 12:836 – 843.

Bouzat, J. L., and K. Johnson. 2004. Genetic structure among closely spaced leks in a peripheral population of lesser prairie-chickens. Molecular Ecology 13:499 - 505.

Boyce, M. S. 1990. The Red Queen visits sage grouse leks. American Zoologist 30:263 – 270.

Boyko, A. R., R. M. Gibson, and J. R. Lucas. 2004. How predation risk affects the temporal dynamics of avian leks: greater sage grouse versus golden eagles. American Naturalist 163:154 - 165.

Braun, C. E., O. O. Oedekoven, and C. L. Aldridge. 2002. Oil and gas development in western North America: effects on sagebrush steppe avifauna with particular emphasis on Sage Grouse. Transactions of the 67th North American Wildlife and Natural Resources Conference 67:337 - 349.

Bush, K. L., M. D. Vinsky, C. L. Aldridge, and C. A. Paszkowski. 2005. A comparison of sample types varying in invasiveness for use in DNA sex determination in an endangered population of greater sage-grouse (*Centrocercus urophasianus*). Conservation Genetics 6:867 - 870.

Bush, K. L. 2008. A pressure-operated drop net for capturing Greater Sage-Grouse. Journal of Field Ornithology 79:64 - 70.

Caizergues, A., S. Dubois, G. Mondor, and J. Y. Rasplus. 2001. Isolation and charcterisation of microsatellite loci in black grouse (*Tetrao tetrix*). Molecular Ecology Notes 1:36 - 38.

Caizergues, A., O. Rätti, P. Helle, L. Rotelli, L. Ellison, and J-Y. Rasplus. 2003a. Population genetic structure of male black grouse (*Tetrao tetrix* L.) in fragmented vs. continuous landscapes. Molecular Ecology 12:2297 - 2305.

Caizergues, A., A. Bernard-Laurent, J. F. Brenot, L. Ellison, and J. Y. Rasplus. 2003b. Population genetic structure of rock ptarmigan *Lagopus mutus* in northern and western Europe. Molecular Ecology 12:2267 – 2274.

Casgrain, P., and P. Legendre. 2001. *The R Package for Multivariate and Spatial Analysis,* Version 4.0. User's Manual. Department des Sciences Biologiques, Université de Montreal. Available at: http://www.fas.umontreal.ca/BIOL/legendre.

Cheng, H. H., I. Levin, R. L. Vallejo, H. Khatib, J. B, Dodgson, L. B. Crittenden, and J. Hillel. 1995. Development of a genetic map of the chicken with high-utility markers. Poultry Science 74:1855 - 1874.

Connelly, J. W., S. T. Knick, M. A. Schroeder, and S. J. Stiver. 2004. Conservation assessment of Greater Sage-grouse and sagebrush habitats. Western Association of Fish and Wildlife Agencies. Unpublished Report. Cheyenne, Wyoming.

Coulon, A., J. F. Cosson, J. M. Angibault, B. Cargnelutti, M. Galan, N. Morellet, E. Petit, S. Aulagnier, and A. J. M. Hewison. 2004 Landscape connectivity influences gene flow in a roe deer population inhabiting a fragmented landscape: an individual-based approach. Molecular Ecology 13:2841 - 2850.

Crozier, R. H. 1997. Preserving the information content of species: genetic diversity, phylogeny and conservation worth. Annual Review of Ecology and Systematics 28:243 - 268.

Dalke, P. D., D. B. Pyrah, D. C. Stanton, J. E. Crawford, and E. F. Schlatterer. 1963. Ecology, productivity, and management of sage grouse in Idaho. Journal of Wildlife Management 27:811 - 840.

Dunn, P. O. and C. E. Braun. 1985. Natal dispersal and lek fidelity of sage-grouse. Auk 102:621 – 627.

DuVal, E. H. 2007. Adaptive advantages of cooperative courtship for subordinate male lance-tailed manakins. The American Naturalist 169:423 – 432.

ENG, R.L. 1955. A method for obtaining sage grouse age and sex ratios from wings. Journal of Wildlife Management: 19:262 - 272.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611 - 2620.

Frankham, R. 2003 Genetics and conservation biology. Comptes Rendus Biologies 326:175 – 184.

Gibson, R. M., D. Pires, K. S. Delaney, and R. K. Wayne. 2005. Microsatellite DNA analysis shows that greater sage grouse leks are not kin groups. Molecular Ecology 14:4453 - 4459.

Giesen, K. M., T. J. Schoenberg, and C. E. Braun. 1982. Methods for trapping sage grouse in Colorado. Wildlife Society Bulletin 10:223 - 231.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html. Updated from Goudet (1995).

Hamerstrom, F. and F. Hamerstrom. 1961. Status and problems of North American grouse. Wilson Bulletin 73:284 – 294.

Hardy, O. J. and X. Vekemans. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Molecular Ecology Notes 2:618 - 620.

Höglund, J., and R. V. Alatalo. 1995. Leks. Princeton University Press, Princeton, New Jersey.

Höglund, J. R., V. Alatalo, A. Lundberg, P. T. Rintamäki, and J. Lindell. 1999. Microsatellite markers reveal the potential for kin-selection on black grouse leks. Proceedings of the Royal Society of London B 266:813 - 816.

Höglund, J. and L. Shorey. 2003. Local genetic structure in a white-bearded manakin population. Molecular Ecology 12:2457 - 2463.

Johnson, J. A., J. E. Toepfer, and P. O. Dunn. 2003. Contrasting patterns of mitochondrial and microsatellite population structure in fragmented populations of greater prairie chickens. Molecular Ecology 12:3335 - 3347.

Johnson, D. H. 2001. Habitat fragmentation effects on birds in grasslands and wetlands: a critique of our knowledge. Great Plains Research 11:211 – 213.

Knopp, T., M. Heimovirta, H. Kokko, and J. Merilä. 2008. Do male moor frogs (*Rana arvalis*) lek with kin? Molecular Ecology 17:2522 - 2530.

Kokko, H. 1997. The lekking game: can female choice explain aggregated male displays? Journal of Theoretical Biology 187:57 - 64.

Kokko, H., and J. Lindström. 1996. Kin selection and the evolution of leks: whose success do young males maximize? Proceedings of the Royal Society of London B 263: 919 - 923.

Kraaijeveld-Smit, F. J. L., T. J. C. Beebee, R. A. Griffiths, R. D. Moore, and L. Schley. 2005. Low gene flow but high genetic diversity in the threatened Mallorcan midwife toad *Alytes muletensis*. Molecular Ecology 14:3307 - 3315.

Lebigre, C., R. V. Alatalo, H. Sitari, and S. Parri. 2007. Restrictive mating by females on black grouse leks. Molecular Ecology 16:4380 – 4389.

Loiselle, B. A., T. B. Ryder, R. Durães, W. Tori, J. G. Blake, and P. G. Parker. 2007. Kin selection does not explain male aggregation at leks of 4 manakin species. Behavioral Ecology 18:287 - 291.

Lungle, K., and S. Pruss. 2008. Recovery Strategy for the Greater Sage-Grouse (*Centrocercus urophasianus urophasianus*) in Canada. *In* Species at Risk Recovery Strategy Series. Parks Canada Agency. Ottawa.

Madden, J. R., T. J. Lowe, H. V. Fuller, R. L. Coe, K. K. Dasmahapatra, W. Amos, and F. Jury. 2004. Neighbouring male spotted bowerbirds are not related, but do maraud each other. Animal Behaviour 68:751 - 758.

Mantel, N. 1967. The detection of disease clustering and generalized regression approach. Cancer Research 27:209 - 220.

Martin, C. A., J. C. Alonso, J. Alonso, C. Pitra, and D. Lieckfeldt. 2002. Great bustard population structure in central Spain: concordant results from genetic analysis and dispersal study. Proceedings of the Royal Society of London, B 269:119 - 125.

McDonald, D. B. and W. K. Potts. 1994. Cooperative display and relatedness among males in a lekking bird. Science 266:1030 - 1032.

Oyler-McCance, S. J., S. E. Taylor, and T. W. Quinn. 2005. A multilocus population genetic survey of the greater sage-grouse across their range. Molecular Ecology 14:1293 - 1310.

Paetkau, D. and C. Strobeck. 1994. Microsatellite analysis of genetic variation in black bear populations. Molecular Ecology 3:489 – 495.

Park, S. D. E. 2001 Trypanotolerance in West African cattle and the population genetic effects of selection. PhD thesis, University of Dublin, Ireland.

Peakall, R. and P. E. Smouse. 2001. GENALEX version 5.1. Genetic analysis in Excel. Population genetic software for teaching and research. http://www.anu.edu.au/BoZo/GenAlEx. Australian National University, Canberra, Australia.

Petrie, M., A. Krupa, and T. Burke. 1999. Peacocks lek with relatives even in the absence of social and environmental cues. Nature 401:155 - 157.

Piertney, S. B. and J. F. Dallas. 1997. Isolation and characterization of hypervariable microsatellites in the red grouse. *Lagopus lagopus scoticus*. Molecular Ecology 6:93 - 95.

Piertney, S. B. and J. Höglund. 2001. Polymorphic microsatellite DNA markers in black grouse (*Tetrao tetrix*). Molecular Ecology Notes 1:303 - 304.

Piertney, S. B., X. Lambin, A. D. C. Maccoll, K. Lock, P. J. Bacon, J. F. Dallas, F. Leckie, F. Mougeot, P. A. Racey, and R. Moss. 2008. Temporal changes in kin structure through a population cycle in a territorial bird, the red grouse *Lagopus lagopus scoticus*. Molecular Ecology 17:2544 - 2511.

Pritchard, J. K., M. Stephens, and P. Donelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945 - 959.

Queller, D. C., and K. F. Goodnight. 1989. Estimating relatedness using genetic markers. Evolution 43:258 – 275.

Raymond, M., and F. Rousset. 1995. Genepop version 3.1d: population genetics software for exact test and ecumenism. Journal of Heredity 86:248 - 249.

Sœther, S. A. 2002. Kin selection, female preference and the evolution of leks: direct benefits may explain kin structuring. Animal Behaviour 63:1017 - 1019.

Schroeder, M. A., and C. E. Braun. 1991 Walk-in traps for capturing greater prairie-chickens on leks. Journal of Field Ornithology 62:378 - 385.

Segelbacher, G., R. J. Paxton, G. Steinbrueck, P. Trontelj, and I. Storch. 2000. Characterisation of microsatellites in capercaillie (*Tetrao urogallus*) (AVES). Molecular Ecology 9:1934 - 1935.

Segelbacher, G. and G. Steinbrück. 2001. Bird faeces for sex identification and microsatellite analysis. Vogelwarte 41:139 – 142.

Segelbacher, G., J. Höglund, and I. Storch. 2003. From connectivity to isolation: genetic consequences of population fragmentation in capercaillie across Europe. Molecular Ecology 12:1773 - 1780.

Segelbacher, G., P. Wegge, A. V. Sivkov and J. Höglund. 2007. Kin groups in closely spaced capercaillie leks. Journal of Ornithology 148:79 - 84.

Sherman, P. W. 1999. Birds of a feather lek together. Nature 401:119 - 120.

Sherwin W. B. and C. Moritz. 2000. Managing and monitoring genetic erosion. *In* Genetics, Demography and Viability of Fragmented Populations (Young A. J. and G. Clarke, Eds). Cambridge University Press, New York.

Spielman, D. B. W. Brook, and R. Frankham. 2004. Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Sciences 101:15261 – 15264.

Taberlet, P. and G. Luikart. 1999. Non-invasive genetic sampling and individual identification. Biological Journal of the Linnean Society 68:41 - 55.

Taylor, S. E., S. J. Oyler-McCance, and T. W. Quinn. 2003. Isolation and characterization of microsatellite loci in greater sage-grouse (*Centrocercus urophasianus*). Molecular Ecology Notes 3:262 - 264.

Van Oosterhout, C., Hutchingson, W. F., Wills, D. P. M., and Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535 – 538.

Veit, M. L., R. J. Robertson, P. B. Hamel, and V. L. Friesen. 2005. Population genetic structure and dispersal across a fragmented landscape in cerulean warblers (*Dendroica cerulea*). Conservation Genetics 6:159 – 174.

Weir, B. S. and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358-1370.

Wiley, R. H. 1973. Territoriality and non-random mating in the sage grouse, *Centrocercus urophasianus*. Animal Behaviour Monographs 6:87 - 169

Table 3-1. Genetic characteristics of active Sage-Grouse leks in Alberta from 1998 – 2007. *n*, number of individuals analyzed; *AR*, allelic richness or number of alleles corrected to a sample size of six; H_O, mean observed heterozygosity; *R*, average relatedness of individuals; F_{IS}, inbreeding of individuals relative to their lek. Values in parentheses are ranges of annual averages.

Lek	*n*	*AR*	H_O	*R*	F_{IS}
1/9	6	2.6	0.69	0.64	-0.33
2/24	26	4.4 (2.4 – 2.7)	0.70 (0.68 – 0.79)	0.02 (-0.08 – 0.2)	0.01 (-0.2 – 0.07)
10/11	84	4.5 (2.3 – 2.8)	0.69 (0.65 – 0.73)	0.01 (-0.01 – 0.08)	-0.01 (-0.02 – 0.1)
16	171	4.7 (2.3 – 2.7)	0.69 (0.67 – 0.71)	-0.01 (-0.01 – 0.003)	0.03 (0.009 – 0.05)
22	41	4.1 (2.2 – 2.5)	0.65 (0.62 – 0.72)	0.09 (-0.2 – 0.2)	0.01 (-0.1 – 0.04)
30	67	4.5 (2.3 – 2.7)	0.66 (0.62 – 0.72)	0.01 (-0.3 – 0.07)	0.05 (-0.06 – 0.1)
31	77	4.6 (2.3 – 2.7)	0.69 (0.63 – 0.72)	-0.01 (-0.03 – 0.04)	0.02 (-0.02 – 0.1)
34	74	4.6 (2.3 – 2.7)	0.69 (0.68 – 0.71)	0.02 (-0.09 – 0.04)	0.01 (-0.04 – 0.01)
35	37	4.3 (2.3 – 2.7)	0.69 (0.66 – 0.81)	0.06 (0.02 – 0.32)	-0.03 (-0.3 – 0.1)
Global Alberta Average	604	4.7	0.68	-0.01	0.03

Table 3-2. Correlation between average lek-to-lek relatedness and geographic distance between leks by sex, year, and combined. See figure 2 for the associated isolation-by-distance plots for both sexes combined for each year of the study (1998 – 2007) and across all years. * denotes significant difference from zero, $\alpha = 0.05$.

Year	Sexes Combined			Males			Females		
	Mantel r	*n* (birds)	*N* (leks)	Mantel r	*n* (birds)	*N* (leks)	Mantel r	*n* (birds)	*N* (leks)
1998	0.14	68	6	-0.78	41	5	-0.68*	25	4
1999	-0.11	81	5	-0.32	32	4	-0.79	47	4
2000	-0.40	87	6	0.08	31	5	-0.28	55	5
2001	-0.54	91	5	-0.47	28	5	-0.20	63	5
2002	-0.46*	99	6	0.04	42	6	-0.58*	57	5
2003	-0.45*	109	8	-0.37*	55	8	0.41	53	5
2004	-0.36*	96	9	-0.34*	65	9	0.98	29	3
2005	0.11	186	8	0.07	123	8	0.02	61	6
2006	0.26	166	7	0.15	111	7	-0.25	48	5
2007	0.15	48	6	0.17	47	6	N/A	N/A	N/A
Global Alberta Average	-0.09	604	9	-0.34	375	9	0.10	229	8

Table 3-3. Average relatedness for males, females, and both sexes combined for Sage-Grouse on nine leks in Alberta (1998 – 2007). Standard errors were generated by jackknife resampling in SPAGEDI. Global Alberta averages were calculated by combining all birds across years and standard errors were generated by jackknife resampling in SPAGEDI. Means across leks were calculated by taking the average of the lek averages and standard errors were calculated based on the range in leks. * denotes significant difference from zero, $\alpha = 0.05$.

Lek	Sexes Combined		Males		Females	
	R (SE)	*n*	*R* (SE)	*n*	*R* (SE)	*n*
1/9	0.64 (0.09)*	6	0.64 (0.09)*	6	N/A	0
2/24	0.02 (0.02)	26	0.04 (0.03)	13	0.0002 (0.03)	13
10/11	0.01 (0.01)	84	0.02 (0.01)*	48	0.006 (0.01)	36
16	-0.008 (0.008)	171	-0.01 (0.01)	97	0.02 (0.02)	74
22	0.09 (0.04)*	41	0.08 (0.04)*	32	0.18 (0.08)*	9
30	0.01 (0.01)*	67	0.007 (0.01)	54	0.07 (0.04)*	13
31	-0.007 (0.01)	77	0.02 (0.02)*	46	-0.006 (0.02)	31
34	0.02 (0.02)	74	0.03 (0.02)*	43	0.03 (0.02)*	31
35	0.06 (0.02)*	37	0.09 (0.03)*	30	0.04 (0.07)	7
Mean Across Leks	0.09 (0.003)*	604	0.1 (0.003)*	375	0.04 (0.004)	229
Global Alberta Average	-0.002 (0.001)*	604	0.001 (0.005)	375	0.01 (0.007)*	229

Table 3-4. Mean relatedness of Alberta Sage-Grouse within and between leks by year and overall (all years combined) for both sexes combined, males, and females. Standard errors were generated by jackknife resampling in SPAGEDI. Global Alberta averages were calculated by combining all birds across years and standard errors were generated by jackknife resampling in SPAGEDI. Means across leks were calculated by taking the average of the lek averages and standard errors were calculated based on the range in years. * denotes significant difference from zero, $\alpha = 0.05$. * denotes significant difference from zero, $\alpha = 0.05$.

Year	Sexes Combined		Males		Females	
	Within Lek *R* (SE)	Between Lek *R* (SE)	Within Lek *R* (SE)	Between Lek *R* (SE)	Within Lek *R* (SE)	Between Lek *R* (SE)
1998	0.03 (0.05)	-0.02 (0.002)*	0.07 (0.06)*	-0.03 (0.004)*	-0.03 (0.05)	-0.04 (0.005)*
1999	-0.001 (0.05)	-0.02 (0.002)*	0.004 (0.08)	-0.04 (0.003)*	-0.02 (0.04)	-0.03 (0.003)*
2000	-0.004 (0.06)	-0.01 (0.002)*	-0.01 (0.09)	-0.04 (0.006)*	0.06 (0.05)*	-0.02 (0.004)*
2001	0.01 (0.03)	-0.01 (0.002)*	0.07 (0.08)	-0.05 (0.007)*	0.007 (0.03)	-0.02 (0.002)*
2002	0.03 (0.04)	-0.01 (0.001)*	0.01 (0.06)	-0.02 (0.003)*	0.02 (0.04)	-0.02 (0.003)*
2003	0.02 (0.04)	-0.01 (0.003)*	0.05 (0.06)	-0.02 (0.003)*	-0.002 (0.05)	-0.02 (0.004)*
2004	0.09 (0.06)*	-0.01 (0.003)*	0.08 (0.07)*	-0.03 (0.004)*	-0.01 (0.03)	-0.03 (0.007)*
2005	0.09 (0.05)*	-0.005 (0.003)*	0.09 (0.05)*	-0.009 (0.003)*	0.07 (0.08)	-0.02 (0.007)*
2006	0.09 (0.06)*	-0.01 (0.001)*	0.12 (0.06)*	-0.02 (0.001)*	0.12 (0.07)*	-0.03 (0.006)*
2007	0.01 (0.04)	-0.05 (0.009)*	0.01 (0.04)	-0.05 (0.009)*	0.006 (0.18)	N/A
Mean Across Leks	0.04 (0.005)*	-0.02 (0.003)*	0.05 (0.007)*	-0.03 (0.004)*	0.03 (0.007)	-0.03 (0.005)*
Global Alberta Average	0.02 (0.002)	-0.04 (0.002)*	0.03 (0.002)*	-0.006 (0.001)*	0.04 (0.004)	-0.04 (0.002)*

Table 3-5. Comparison of genetic diversity between grouse studies using average heterozygosity for all loci and for the subset of loci used in common with this study. Averages are given for single regions/populations/leks and ranges are given if multiple regions/populations/leks were studied. Number of common loci between studies is given in parentheses.

Species/Study	Location/ Heterozygosity type	Average *H* for study	Average *H* for common loci in study species	Average *H* for common loci in Alberta Sage-Grouse
Sage-Grouse, Oyler-McCance et al. (2005)	Range-wide, H_E	0.29 – 0.86	0.45 – 0.75 (4)	0.75 (4)
Sage-Grouse, Semple et al. (2001)	California, H_O	0.64	0.62 (1)	0.75 (1)
Sage-Grouse, Gibson et al. (2005)	California (two time periods), H_O	0.49 – 0.53	0.59 – 0.64 (3)	0.74 (3)
Black Grouse, Caizergues et al. (2003a)	Alps, H_O	0.74	0.73 (6)	0.68 (6)
	Finland, H_O	0.75	0.79 (6)	0.68 (6)
Black Grouse, Lebigre et al. (2007)	Finland, H_O	0.73	0.79 (5)	0.72 (5)
Rock Ptarmigan, Caizergues et al. (2003b)	Norway, H_O	0.81	0.86 (2)	0.73 (2)
	Pyrenees, H_O	0.64	0.58 (2)	0.73 (2)
	Alps, H_O	0.84	0.86 (2)	0.73 (2)
Lesser Prairie Chicken, Bouzat and Johnson (2004)	New Mexican leks, H_O	0.53 – 0.55	0.60 – 0.89 (1)	0.66 – 0.83 (1)
Greater Prairie Chicken, Bouzat et al. (1998)	Illinois, Kansas, Minnesota, and Nebraska, H_O	0.57 – 0.65	0.75 – 0.89 (1)	0.72 (1)

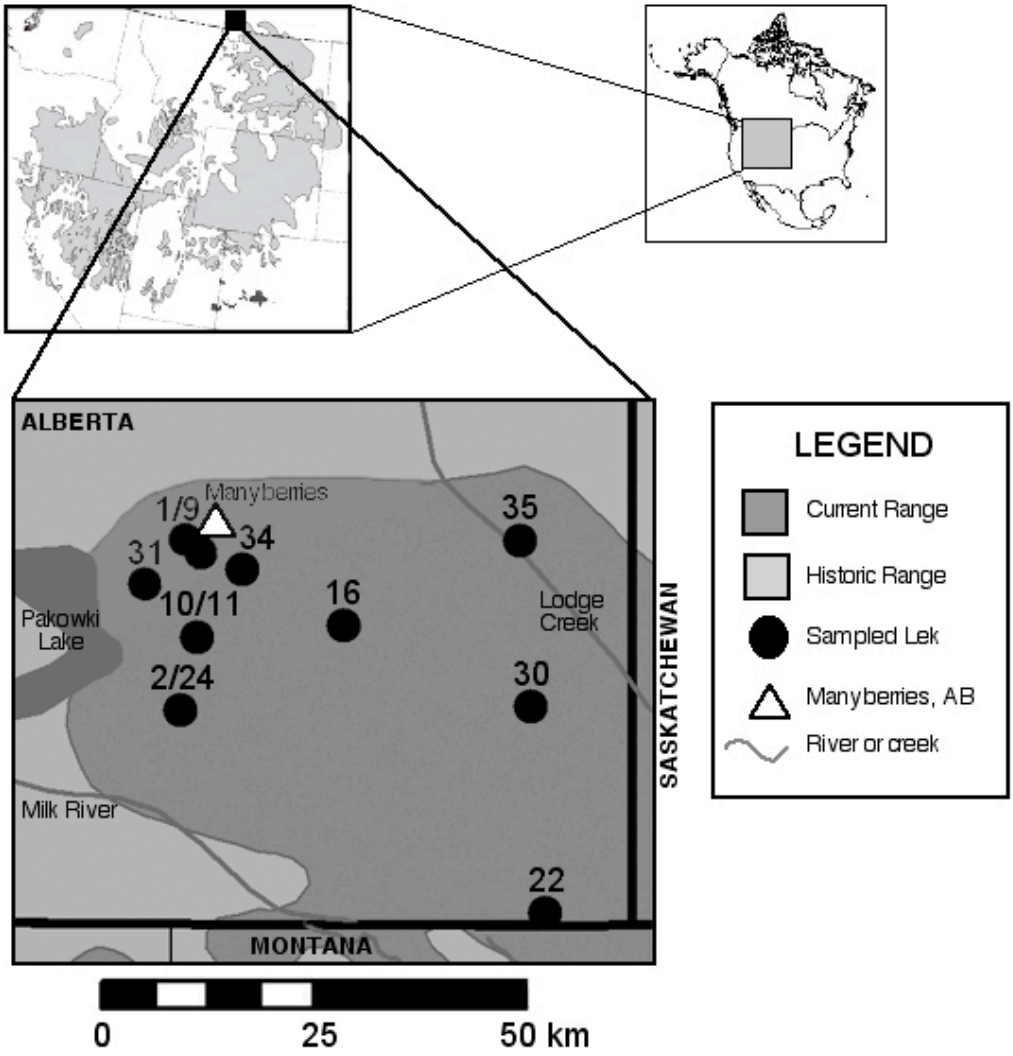

Figure 3-1. Map of the study area in Alberta, Canada with sampled Sage-Grouse leks highlighted.

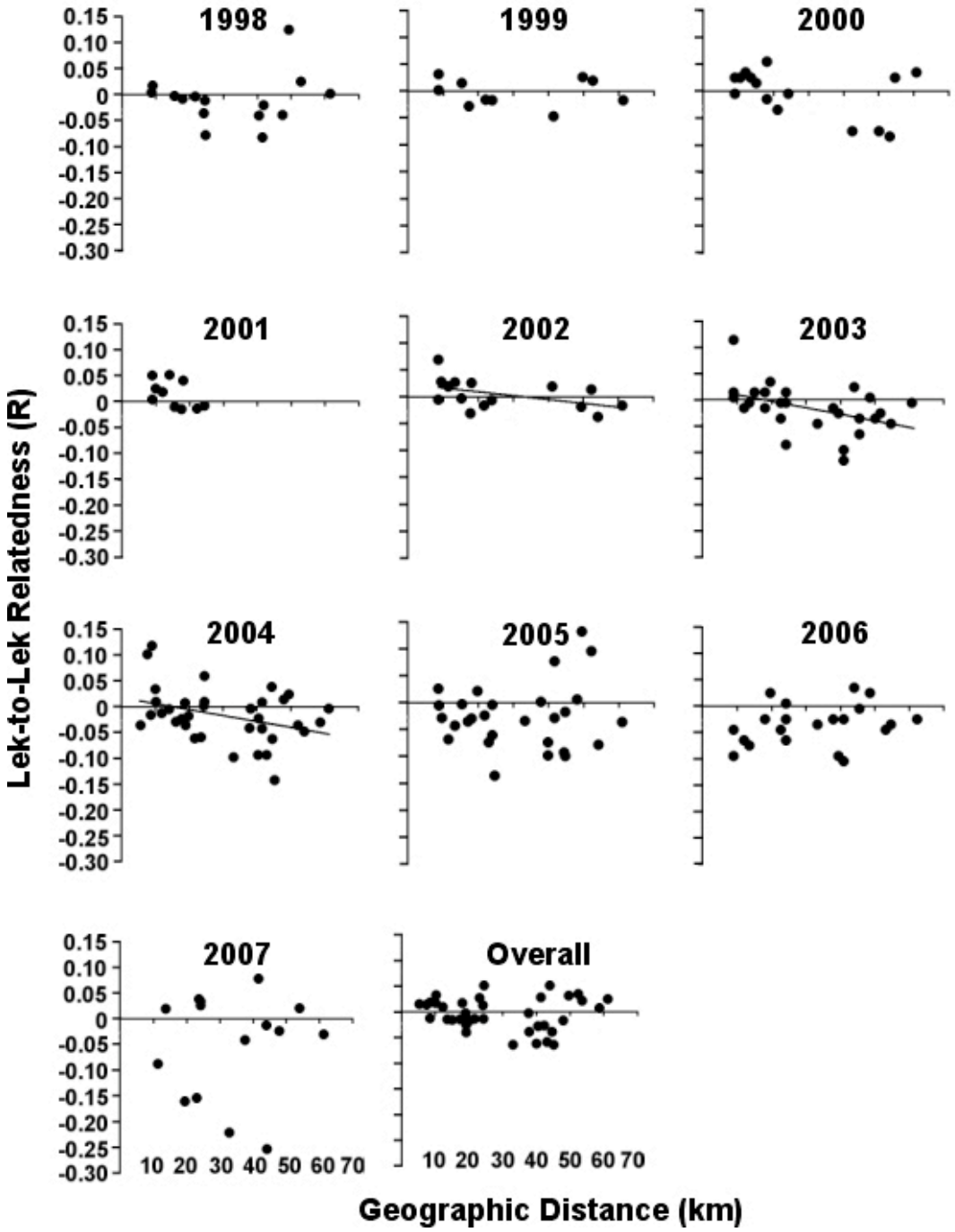

Figure 3-2. Average lek-to-lek relatedness plotted versus geographic distance between Sage-Grouse leks in Alberta for each year of the study (1998 – 2007) and overall.

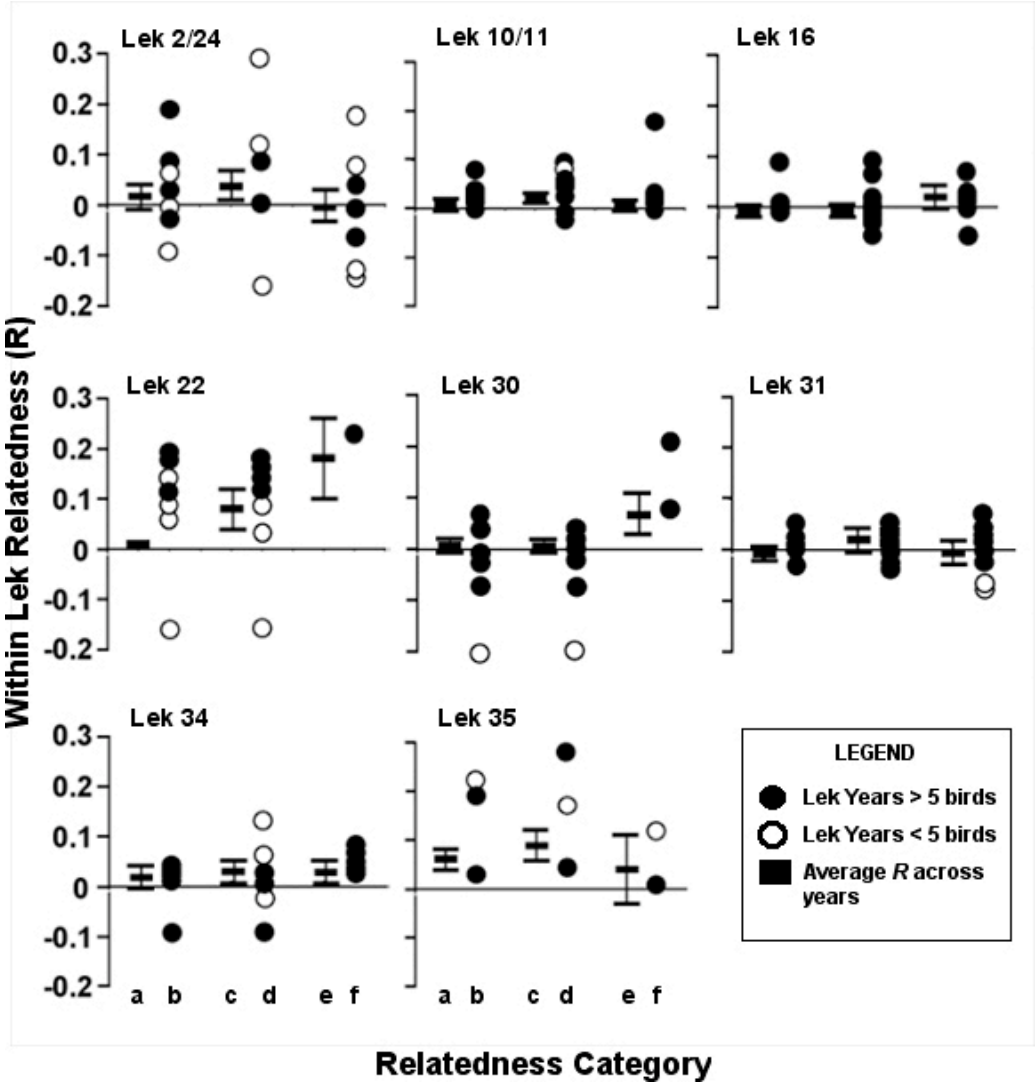

Figure 3-3. Average and annual within-lek relatedness for Alberta Sage-Grouse leks from 1998 - 2007. Relatedness within each lek is presented as (a) average $R \pm$ SE for both sexes combined across all years, (b) average R for both sexes combined for each year with greater than two individuals sampled, (c) male average $R \pm$ SE across all years, (d) male average R for each year with greater than two individuals sampled, (e) female average $R \pm$ SE across all years, and (f) female average R for each year with greater than two individuals sampled.

CHAPTER 4

The Secret Sex Lives of Sage-Grouse: Multiple Paternity, Reduced Variance in Male Mating Success, and Intraspecific Nest Parasitism Revealed Through Genetic Analysis[3]

1. Introduction

Variance in reproductive success is predicted to be greater in males than females for species exhibiting polygyny because female reproductive success is limited by resource availability while male success is only limited by partner availability (Bateman 1948). In polygynous mating systems, such as the lekking system (where males congregate on communal display grounds and females only visit to mate and then raise the young on their own), female choice is relatively unconstrained (Wiley 1973; Gibson and Bradbury 1986; Gibson et al. 1991) resulting in greatly skewed male mating-success (Wiley 1973; Borgia 1985; Alatalo and Lundberg 1986; Wiley 1991; Höglund and Alatalo 1995; Alberts et al. 2003; Say et al. 2003; Reynolds et al. 2007). Variance in male reproductive success greatly influences the opportunity for sexual selection (Wade 1979; Wade and Arnold 1980) and effective population size (N_e; Wright 1938; Nunney 1993) and thereby has important implications for genetic drift and the evolutionary dynamics of taxa characterized by polygynous mating systems. However, patterns of genetic paternity often differ from behavioral observations of male mating success, revealing different intensities of sexual selection (Jones et al. 2001; Whittingham and Dunn 2005) and offering new insights into mating systems (Lanctot et al. 1997; Gemmell et al. 2001). Multiple factors can affect the accuracy of paternity assessment based on field observations of lekking species, including incomplete coverage of known breeding sites in time or space, the existence of unknown breeding sites, or undocumented matings away from

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breeding sites (Wilmer et al. 1999; Gemmell et al. 2001; Semple et al. 2001). Missing behavioral data should especially make alternative male mating strategies and multiple mating by females difficult to document.

Multiple paternity within clutches or litters is expected to be rare in all lekking species because females are believed to mate only once during a breeding season (Wiley 1973; Alatalo et al. 1996). However, polyandry (females mating with multiple males) has been found using genetic and behavioral methods in several lekking species: black grouse (*Tetrao tetrix*; Lebigre et al. 2007), buff-breasted sandpiper (*Tryngits subruficollis*; Lanctot et al. 1997), cock-of-the-rock (*Rupicola rupicola*; Trail 1985), great snipe (*Gallinago media*; Fiske and Kålås 1995), peafowl (*Pavo cristatus*; Petrie et al. 1992), ruff (*Philomachus pugnax*; Lank et al. 2002), and greater sage-grouse (*Centrocercus urophasianus*; Semple et al. 2001). Polyandry is believed to provide genetic benefits to both mother and offspring, such as improving the likelihood that a female will acquire "good" genes for her offspring, increasing the genetic diversity among a female's offspring, and assuring eggs are fertilized if some males have poor quality sperm (Kempenaers et al. 1992; Wagner 1992; Yasui 1998). But because there are also costs associated with polyandry, such as increased energy expended on travel or elevated predation risk (Gibson and Bachman 1992), females are expected to mate with multiple males only when benefits outweigh costs.

Greater sage-grouse (hereafter sage-grouse) are a good model for studying variance in reproductive success and mating patterns in lekking species because they have highly skewed observed mating success among males, are well studied, and are easy to sample. Researchers have found that only a few males perform the majority of copulations on individual leks (e.g., Wiley 1973). Females visit one or more leks on several consecutive mornings and copulate only once with a single male (Wiley 1973; Gibson et al. 1991). However, males around the edges of leks also display to females and can follow females off-lek (Gibson 1996). Males have been reported to display to females away from leks (Dunn and Braun 1985) and yearling males (males hatched the previous spring that are physiologically capable of reproducing, but are assumed not to breed based on reduced testis size; Eng

1963) can walk or fly onto leks accompanying one or more females (K. L. Bush, unpubl. data). Furthermore, most visiting females are never observed to mate even on intensively monitored leks (Semple et al. 2001). Therefore, the breeding system in sage-grouse may be more complex than previously thought. Consistent with this idea, a small-scale paternity study on sage-grouse in California found only 40% of broods were fathered by territorial males from focal leks, while 40% were fathered by males from other leks or males off-lek, and 20% of broods exhibited multiple paternity (Semple et al. 2001). This study by Semple and colleagues examined only 10 broods, making it necessary to assess the generality of these results with a larger study conducted in a different geographic region.

I used polymorphic microsatellites to study the mating system and parentage of sage-grouse in Alberta and address two main questions. First, do a limited number of sage-grouse males father the majority of offspring in a given year as predicted by most behavioral studies (e.g. Wiley 1973) or is paternity more evenly distributed across the population? Second, do sage-grouse broods exhibit multiple paternity? I anticipated some variance in male mating success, as sage-grouse are a highly sexually dimorphic species where copulation rates on leks are known to be highly skewed among males (Wiley 1973). However, Semple et al. (2001) found a greater spread in paternity than suggested by previous behavioural studies. I also expected to document cases of multiple paternity within broods because the pattern has been reported in most lekking species studied using genetic methods (Lanctot et al. 1997; Semple et al. 2001; Lank et al. 2002; Lebigre et al. 2007).

2. Methods

2.1. Study location and sample collection

This study was conducted on sage-grouse from multiple leks in southeastern Alberta, Canada near Manyberries (Fig 4-1; 4,000 km^2; Aldridge and Brigham 2003). Birds of both sexes were captured using walk-in funnel traps (Schroeder and Braun 1991), night lighting (Giesen et al. 1982), and drop-nets (Bush 2008) during the lekking season (mid-March to mid-May). Blood, feather,

and mouth swab samples were collected from captured adult sage-grouse between 1998-2006. Vehicular and predator mortalities were opportunistically sampled and molted feather were collected on leks from 2003-2007. All captured birds were aged using the Eng (1955) procedure. "Juveniles" were young hatched in the study year, "yearlings" were birds entering their first breeding season, and "adults" were birds entering their second or subsequent breeding seasons (Dalke et al. 1963). Captured females were fitted with radiotransmitters (Aldridge and Brigham 2002) to locate nests. Females were located every other day (Aldridge and Brigham 2002) to determine the date of nest initiation and hatch/abandonment/predation. Clutches were sampled after hatching, predation, or abandonment as hatched eggshells, predated eggshells, intact eggs, and dead chicks. Collected clutches contained 1 - 14 eggs. Eggs were stored in zip-lock bags with desiccant at room temperature until DNA could be extracted (Bush et al. 2005). For hatched eggs, only membranes from the egg bottom (the pointed end of the egg) were sampled. For predated eggs, either membranes around the entry point (intact eggs) or all large fragments (broken and crushed shells) were sampled to ensure all individuals were detected (Bush et al. 2005). I use the term "offspring" for samples from all eggs and chicks regardless of hatching success and "successful offspring" for chicks that hatched. Survivorship after hatch was not known for the majority of chicks. In total, I collected 1,422 adult samples (327 from blood, plucked feathers, mouth swabs, and road kills and 1,095 from molted feathers); 1,391 of these samples were from the nine known active leks in Alberta and 31 samples were collected off-lek). I collected 1,420 offspring samples (from 95 known mothers and nine unknown mothers) from 191 broods. Annual lek counts (maximum number of males counted on a lek in a morning) ranged from 1 – 35 during the study with average lek count of 11.6 males (lek 1/9 = 3.3, lek 2/24 = 5.8, lek 10/11 = 8.5, lek 16 = 27.4, lek 22 = 10.1, lek 30 = 18.5, lek 31 = 16.6, lek 34 = 8.6, lek 35 = 5.8).

Visits to leks by radio-tracked females between capture and start of incubation were documented by monitoring four focal leks (10/11, 16, 31, and 34; Fig. 4-1) every morning during the lekking period. This method did not detect all

lek visits because some may have occurred in the evening, focal leks were not monitored daily, and not all nine active leks were monitored yearly.

2.2. Microsatellite genotyping

DNA was extracted using Qiagen DNeasy® Tissue and QIAamp® DNA Micro kits and samples were DNA sexed using methods described by Bush et al. (2005). Thirteen microsatellite loci developed from sage-grouse (SGCA9-2 [redesigned primer set; S. Taylor, personal communication] and SGCA5; Taylor et al. 2003), capercaillie (*Tetrao urogallus*; TUT3, TUT4, TUD1, and TUD3; Segelbacher et al. 2000), black grouse (BG6 and BG15; Piertney and Höglund 2001; TTD6 and TTT1; Caizergues et al. 2001; TTT3; Caizergues et al. 2003), red grouse (*Lagopus lagopus*; LLSD8; Piertney and Dallas 1997), and domestic chicken (*Gallus gallus*; ADL230; Cheng et al. 1994) were used as described in Bush et al. (2005). Forward primers were fluorescently labeled with 6-FAM, TET, and HEX (Applied Biosystems). I followed PCR cycling conditions outlined for each microsatellite in the original publications using Perkin Elmer Cetus GeneAmp PCR System 9600® and Eppendorf Mastercycler® ep machines. All non-invasive samples were run in triplicate (modified multiple tubes approach) as outlined in Bush et al. (2005). PCR products were visualized using ABI 377® and ABI 3730® automated sequencers with GENESCAN ANALYSIS3.1®, GENOTYPER®2.0, and GeneMapper 4.0® software (Applied Biosystems).

2.3. Duplicate samples

I determined DNA quality of each non-invasive sample by amplifying five microsatellites (TUT3, TUT4, SGCA5, SGCA9-2, and TTD6) once and assessing peak height (amplification strength) and peak quality (presence/absence and amplitude of stutter peaks) on GENESCAN ANALYSIS electropherograms. Each sample was then assigned as high (high peaks with no stutter), medium (medium height peaks with little to no stutter), or low quality (short peaks and/or those exhibiting stutter) and triplicate PCR replicates were performed with 3, 4, and 5μl DNA, respectively. Duplicate samples were identified using GENALEX version 5.1

(Peakall & Smouse 2001). For all non-invasive samples, the triplicate runs were first compared to one another. If the genotype for a given microsatellite was the same in all three runs, that genotype was retained. If inconsistent genotypes were found (different alleles in different runs), data were considered missing for that locus in all analyses. This approach decreased likelihood of allelic dropout and limited error. Two samples were considered duplicates if they were identical for all loci or differed by no more than one allele at 1 - 2 loci in a manner consistent with allelic drop out. Identification of genotyping errors was performed in MICRO-CHECKER (Van Oosterhout et al. 2004) prior to and post duplicate identification. Probability of identity (PI; the probability of observing two copies of any genetic profile in the population) was calculated in GENALEX.

2.4. Paternity analysis

I tested for deviations from Hardy-Weinberg and linkage equilibrium in GENEPOP, version 3.4 (Raymond and Rousset 1995). After correction for multiple tests (Sokal and Rohlf 1995), no leks were in Hardy-Weinberg or linkage disequilibrium. I tested whether all offspring from all broods matched their putative mothers at all loci by comparing each offspring's genotype with the nesting female's genotype. Errors between mothers and offspring were reduced by genotyping a mother and all of her offspring in the same run and by running females independent of offspring (to ensure female genotypes matched between both runs). Offspring matching a female at 12 or 13 of all 13 loci were considered to belong to the putative mother. All offspring with ≥ 5 mismatches, as there were no offspring with 2 - 4 mismatches, were deemed to be the product of intraspecific nest parasitism (a female laying an egg(s) in a nest incubated by another bird).

I attempted to determine the paternity of all offspring using CERVUS 3.0 (Marshall et al. 1998; Kalinowski et al. 2007). Based on patterns of lek attendance by mothers during the breeding season, all offspring were assigned to no lek (mother never detected on a lek), one, or two leks. All males were assigned to the lek(s) on which they were sampled and assigned years (1999 – 2006) in which

they were capable of reproducing (yearling and older). If males were not known to have died, they were assumed to be capable of fathering offspring until the end of the study (2006). Paternity analyses were then done in a step-wise manner. Offspring from a particular year and lek were tested against (1) all males of reproductive age alive in that year at that lek, (2) all males of reproductive age alive in that year at all leks, and (3) all males of reproductive age alive in that year from all leks and all hatched male offspring that would be at least a yearling in that year. No offspring assigning to a male at > 80% confidence in steps one or two assigned to another male at a greater confidence at a downstream step so only unassigned offspring were carried onto the next step. The allele frequencies for each locus were calculated using the genotypes of all mothers and males potentially alive in a given year for steps 1, 2, and 3. Simulations were performed with 25 000 cycles, 99.0% of loci typed, with an error rate of 1.0% (see Results) to derive a delta value (value that estimates the critical differences between the LOD [natural logarithm of the likelihood ratio scores] between the first and second most likely candidate fathers) for the assignment of paternity at > 95% and > 80% confidence. Field observations based on lek counts suggested that between 20% (1999 – 2002) and 90% (2005 – 2006) of known males were sampled genetically so the proportion of candidate males sampled in the simulations was set to 50% to account for low male detection and the possibility of both unknown leks and off-lek mating. Paternal assignments were accepted if there was either zero or one mismatch between the genotypes of the candidate male and the offspring (given the mother's genotype) and a significant ΔLOD.

Offspring of unknown paternity were assumed to have an unsampled father. I addressed the existence of unsampled fathers in two ways. (1) The genotypes of unsampled males that fathered ≥ 4 offspring in a brood (see below for multiple paternity detection methods) were reconstructed by deducing the paternally derived alleles. In all cases where only one paternally-derived allele was detected in the offspring at a locus, the male was assumed to be a homozygote. This introduced potential error (i.e., the male could be a heterozygote), but it provided more information than excluding homozygous loci

for reconstructed males. The reconstructed paternal genotypes were then compared against one another in GENALEX to see if any unsampled fathers sired more than one brood and if any of the reconstructed genotypes closely matched sampled males (at 11 – 13 of 13 loci). (2) I used COLONY 2.0 (Wang 2008) to identify full-sib families in clutches of unknown paternity. I used the polygamous setting for both sexes, provided data on known maternity and maternal full siblings, and provided two levels of information on potential fathers. First, I included only sampled males to verify CERVUS paternity assignments and to identify multiple clutches fathered by single unsampled males. Separate analyses were performed on (1) each individual female including all clutches and (2) year across leks in COLONY. Second, I included both sampled and unsampled males with reconstructed genotypes to identify clutches with multiple fathers and males that fathered more than one clutch. Once all fathers were determined, I calculated mean annual and overall paternity success (total number of offspring fathered in a given year/number of males) for all males (fathers and non-fathers), sampled fathers, and all fathers (sampled and unsampled males).

I used a combination of three methods to determine multiple paternity. First, I counted the paternally-derived alleles in each clutch with a genotyped mother to identify single (≤ 2 paternal alleles at each locus) and multiple (> 2 paternal alleles at ≥ one locus) paternity. Second, I used CERVUS to identify clutches that had one or more fathers. Finally, I used COLONY to determine whether clutches with unsampled fathers displayed evidence of single or multiple paternity. All clutches with ≤ 3 offspring (n = 26) were conservatively assumed to have one father because multiple paternity could not be accurately assessed and none of these clutches were identified as having more than one father using any of the three methods. These small clutches were the result of a very small complete clutch (n = 1), nest predation at an early stage of egg laying (n = 19), or incomplete sampling of some clutches in 1999-2001 (n = 6).

2.5. Opportunity for selection

I used the opportunity for selection (I = variance/mean^2; Wade and Arnold 1980) to standardize the variance in reproductive success and facilitate comparison between different samples and studies. The upper limit to the strength of directional sexual selection on sexually selected traits is represented by I (Wade and Arnold 1980) and was calculated for both males (I_M) and females (I_F). I calculated I for both sexes across Alberta in each year to assess inter-annual variation. I_F was calculated using only successful breeders, whereas I_M was calculated based on variably inclusive data subsets: (1) using both successful (i.e., sampled and unsampled fathers) and unsuccessful males (I_{M1}), (2) using only sampled fathers and unsuccessful males (I_{M2}), (3) using only successful males; I_{M3}, and (4) using only sampled fathers; I_{M4}. The first two measures provide a range in I_M within which the actual I_M may fall, as the number of unsampled fathers may overestimate the number of males fathering single broods. The last two measures facilitate comparison between males and females, as all females alive during a breeding season laid at least one clutch of eggs and were therefore classified as successful. I quantified success for both sexes (and the respective male subsets) at the annual level in three ways: (1) number of clutches (I_{MC} or I_{FC}), (2) number of offspring (I_{MO} or I_{FO}), and (3) number of successful (hatched) offspring (I_{MH} or I_{FH}).

3. Results

3.1. Duplicate samples

Of the 1095 molted feather samples, 1093 (99.8%) contained enough DNA to amplify 7 – 13 loci in triplicate. Rates of missing data were homogeneous across lek, year, sex, and sample type (i.e. one locus did not fail to amplify for an entire year or in all molted feathers). For all samples that failed to produce the same genotype in two of three replicates, the genotype for that locus was excluded and only genotypes that were identical in three of three replicates were used to identify duplicates. I found low rates of drop out (3.1% occurring in low quality samples) and no false alleles. Of the 1422 adult samples, 604 were unique and

82% of these samples were genotyped at all 13 loci. Probability of identity (PI) for non-relatives and siblings of 0.001 was achieved at four and seven loci respectively.

All 1420 offspring samples contained enough DNA to successfully amplify 7 – 13 loci and 1208 samples were unique. Only predated eggshell fragments resulted in duplicates of one another. I minimized the error rate of offspring samples by running them with their mothers, resulting in a drop out rate near zero. Combined with the higher drop out rate for molted feathers, I set a universal error rate of 1.0% for all analyses requiring a rate.

3.2. Paternity analysis

I found all offspring matched their putative mothers with the exception of 26 eggs found in 10 clutches that were the result of intraspecific nest parasitism ("egg dumping"). There were up to six dumped eggs per clutch (Fig. 4-2) with six (60.0%) parasitized clutches containing more than two non-maternal eggs.

Paternity was assigned to 443 sage-grouse offspring (36.7%) of known maternity (Table 4-1) at 80% confidence, and of these, 175 could be assigned at 95% confidence. Thirty-six sampled males were identified as fathers (24 captured males, 10 males sampled via molted feathers, and two males sampled as offspring in previous years; age 2 and 3 when they fathered offspring; Table 4-1). These 36 males fathered completely, or in part, 63 (33.2%) of the sampled clutches. Unsampled males fathered the remaining clutches (n = 127, 66.8%). The most clutches that any given male fathered during the course of the study was seven (one male) over three years and the most fathered in a given year was three (n = 5 males). Nine unsampled males fathered more than one clutch. None of the known males that fathered offspring was a yearling. Of the 34 males with known lek affiliations, nine sired offspring of females never observed attending their lek. In two of these instances, females were observed on the closest neighboring lek to where males were displaying, but in one case, the female was only observed on a lek 54 km away. Thirteen of 14 males that fathered multiple clutches mated with females that were observed to attend the leks on which these males displayed.

Across all years individual paternity success, in terms of number of offspring produced, ranged from zero to 44, with a maximum of 24 offspring fathered by an individual male (sampled or unsampled) in a single year (Appendix 4-1). Percentage of genetically identified males in the population fathering offspring in a given year ranged from 14.3 to 54.5%, with an overall average of 45.9% (Table 4-1). Of the 191 clutches, 169 (88.5%) had a single father, 13 (6.8%) had two fathers, seven (3.7%) were a mix of eggs belonging to the putative mother with a single father and dumped eggs (single paternity in both clutches), one (0.5%) had two fathers of different species (sage-grouse and sharp-tailed grouse [*Tympanuchus phasianellus jamesi*]), and one (0.5%) was a mix of eggs belonging to the putative mother with two fathers and dumped eggs with single paternity (Fig. 4-3). One hundred and thirty offspring (10.8%) came from clutches with multiple fathers. In clutches with two fathers, paternity by individual males ranged from 11 to 89% (Fig. 4-4).

Of the 1206 eggs, 574 (47.6%) successfully hatched. Unhatched eggs resulted from nest predation or weather-induced nest abandonment. One-hundred and four females laid 191 clutches. Each female produced between one and six clutches with a maximum of 44 offspring and 32 successful offspring (Appendix 4-2). Twenty-four females laid two sampled clutches in a single year, with most of these clutches fathered by two different males (Fig. 4-5). Thirty-seven females laid two or more clutches over their sampled lifetime, with most of these clutches singly fathered by different males (Fig. 4-6).

3.3. Opportunity for selection

The opportunity for selection among all males measured by successful offspring (I_{M1H}) was approximately twice that measured by clutch and offspring (Table 4-2). A similar trend was seen for known males (I_{M2}), with the exception of 2003 – 2006 for successful offspring (Table 4-2). Years of low mean reproduction and high variance (poor production years where only a few males were successful) produced the highest I_M. There were few eggs laid in 2000 and therefore very few males were fathers relative to the number of known, living males. This produced a

high I_M, so inter-annual means were presented with and without 2000 (Table 4-2). Among successful breeders only, I_F was lower than I_{M3} and I_{M4} because females generally had higher mean reproduction and lower variance than males (Table 4-3).

4. Discussion

I examined eight years of paternity data and found that sage-grouse clutches in Alberta primarily exhibited single paternity, with instances of multiple paternity, hybridization, and intraspecific nest parasitism. Most males fathered only one sampled clutch and very few fathered multiple clutches (two or three) in a given year. The opportunity for selection was higher among males than females.

4.1. Paternity analysis

4.1.1. Intraspecific nest parasitism

This study revealed the first evidence of intraspecific nest parasitism in sage-grouse. Ten of 104 (9.6%) females had their nests parasitized by other sage-grouse females suggesting that this is not a rare phenomenon in Alberta. Intraspecific nest parasitism has been reported in three other grouse species; sharp-tailed grouse (Gratson 1989), willow ptarmigan (*Lagopus lagopus*; Martin 1984; Filchagov 1996), and capercaillie (Storch and Segelbacher 2005). No female's nest was the site of egg dumping more than once during her sampled lifetime, but 60% of clutches with dumped eggs contained greater than two non-maternal eggs suggesting that most "egg dumpers" put multiple eggs into one parasitized nest instead of many nests. This is likely because it is difficult to find sage-grouse nests at the low population density observed in Alberta. It is unknown whether parasitic females solely parasitize nests or if they lay nests of their own because none of the reconstructed genotypes of parasitic females matched any known females.

4.1.2. Paternity

No male in Alberta fathered more than three clutches in a given year and approximately half of the sampled male population successfully reproduced during the study period. Percentage of males fathering offspring ranged from 14.3% - 54.5% annually, with an average of 45.9% across years supporting the case that a large proportion of the sampled males in Alberta successfully bred and that a few males were not responsible for the majority of matings on each lek. Only 14 males fathered more than one clutch within years and more than one clutch (between two to seven) across years. None of these males fathered more than one clutch two years in a row. This suggests that either male quality (i.e., secondary sexual characteristics, display, level of disease, etc.) or female preference for male traits varied between years.

The large number of successful fathers has important implications for the genetic health of the population and its effective size (N_e). Polygynous mating systems affect N_e by reducing the number of breeding males and by skewing the proportional representation of male ancestors in the gene pool of future generations (Wright 1931; Kimura and Crow 1963; Leberg 2005). Variance in reproductive success and the sex ratio of breeding adults also primarily determines the rate of genetic drift when populations maintain a constant size (Wright 1938; Nunney1993). Therefore, an increase in the proportion of males breeding in a population decreases variance in breeding success, which ultimately increases N_e (Frankham 1995). Increased values of N_e have positive ramifications for the genetic diversity and sustainability of sage-grouse in Alberta because larger effective population size reduces the potential for inbreeding. More birds are breeding than predicted for a typical lekking system with highly skewed mating success (Wiley 1973; Höglund and Alatalo 1995), which could reflect the use of alternative mating strategies by both sexes.

My data support the idea that inter-lek movement of females and off-lek mating occur in Alberta. Nine males mated with females not documented to attend their lek suggesting that more inter-lek movement is taking place in Alberta than detected based on telemetry (Aldridge 2005). Despite extensive sampling, I

also found that many chicks were fathered by unsampled males. While I did not sample every male on every lek, in 2005 and 2006, I intensively sampled molted feathers and genetically identified 53 (2005) and 43 (2006) more males than were enumerated during lek counts (based on the maximum number of males attending a lek on a single morning during the lekking season; K.L. Bush, unpubl. data; Alberta Fish and Wildlife, unpubl. data). This, and intensive sampling of leks throughout the lekking season, suggests that I did genetically sample most lekking males on my focal leks in the last two years of the study and that some males apparently did not attend leks regularly throughout the breeding season. Also, despite intensive sampling in 2005 and 2006, I still had 17 unsampled fathers in 2005 and seven in 2006. Possible explanations for this result are that some males do not attend leks frequently, do not attend leks at all and mate strictly off lek, or that there are multiple unknown leks in Alberta. Mating by females on alternative leks (leks other than the one(s) females were known to attend) or off-lek may explain why 40% of clutches in the Semple et al. (2001) study on sage-grouse in California and 10% of the clutches in the Lebigre et al. (2007) study on black grouse had unsampled fathers. Off-lek mating could be an alternative mating strategy for males that either cannot obtain territories or copulations on traditional lek sites. Some females may prefer these off-lek encounters due to decreased intra-sexual competition for males and reduced harassment by males, suggesting that alternative mating strategies may be driven by both male and female behaviour.

Most broods had single paternity, but 15 broods (7.9%) exhibited multiple paternity. This level of multiple paternity was lower than the 20% found by Semple et al. (2001) for sage-grouse in California, but they only sampled 10 broods across three years, performed the study at the opposite end of the species' range (southwest versus my study site at the northeast periphery), and were working with a small and isolated population. My annual multiple paternity levels ranged from 0% (1999 and 2000) to 16.7% (2004) revealing that levels vary between years. I also had 51 single fathered clutches with four or fewer eggs, leaving the possibility that I could not detect all cases of multiple paternity in

Alberta. Multiple mating may represent a bet-hedging strategy wherein females mate with several males to lower the probability of producing offspring with males that are genetically incompatible, inferior, or infertile (Fedorka and Mousseau 2002). However, multiple mating does not necessarily translate to multiple paternity. A genetic study on black grouse revealed that only 25% of females observed to mate with more than one male and 9% of females that mated more than once due to disrupted copulations had clutches with multiple paternity (Lebigre et al. 2007). This suggests that multiple mating occurs much more frequently than multiple paternity and that either some males have low fertility or that female grouse utilize some form of post-copulation mechanism, such as sperm competition (Birkhead 1998) or sperm choice (Birkhead et al. 2004), to prevent insemination from all partners. Since fertility of clutches was high across all years (99.2%) in Alberta, sperm competition may be a more likely mechanism in sage-grouse, as females did not need to adjust their behaviour to account for possible male infertility. Semple et al. (2001) also suggested that multiple paternity may occur more commonly in second clutches (re-nesting attempts due to the destruction of the first nest) if the female mated with different males in her first and second breeding attempts. I found no evidence of this scenario, as all cases of multiple paternity in the second clutch involved two different males from the father of the first nest. Levels of multiple paternity were also not elevated in second nests (n = 2) compared to first nests (n = 13) for 24 females that laid two nests in a single season and had multiple paternity in at least one nest. Taken together, multiple paternity in sage-grouse is likely due to a higher instance of multiple mating with sperm competition and not the result of re-mating after nest loss.

4.2. Opportunity for selection

I found that the opportunity for selection was higher among males than females and was generally highest when measured in terms of successful (hatched) offspring. I calculated I_M for behavioral studies that published raw copulation data (how many times every male on a lek(s) bred over a season) for

sage-grouse across the species' range and compared these values to my clutch values (Table 4-2). Annual I_M was high for large leks in southeastern Wyoming (8.7 – 20.3; Wiley 1973), central Wyoming (6.8 – 16.2; G. L. Patricelli and A. H. Krakauer, unpubl. data) and Montana (6.0; Lumsden 1968; Table 4-4). I_M was considerably lower in California (3.0; R. M. Gibson, pers. comm.), Alberta (5.1; J. Carpenter, unpubl. data) and for Gunnison sage-grouse in Colorado (annual I_M = 2.9 – 9.9; J. Stiver, unpubl. data). Because I never sampled all males in the population, unsampled fathers were likely real fathers that infrequently attended leks, belonged to an unknown lek, or mated off-lek, therefore lower values of I_M (3.9; Table 4-2) are likely more accurate for Alberta. These lower values are consistent with field observations of mating behavior from the focal study leks in Alberta, where I_M ranged from 8.3 in 2005 to 1.9 in 2006 (J. Carpenter, unpubl. Data). If only 10 to 30% of the male population are expected to mate in a given year (Wiley 1973), I_M should be between 1.7 to 6.0 times greater than observed (Table 4-2) based on Alberta's population size. Because my results were comparable to the California study and consistent with field observations in Alberta, I_M may vary according to lek size or location within the species' range. Sage-grouse in California share attributes with Alberta's birds, such as small leks and peripheral location on the range, but differ greatly with regards to habitat, climate, and anthropogenic impacts. A higher opportunity for selection in Wyoming and Montana suggests that either larger leks (> 30 males) offered greater opportunity for selection or behavioral observations are not always comparable to genetic data. The idea that large leks create greater opportunity for selection is contrary to patterns uncovered by Kokko et al. (1999) in their study using behavioral data from 71 leks from various avian and mammalian species. They found that reproductive inequality decreases with increasing lek size. A trend in decreasing variance with increasing lek size was also observed by Widemo and Owens (1995) and Keller and Krieger (1996) in avian lekking species. Therefore, sage-grouse in Alberta differ in terms of opportunity for selection from sage-grouse in central parts of the range and have small leks with lower I_M.

When considering successful birds only (birds known to reproduce), I_F was lower than I_M and showed less variation between years. If I consider that fathers, including both sampled and unsampled males, are the most accurate representation of I_M, then I_M is approximately double I_F for all three categories (Table 4-3), suggesting that opportunity for selection is higher in males. This is consistent with theoretical predictions that polygyny in conjunction with high variance in male mating success results in intense sexual selection and dimorphism (Bateman 1948, Clutton-Brock and Vincent 1991, Arnold 1994, Shuster and Wade 2003).

How do sage-grouse compare in terms of variance in breeding success with other galliforms and lekking avian species? I compared I_M values for sage-grouse in Alberta to those for galliforms and lekking avian species. I used I_M to examine multiple taxa because it is a standardized measure that allows comparison between species with widely different fecundities (Wade and Arnold 1980). It is also a potential predictor of the position of a population in the monogamy – polygyny continuum of mating systems or gender-based monomorphism – dimorphism scale for sexually selected traits (Vanpé et al. 2008). Among galliform and lekking avian species for which breeding success has been assessed using genetic and/or field observations, sage-grouse from Alberta rank highly in I_M (Table 4-4). They display similar I_M to other lekking grouse; greater prairie-chicken (4.1; *Tympanuchus cupido*), capercaillie (3.2), and sharp-tailed grouse (2.4). Therefore, sage-grouse fall into the polygynous mating system range and possess sexually dimorphic sexually selected traits. The relatively high I_M (3.9) also suggests that there is the potential for strong sexual selection on sage-grouse males. However, while they have high I_M compared to other lekking birds, they have low I_M (both genetic and observational) compared to sage-grouse in central parts of the species' range, possibly due to lek size.

5. Conservation Implications

Despite their small numbers and restricted habitat, sage-grouse in Alberta are genetically diverse and do not exhibit evidence of inbreeding. I have

previously found that this is partly due to high levels of gene flow from other parts of the northern Montana population (the population to which they belong [Alberta, Saskatchewan, and northern Montana]; chapter 2), but this study provides further evidence for why the birds are genetically diverse. If sage-grouse leks in Alberta functioned as previously thought, with a small proportion of males obtaining most of the copulations (Wiley 1973), genetic drift would be accelerated due to a small effective population size. Instead, it appears a large subset of males breed at least once during their lifespan, with only a few males being more successful. This pattern causes genetic diversity to be lost at a slower rate from the population. Alternatively, because the habitat available in Alberta is so small, suboptimal, and naturally fragmentated, high rates of male influx and turnover may have led to reduced opportunity for polygyny. Sage-grouse in Alberta currently exhibit both gene flow and reduced variance in reproductive success, but if gene flow from the rest of the population stops or usable habitat is further reduced, sage-grouse will not be able to sustain current genetic diversity levels for long. Therefore, the landscape needs to be managed to maintain connectivity. Future research needs to determine where unsampled males breed (on-lek, off-lek, or unsampled/unknown leks). Leks are the primary focus of current sage-grouse conservation because they are the mating and nesting hubs for the species (Connelly et al. 2004), but if mating occurs off-lek and birds move great distances between leks to select a mate, a broader-based, less lek-centric approach to habitat conservation should be adopted.

6. Literature Cited

Alatalo RV, Lundberg A (1986) The sexy son hypothesis: data from the Pied Flycatcher *Ficedula hypoleuca*. Anim Behav 34: 1454–1462.

Alatalo RV, Höglund J, Lundberg A, Sutherland WJ (1992) Evolution of black grouse leks: female preferences benefit males in larger leks. Behav Ecol 3: 53-59.

Alatalo RV, Burke T, Dann J, et al. (1996) Paternity, copulation disturbance and female choice in lekking black grouse. Anim Behav 52: 861-873. Doi:10.1006/anbe.1996.0234

Alberts SC, Watts HE, Altmann J (2003) Queuing and queue-jumping: long-term patterns of reproductive skew in male savannah baboons, Papio cynocephalus. Anim Behav 65: 821–840. Doi:10.1006/anbe.2003.2106

Aldridge CL (2005) Identifying habitats for persistence of greater sage-grouse (*Centrocercus urophasianus*) in Alberta, Canada. Ph.D. dissertation, University of Alberta, Edmonton.

Aldridge CL, Brigham RM (2003) Distribution, abundance, and status of the greater sage-grouse, *Centrocercus urophasianus*, in Canada. Can Field-Nat 117: 25-34.

Aldridge CL, Brigham RM (2002) Sage-grouse nesting and brood habitat use in southern Canada. J Wildlife Manage 66: 433-444.

Andersson M (1989) Sexual selection and cues for female choice in leks of Jackson's widowbird *Euplectes jacksoni*. Behav Ecol Sociobiol 25: 89-97.

Arnold SJ (1994) Bateman's principles and the measurement of sexual selection in plants and animals. Am Nat 144: S126-S149.

Ballard WB, Robel RJ (1974) Reproductive importance of dominant male greater prairie chickens. Auk 91: 75-85.

Bateman AJ (1948) Intra-sexual selection in *Drosophila*. Heredity 2: 349-368.

Beehler B (1983) Lek behavior of the lesser bird of paradise. Auk 100: 992-995.

Benson DP (2002) Low extra-pair paternity in white-tailed ptarmigan. Condor 104: 192-197. Doi: 10.1650/0010-5422(2002)104[0192:LEPPIW]2.0.CO;2

Birkhead TR (1998) Sperm competition in birds. Rev Reprod 3: 123-129. Doi: 10.1530/ror.0.0030123

Birkhead TR, Chaline N, Biggins JD, Burke T, Pizzari T (2004) Nontransitivity of paternity in a bird. Evolution 58: 416-420. Doi: 10.1554/03-488

Borgia G (1985) Bower quality, number of decorations and mating success of male Satin Bowerbirds (*Ptilonorhynchus violaceus*): An experimental analysis. Anim Behav 33: 266–271.

Bush KL (2008) A pressure-operated drop net for capturing Greater Sage-Grouse. J Field Ornithol 79: 64-70. Doi: 10.1111/j.1557-9263.2008.00146.x

Bush KL, Vinsky MD, Aldridge CL, Paszkowski CA (2005) A comparison of sample types varying in invasiveness for use in DNA sex determination in an endangered population of greater sage-grouse (*Centrocercus urophasianus*). Conserv Genet 6: 867-870. Doi: 10.1007/s10592-005-9040-6

Caizergues A, Rätti O, Helle P, et al. (2003) Population genetic structure of male black grouse (*Tetrao tetrix* L.) in fragmented vs. continuous landscapes. Mol Ecol 12: 2297-2305. Doi: 10.1046/j.1365-294X.2003.01903.x

Caizergues A, Dubois S, Mondor G, Rasplus J-Y (2001) Isolation and charcterisation of microsatellite loci in black grouse (*Tetrao tetrix*). Mol Ecol Notes 1: 36-38. Doi: 10.1046/j.1471-8278.2000.00015.x

Cheng HH, Levin I, Vallejo RL, *et al*. (1995) Development of a genetic map of the chicken with high-utility markers. Poultry Sci 74: 1855-1874.

Clutton-Brock TH, Vincent ACJ (1991) Sexual selection and the potential reproductive rates of males and females. Nature 351: 58-60.

Connelly JW, Knick ST, Schroeder MA, Stiver SJ (2004) Conservation Assessment of Greater Sage-grouse and Sagebrush Habitats. Western Association of Fish and Wildlife Agencies. Unpublished Report. Cheyenne, Wyoming.

Dalke PD, Pyrah DB, Stanton DC, Crawford JE, Schlatterer EF (1963) Ecology, productivity, and management of sage grouse in Idaho. J Wildl Manage 27: 811-840.

Dunn PO, Braun CE (1985) Natal dispersal and lek fidelity of sage grouse. Auk 102: 621-627.

DuVal EH, Kempenaers B (2008) Sexual selection in a lekking bird: the relative opportunity for selection by female choice and male competition. P Roy Soc Lond B Bio 275: 1995–2003. Doi:10.1098/rspb.2008.0151

Eng RL (1955) A method for obtaining sage grouse age and sex ratios from wings. J Wildl Manage 19: 262-272.

Eng RL (1963) Observations on the breeding biology of male sage grouse. J Wildlife Manage 27: 841-846.

Fedorka KM, Mousseau, TA (2002) Material and genetic benefits of female multiple mating and polyandry. Anim Behav 64: 361-367. Doi: 10.1006/anbe.2002.3052

Filchagov AV (1996) Two clutches of willow grouse (*Lagopus lagopus*) in the same nest. Gibier Faune Sauvage, Game Wildl 13: 75-78.

Fiske P, Kålås JA (1985) Mate sampling and copulation behaviour of great snipe females. Anim Behav 49: 209-219.

Frankham R (1995) Conservation genetics. Annu. Rev. Genet. 29: 305–327.

Gemmell NJ, Burg TM, Boyd IL, Amos W (2001) Low reproductive success in territorial male Antarctic fur seals (*Arctocephalus gazella*) suggests the existence of alternative mating strategies. Mol Ecol 10: 451-460. Doi: 10.1046/j.1365-294X.2001.01186.x

Gibson RM (1996) A re-evaluation of hotspot settlement in lekking sage grouse. Anim Behav 52: 993-1005. Doi:10.1006/anbe.1996.0247

Gibson RM, Bachman GC (1992) The costs of female choice in a lekking bird. Behav Ecol 3: 300-309.

Gibson RM, Bradbury JW, Vehrencamp SL (1991) Mate choice in lekking sage grouse revisited: the roles of vocal display, female site fidelity and copying. Behav Ecol 2: 165-180.

Gibson RM, Bradbury JW (1986) Male and female mating strategies on sage grouse leks. In: *Ecological Aspects of Social Evolution* (eds Rubenstein DI, Wrangham RW), pp. 379-398, Princeton University Press, Princeton, NJ.

Gibson RM, Bradbury JW (1985) Sexual selection in lekking sage grouse: phenotypic correlates of male mating success. Behav Ecol Sociobiol 18: 117-123. Doi: 10.1007/BF00299040

Giesen KM, Schoenberg TJ, Braun CE (1982) Methods for trapping sage grouse in Colorado. Wildlife Soc B 10: 223-231.

Gratson MW (1989) Intraspecific nest parasitism by sharp-tailed grouse. Wilson Bull 101: 126-127.

Gratson MW, Gratson GK, Bergerud AT (1991) Male dominance and copulation disruption do not explain variance in male mating success on sharp-tailed grouse (*Tympanuchus phasianellus*) leks. Behaviour 118: 187-213.

Hannon SJ, Dobush G (1997) Pairing status of male willow ptarmigan: is polygyny costly to males? Anim Behav 53: 369-380. Doi:10.1006/anbe.1996.0328

Hill WL (1991) Correlates of male mating success in the ruff *Philomachus pugnax,* a lekking shorebird. Behav Ecol Sociobiol 29: 367-372. Doi: 10.1007/BF00165962

Höglund J, Lundberg A (1987) Sexual selection in a monomorphic lek-breeding bird: correlates of male mating success in the great snipe *Gallinago media*. Behav Ecol Sociobiol 21: 211-216. Doi: 10.1007/BF00292501

Höglund J, Alatalo RC (1995) Leks. Princeton University Press, Princeton, NJ.

Jenkins D, Watson A, Miller GR (1963) Population studies on red grouse, *Lagopus lagopus scoticus* (Lath.) in north-east Scotland. J Anim Ecol 32: 317-376.

Jones AG, Walker D, Kvarnemo C, Lindstrom K, Avise JC (2001) How cuckoldry can decrease the opportunity for sexual selection: Data and theory from a genetic parentage analysis of the Sand Goby, *Pomatoschistus minutus*. P Natl Acad Sci USA 98: 9151–9156.

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16: 1099-1106. Doi: 10.1111/j.1365-294X.2007.03089.x

Keller L, Krieger MJB (1996) Mating success of male birds. Nature 380: 208-209. Doi: 10.1038/380208a0

Kempenaers B, Verheyen GR, Van den Broeck M, et al. (1992) Extra-pair paternity results from female preference for high quality males in the blue tit. Nature 357: 494-496. Doi: 10.1038/357494a0

Kimura M, Crow JF (1963) The measurement of effective population number. Evolution 17: 279-288.

Kokko H, Mackenzie A, Reynolds JD, et al. (1999) Measures of inequality are not equal. Am Nat 72: 358-382. Doi: 10.1086/303235

Krakauer AH (2008) Sexual selection and the genetic mating system of wild turkeys. Condor 110: 1-12. Doi: 10.1525/cond.2008.110.1.1

Kruijt JP, de Vos GJ (1988) Individual variation in reproductive success in male black grouse, *Tetrao tetrix* L. Pages 279-290. In Clutton-Brock TH Ed. Reproductive Success. University of Chicago Press, Chicago.

Lanctot RB, Scribner KT, Kempenaers B, Weatherhead PJ (1997) Lekking without paradox in the buff-breasted sandpiper. Am Nat 149: 1051-1070. Doi: 10.1086/286038

Lank DB, Smith CM, Hanotte O, Ohtonen A, Bailey S, Burke T (2002) High frequency of polyandry in a lek mating system. Behav Ecol 13: 209-215.

Leberg P (2005) Genetic approaches for estimating the effective size of populations. J Wildlife Manage 69: 1385-1399. Doi: 10.2193/0022-541X(2005)69[1385:GAFETE]2.0.CO;2

Lebigre C, Alatalo RV, Siitari H, Parri S (2007) Restrictive mating by females on black grouse leks. Mol Ecol 16: 4380-4389. Doi: 10.1111/j.1365-294X.2007.03502.x

Lill A (1974) Sexual behavior of the lek-forming white-bearded manakin (*Manacus manacus trinitatis* Harteft). Z Tierz Zuchtungsbio 36: 1-36.

Lumsden HG (1968) The displays of the sage grouse. Ontario Department of Lands and Forests Research Report (Wildlife) no. 83.

Marshall TC, Slate J, Kruuk EB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7: 639-655. Doi: 10.1046/j.1365-294x.1998.00374.x

Martin K (1984) Intraspecific nest parasitism in willow ptarmigan. J Field Ornithol 55: 250-251.

McDonald DB (1989) Correlates of male mating success in a lekking bird with male-male cooperation. Anim Behav 37: 1007-1022.

Müller F (1979) A 15-year study of a capercaillie lek in the western Rhön-Mountains (W. Germany). In Behaviour of woodland grouse, woodland grouse symposium (Lovel TEI Ed). Pages 120-130. World Pheasant Association, Bures, Suffolk, England.

Nunney L (1993) The influence of mating system and overlapping generations on effective population size. Evolution 47: 1329–1341.

Payne R, Payne K (1977) Social organization and mating success in local song populations of village indigo birds, *Vidua chalybeata,* Z Tierz Zuchtungsbio 45: 113-173.

Peakall R, Smouse PE (2001) GENALEX version 5.1. Genetic analysis in Excel. Population genetic software for teaching and research. http://www.anu.edu.au/BoZo/GenAlEx. Australian National University, Canberra, Australia.

Petrie M, Hall M, Halliday T, Budget H, Pierpoint C (1992) Multiple mating in a lekking bird: why do peahens mate with more than one male and with the same male more than once? Behav Ecol Sociobiol 31: 52-58.

Petrie M, Halliday T, Sanders C (1991) Peahens prefer peacocks with elaborat traits. Anim Behav 41: 323-331.

Piertney SB, Höglund J (2001) Polymorphic microsatellite DNA markers in black grouse (*Tetrao tetrix*). Mol Ecol Notes 1: 303-304, Doi: 10.1046/j.1471-8278.2001.00118.x

Piertney SB, Dallas JF (1997) Isolation and characterization of hypervariable microsatellites in the red grouse. *Lagopus lagopus scoticus*. Mol Ecol 6: 93-95. Doi: 10.1046/j.1365-294X.1997.00154.x

Pruett-Jones SG (1988) Lekking versus solitary display: temporal variations in dispersion in the buff-breasted sandpiper. Anim Behav 36: 1740-1752.

Raymond M, Rousset F (1995) Genepop version 3.1d: population genetics software for exact test and ecumenism. J Hered 86: 248-249

Reynolds SM, Dryer K, Bollback J, Uy JAC, Patricelli GL, Robson T, Borgia G, Braun MJ (2007) Behavioral paternity predicts genetic paternity in satin bowerbirds (*Ptilonorhynchus violaceus*), a species with a non-resource-based mating system. Auk 124: 857-867. Doi: 10.1642/0004-8038(2007)124[857:BPPGPI]2.0.CO;2

Robel RJ (1966) Booming territory size and mating success of the greater prairie-chicken (*Tympanuchus cupido pinnatus*). Anim Behav 14: 328-331.

Rodríguez-Teijeiro JD, Puigcerver M, Gallego S, Cordero PJ, Parkin DT (2003) Pair bond and multiple paternity in the polygamous common quail *Coturnix coturnix*. Ethology 109: 291-302. Doi: 10.1046/j.1439-0310.2003.00873.x

Say L, Naulty F, Hayden TJ (2003) Genetic and behavioural estimates of reproductive skew in male fallow deer. Mol Ecol 12: 2793-2800. Doi: 10.1046/j.1365-294X.2003.01945.x

Schroeder MA, Braun CE (1991) Walk-in traps for capturing greater prairie-chickens on leks. J Field Ornithol 62: 378-385

Segelbacher G, Paxton RJ, Steinbrueck G, Trontelj P, Storch I (2000) Characterisation of microsatellites in capercaillie (*Tetrao urogallus*) (AVES). Mol Ecol 9: 1934-1935. Doi: 10.1046/j.1365-294x.2000.0090111934.x

Semple K, Wayne RK, Gibson RM (2001) Microsatellite analysis of female mating behaviour in lek-breeding sage grouse. Mol Ecol 10: 2043-2048. Doi: 10.1046/j.0962-1083.2001.01348.x

Shuster SM, Wade MJ (2003) Mating Systems and Strategies. Princeton University Press, Princeton, New Jersey.

Sokal RR, Rohlf FJ (1995) Biometry, the principles and practice of statistics in biological research, 3rd edition. W.H. Freeman and Company, New York

Stiver JR, Apa AD, Remington TE, Gibson RM (2008) Polygyny and female breeding failure reduce effective population size in the lekking Gunnison sage-grouse. Biol Conserv 141: 472-481. Doi:10.1016/j.biocon.2007.10.018

Storch I, Segelbacher G (2005) Two grouse clutches in the same nest: evidence for nest site adoption in capercaillie (*Tetrao urogallus*). J Ornithol 146: 85-88. Doi: 10.1007/s10336-004-0064-3

Taylor SE, Oyler-McCance SJ, Quinn TW (2003) Isolation and characterization of microsatellite loci in greater sage-grouse (*Centrocercus urophasianus*). Mol Ecol Notes 3: 262-264. Doi: 10.1046/j.1471-8286.2003.00424.x

Trail PW (1985) Courtship disruption modifies mate choice in a lek-breeding bird. Science 227: 778-780.

Trail PW (1990) Why should lek-breeders be monomorphic? Evolution 44: 1837-1852.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4: 535-538. Doi: 10.1111/j.1471-8286.2004.00684.x

Vanpé C, Kjellander P, Gallin M, et al. (2008) Mating system, sexual dimorphism, and the opportunity for sexual selection in a territorial ungulate. Behav Ecol 19: 309-316. Doi:10.1093/beheco/arm132

Wade MJ (1979) Sexual selection and variance in reproductive success. Am Nat 114: 742–747.

Wade M J, Arnold SJ (1980) The intensity of sexual selection in relation to male sexual behaviour, female choice, and sperm precedence. Anim Behav 28: 446–461.

Wagner RH (1992) Behavioural and breeding-habitat related aspects of sperm competition in razorbills. Behaviour 123: 1-25.

Wang J (2008) COLONY 2.0 Manual. Downloadable from http://www.zoo.cam.ac.uk/ioz/index.htm

Whittingham LA, Dunn PO (2005) Effects of extra-pair and within-pair reproductive success on the opportunity for selection in birds. Behav Ecol 16:138–144. Doi: 10.1093/beheco/arh140

Widemo F, Owens IPF (1995) Lek size, male mating skew and the evolution of lekking. Nature 373: 148-151. Doi: 10.1038/373148a0

Wiley RH (1973) Territoriality and non-random mating in Sage Grouse, *Centrocercus urophasianus*. Anim Behav Mono 6: 85-109.

Wiley RH (1991) Lekking in birds and mammals: Behavioral and evolutionary issues. Adv Stud Behav 20: 201–291.

Wilmer JW, Allen PJ, Pomeroy PP, Twiss SD, Amos W (1999) Where have all the fathers gone? An extensive microsatellite analysis of paternity in the grey seal (*Halichoerus grypus*). Mol Ecol 8: 1417–1429. Doi: 10.1046/j.1365-294x.1999.00705.x

Wright S (1931) Evolution in Mendelian populations. Genetics 16: 97-159

Wright S (1938) Size of population and breeding structure in relation to evolution. Science 87: 430–431.

Yasmin S, Yahya HAS (1996). Correlates of mating success in Indian peafowl. Auk 113: 490-492.

Yasui, Y. 1998. The ''genetic benefits'' of multiple mating reconsidered. Trends Ecol Evol 13: 246–250. Doi: 10.1016/S0169-5347(98)01383-4

Table 4-1. Paternity assignment for sage-grouse offspring in Alberta (1999 - 2006)

Year	Number of Offspring (Number of Sampled Clutches)	Number of Offspring with Assigned Paternity	Number of Sampled Fathers	Number of Fathers (Sampled + Unsampled)	Mean Paternity Success		
					All Males (fathers + non-fathers)	Known Fathers (sampled males)	All Fathers (sampled + unsampled)
1999	84 (20)	32	4	20	1.62	8.00	4.20
2000	22 (8)	4	1	7	0.52	4.00	3.14
2001	138 (26)	41	8	27	2.26	5.13	5.11
2002	225 (34)	50	5	37	2.71	10.00	6.08
2003	242 (32)	96	6	27	2.95	16.00	8.96
2004	165 (24)	41	4	25	1.79	10.25	6.60
2005	196 (29)	91	9	30	1.32	10.11	6.53
2006	134 (18)	88	10	19	1.01	8.80	7.05
Overall	1206 (191)	443	36	174	3.18	12.31	6.93

Table 4-2. Opportunity for selection (I_M) for male reproductive success measured at the clutch, offspring, and successful offspring levels in Alberta (1999 - 2006). "All Males" include sampled and unsampled fathers and all males counted via lek counts. "Known Males" include only sampled fathers and all males enumerated via lek counts. "Mean" reproductive success is the mean of annual values with standard error in parentheses. Total was also calculated excluding 2000 because it appears to be an aberrant year with few nests laid and few fathers.

Year	All Males						Known Males					
	Clutch		Offspring		Successful Offspring		Clutch		Offspring		Successful Offspring	
	I_{M1C}	Mean (variance)	I_{M1O}	Mean (variance)	I_{M1H}	Mean (variance)	I_{M2C}	Mean (variance)	I_{M2O}	Mean (variance)	I_{M2H}	Mean (variance)
1999	6.1	0.2 (0.2)	9.0	0.7 (4.6)	21.3	0.4 (3.4)	38.3	0.05 (0.1)	44.4	0.3 (3.3)	80.6	0.2 (2.6)
2000*	140.0	0.06 (0.4)	145.0	0.2 (6.2)	248.0	0.03 (0.2)	248.0	0.01 (0.02)	248.0	0.03 (0.2)	0**	0 (0)
2001	3.6	0.2 (0.2)	4.1	1.2 (5.6)	11.6	0.6 (3.7)	16.6	0.05 (0.05)	17.4	0.3 (1.8)	38.2	1.0 (1.1)
2002	1.8	0.4 (0.3)	2.3	2.6 (15.3)	23.6	1.5 (2.2)	19.4	0.07 (0.08)	20.8	0.6 (6.3)	91.0	0.9 (0.9)
2003	3.8	0.3 (0.4)	4.0	2.4 (23.6)	8.0	1.3 (14.4)	16.0	0.1 (0.2)	15.4	1.0 (16.8)	17.2	0.8 (11.9)
2004	3.8	0.2 (0.2)	4.1	1.8 (12.7)	8.3	0.9 (2.6)	25.3	0.06 (0.09)	26.6	0.4 (5.1)	23.4	0.29 (1.9)
2005	3.4	0.3 (0.3)	3.5	2.1 (14.9)	9.1	1.0 (9.1)	12.7	0.2 (0.3)	12.9	1.0 (11.8)	17.9	0.7 (7.6)
2006	4.6	0.2 (0.2)	5.2	1.5 (11.6)	6.2	1.4 (11.4)	9.3	0.1 (0.1)	9.9	1.0 (9.5)	9.9	1.0 (9.5)
Mean	20.9 (17.0)		22.2 (17.6)		42.0 (29.5)		48.2 (28.7)		49.4 (28.6)		34.8 (11.8)	
Mean (excluding 2000)	3.9 (0.6)		4.6 (0.9)		12.6 (2.8)		19.7 (4.0)		21.1 (4.6)		39.7 (11.8)	

* Very few nests were produced in 2000

** No sampled fathers had successful offspring

Table 4-3. Opportunity for selection for female (I_F) and male (I_M) successful breeders based on clutches, offspring, and successful offspring produced in Alberta (1999 - 2006). "Mean" reproductive success is the mean of all years with standard error in parentheses.

Year	Mothers						Fathers (sampled + unsampled)						Fathers (sampled only)					
	Clutch		Offspring		Successful Offspring		Clutch		Offspring		Successful Offspring		Clutch		Offspring		Successful Offspring	
	I_{FC}	Mean (variance)	I_{FO}	Mean (variance)	I_{FH}	Mean (variance)	I_{M3C}	Mean (variance)	I_{M3O}	Mean (variance)	I_{M3H}	Mean (variance)	I_{M4C}	Mean (variance)	I_{M4O}	Mean (variance)	I_{M4H}	Mean (variance)
1999	0.08	1.1 (0.1)	0.2	4.2 (3.3)	1.2	2.1 (5.2)	0.2	1.1 (0.2)	0.7	4.2 (12.8)	2.9	2.4 (16.1)	6.0	0.3 (0.5)	7.0	1.6 (18.0)	13.5	1.1 (14.9)
2000	0	1.0 (0)	0.3	4.4 (5.4)	2.8	1.8 (8.5)	0	1.0 (0)	0.3	3.7 (4.6)	7.0	0.6 (2.3)	0	0.1 (0.1)	7.0	0.6 (2.3)	0	0 (0)
2001	0.07	1.1 (0.08)	0.2	5.8 (5.3)	1.8	2.6 (12.3)	0.04	0.9 (0.03)	0.2	5.2 (4.1)	1.9	2.5 (12.0)	3.1	0.2 (0.2)	3.3	1.4 (6.7)	8.2	0.7 (4.4)
2002	0.1	1.3 (0.2)	0.4	8.6 (27.1)	6.2	1.1 (7.2)	0.1	1.0 (0.1)	0.3	6.4 (13.7)	9.2	0.8 (5.2)	7.4	0.2 (0.2)	8.0	1.4 (14.6)	37.0	0.2 (2.2)
2003	0.1	1.3 (0.2)	0.1	9.7 (12.8)	0.6	5.3 (16.8)	0.3	1.1 (0.4)	0.4	8.6 (31.1)	1.6	4.8 (35.6)	3.9	0.4 (0.7)	3.7	3.7 (50.9)	4.2	3.0 (37.0)
2004	0.1	1.2 (0.2)	0.2	8.7 (12.6)	0.9	4.5 (18.8)	0.3	0.9 (0.2)	0.4	6.6 (15.8)	1.5	3.4 (17.2)	6.2	0.2 (0.3)	6.5	1.6 (17.6)	5.6	1.1 (6.6)
2005	0.09	1.1 (0.1)	0.2	7.5 (10.0)	1.3	3.7 (17.7)	0.4	1.0 (0.4)	0.4	6.5 (18.2)	2.2	3.2 (22.4)	3.4	0.5 (0.7)	3.5	3.0 (31.8)	5.1	2.1 (21.7)
2006	0	1.0 (0)	0.1	7.4 (5.4)	0.2	6.8 (10.5)	0.2	1.0 (0.2)	0.3	7.1 (16.1)	0.5	6.4 (22.3)	1.2	0.6 (0.4)	1.4	4.6 (29.1)	1.4	4.6 (29.1)
Mean	0.07 (0.02)		0.2 (0.04)		1.9 (0.7)		0.2 (0.05)		0.4 (0.05)		3.4 (1.1)		3.9 (0.9)		5.1 (0.8)		9.4 (4.2)	

Table 4-4. Values for male opportunity for selection (I_M; standardized variance in male breeding success) for galliform and lekking avian species from studies that have assessed breeding success using genetic and field observations. I_M values are listed from highest (top) to lowest (bottom) and are means for each study.

Avian Species	I_M	Type of Study	Citation
Long-tailed manakin (*Chiroxiphia linearis*)	21.5	Field	McDonald 1989
Greater sage-grouse (*Centrocercus urophasianus*)	16.3	Field	Wiley 1973
Greater sage-grouse (*Centrocercus urophasianus*)	11.5	Field	G. L. Patricelli and A. H. Krakauer, unpubl. data
Lance-tailed manakin (*Chiroxiphia lanceolata*)	9.3	Genetic	DuVal and Kempenaers 2008
Black grouse (*Tetrao tetrix*)	7.6	Field	Kruijt and de Vos 1988
Lesser bird of paradise (*Paradisaea minor*)	7.3	Field	Beehler 1983
Guianian cock-of-the-rock (*Rupicola rupicola*)	6.7	Field	Trail 1990
Gunnison sage-grouse (*Centrocercus minimus*)	6.4	Field	J. Stiver, unpubl. data
Greater sage-grouse (*Centrocercus urophasianus*)	6.0	Field	Lumsden 1968
Black grouse (*Tetrao tetrix*)	5.7	Field	Alatalo et al. 1992
Village indigobird (*Vidua chalybeata*)	5.5	Field	Payne and Payne 1977
Buff-breasted Sandpiper (*Tryngites subruficollis*)	5.3	Genetic	Lancetot et al. 1997
Wild turkey (*Meleagris gallopavo*)	5.2	Genetic	Krakauer 2008
Gunnison sage-grouse (*Centrocercus minimus*)	5.1	Field - Simulations	Stiver et al. 2008
Greater sage-grouse (*Centrocercus*	5.1	Field	J. Carpenter, unpubl. data

urophasianus)			
Greater prairie-chicken (*Tympanuchus cupido*)	4.1	Field	Robel 1966
Greater sage-grouse (*Centrocercus urophasianus*)	3.9	Genetic	This study
White-bearded manakin (*Manacus manacus*)	3.8	Field	Lill 1974
Capercaillie (*Tetrao urogallus*)	3.2	Field	Müller 1979
Greater sage-grouse (*Centrocercus urophasianus*)	3.0	Field	R. M. Gibson, unpubl. data
Buff-breasted Sandpiper (*Tryngites subruficollis*)	2.6	Field	Pruett-Jones 1988
Jackson's widowbird (*Euplectes jacksoni*)	2.5	Field	Andersson 1989
Sharp-tailed grouse (*Tympanuchus phasianellus*)	2.4	Field	Gratson et al. 1991
Ruff (*Philomachus pugnax*)	2.0	Field	Hill 1991
Greater prairie-chicken (*Tympanuchus cupido*)	1.9	Field	Ballard and Robel 1974

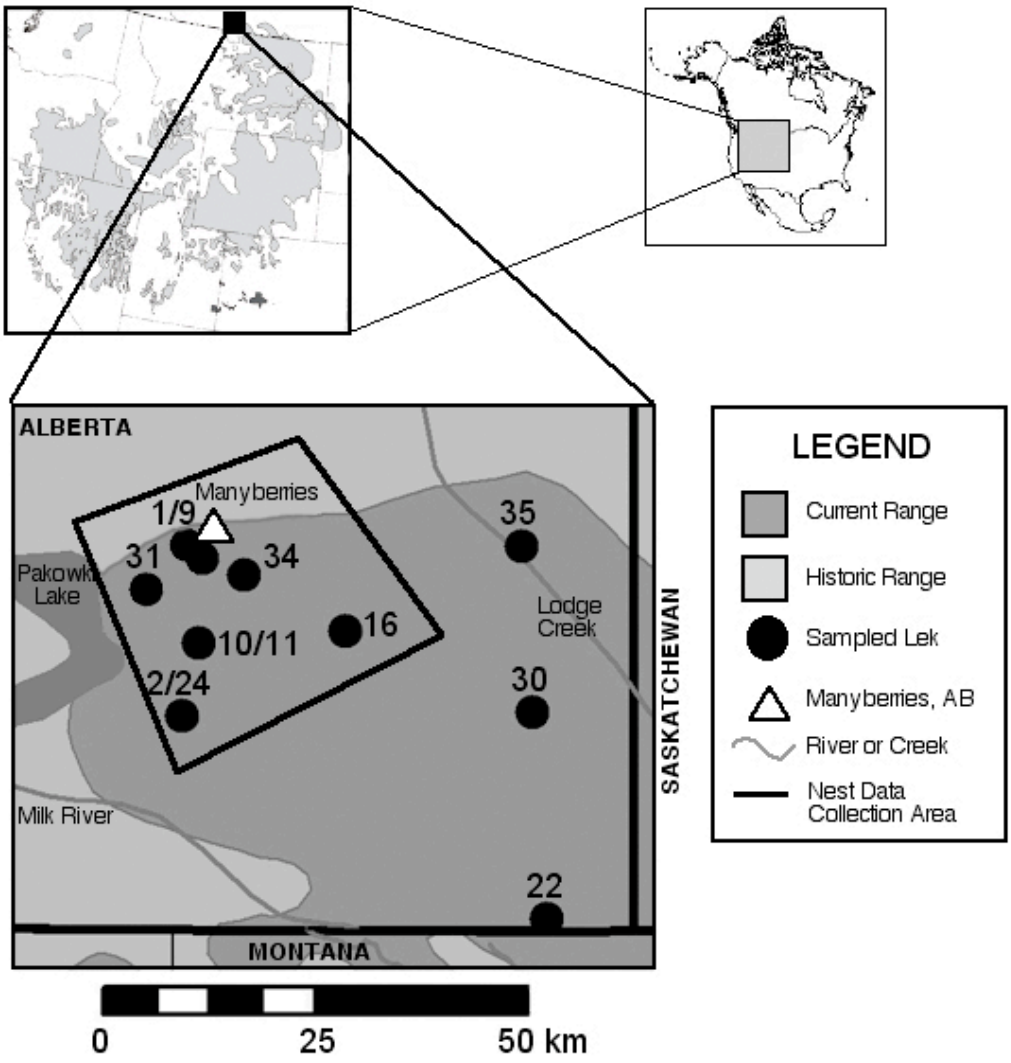

Figure 4-1. Map of the Alberta Sage-Grouse study area with sampled leks highlighted

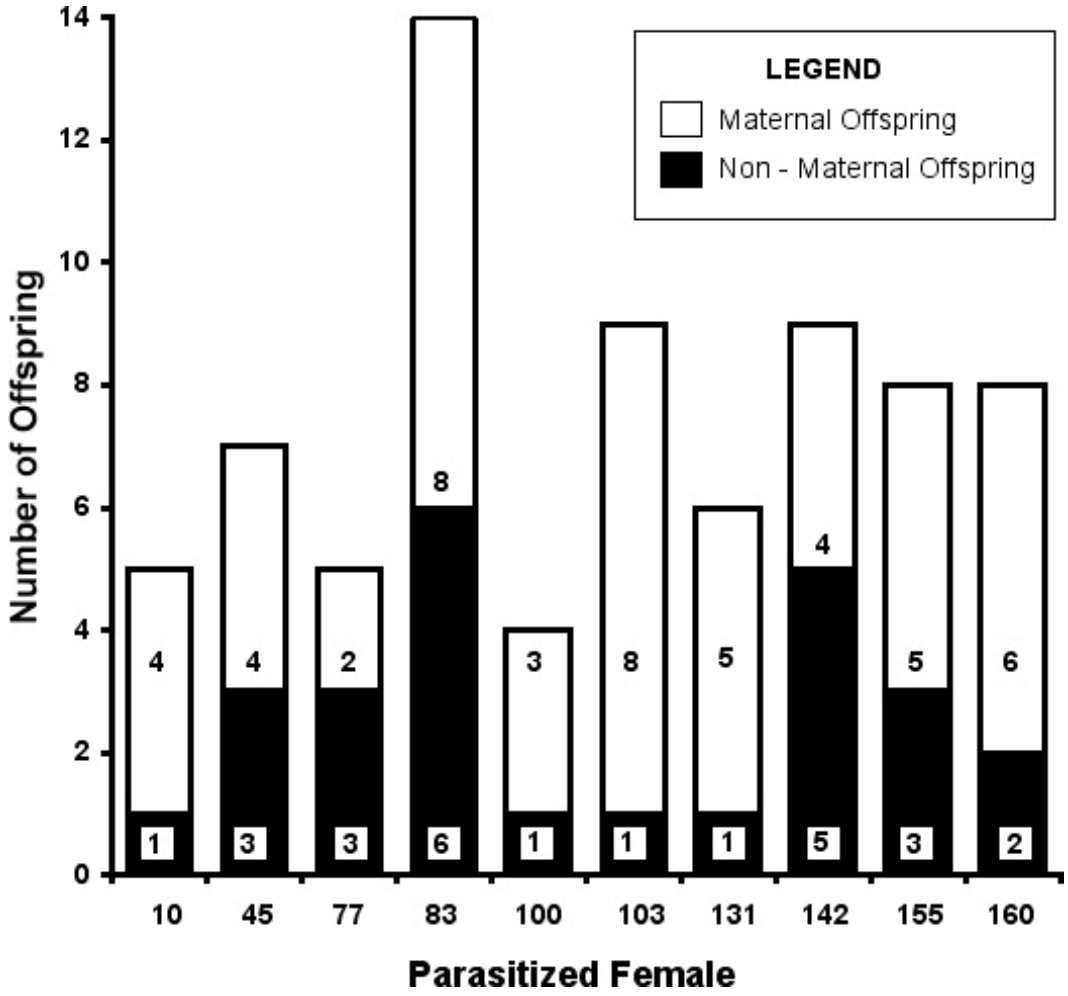

Figure 4-2. Incidence of intraspecific nest parasitism in sage-grouse in Alberta (1999 – 2006) showing number of both maternal (white) and non-maternal (black) offspring in each clutch.

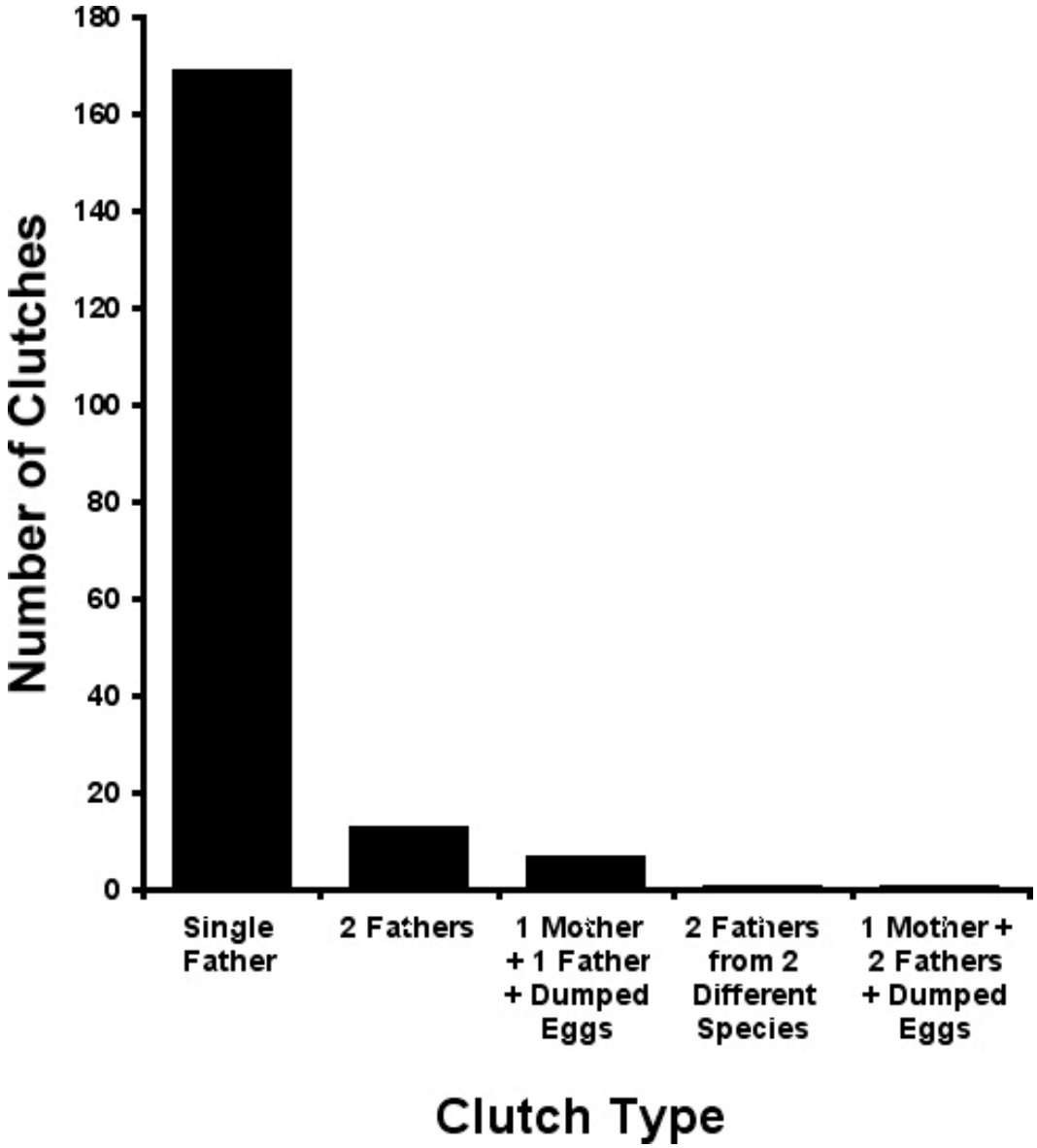

Figure 4-3. Distribution of clutches displaying different combinations of parentage based on patterns for 191 sage-grouse clutches in Alberta (1999 – 2006)

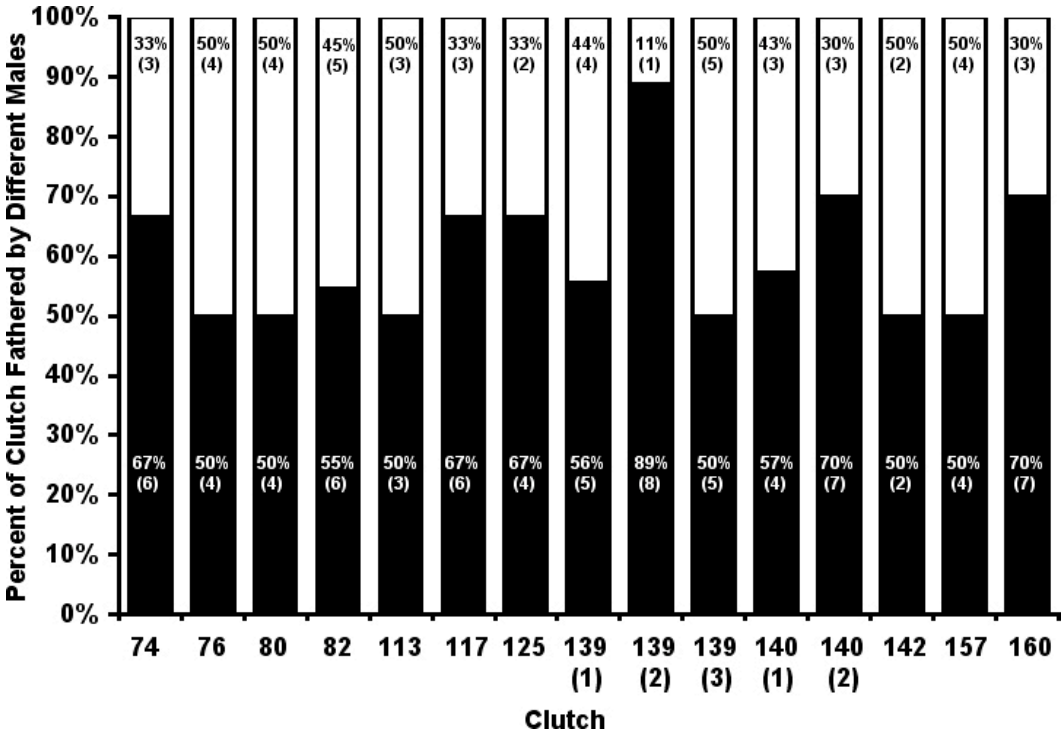

Figure 4-4. Distribution of paternity for clutches with two fathers. Black represents the more successful male and white represents the less successful male measured in terms of fathering offspring in the clutch. Numbers on the x-axis represent the identification number of individual females.

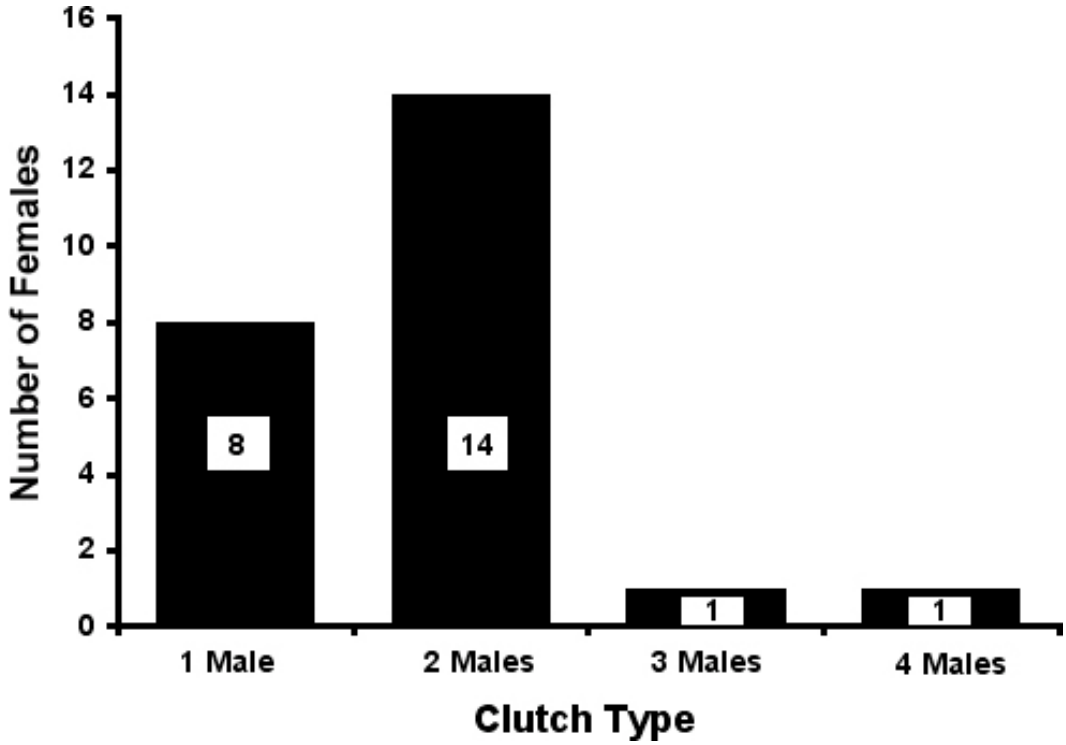

Figure 4-5. Number of pairs of clutches (nest and re-nest attempt) with one, two, three, and four fathers for female sage-grouse that laid two clutches in a given year.

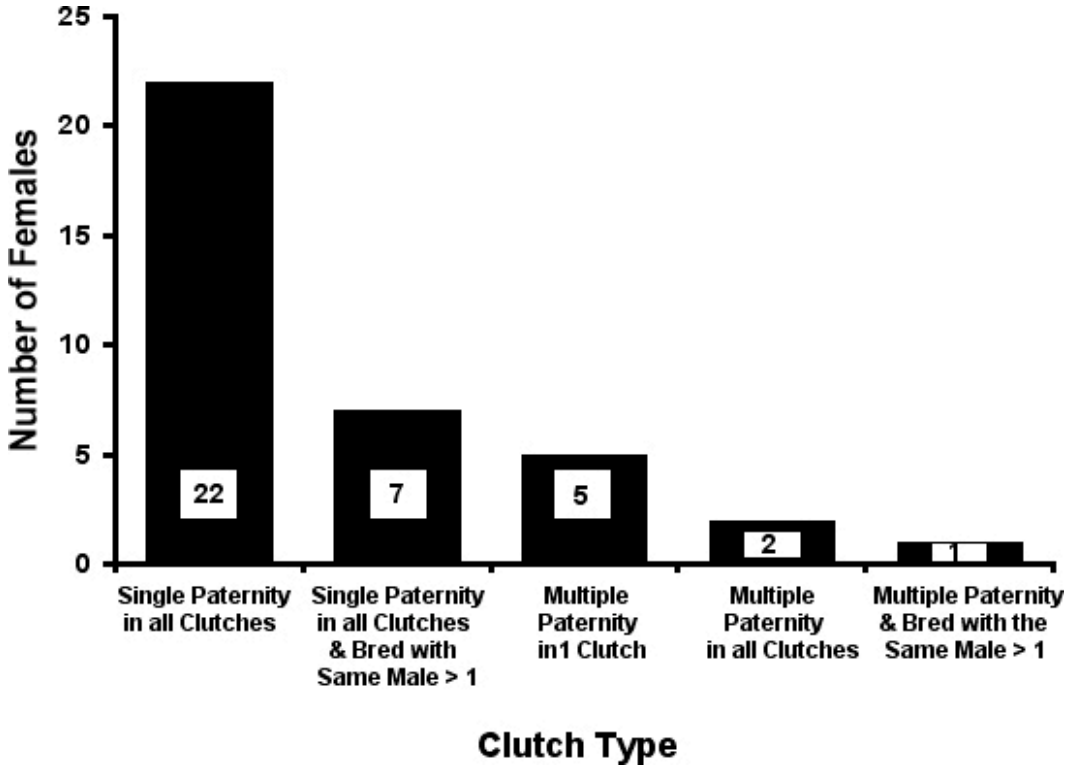

Figure 4-6. Number of sage-grouse females that laid more than one clutch over multiple years in each of five paternity classes: (1) single paternity by different males in all clutches, (2) single paternity in all clutches, but bred with the same male more than once, (3) multiple paternity in one clutch, (4) multiple paternity in all clutches, and (5) multiple paternity in at least one clutch and bred with the same male for more than one clutch.

Appendix 4-1. Success of male sage-grouse in Alberta fathering clutches and offspring across their lifetimes.

Number of clutches fathered	Number of males that fathered clutches	Number of clutches in each category*	Number of hatched clutches	Percent hatched clutches	Number of offspring	Number of hatched offspring	Percent hatched offspring	Average number of offspring per male	Average number of hatched offspring per male
1	150	150	55	36.7	795	318	40.0	5.3	2.1
2	17	34	20	58.8	216	129	59.7	12.7	7.6
3	3	9	3	33.3	62	23	37.1	20.7	7.7
4	2	8	6	75.0	60	48	80.0	30.0	24.0
5	1	5	4	80.0	29	22	75.9	29.0	22.0
6	0	0	0	0	0	0	0	0	0
7	1	7	5	71.4	44	34	77.3	44.0	34.0
Total	174	213	93	43.7	1206	574	47.6	6.9	3.3

* There are more clutches fathered than clutches laid because some clutches exhibit multiple paternity

Appendix 4-2. Success of female sage-grouse in Alberta at producing clutches and offspring across their lifetimes.

Number of clutches laid	Number of females that laid clutches*	Number of clutches in each category	Number of hatched clutches	Percent hatched clutches	Number of offspring	Number of hatched offspring	Percent hatched offspring	Average number of offspring per female	Average number of hatched offspring per female
1	58	58	20	34.5	332	127	38.3	5.7	2.2
2	21	42	15	35.7	254	115	45.3	12.1	5.5
3	15	45	21	46.7	340	156	45.9	22.7	10.4
4	6	24	11	45.8	136	81	59.6	22.7	13.5
5	2	10	6	60.0	66	44	66.7	33.0	22.0
6	2	12	7	58.3	78	51	65.4	39.0	25.5
Total	104	191	80	41.9	1206	574	47.6	11.6	5.5

* Does not include females that parasitized other female's nests

CHAPTER FIVE

Museum Specimens Reveal Little Genetic Change Over Time (1895 – 2007) in Endangered Greater Sage-Grouse (*Centrocercus urophasianus*) in Canada[4]

1. Introduction

Anthropogenic habitat loss causes formerly continuous populations to become fragmented and/or isolated resulting in population declines and disrupted gene flow (Frankham et al. 2002). Populations that further undergo severe demographic contractions (bottlenecks) are expected to lose considerable genetic variation due to genetic drift and experience reductions in effective population size (N_e; Wright 1969; Nei et al. 1975; Chakraborty and Nei 1977; Gilpin and Soulé 1986; Lacy 1997; Soulé 1987; Soulé and Mill 1998; Reed 2007). N_e can be defined as the size of an ideal population with the same rate of genetic change as a real, study population (Wright 1931) and indicates the level of inbreeding and the amount of genetic variation lost from populations due to random genetic drift (Nei et al. 1975; Chakraborty and Nei 1977). N_e is fundamental to estimating the impact of inbreeding, drift, and selection on a population and is always smaller than census counts (Wright 1931, 1938; Kimura and Crow 1963; Frankham 1995; Brodie 2007). A species' mating system strongly impacts N_e, because populations with a large variance in reproductive success, such as polygynous or lekking species, have reduced N_e (Wright 1938; Nunney 1991, 1993), which has important ramifications for declining or threatened species.

Studies that compare a population before and after a population decline are rare (e.g., Bouzat et al. 1998a, 1998b; Pertoldi et al. 2001; Martínez-Cruz 2007), as they rely primarily on the existence of museum specimens that pre-date the decline. However, such temporal sampling offers invaluable information that supplies a genetic baseline for evaluating the current genetic state of a species or population. Alternatives to temporal sampling are less desirable as they base

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comparisons on other contemporary populations or species with different demographic histories that have not suffered significant declines or they only study the declining population and assume that genetic loss has occurred from earlier genetic bottlenecks (Bouzat 2001). To examine genetic diversity and structure before and during a decline and after a bottleneck event, I investigated a gamebird species that is well represented in North American museum collections, the greater sage-grouse (*Centrocercus urophasianus*; hereafter sage-grouse).

Sage-grouse are a lekking galliform species in which males congregate on communal display grounds (leks) and females select a mate, breed, and then incubate eggs and raise young on their own (Wiley 1973; Gibson 1996). Sage-grouse are endangered at the provincial (Alberta Sage-Grouse Recovery Action Group 2005) and federal (Lungle and Pruss 2008) levels in Canada where they are located at the northern periphery of the species' range. Sage-grouse in Canada have declined by 66-92% over the last 35 years (Aldridge and Brigham 2003) with a population crash in 1994 (Fig. 5-1). The current estimated population size is approximately 300 – 400 birds based on 2006 – 2009 lek counts, which has dropped substantially from the 1999 estimation of 813-1204 birds (Aldridge and Brigham 2003). Historically, the species inhabited three Canadian provinces (Alberta, Saskatchewan, and British Columbia) and 14 U.S. states, but presently occurs only in southeastern Alberta, southwestern Saskatchewan, and 11 U.S. states (Schroeder *et al* 2004). Rangewide, the amount of sagebrush habitat decreased by greater than 50% (Schroeder *et al.* 2004) and in Canada, potential habitat declined from 100 000 km^2 to 6000 km^2 (Aldridge & Brigham 2003). Only 6% of potential habitat remains in Canada, where there are currently two disjunct regions supporting sage-grouse separated by more than 100 km (Fig. 5-2). Suggested causes for the decline include oil and gas development (Braun et al. 2002), intensive livestock grazing (Aldridge et al. 2004), wildlife viewing, changes in the predator community, climate change, and widespread destruction of habitat in neighboring Montana (Alberta Sage-Grouse Recovery Action Group 2005). Throughout most of the species' range, sage-grouse are associated with big sagebrush (*Artemisia tridenta*), but in Canada, the species is dependent on silver

sagebrush (*A. cana*), as its main food and source of cover (Alberta Sage-Grouse Recovery Action Group 2005). The distribution of silver sagebrush is naturally patchy, so birds have adapted to move large distances to find suitable habitat.

While the current status of sage-grouse in Canada is bleak, recent genetic work has revealed a less desperate picture. Sage-grouse in Canada are part of a much larger and demographically stable population, the northern Montana population, which includes Canada and all birds north of the Missouri River in Montana (chapter 2). There is evidence of population structure (northern and southern subpopulations), but there is also gene flow connecting subpopulations and diversity levels are high (chapter 2). Sage-grouse in Alberta show no evidence of inbreeding and limited kin association, a suspected mechanism behind lek formation and maintenance, which would increase relatedness within leks and the potential for inbreeding (chapter 3). Also, a greater proportion of male sage-grouse in Alberta father offspring than expected for lekking galliforms, which decreases variance in reproductive success, and should increase N_e.

To investigate the existence of genetic impacts connected with a demographic decline, individuals from the entire time span of the decline should be assessed. Therefore, I expanded my contemporary research to include Canadian sage-grouse samples from 1895 to present to investigate four main questions: (1) Has genetic diversity declined over time? (2) Has the sage-grouse population in Canada become more genetically structured with decreases in available habitat? (3) Is there evidence for a genetic bottleneck accompanying the demographic bottleneck in the 1990s? (4) What is the current and past effective population size? While the demographic decline in Canada has been severe, contemporary genetic diversity is comparable to regions not experiencing drastic declines (chapter 2). This suggests that diversity has been maintained through immigration from the southern part of the population and that diversity has not declined significantly. Sage-grouse in Canada presently show little genetic structure even in the presence of habitat loss and degradation, therefore historic birds that inhabited less fragmented habitat should exhibit little structure as well. I expected to find a genetic signature from the population crash in 1994 due to its

severity and the fact there has been no recovery in the past 15 years (Fig. 5-1). Finally, I expected a reduction in N_e, as it is tied to population size and should mirror the documented population decline.

2. Materials and methods

2.1. Study location and sample collection

Contemporary samples were collected from sage-grouse in the extreme southeastern corner of Alberta and southwestern corner of Saskatchewan, Canada (Fig. 5-2). Birds were captured using walk-in funnel traps (Schroeder and Braun 1991), night lighting (Giesen et al. 1982), rocket nets (Giesen et al. 1982), and drop-nets (Bush 2008). Blood, feather, and mouth swab samples were collected from captured adult sage-grouse between 1998 – 2007. Vehicular and predator mortalities were opportunistically sampled and molted feathers were collected on leks from 2003 – 2007. In total, I collected 1,422 samples from Alberta (327 blood, plucked feather, mouth swab, and road kill and 1,095 molted feathers) and 503 samples from Saskatchewan (9 blood and kill-site samples and 494 molted feathers). Most samples were collected on leks.

Historic Canadian DNA samples (1895 – 1991) were collected from museums, government collections, and university collections (Alberta Fish and Wildlife, Augustana, Canadian Museum of Nature, Etzikom Museum, Grand Coteau Museum, Grasslands National Park, Jasper Centre Museum, Manitoba Museum, Police Point Park Interpretive Nature Centre, Royal Alberta Museum, Royal British Columbia Museum, Royal Ontario Museum, Royal Saskatchewan Museum, Saskatchewan Environment, Swift Current Museum, University of Alberta Museum of Zoology, University of British Columbia, University of Calgary, University of Regina, and University of Saskatchewan Museum of Natural Sciences) and private individuals (see acknowledgements section; Appendix A). Most samples were taken as plucked feathers from mounted specimens or study skins; five to ten feathers were plucked from underneath the wing and stored in labeled paper envelopes; five feather tips were used in each DNA extraction. Bones were drilled with individual sterilized drill bits from

skeletal mounts and partial skeletons and the resulting dust was stored in labeled 1.5 ml microcentrifuge tubes. In total, 238 historic samples were collected; 124 from Alberta and 114 from Saskatchewan; 113 originated from before 1965 (60 from Alberta and 53 from Saskatchewan) and 125 from between 1966 – 1991 (64 from Alberta and 61 from Saskatchewan). Samples from Alberta and Saskatchewan were primarily concentrated post and pre-1965 respectively because hunting seasons were open from 1967 to 1995 in Alberta and prior to 1938 in Saskatchewan (Lungle and Pruss 2008). The difference in timing of specimen collection between provinces is likely not a concern, as the most severe decline started in the late-1980s (Fig. 5-1). Many samples were also given date ranges or classified as pre-1965 or post-1965 because they ultimately originated from private collections and precise information was never recorded.

Samples with location information were plotted on maps for comparison to current sage-grouse sampling locations and the estimated historic range (Fig. 5-2). Schroeder et al. (2004) determined historic sage-grouse range based on museum specimens, published observations, and potential presettlement distribution of sagebrush habitat. In Canada, the historic range distribution was based on silver sagebrush distribution and published observations, however the authors state that the authenticity of some of these records is uncertain (Schroeder et al. 2004).

2.2. Microsatellite genotyping

DNA was extracted using Qiagen DNeasy® Tissue and QIAamp® DNA Micro kits (all historic samples) using modifications from Bush et al. (2005). All samples were sexed using DNA methods following Bush et al. (2005). Thirteen microsatellite loci developed from sage-grouse (SGCA9-2 [redesigned primer set; S. Taylor, personal communication] and SGCA5; Taylor et al. 2003), capercaillie (*Tetrao urogallus*; TUT3, TUT4, TUD1, and TUD3; Segelbacher et al. 2000), black grouse (*Tetrao tetrix*; BG6 and BG15; Piertney and Höglund 2001; TTD6 and TTT1; Caizergues et al. 2001; TTT3; Caizergues et al. 2003), red grouse (*Lagopus lagopus*; LLSD8; Piertney and Dallas 1997), and domestic chicken

(*Gallus gallus*; ADL230; Cheng et al. 1994) were used. I assessed the presence of null alleles by examining 20 sage-grouse females and their known offspring (full nests; offspring were not included in the general analyses). I detected no null alleles, therefore the 13 loci were used for all analyses. Microsatellite PCRs (15μl total volume with 3, 4, or 5μl extracted DNA) were carried out as described in Bush et al. (2005). Forward primers were fluorescently labeled with 6-FAM, TET, and HEX (Applied Biosystems). I followed the PCR cycling conditions outlined for each microsatellite in the original publications using Perkin Elmer Cetus GeneAmp PCR System 9600® and Eppendorf Mastercycler® ep machines. All non-invasive and historic samples were run in triplicate as outlined in Bush et al. (2005). The PCR products were visualized using an ABI 377® automated sequencer with genescan analysis3.1® software (Applied Biosystems). Alleles were scored using genotyper®2.0 software (Applied Biosystems).

Duplicate samples were identified using Microsoft Excel Microsatellite toolkit (Park 2001; see chapter 3). For all non-invasive samples, identification of genotyping errors was performed in MICRO-CHECKER (Van Oosterhout et al. 2004) and probability of identity (PI) was calculated in GENALEX version 5.1 (Peakall and Smouse 2001).

2.3. Genetic diversity and structure

I used the Bayesian program STRUCTURE (Pritchard et al. 2000) to investigate spatial genetic substructure within Canada at multiple temporal scales. Previous research using STRUCTURE showed that Alberta birds are part of the northern Montana sage-grouse population (Alberta, Saskatchewan, and Blaine, Choteau, Phillips, and Valley counties in Montana) and belong to a subpopulation that occurs north of the Milk River (Alberta, Saskatchewan, and north Blaine, Phillips, and Valley counties; chapter 2). To assess whether contemporary and historic sage-grouse form a single population, I analyzed all contemporary and historic samples together. I also determined if genetic structure varied within or between time periods (contemporary [1998 – 2007], historic [1895 – 1991], pre-1965 historic [1895 – 1965], and post-1965 historic [1966 – 1991]; Table 1) by

running each time-period separately. I ran 20 independent simulations for each K (1-19) with 100,000 burn-in iterations and 1,000,000 data repetitions assuming an admixture model and no prior population information. I used the method of Evanno et al. (2005), which calculates ΔK, a measure of the second order rate of chance in the likelihood of K, to estimate the true K, or number of clusters.

I calculated all genetic diversity measures at the Canada, Alberta, and Saskatchewan levels for five different temporal periods (contemporary, historic, pre-1965 historic, post-1965 historic, and contemporary and historic combined). I calculated expected (H_E) and observed (H_O) heterozygosity for each locus and tested for deviations from Hardy-Weinberg and linkage equilibrium in GENEPOP, version 3.4 (Raymond and Rousset 1995). Number of alleles per locus (A) were calculated in Microsatellite toolkit. Allelic richness (AR; number of alleles corrected to the smallest sample size) and the inbreeding coefficient F_{IS} were calculated in FSTAT, version 2.9.3 (Goudet 2001). Difference in genetic diversity between each time period was tested using a Wilcoxon signed ranks test, which pairs the data by locus. To estimate the rate of decline in genetic diversity in a closed population, I simulated a bottleneck in BOTTLESIM (Kuo and Janzen 2003) using lek count data collected since 1968 (Fig. 5-1) and genotypes recorded for 1895 - 1965. When lek count data were available for only Alberta, the number of males was doubled to account for Saskatchewan. While pre-1990 counts for Saskatchewan suggest a much larger population size than Alberta, they are likely inaccurate because they are from a single year, most of the locations were very close to one another, but were classified as unique leks, and some locations appear to represent off-lek observations (chapter 1). Once consistent lek counts were started in 1994, counts in Saskatchewan were similar to those in Alberta (chapter 1; Fig. 5-1). The number of females was considered to be equal to the male census size. Parameters in the simulation were set for random mating, generation overlap, one year to maturity, and an average lifespan of three years (Connelly et al. 2004)

2.4. Effective population size and bottleneck tests

I estimated N_e in NEESTIMATOR 1.3 (Peel et al. 2004) using the temporal method where N_e is estimated from changes in allele frequencies (F) between samples taken at different times (Waples 1989). Essentially, differences in allele frequencies between samples are used to measure genetic drift. A moments-based statistic was used to estimate F (Pollack 1983; Waples 1989 equation 9):

$$F_k = \frac{1}{K-1}\sum_{i=1}^{K}\frac{(x_i - y_i)^2}{\left(\frac{(x_i + y_i)}{2}\right)'}$$

where K is the number of alleles and i is the frequency of a given allele during the time period when the first (x) and second (y) sample were collected. F was then converted to an estimate of effective population size using:

$$\widehat{N}_e = \frac{t}{2(F - 1/S)'}$$

where t is the number of elapsed generations between time periods and S is the sample size. I also employed point estimate methods using heterozygote excess (Pudovkin et al. 1996) and linkage disequilibrium (Bartley et al. 1992) in NEESTIMATOR 1.3. Linkage disequilibrium occurs when alleles do not occur independently at different loci, which can arise in small populations through random genetic drift (Hill 1981; Bartley et al. 1992) or a variety of other mechanisms. Heterozygote excess occurs in small populations through chance when males and females differ in allele frequencies resulting in offspring that are more heterozygous than expected based on overall population allele frequencies (Pudovkin et al. 1996). Instead of strictly measuring N_e, both point estimate methods calculate the effective number of breeding individuals (Leberg 2005), which has useful applications in conservation.

Generation time was required to estimate N_e using the temporal method, therefore I used an estimation for overlapping generations where generation time equals the mean age of the parents when offspring are produced (Hill 1979). Because I could not age the majority of samples (molted feathers), could only age captured individuals as yearlings or adults, and could not determine relative fecundity per age group, I set generation time to either one or two years, as most females reproduce in their first year, but most males are not believed to reproduce until their second (Eng 1963). My three temporal categories (1895 – 1965, 1966 – 1991, and 1998 – 2007) were uneven in length so I determined the median date in each time span (1930, 1978, and 2002), which I then used to calculate generation time between the three categories (1895 – 1965, 1966 – 1991, and 1998 – 2007).

I used BOTTLENECK 1.2.02 (Piry et al. 1999) to test for the occurrence of a population contraction by detecting heterozygosity excess. Heterozygosity excess is expected to follow a population bottleneck event, therefore a population can be assessed for a recent loss in genetic diversity without needing to know pre-bottleneck heterozygosity (Cornuet and Luikart 1996; Luikart and Cornuet 1998; Luikart et al. 1998). The occurrence of heterozygosity excess was tested using the two-phase model (TPM; Dirienzo et al. 1994) with 95% single-step mutations, 5% multiple-step mutations, and a variance among multiple steps of 12 as recommended by Piry et al. (1999) for microsatellite data. I used the Wilcoxon signed-rank test to determine if there was a deviation from 50:50 heterozygosity deficiency/excess (Cornuet and Luikart 1996) because it is the most appropriate and powerful test when using fewer than 20 microsatellite loci (Piry et al. 1999).

3. Results

3.1. Genetic diversity and structure

The duplicate analysis performed on the contemporary samples revealed 604 unique individuals in Alberta (chapter 3) and 242 individuals in Saskatchewan. Most loci were in Hardy-Weinberg and linkage disequilibrium at the Canada level for all time periods after corrections for multiple comparisons. However, at the contemporary lek-level (historic samples could not be assigned to

specific leks), all loci were in equilibrium (chapter 3). All loci were retained because the disequilibrium was due to differences between leks (chapter 3).

Using STRUCTURE, I identified an increase in ln Pr (X/*K*) up to $K = 13$, after which the value plateaued. The estimated posterior probability of *K* was 1 at $K = 13$, with higher and lower values of *K* having lower *P*-values. Analysis of ΔK revealed maximum values at 1 ($\Delta K_1 = 34.8$ vs. the next highest $\Delta K_4 = 2.2$), which was consistent with graphical displays of population admixture from STRUCTURE where cluster assignment appeared random at values of *K* greater than 1. An overall *K* of 1, or a single genetic cluster, was also consistent with a lack of substructure when locations and/or time periods were analyzed separately.

All microsatellite loci were polymorphic with number of alleles ranging from 3 – 27. Observed heterozygosity was lowest in the earliest time period (pre-1965) and highest in 1966 – 1991 for all three locations (Canada, Alberta, and Saskatchewan; Table 5-1). Allelic richness tended to be highest in the two earliest time periods (Table 5-1). The only significant difference between time periods was observed for *AR* for Alberta pre-1965 vs. contemporary (Wilcoxon signed ranks test; $P = 0.02$) and Alberta 1966 – 1991 vs. contemporary (Wilcoxon signed ranks test; $P = 0.002$), with the contemporary time period having the lowest *AR*.

Using genotypic data from birds collected prior to 1965 and lek count data gathered since 1968, I simulated the expected loss of genetic diversity over the past 40 years. Given the observed demographic decline and the diversity present 40 years ago, 93.7% of allelic diversity ($A = 10.3$; $AR = 6.3$), 98.6% of H_O (0.65), and 98.2% of H_E (0.72) were predicted to be maintained. Contemporary *AR* and H_O were slightly higher and H_E was slightly lower than these estimates (Table 5-1).

3.2. Effective population size and bottleneck tests

Using the moment-based estimator, harmonic mean N_e for Canada was estimated at infinity (>1000) when comparing 1895 – 1965 and 1966 – 1991 using either generation time. For 1895 – 1965 versus 1998 – 2007, harmonic mean N_e was 287.8 (95% CI = 233.1 – 349. 2) and 143.9 (95% CI = 116.5 –

174.6) for generation times of one and two years, respectively. For 1966 – 1991 versus 1998 – 2007, harmonic mean N_e was 93.6 (95% CI = 75.6 – 113.7) and 46.8 (95% CI = 37.8 – 56.9) for generation times of one and two years, respectively. When I used the point count methods (linkage disequilibrium and heterozygote excess) to examine the effective number of breeders (N_{eb}), the heterozygote excess method estimated N_{eb} to be infinity for all time periods and the linkage disequilibrium method produced values ranging from 440.7 (95% CI = 420.5 – 462.3) for the contemporary time period to 779.1 (95% CI = 368.1 – infinity) for 1895 – 1965. Results from the heterozygote method are likely more accurate, as both methods appear to be estimating the number of breeders for the northern Montana population as a whole, instead of Canada alone, and the northern Montana population currently contains >1000 breeding individuals. When I tested for bottlenecks, no region or time period displayed a significant heterozygosity excess (Table 5-1).

4. Discussion

Using historic (1895 – 1991) and contemporary samples, I documented that sage-grouse in Canada have not experienced reduced genetic diversity, increased population structure, or genetic bottlenecks despite significant demographic declines in the last 40 years. Both effective population size and effective number of breeders decreased with time, but the effective number of breeders was high given the estimated population size. This is likely due to lower than expected variance in reproductive success and gene flow from the rest of the northern Montana population. Presently, it appears that genetic variability in Canada is being maintained through migration from southern parts of the northern Montana sage-grouse population and the low expected decline in genetic diversity based on simulations using historic genotypes.

4.1. Genetic diversity and structure

For all time periods (contemporary, historic, pre-1965, and 1966 – 1991) and regions (Canada, Alberta, and Saskatchewan), the most likely number of

genetic clusters was one indicating a single panmictic population. Despite anthropogenic fragmentation over the past 110 years (Schroeder et al. 2004; Lungle and Pruss 2008) and a severe reduction in suitable habitat in Canada (Figure 5-2; Aldridge and Brigham 2003; Schroeder et al. 2004; Alberta Sage-Grouse Recovery Action Group 2005), a lack of structure indicates that birds are capable of crossing or circumventing disturbance. It also suggests that at no time has any part of Canada has been isolated from the rest of the northern Montana population and that gene flow continues to occur throughout the population (chapter 2). A similar lack of fragmentation-induced genetic structure has been found in capercaillie (*Tetrao urogallus*; Segelbacher et al. 2008) and Spanish imperial eagles (*Aquila adalberti*; Martínez-Cruz et al. 2007). However, this is not always the case. An isolated Dutch population of black grouse (*Tetrao tetrix*) showed altered genetic structure between historic (1893 – 1941) and contemporary (1991 - 2005) samples (Larsson et al. 2008). Wisconsin greater prairie-chickens (*Tympanuchus cupido*) revealed significant genetic subdivision in contemporary (1998 – 2000) compared to historic (1951 – 1954) samples (Johnson et al. 2004). All of these species have undergone habitat loss so it is possible that the presence or absence of population structure reflects the ability of a species to cope with disturbance and its dispersal ability, or is an artifact of sampling (i.e., not sampling an entire population, too small of a geographic area, etc.).

Genetic diversity did not vary temporally despite substantial demographic declines. Contrary to expectations, H_O was the lowest in the oldest time period (pre-1965) for all three regions (Canada, Alberta, and Saskatchewan). This may be an artifact of sample size, but the trend remains the same when data are corrected to the lowest sample size (Table 5-1; Canada = 117, Alberta = 60, Saskatchewan 53). It could also be an artifact of how grouse were sampled, sampling locations for the pre-1965 samples (i.e., based on museum records, up to 10 of these specimens were obtained from single locations over the span of one or two days), or sampling error. While contemporary samples show slightly lower diversity, values are within the expected range generated by simulations using

pre-decline genotypes and lek counts over the last 40 years. This suggests that the majority of the genetic diversity should have been retained, even in a closed system. However, Canada is not part of a closed system and exchange of individuals occurs with the rest of the northern Montana population (chapter 2). The southern subpopulation (south of the Milk River) contains one of the largest and healthiest segments in the species' range (Connelly et al. 2004) and immigrants from this region appear to introduce new genetic material, as contemporary Canadian birds contain multiple alleles absent from the earlier time periods.

Temporal studies on avian species have found evidence for both decreased genetic variation over time and for no significant change. Dutch black grouse (Larsson et al. 2008) and greater prairie-chickens in Wisconsin (Bellinger et al. 2003; Johnson et al. 2004) and Illinois (Bouzat et al. 1998a; Bouzat 2001) showed genetic impoverishment in contemporary samples compared to museum specimens. Mainland dwelling New Zealand robins (*Petroica australis*) showed evidence of genetic impoverishment over time whereas island dwelling birds did not (Taylor et al. 2007). In capercaillie, genetic variability did not differ between historic and present-day sampling periods, but allelic richness was higher in historic samples (Segelbacher et al. 2008). Saddlebacks (*Philesturnus carunculatus*; Taylor et al. 2007) and Spanish imperial eagles (Martínez-Cruz et al. 2007) also did not show a decrease in genetic variability. Similar to population structure, differences may be due not only to sampling, but to the species' dispersal patterns, physiology, behaviour, reproductive biology, or habitat needs that translate into interspecific differences in the ability to cope with fragmentation, disturbance, predation, or exploitation. In non-avian species, typically more mobile species (arctic fox [*Alopex lagopus*], Nyström et al. 2006) displayed less loss in genetic variation compared to more sedentary and/or heavily exploited or persecuted species (African elephants [*Loxodonta Africana africana*], Whitehouse and Harley 2001; black-footed ferrets [*Mustela nigripes*]; Wisely et al. 2002; New Zealand snapper [*Pagrus auratus*], Hauser et al. 2002; sea otters [*Enhydra lutris*], Larson et al. 2002; grizzly [*Ursus arctos*], Miller and Waits

2003; Scandinavian wolves [*Canis lupus*], Flagstad et al. 2003). This suggests that dispersal ability and exploitation level influence a species' ability to maintain genetic diversity.

4.2. Effective population size and bottleneck tests

Effective population is expected to be lower in lekking species because of the high variance in reproductive success among males (Nunney 1993), as only a few males are typically observed to mate on leks (e.g., Wiley 1973). However, I have previously found that variance in reproductive success among sage-grouse males from Alberta is lower than expected based on estimates from other parts of the range and almost 50% of sampled males fertilized eggs in their lifetime (chapter 4). I found that N_e using the moment-based estimator decreased with time, which was expected because a decrease in census size is almost always accompanied by a decrease in N_e (Frankham 1995). I also found that generation time (one or two years) had a large impact on the estimate, but considering the reproductive biology of the species, the true generation time is likely somewhere between one and two years resulting in a true N_e of between 46.8 and 93.6 or 12.3 to 24.8% of the census estimate. This range in N_e is consistent, but slightly higher than that of greater prairie chickens, which ranged from 5.1 to 15.2% of the census estimates (Johnson et al. 2004). Gene flow currently occurs in this sage-grouse population (chapter 2), which may account for the difference. Both point count methods (linkage disequilibrium and heterozygote excess) gave high estimates for the number of effective breeders for all time periods, suggesting a large proportion of Canadian birds breed and/or there are considerably more birds in Canada than indicated by lek counts. Both findings are anticipated based on level of reproductive success observed in Alberta (chapter 4) and Canada's connectivity to the rest of the Northern Montana population (chapter 2). While the most contemporary estimate of N_e is low compared to the population size for Canada, the number of effective breeders is high given the estimated census population size. This is likely due to the high level of reproductive success observed in Alberta and gene flow from the rest of the population, which have

positive implications for the maintenance of genetic diversity and sustainability of sage-grouse in Canada.

Populations that have recently undergone a demographic bottleneck are expected to exhibit heterozygote excess at most microsatellite loci. I found no evidence of heterozygote excess during any time period or in any region suggesting that a genetic bottleneck had not accompanied the demographic bottleneck. Apart from the actual population decline, I saw no corresponding negative genetic (this study) or biological (e.g., fertility; chapter 4) impacts. In Alberta, sage-grouse exhibited 99.2% fertility from 1999 – 2006 (chapter 4). In contrast, greater prairie-chickens in Illinois that suffered a bottleneck had reduced fertility (< 80%; Westemeier et al. 1998) compared to the normal species average of approximately 90% (Westemeier et al. 1998; Bellinger et al. 2003). Taxonomically diverse imperiled species or populations have exhibited genetic bottlenecks in contemporary samples: European tree frog (*Hyla arborea*; Andersen et al. 2004), bumblebee (*Bombus muscorum*; Darvill et al. 2006), and Bechstein's bat (*Myotis bechsteinii*; Durrant et al. 2009). Lack of evidence for a bottleneck in sage-grouse is consistent with the findings of many other studies on declining species and populations: capercaillie (Segelbacher et al. 2008), black grouse (Larsson et al. 2008), greater prairie-chicken in Wisconsin (Bellinger et al. 2003), Bennett's wallaby (*Macropus rufogriseus rufogriseus*; Le Page et al. 2000), African elephants (Whitehouse and Harley 2001), North Atlantic right whale (*Eubalaena glacialis*; Waldick et al. 2002), banner-tailed kangaroo rat (*Dipodomys spectabilis*, Busch et al. 2007), peregrine falcon (*Falco peregrinus*; Brown et al. 2007), saddleback (Taylor et al. 2007), and New Zealand robin (*Petroica australis*; Taylor et al. 2007). I likely did not detect a genetic bottleneck because Canada is only part of the northern Montana population and gene flow is occurring between all regions of the population (chapter 2). Also, the loss of genetic diversity was predicted to be small based on simulations. Therefore, despite a substantial demographic decline, gene flow from healthier parts of the population and high levels of genetic variability present prior to the decline are maintaining genetic diversity in Canada.

Because sage-grouse are experiencing declines throughout their range (Connelly et al. 2004; Schroeder et al. 2004), my results should be compared to patterns in other parts of the range to determine the genetic resiliency of the species. In particular, peripheral (South and North Dakota) and isolated peripheral (Washington, California, and Utah) populations and Gunnison sage-grouse (*Centrocercus minimus*; Colorado) should be examined to assess their loss of genetic diversity over time and to determine if they are connected to larger, more demographically stable populations or regions that may provide genetic rescue, similar to that observed in Canada (chapter 2). Genetic data will provide essential information for managing this declining species threatened by continued habitat loss, particularly in peripheral and isolated regions that are at the highest risk of extirpation.

Literature Cited

Alberta Sage-Grouse Recovery Action Group. 2005. Alberta Greater Sage-Grouse Recovery Plan. Alberta Sustainable Resource Development, Fish and Wildlife Division, Alberta Species at Risk Recovery Plan No. 8. Edmonton, Alberta.

Aldridge, C.L., Boyce, M.S., and Baydack, R.K. 2004. Adaptive management of prairie grouse: how do we get there? Wildlife Society Bulletin **32**: 92-103.

Aldridge, C.L. and Brigham, R.M. 2003. Distribution, abundance, and status of the greater sage-grouse, *Centrocercus urophasianus*, in Canada. Canadian Field Naturalist **117**: 25-34.

Anderson, L.W., Fog, K., and Damgaard, C. 2004. Habitat fragmentation causes bottlenecks and inbreeding in the European tree frog (*Hyla arborea*). Proceedings of the Royal Society of London, Series B **271**: 1293–1302. Doi: 10.1098/rspb.2004.2720.

Bartley, D., Bagley, M., Gall, G., and Bentley, B. 1992. The use of linkage disequilibrium data to estimate effective size of hatchery and natural fish populations. Conservation Biology **6**: 365–375.

Bellinger, M.R., Johnson, J.A., Toepfer, J., and Dunn, P. 2003. Loss of genetic variation in Greater Prairie Chickens following a population bottleneck in Wisconsin, U.S.A. Conservation Biology **17**: 717–724. Doi: 10.1046/j.1523-1739.2003.01581.x

Bouzat, J.L. 2001. The importance of control populations for the identification and management of genetic diversity. Genetics **110**: 109–115. Doi: 10.1023/A:1017985522650

Bouzat, J.L., Lewin, H.A., and Paige, K.N. 1998a. The ghost of genetic diversity past: historical DNA analysis of the greater prairie chicken. American Naturalist **152**: 1–6. Doi: 10.1086/286145

Bouzat, J.L., Cheng, H.H., Lewin, H.A., Westemeier, R.L., Brawn, J.D., and Paige, K.N. 1998b. Genetic evaluation of a demographic bottleneck in the greater prairie chicken. Conservation Biology **12**: 836–843.

Brodie III, E.D. 2007. Population size is not genetic quality. Animal Conservation **10**: 288–290. Doi: 10.1111/j.1469-1795.2007.00134.x

Brown, J.W., Van Coeverden De Groot, P.J., Birt, T.P., Seutin, G., Boag, P.T., and Friesen, V.L. 2007. Appraisal of the consequences of the DDT-induced bottleneck on the level and geographic distribution of neutral genetic variation in Canadian peregrine falcons, *Falco peregrinus*. Molecular Ecology **16**: 327–343. Doi: 10.1111/j.1365-294X.2007.03151.x

Busch, J.D., Waser, P.M., and DeWoody, J.A. 2007. Recent demographic bottlenecks are not accompanied by a genetic signature in banner-tailed kangaroo rats (*Dipodomys spectabilis*). Molecular Ecology **16**: 2450–2462. Doi: 10.1111/j.1365-294X.2007.03283.x

Bush, K.L. 2008. A pressure-operated drop net for capturing Greater Sage-Grouse. Journal of Field Ornithology **79**: 64-70. Doi: 10.1111/j.1557-9263.2008.00146.x

Bush, K.L., Vinsky, M.D., Aldridge, C.L., and Paszkowski, C.A. 2005. A comparison of sample types varying in invasiveness for use in DNA sex determination in an endangered population of greater sage-grouse (*Centrocercus urophasianus*). Conservation Genetics **6**: 867-870. Doi: 10.1007/s10592-005-9040-6

Braun, C.E., Oedekoven, O.O., and Aldridge, C.L. 2002. Oil and gas development in western North America: effects on sagebrush steppe avifauna with particular emphasis on Sage Grouse. Transactions of the 67th North American Wildlife and Natural Resources Conference **67**: 337-349.

Caizergues, A., Rätti, O., Helle, P., Rotelli, L., Ellison, L., and Rasplus, J-.Y. 2003. Population genetic structure of male black grouse (*Tetrao tetrix* L.) in fragmented vs. continuous landscapes. Molecular Ecology **12**: 2297-2305. Doi: 10.1046/j.1365-294X.2003.01903.x

Caizergues, A., Dubois, S., Mondor, G., and Rasplus J-.Y. 2001. Isolation and charcterisation of microsatellite loci in black grouse (*Tetrao tetrix*). Molecular Ecology Notes **1**: 36-38. Doi: 10.1046/j.1471-8278.2000.00015.x

Chakraborty, R. and Nei, M. 1977. Bottleneck effects on average heterozygosity and genetic distance with the stepwise mutation model. Evolution **31**: 347–356.

Cheng, H.H., Levin, I., Vallejo, R.L., Khatib, H., Dodgson, J.B., Crittenden, L.B., and Hillel, J. 1995. Development of a genetic map of the chicken with high-utility markers. Poultry Science **74**: 1855-1874.

Connelly, J.W., Knick, S.T., Schroeder, M.A., and Stiver, S.H. 2004. Conservation Assessment of Greater Sage-grouse and Sagebrush Habitats. Western Association of Fish and Wildlife Agencies. Unpublished Report. Cheyenne, Wyoming.

Cornuet, J.M. and Luikart, G. 1996. Description and evaluation of two tests for detecting recent bottlenecks. Genetics **144**: 2001–2014.

Darvill, B., Ellis, J.S., Lye, G.C., and Goulson, D. 2006. Population structure and inbreeding in a rare and declining bumblebee, *Bombus muscorum* (Hymenoptera: Apidae). Molecular Ecology **15**: 601–611. Doi: 10.1111/j.1365-294X.2006.02797.x

Durrant, C.J., Beebee, T.J.C., Greenaway, F., and Hill, D.A. 2009. Evidence of recent population bottlenecks and inbreeding in British populations of Bechstein's bat, *Myotis bechsteinii*. Conservation Genetics **10**: 489–496. Doi: 10.1007/s10592-008-9639-5

Eng, R.L. 1963. Observations on the breeding biology of male sage grouse. Journal of Wildlife Management **27**: 841-846.

Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology **14**: 2611-2620. Doi: 10.1111/j.1365-294X.2005.02553.x

Flagstad, Ø., Walker, C.W., Vilá, C., Sundqvist, A.K., Fernholm, B., Hufthammer, A.K., Wiig, Ø., Koyola, I., and Ellegren, H. 2003. Two centuries of the Scandinavian wolf population: patterns of genetic variability and migration during an era of dramatic decline. Molecular Ecology **12**: 869–880. Doi: 10.1046/j.1365-294X.2003.01784.x

Frankham, R., Ballou, J.D., and Briscoe, D.A. 2002. Introduction to Conservation Genetics. Cambridge University Press, Cambridge.

Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: a review. Genetical Research **66**: 95-106.

Gibson, R.M. 1996. Female choice in sage grouse: the roles of attraction and active comparison. Behavioural Ecology and Sociobiology **39**: 55-59. Doi:10.1006/anbe.1996.0247

Giesen, K.M., Schoenberg, T.J., and Braun, C.E. 1982. Methods for trapping sage grouse in Colorado. Wildlife Society Bulletin **10**: 223-231.

Gilpin, M.E. and Soulé, M.E. 1986. Minimum viable populations: processes of species extinction. In: Conservation Biology: the Science of Scarcity and Diversity (ed. Soulé, M.E.), pp 19 – 34. Sinauer Associates, Sunderland, MA.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html. Updated from Goudet (1995).

Hauser, L., Adcock, G.J., Smith, P.J., Bernal Ramírez, J.H., and Carvalho, G.R. 2002. Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). Proceedings of the National Academy of Sciences, USA **99**: 1 1742–11747. Doi: 10.1073/pnas.172242899

Hill, W.G. 1981. Estimation of effective population size from data on linkage disequilibrium. Genetical Research (Cambridge) **38**: 209-216

Hill, W.G. 1979. A note on effective population size with overlapping generations. Genetics **92**: 317–322.

Johnson, J.A., Bellinger, M.R., Toepfer, J.E., and Dunn, P. 2004. Temporal changes in allele frequencies and low effective population size in greater prairie-chickens. Molecular Ecology **13**: 2617–2630. Doi: 10.1111/j.1365-294X.2004.02264.x

Kimura, M. and Crow, J.F. 1963. The measurement of effective population number. Evolution **17**: 279–288.

Kuo, C.H. and Janzen, F.J. 2003 BOTTLESIM: a bottleneck simulation program for long-lived species with overlapping generations. Molecular Ecology Notes **3**: 669–673. Doi: 10.1046/j.1471-8286.2003.00532.x

Lacy, R.C. 1997. Importance of genetic variation to the viability of mammalian populations. Journal of Mammalogy **78**: 320–335.

Larson, S., Jameson, R., Etnier, M., Fleming, M., and Bentzen, P. 2002. Loss of genetic diversity in sea otters (*Enhydra lutris*) associated with the fur trade of the 18th and 19th centuries. Molecular Ecology **11**: 1899–1903. Doi: 10.1046/j.1365-294X.2002.01599.x

Larsson, J.K., Jansman, H.A.H., Segelbacher, G., Höglund, J., and Koelewijn, H.P. 2008. Genetic impoverishment of the last black grouse (*Tetrao tetrix*) population in the Netherlands: detectable only with a reference from the past. Molecular Ecology **17**: 1897–1904. Doi: 10.1111/j.1365-294X.2008.03717.x

Leberg, P. 2005. Genetic approaches for estimating the effective size of populations. Journal of Wildlife Management **69**: 1385–1399.

Le Page, S.L., Livermore, R.A., Cooper, D.W., and Taylor, A.C. 2000. Genetic analysis of a documented population bottleneck: introduced Bennett's wallabies (*Macropus rufogriseus rufogriseus*) in New Zealand. Molecular Ecology **9**: 753–763. Doi: 10.1046/j.1365-294x.2000.00922.x

Luikart, G. and Cornuet, J. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conservation Biology **12**: 228–237.

Luikart, G., Sherwin, W.B., Steele, B.M., and Allendorf, F.W. 1998. Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. Molecular Ecology **7**: 963-974. Doi: 10.1046/j.1365-294x.1998.00414.x

Lungle, K. and Pruss, S. 2008. Recovery Strategy for the Greater Sage-Grouse (*Centrocercus urophasianus urophasianus*) in Canada. *In* Species at Risk Recovery Strategy Series. Parks Canada Agency. Ottawa.

Martínez-Cruz, B., Godoy, J.A., and Negro, J.J. 2007. Population fragmentation leads to spatial and temporal genetic structure in the endangered Spanish imperial eagle. Molecular Ecology **16**: 477–486. Doi: 10.1111/j.1365-294X.2007.03147.x

Miller, C.R. and Waits, L.P. 2003. The history of effective population size and genetic diversity in the Yellowstone grizzly (*Ursus arctos*): implications for conservation. Proceedings of the National Academy of Sciences, USA **100**: 4334–4339. Doi: 10.1073/pnas.0735531100

Nei, M., Maruyama, T., and Chakraborty, R. 1975. The bottleneck effect and genetic variability in populations. Evolution **29**: 1-10.

Nunney, L. 1993. The influence of mating systems and overlapping generations on effective population size. Evolution **47**: 1329–1341.

Nunney, L. 1991. The influence of age structure and fecundity on the effective population size. Proceedings of the Royal Society of London. Series B, Biological Sciences **246**: 71–76.

Nyström, V., Angerbjörn, A., and Dalén, L. 2006. Genetic consequences of a demographic bottleneck in the Scandinavian arctic fox. OIKOS **114**: 84–94. Doi: 10.1111/j.2006.0030-1299.14701.x

Park, S.D.E. 2001. Trypanotolerance in West African cattle and the population genetic effects of selection. PhD thesis, University of Dublin, Ireland.

Peakall, R. and Smouse, P.E. 2001. GENALEX version 5.1. Genetic analysis in Excel. Population genetic software for teaching and research. http://www.anu.edu.au/BoZo/GenAlEx. Australian National University, Canberra, Australia.

Peel, D., Ovenden, J.R., and Peel, S.L. 2004. NEESTIMATOR: software for estimating effective population size, Queensland Government, Department of Primary Industries and Fisheries, Brisbane, Australia, version 1.2.

Pertoldi, C., Moller Hansen, M., Loeschcke, V., Bo Madsen, A., Jacobsen, L., and Baagoe, H. 2001. Genetic consequences of population decline in the European otter (*Lutra lutra*): an assessment of microsatellite DNA in Dannish otters from 1883 to 1993. Proceedings of the Royal Society of London. Series B, Biological Sciences **268**: 1775–1781. Doi: 10.1098/rspb.2001.1762.

Piertney, S.B. and Höglund, J. 2001. Polymorphic microsatellite DNA markers in black grouse (*Tetrao tetrix*). Molecular Ecology Notes **1**: 303-304. Doi: 10.1046/j.1471-8278.2001.00118.x

Piertney, S.B. and Dallas, J.F. 1997. Isolation and characterization of hypervariable microsatellites in the red grouse. *Lagopus lagopus scoticus*. Molecular Ecology **6**: 93-95. Doi: 10.1046/j.1365-294X.1997.00154.x

Piry, S., Luikart, G., and Cornuet, J. 1999. BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity **90**: 502–503.

Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics **15**: 945-959.

Pudovkin, A.I., Zaykin, D.V., and Hedgecock, D. 1996. On the potential for estimating the effective number of breeders from heterozygote excess in progeny. Genetics **144**: 383-387.

Raymond, M. and Rousset, F. 1995 Genepop version 3.1d: population genetics software for exact test and ecumenism. Journal of Heredity **86**: 248-249.

Reed, D.H. 2007. Extinction of island endemics: it is not inbreeding depression. Animal Conservation **10**: 145–146. Doi: 10.1111/j.1469-1795.2007.00108.x

Rienzo, AD., Peterson, A.C., Garza, J.C., Caldes, A.M., Slatkin, M., and Freimer, M.B. 1994. Mutational processes of simple-sequence repeat loci in human populations. Proceedings of the National Academy of Sciences of the USA **91**: 3166–3170.

Schroeder, M.A., Aldridge, C.L., Apa, A.D., Bohne, J.R., Braun, C.E., Bunnell, S.D., Connelly, J.W., Diebert, P.A., Gardner, S.C., Hilliard, M.A., Kobriger, G.D., McAdam, S.M., McCarthy, C.W., McCarthy, J.J., Mitchell, D.L., Rickerson, E.V., and Stiver, S.J. 2004. Distribution of sage-grouse in North America. Condor **106**: 363-376. Doi: 10.1650/7425

Schroeder, M.A. and Braun, C.E. 1991. Walk-in traps for capturing greater prairie-chickens on leks. Journal of Field Ornithology **62**: 378-385.

Segelbacher, G., Manel, S., and Tomiuk, J. 2008. Temporal and spatial analyses disclose consequences of habitat fragmentation on the genetic diversity of capercaillie (*Tetrao urogallus*). Molecular Ecology **17**: 2356–2367. Doi: 10.1111/j.1365-294X.2008.03767.x

Segelbacher, G., Paxton, R.J., Steinbrueck, G., Trontelj, P., and Storch, I. 2000. Characterization of microsatellites in capercaillie (*Tetrao urogallus*) (AVES). Molecular Ecology **9**: 1934-1935. Doi: 10.1046/j.1365-294x.2000.0090111934.x

Soulé, M.E. and Mill, L.S. 1998. No need to isolate genetics. Science **282**: 1658–1659. Doi: 10.1126/science.282.5394.1658

Taylor, S.S., Jamieson, I.G., and Wallis, G.P. 2007. Historic and contemporary levels of genetic variation in two New Zealand passerines with different histories of decline. Journal of Evolutionary Biology **20**: 2035–2047. Doi: 10.1111/j.1420-9101.2007.01362.x

Taylor, S.E., Oyler-McCance, S.J., and Quinn, T.W. 2003. Isolation and characterization of microsatellite loci in greater sage-grouse (*Centrocercus urophasianus*). Molecular Ecolology Notes **3**: 262-264. Doi: 10.1046/j.1471-8286.2003.00424.x

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., and Shipley, P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes **4**: 535–538. Doi: 10.1111/j.1471-8286.2004.00684.x

Waldick, R.C., Kraus, S., Brown, M., and White, B.N. 2002. Evaluating the effects of historic bottleneck events: an assessment of microsatellite variability in the endangered, North Atlantic right whale. Molecular Ecology **11**: 2241–2249. Doi: 10.1046/j.1365-294X.2002.01605.x

Waples, R.S. 1989. Temporal variation in allele frequencies: testing the right hypothesis. Evolution **43**: 1236–1251.

Westemeier, R.L., Brawn, J.D., Simpson, S.A., Esker, T.L., Jansen, R.W., Walk, J.W., Kershner, E.L., Bouzat, J.L., and Paige, K.N. 1998. Tracking the long-term decline and recovery of an isolated population. Science **282**: 1695–1698. Doi: 10.1126/science.282.5394.1695

Whitehouse, A.M. and Harley, E.H. 2001. Post-bottleneck genetic diversity of elephant populations in South Africa, revealed using microsatellite analysis. Molecular Ecology **10**: 2139–2149. Doi: 10.1046/j.0962-1083.2001.01356.x

Wiley, R.H. 1973 Territoriality and non-random mating in the sage grouse, *Centrocercus urophasianus*. Animal Behaviour Monographs **6**: 87-169.

Wisely, S.M., Buskirk, S.W., Fleming, M.A., McDonald, D.B., and Ostrander, E.A. 2002. Genetic diversity and fitness in black-footed ferrets before and during a bottleneck. Journal of Heredity **93**: 231–237.

Wright, S. 1938. Size of population and breeding structure in relation to evolution. Science **87**: 430–431.

Wright, S. 1931. Evolution in Mendelian populations. Genetics **16**: 97–159.

Table 5-1. Genetic diversity in historic and contemporary (1998 – 2007) Canadian sage-grouse separated into three regions: Canada (Alberta and Saskatchewan combined), Alberta only, and Saskatchewan only. Values are given for observed heterozygosity (H_O), expected heterozygosity (H_E), number of alleles (A), number of alleles corrected to the smallest sample size (AR), and the inbreeding coefficient (F_{IS}). Values in parentheses for H_O, H_E, and F_{IS} are corrected to the smallest sample size for each group of three (Canada [117], Alberta [60], and Saskatchewan [53]), as is AR. All diversity values are given ± standard error. Probability of heterozygosity excess was determined using the Wilcoxon signed-rank test to identify bottlenecks using the two-phase model (TPM).

Location and Time Period	n	H_O	H_E	A	AR	F_{IS}	Probability of Heterozygosity Excess
Canada Pre-1965	113	0.66 ± 0.05 (0.66 ± 0.05)	0.73 ± 0.03 (0.72 ± 0.03)	11.0 ± 1.4	6.7 ± 0.63	0.07 ± 0.06 (0.06 ± 0.06)	0.96
Canada 1965-1991	125	0.70 ± 0.03 (0.70 ± 0.03)	0.76 ± 0.03 (0.76 ± 0.03)	11.5 ± 1.4	6.4 ± 0.7	0.05 ± 0.05 (0.05 ± 0.05)	0.83
Canada - Contemporary	860	0.68 ± 0.03 (0.67 ± 0.04)	0.71 ± 0.04 (0.71 ± 0.04)	14.3 ± 1.6	6.5 ± 0.7	0.04 ± 0.03 (0.09 ± 0.03)	0.99
Alberta Pre-1965	60	0.68 ± 0.03 (0.68 ± 0.03)	0.75 ± 0.03 (0.75 ± 0.03)	9.9 ± 1.1	8.1 ± 0.8	0.09 ± 0.04 (0.09 ± 0.04)	0.92
Alberta 1965-1991	64	0.71 ± 0.03 (0.71 ± 0.03)	0.78 ± 0.03 (0.77 ± 0.03)	9.9 ± 1.1	8.1 ± 0.8	0.09 ± 0.03 (0.07 ± 0.03)	0.34
Alberta - Contemporary	618	0.68 ± 0.03 (0.69 ± 0.04)	0.71 ± 0.04 (0.70 ± 0.04)	12.4 ± 1.4	6.9 ± 0.8	0.03 ± 0.03 (0.09 ± 0.03)	0.97
Saskatchewan Pre-1965	53	0.64 ± 0.07 (0.64 ± 0.07)	0.70 ± 0.05 (0.70 ± 0.05)	8.3 ± 1.2	6.5 ± 0.7	0.08 ± 0.1 (0.08 ± 0.1)	0.85
Saskatchewan 1965-1991	61	0.68 ± 0.04 (0.68 ± 0.04)	0.74 ± 0.03 (0.73 ± 0.03)	8.8 ± 1.1	6.7 ± 0.6	0.07 ± 0.6 (0.08 ± 0.06)	0.55
Saskatchewan - Contemporary	242	0.66 ± 0.04 (0.68 ± 0.04)	0.70 ± 0.04 (0.70 ± 0.04)	11.1 ± 1.4	6.4 ± 0.7	0.05 ± 0.05 (0.04 ± 0.04)	0.96

Appendix 5-1. Samples from historical sage-grouse used in this study. Samples were obtained from the following museums: Canadian Museum of Nature (CMN), Etzikom Museum (EM), Grand Coteau Museum (GCM), Jasper Centre Museum (JCM), Manitoba Museum (MM), Royal Alberta Museum (RAM), Royal British Columbia Museum (RBCM), Royal Ontario Museum (ROM), Royal Saskatchewan Museum (RSM), Swift Current Museum (SCM), University of Alberta Museum of Zoology (UA), University of Saskatchewan Museum of Natural Sciences (US); and private collections: Alberta Fish and Wildlife (F&W), Augustana (AUG), Grasslands National Park (GNP), Police Point Park Interpretive Nature Centre (PPP), Saskatchewan Environment (SE), University of British Columbia (UBC), University of Calgary (UC), and University of Regina (UR). Province codes are Alberta (AB) and Saskatchewan (SK). Many specimens had unknown exact collection dates so best estimates are given based on the data that was available.

	Source	Accession/Sample Number	Province	Location	Year of Collection	Sample Source
1	CMN	CMNAV-1965	SK	Unknown	1895	Skin
2	CMN	CMNAV-1966	SK	Unknown	1895	Skin
3	CMN	CMNAV-1968	SK	Unknown	1895	Skin
4	CMN	CMNAV-24865	SK	Unknown	1931	Skin
5	CMN	CMNAV-24866	SK	Unknown	1931	Skin
6	CMN	CMNAV-24867	SK	Unknown	1931	Skin
7	CMN	CMNAV-24917	SK	Val Marie	1931	Skin
8	CMN	CMNAV-25927	SK	East End	1935	Skin
9	CMN	CMNAV-26654	SK	East End	1935	Skin
10	CMN	CMNAV-30490	AB	Milk River	1945	Skin
11	CMN	CMNAV-30491	AB	Milk River	1945	Skin
12	CMN	CMNAV-30492	SK	Divide	1945	Skin
13	CMN	CMNAV-30493	SK	Divide	1945	Skin
14	CMN	CMNAV-30494	SK	Divide	1945	Skin
15	CMN	CMNAV-33083	SK	Robsart	1948	Skin
16	CMN	CMNAV-33084	SK	Divide	1948	Skin

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17	CMN	CMNAV-33085	SK	Robsart	1948	Skin
18	CMN	CMNAV-52098	SK	Consul	1960	Skin
19	CMN	CMNAV-52099	SK	Consul	1960	Skin
20	CMN	CMNAV-24864	SK	Unknown	1931	Mount
21	CMN	CMNAV-B429	AB	Medicine Hat	1948	Mount
22	CMN	CMNAV-33024	SK	Unknown	1939	Skin
23	CMN	CMNAV-NOCODE1	SK	Consul	1960s	Mount
24	CMN	CMNAV-NOCODE2	SK	Consul	1960s	Mount
25	CMN	CMNAV-NOCODE3	SK	Consul	1960s	Mount
26	EM	HIST-63	AB	Manyberries Area	< 1965	Mount
27	EM	HIST-64	AB	Manyberries Area	< 1965	Mount
28	EM	HIST-65	AB	Manyberries Area	< 1965	Mount
29	GCM	1930.82.3	SK	Unknown	< 1937	Mount
30	GCM	1930.82.6	SK	Unknown	< 1937	Mount
31	GCM	1932.15.2	SK	Unknown	< 1937	Mount
32	GCM	1932.15.3	SK	Unknown	< 1937	Mount
33	GCM	1932.18.1	SK	Unknown	< 1937	Mount
34	GCM	GC-6	SK	Unknown	< 1937	Mount
35	JCM	HIST-43	AB	Southwest of Onefour	1979	Mount
36	MM	MM-1-2-1773	SK	Val Marie	1942	Skin
37	MM	MM-1-2-2428	SK	Unknown	1965	Skin
38	RAM	Z66.56.15	AB	Manyberries	1966	Mount
39	RAM	Z66.56.17	AB	Comrey	1966	Mount
40	RAM	Z66.56.18	AB	Comrey	1966	Mount
41	RAM	Z66.56.19	AB	Comrey	1966	Mount
42	RAM	Z67.24.15	AB	Manyberries	1967	Mount
43	RAM	Z79.114.335	AB	Manyberries	1973	Mount

44	RAM	Z79.114.336	AB	Manyberries	1973	Mount
45	RAM	Z79.114.800	SK	Southern Saskatchewan	1957	Mount
46	RAM	Z73.56.24	AB	Southeast of Comrey	1973	Skin
47	RAM	Z73.46.25	AB	Southeast of Comrey	1973	Skin
48	RAM	Z80.120.1	SK	White Mud	1930	Skin
49	RAM	Z80.120.2	SK	White Mud	1939	Skin
50	RAM	Z83.44.69	AB	West of Onefour	1983	Skin
51	RAM	Z83.44.70	AB	West of Onefour	1983	Skin
52	RAM	Z71.53.1	AB	Southern Alberta	1970	Partial Skeleton
53	RAM	Z71.53.2	AB	Southern Alberta	1970	Partial Skeleton
54	RAM	Z71.53.3	AB	Southern Alberta	1970	Partial Skeleton
55	RAM	Z71.53.4	AB	Southern Alberta	1970	Partial Skeleton
56	RAM	Z71.53.5	AB	Southern Alberta	1970	Partial Skeleton
57	RAM	Z83.44.82	AB	East of Onefour	1983	Skeleton
58	RAM	Z89.51.7	AB	Southwest of Manyberries	1989	Skin
59	RAM	Z01.17.1	AB	Unknown	1960s	Mount
60	RAM	Z62.1.102	AB	Unknown	1960s	Mount
61	RAM	Z79.114.222	AB	Unknown	1970/71	Mount
62	RAM	Z79.114.328	AB	Unknown	1970/71	Mount
63	RBCM	RBCM-009615	SK	Val Marie	1930	Skin
64	RBCM	RBCM-009616	SK	Cadillac	1929	Skin
65	RBCM	RBCM-012647	SK	White Mud	1939	Skin
66	RBCM	RBCM-012648	SK	White Mud	1939	Skin
67	RBCM	RBCM-018803	AB	South of Manyberries	1955	Skin
68	RBCM	RBCM-021840	AB	Southwest of Manyberries	1968	Skin
69	RBCM	RBCM-021841	AB	Southwest of Manyberries	1969	Skin
70	RBCM	RBCM-021842	AB	Southwest of Manyberries	1969	Skin
71	RBCM	RBCM-021843	AB	Southwest of Manyberries	1968	Skin

72	RBCM	RBCM-021844	AB	Southwest of Manyberries	1968	Skin
73	RBCM	RBCM-021845	AB	Southwest of Manyberries	1969	Skin
74	RBCM	RBCM-021846	AB	Southwest of Manyberries	1969	Skin
75	RBCM	RBCM-021847	AB	Southwest of Manyberries	1969	Skin
76	RBCM	RBCM-021848	AB	Southwest of Manyberries	1969	Skin
77	RBCM	RBCM-021849	AB	Southwest of Manyberries	1969	Skin
78	RBCM	RBCM-021850	AB	Southwest of Manyberries	1969	Skin
79	RBCM	RBCM-021851	AB	Southwest of Manyberries	1969	Skin
80	RBCM	RBCM-021852	AB	Southwest of Manyberries	1969	Skin
81	RBCM	RBCM-021853	AB	Southwest of Manyberries	1969	Skin
82	RBCM	RBCM-021854	AB	Southwest of Manyberries	1969	Skin
83	RBCM	RBCM-021855	AB	Southwest of Manyberries	1972	Skin
84	RBCM	RBCM-021856	AB	Southwest of Manyberries	1969	Skin
85	RBCM	RBCM-021857	AB	Southwest of Manyberries	1972	Skin
86	RBCM	RBCM-021858	AB	Southwest of Manyberries	1972	Skin
87	RBCM	RBCM-021859	AB	Southwest of Manyberries	1972	Skin
88	RBCM	RBCM-021860	AB	Southwest of Manyberries	1972	Skin
89	RBCM	RBCM-021861	AB	Southwest of Manyberries	1972	Skin
90	RBCM	RBCM-021862	AB	Southwest of Manyberries	1972	Skin
91	RBCM	RBCM-021863	AB	Southwest of Manyberries	1972	Skin
92	RBCM	RBCM-018803	AB	Unknown	1955	Mount
93	ROM	ROM-33024	SK	Cypress Hills	1939	Skin
94	ROM	ROM-33025	SK	Cypress Hills	1939	Skin
95	ROM	ROM-33026	SK	Cypress Hills	1939	Skin
96	ROM	ROM-33027	SK	Cypress Hills	1939	Skin
97	ROM	ROM-33192	SK	Dollard, White Mud	1939	Skin
98	ROM	ROM-36822	SK	Frenchman River	1910	Skin
99	ROM	ROM-36823	SK	Frenchman River	1910	Skin

100	ROM	ROM-82720	SK	Dollard, White Mud	1935	Skin
101	ROM	ROM-82721	SK	Val Marie	1929	Skin
102	ROM	ROM-82722	SK	White Mud Creek	1935	Skin
103	ROM	ROM-86094	SK	Dollard, White Mud	1935	Skin
104	ROM	ROM-110718	AB	North of Orion	1971	Skin
105	ROM	ROM-110719	SK	Northwest of Consul	1971	Skin
106	ROM	ROM-110799	AB	North of Orion	1971	Skeleton
107	ROM	ROM-110800	SK	North of Consul	1971	Skeleton
108	ROM	ROM-145475	SK	Southeast of Consul	1982	Skeleton
109	ROM	ROM-145476	SK	South of Consul	1982	Skin
110	ROM	ROM-145905	SK	Southeast of Val Marie	1966	Skin
111	ROM	ROM-145906	SK	Southeast of Val Marie	1961	Skin
112	ROM	ROM-145907	SK	Southeast of Val Marie	1961	Skin
113	ROM	ROM-145908	SK	Southeast of Val Marie	1961	Skin
114	ROM	ROM-145909	SK	Southeast of Val Marie	1961	Skin
115	ROM	ROM-145910	SK	Southeast of Val Marie	1961	Skin
116	ROM	ROM-145911	SK	Southeast of Val Marie	1961	Skin
117	ROM	ROM-145914	SK	Southeast of Val Marie	1966	Skin
118	ROM	ROM-145915	SK	Southeast of Val Marie	1966	Skin
119	ROM	ROM-145916	SK	Southeast of Val Marie	1966	Skin
120	ROM	ROM-145917	SK	Southeast of Val Marie	1966	Skin
121	ROM	ROM-145918	SK	Southeast of Val Marie	1966	Skin
122	ROM	ROM-145937	SK	Southeast of Val Marie	?	Skeleton
123	ROM	ROM-35.10.12.2	SK	Dollard, White Mud	1935	Skin
124	ROM	ROM-33.10.12.1	SK	Dollard, White Mud	1935	Skin
125	ROM	ROM-9079	AB	Unknown	>1965	Skin
126	RSM	RSM00369.001	SK	Pinto Creek	1914	Skin
127	RSM	RSM002304.001	SK	Val Marie	1929	Skin

128	RSM	RSM002307.001	SK	Val Marie	1929	Skin
129	RSM	RSM05948.001	SK	Maple Creek	1955	Mount
130	RSM	RSM05975.001	SK	Maple Creek	1955	Mount
131	RSM	RSM05977.001	SK	Maple Creek	1955	Skin
132	RSM	RSM05978.001	SK	Maple Creek	1955	Skin
133	RSM	RSM05979.001	SK	Maple Creek	1955	Skin
134	RSM	RSM05980.001	SK	Maple Creek	1955	Skin
135	RSM	RSM09547.001	SK	Divide	1965	Skin
136	RSM	RSM09548.001	SK	Consul	1965	Skin
137	RSM	RSM09549.001	SK	Divide	1965	Skin
138	RSM	RSM09550.001	SK	Consul	1965	Skin
139	RSM	RSM11219.001	SK	Maple Creek	1979	Skin
140	RSM	RSM11807.001	SK	Val Marie	1929	Skin
141	RSM	RSM11857.001	SK	Val Marie	1929	Skin
142	RSM	RSM13565.001	SK	Maple Creek	1979	Skin
143	RSM	RSM-NoInfoM1	SK	Unknown	?	Mount
144	RSM	RSM-NoInfoM2	SK	Unknown	?	Mount
145	RSM	RSM-NoInfoM3	SK	Unknown	?	Mount
146	RSM	RSM-NoInfoF1	SK	Unknown	?	Mount
147	RSM	RSM-NoInfoF2	SK	Unknown	?	Mount
148	SCM	SCM-1	SK	Unknown	> 1965	Mount
149	SCM	SCM-2	SK	Unknown	> 1965	Mount
150	SCM	SCM-3	SK	Unknown	> 1965	Mount
151	SCM	SCM-4	SK	Unknown	> 1965	Mount
152	UA	UofAMZ-992	AB	Wildhorse	1951	Skin
153	UA	UofAMZ-1839	AB	Northwest of Wildhorse	1962	Skin
154	UA	UofAMZ-1840	AB	Northwest of Wildhorse	1962	Skin
155	UA	UofAMZ-4264	AB	South of Manyberries	1955	Skin

156	UA	UofAMZ-6564	AB	East of Milk River	1950	Skin
157	UA	UofAMZ-6565	SK	Kincaid	1953	Skin
158	UA	UofAMZ-6566	AB	Wildhorse	1948	Skin
159	UA	UofAMZ-7899	AB	Lodge Creek	1971	Mount
160	UA	UofAMZ-993	AB	Unknown	1960s	Skin
161	UA	UofAMZ-1042	AB	Unknown	1960s	Skin
162	UA	UofAMZ-1043	AB	Unknown	1960s	Skin
163	UBC	UBC-3889	SK	White Mud	1932	Skin
164	UBC	UBC-3919	SK	Dollard, White Mud	1944	Skin
165	UBC	UBC-3920	SK	Cypress Hills	1932	Skin
166	UBC	UBC-12438	SK	Consul	1965	Skin
167	US	UofS-1	SK	Govenlock	1967	Skin
168	US	UofS-2	SK	Govenlock	1967	Skin
169	US	UofS-3	SK	Govenlock	1967	Skin
170	US	UofS-4	SK	Val Marie	1944	Skin
171	UR	UofR-1	SK	Unknown	1971	Skin
172	UC	UofC-258	AB	Manyberries	1967	Skin
173	UC	UofC-454	AB	Between Manyberries and Wildhorse	1968	Skin
174	AUG	HIST-34	AB	Unknown	< 1965	Mount
175	F&W	HIST-3	AB	Q Ranch	1969	Mount
176	F&W	HIST-4	AB	Unknown	1960s	Skin
177	F&W	HIST-5	AB	Unknown	1960s	Skin
178	F&W	HIST-37	AB	Unknown	Late 1970s	Tail Fan
179	F&W	HIST-38	AB	Onefour	1982/83	Wing
180	F&W	HIST-39	AB	Unknown	Late 1970s	Mount
181	F&W	HIST-59	AB	Manyberries	1983	Mount
182	F&W	HIST-60	AB	Manyberries	1983	Mount
183	F&W	HIST-61	AB	Unknown	~ 1965	Mount

184	F&W	HIST-62	AB	Unknown	~ 1965	Mount
185	GNP	HIST-66	SK	Unknown	?	Mount
186	PPP	HIST-41	AB	Unknown	~ 1965	Mount
187	PPP	HIST-42	AB	Unknown	~ 1965	Mount
188	SE	HIST-17	SK	Near AB border	1972	Mount
189	Private	HIST-1	AB	Manyberries	~ 1965	Mount
190	Private	HIST-2	AB	Manyberries	~ 1965	Mount
191	Private	HIST-6	AB	South of Manyberries	1980	Mount
192	Private	HIST-7	AB	Wildhorse	1989	Mount
193	Private	HIST-8	AB	Wildhorse	1989	Mount
194	Private	HIST-9	AB	East of Pakowki Lake	1989	Mount
195	Private	HIST-10	AB	Southwest of Manyberries	1991	Mount
196	Private	HIST-11	AB	Onefour	1975	Mount
197	Private	HIST-12	AB	Lodge Creek	~ 1965	Mount
198	Private	HIST-13	AB	Manyberries	1980s	Mount
199	Private	HIST-14	AB	Milk River	1985	Mount
200	Private	HIST-15	AB	Manyberries	1980	Mount
201	Private	HIST-16	AB	Manyberries	1975-1985	Mount
202	Private	HIST-18	AB	Manyberries	1960s	Mount
203	Private	HIST-19	AB	Manyberries	1960s	Mount
204	Private	HIST-20	AB	Southeast of Manyberries	Early 1970s	Mount
205	Private	HIST-21	AB	Manyberries	1981-1985	Mount
206	Private	HIST-22	SK	Unknown	> 1965	Mount
207	Private	HIST-23	SK	Unknown	> 1965	Mount
208	Private	HIST-24	AB	Manyberries	1970-1990	Mount
209	Private	HIST-25	AB	Manyberries	1990s	Mount
210	Private	HIST-26	AB	Manyberries	1950s	Mount
211	Private	HIST-27	AB	Manyberries	1990s	Mount

212	Private	HIST-28	AB	Manyberries	1990s	Mount
213	Private	HIST-29	AB	Manyberries	1990s	Mount
214	Private	HIST-30	AB	Manyberries	1990s	Mount
215	Private	HIST-31	SK	Sandhills	< 1980	Mount
216	Private	HIST-32	AB	North of Montana border	1988	Mount
217	Private	HIST-33	AB	North of Montana border	1986	Mount
218	Private	HIST-35	AB	East of Manyberries	1983	Mount
219	Private	HIST-36	SK	Claydon	< 1965	Mount
220	Private	HIST-40	AB	South of Cypress Hills	1976	Mount
221	Private	HIST-44	SK	East of Fox Valley	1987	Mount
222	Private	HIST-45	AB	Onefour	1979	Mount
223	Private	HIST-46	AB	Onefour	1979	Mount
224	Private	HIST-47	AB	Pakowki Lake	< 1965	Mount
225	Private	HIST-48	AB	Pakowki Lake	< 1965	Mount
226	Private	HIST-49	AB	Northeast of Manyberries	1965	Mount
227	Private	HIST-50	AB	Northeast of Manyberries	1965	Mount
228	Private	HIST-51	AB	South of Manyberries	1984	Mount
229	Private	HIST-52	AB	Manyberries	1970s	Mount
230	Private	HIST-53	AB	Manyberries	1970s	Mount
231	Private	HIST-54	AB	Lodge Creek	1990s	Tail Fan
232	Private	HIST-55	AB	Sage Creek	1990s	Mount
233	Private	HIST-56	AB	By Saskatchewan & Montana borders	~ 1965	Mount
234	Private	HIST-57	AB	North of Wildhorse	~ 1965	Mount
235	Private	HIST-58	SK	Anerley	1960s	Mount
236	Private	HIST-70	AB	Cypress Hills	1979	Mount
237	Private	HIST-71	SK	Val Marie	< 1965	Mount
238	Private	HIST-72	SK	Val Marie	< 1965	Mount

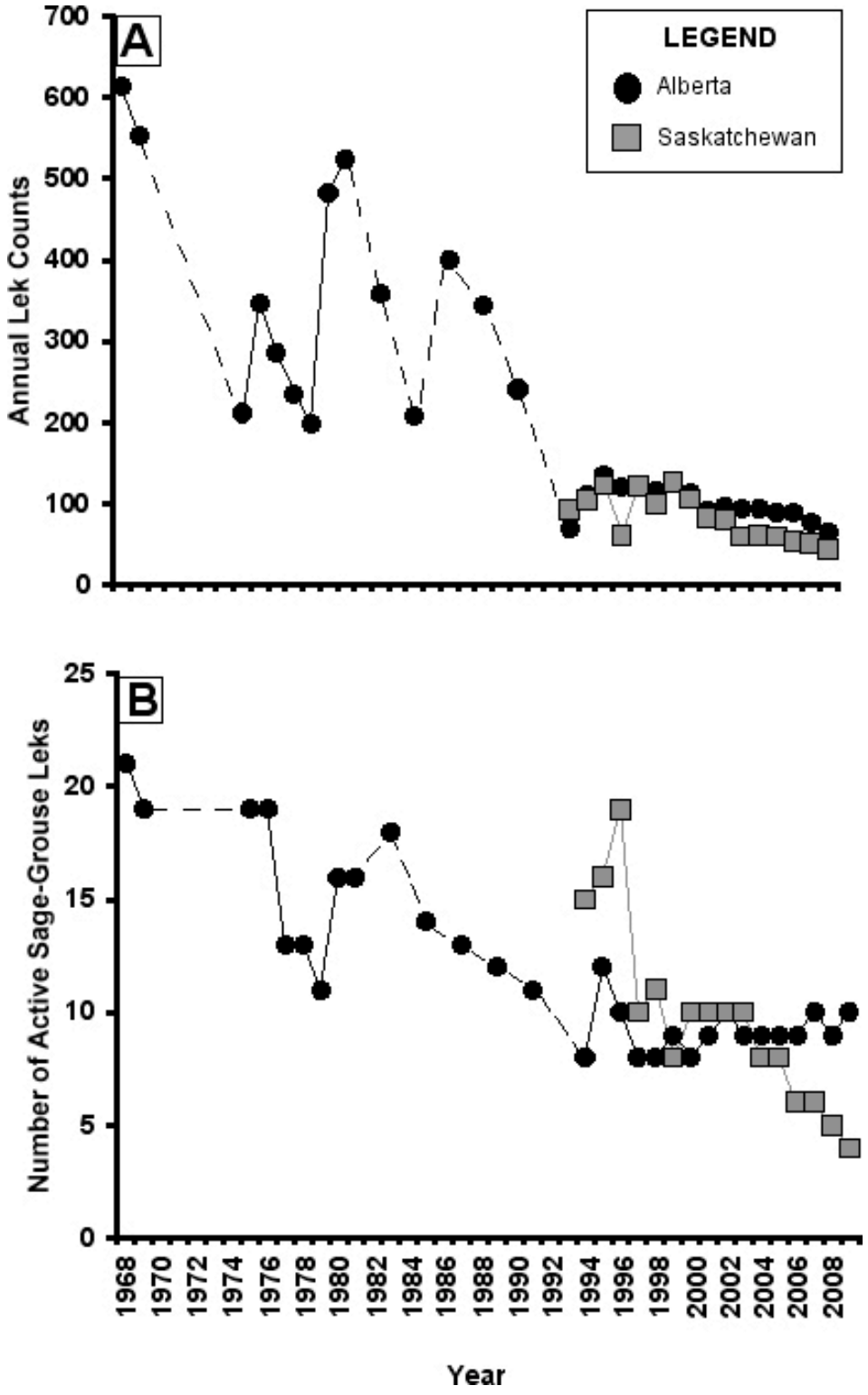

Figure 5-1. Temporal trends in (A) annual lek counts and (B) number of active sage-grouse leks for both Alberta (black circles) and Saskatchewan (grey squares).

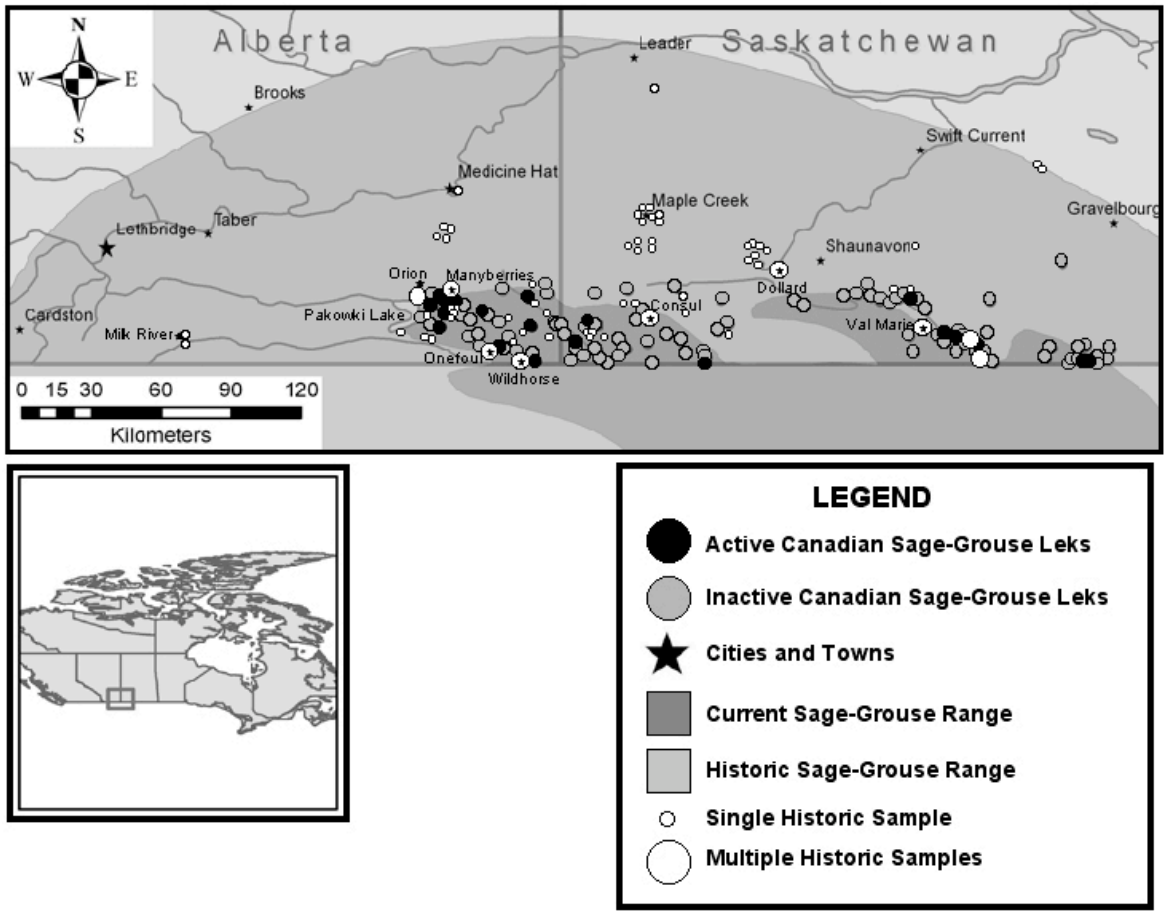

Figure 2. Active (black) and inactive (grey) sage-grouse leks in Canada juxtaposed over the historic and current range. Historic sampling sites are shown as small (single sample) or large (multiple samples) white circles. Locations of active (Alberta and Saskatchewan) and inactive (Alberta) leks are based on intensive government lek counts each spring.

CHAPTER SIX

Thesis Summary and Management Recommendations

1. Summary

Greater Sage-Grouse (*Centrocercus urophasianus*; hereafter Sage-Grouse) are endangered in Canada and have experienced dramatic declines due to habitat alteration, fragmentation, and destruction in both Canada and the United States (Alberta Sage-Grouse Recovery Action Group 2005; Lungle and Pruss 2008). For my thesis, I set out to determine genetic diversity, structure, relatedness, and gene flow in Canada and to identify genetic impacts from reduced population size. One purpose of this was to provide managers with information to better manage the species in Canda. I also wanted to examine the species' behaviour using genetic methods to provide additional insight into the basic biology and ecology of Sage-Grouse.

Overall, my research has revealed new information on Sage-Grouse ecology and behaviour. Sage-Grouse in northern Montana were expected to be highly structured, and perhaps isolated to some extent, due to both anthropogenic and natural fragmentation. However, I did not find this revealing that Sage-Grouse are more mobile than previously thought. No birds from the northern Montana population have ever been observed crossing areas of inappropriate or non-habitat, but individuals were genetically detected up to 316 km away from their natal lek suggesting a greater propensity for dispersal than observed for the species using telemetry (dispersal maximum of 30 km, Dunn and Braun 1986; migration maximum of 161 km, Patterson 1952). Most telemetry studies have also found that females disperse father than males (Dunn and Braun 1985; Dunn and Braun 1986), but I found the opposite with males dispersing between 5 to 316 km and females dispersing 8 and 61 km (chapter 2). This is contrary to the expectation that females of polygynous avian species disperse farther than males and that males attempting to breed for the first time establish territories in their natal areas because of familiarity (reviewed in Greenwood 1980). In terms of

behaviour, I found that male kin association does not drive lek formation or maintenance in Alberta (chapter 3) or northern Montana (chapter 2) Sage-Grouse, as males on individual leks had relatedness close to zero. These findings contradict the kin selection hypothesis for lekking species, where low ranking males become a member of leks because they may indirectly and directly increase their own fitness by joining male relatives (Kokko and Lindström 1996; Sherman 1999). The findings of low within-lek relatedness also contradict other studies on lekking grouse where they have found evidence for kin selection (Bouzat and Johnson 2004; Segelbacher et al. 2007). Gibson et al. (2005) found similar low levels of within-lek relatedness for Sage-Grouse in California so alternative mechanisms for lek formation and maintenance need to be explored for the species to find the true cause. These range from anticipating future breeding opportunities (Wiley 1973), unpredictable female copying behavior (Kokko 1997), reduced predation risk (Boyko et al. 2004), parasite-host co-evolution (Boyce 1990), to increased mating opportunity (Höglund and Alatalo 1995). Contrary to Wiley (1973), one or a few males on each lek do not appear to father the majority of offspring on a lek in a given year based on my paternity results. Instead, an average of 45.9% of sampled males in Alberta fathered offspring suggesting a greater proportion of the population reproduce than predicted for lekking species. I found evidence for intraspecific nest parasitism, which has not been previously documented in Sage-Grouse. I also found multiple paternity, which was not expected to be common in lekking species because females are believed to mate only once (Wiley 1973) Because of my research, we now know more about the basic biology of Sage-Grouse, have new insights into their behaviour, and have better scientific knowledge from which to generate management strategies for the northern Montana population.

While all of the research in my thesis is specific to Canada or the northern Montana population, much of it is applicable to other parts of the species' range. For instance, my findings on a lack of within-lek relatedness were similar to those found in California (Gibson et al. 2005), which is at the opposite end of the species' range. My findings on paternity were also similar to a population in

California where they found multiple paternity and a greater distribution in mating success than observed for Sage-Grouse in most field studies (Semple et al. 2001). There have been no population-specific studies on diversity, structure, and gene flow conducted in other parts of the species' range, but it seems likely that if Sage-Grouse in northern Montana are mobile and have a propensity to disperse, the entire species should exhibit this trait. However, birds in other populations may not be dispersing as far because many of the populations are considerably smaller (see Connelly et al. 2004). It also seems plausible that there will be little genetic structure and high genetic diversity in other populations that are not isolated from the rest of the range because of the mobility of Sage-Grouse and their apparent resilience to disturbance.

In each chapter I found many interesting results that either support data collected across the range or provide new information on the ecology, biology, and behaviour of Sage-Grouse. In chapter 2, I determined genetic diversity, structure, relatedness, and gene flow for the northern Montana Sage-Grouse population. I determined that northern Montana (northern Montana, Alberta, and Saskatchewan) supported a single population of birds that exhibited significant isolation by distance and the Milk River area demarcating two subpopulations (north and south of the Milk River). Both subpopulations exhibited high genetic diversity with no evidence that peripheral regions were genetically depauperate or highly structured. However, the Milk and Missouri rivers and a large patch of agriculture in Saskatchewan appear to be significant barriers to dispersal. Both sexes of Sage-Grouse disperse, but males disperse more frequently and further. Leks were also composed primarily of non-kin providing no evidence from this population to support the proposal that leks form in grouse because of male kin association.

In chapter 3, I expanded on the last observation and assessed the degree of sex-specific relatedness within and between leks in Alberta. I found that birds in Alberta possessed high genetic diversity, with the exception of lek 1/9, and that diversity did not change over time. I did not observe isolation-by-distance among leks and most leks were not differentiated from one another, suggesting gene flow

occurs across the Alberta range. Males and females did not show differential isolation-by-distance, indicating dispersal was not sex-specific. Overall relatedness was close to zero for both sexes at the Alberta, lek, and year levels suggesting neither sex forms strong kin associations. However, within-lek relatedness during certain years was greater than zero suggesting inter-annual variation in intra-sexual relatedness was likely due to the small population size and chance. I also found no evidence that Sage-Grouse follow the typical avian pattern of male philopatry.

In chapter 4, I determined patterns in paternity, polygamy (males and females mating with multiple mates), and variance in reproductive success among individuals for Sage-Grouse in Alberta. I found that most clutches had a single father and mother, but there was evidence of multiple paternity, intra-specific nest parasitism, and hybridization. Annually, most males fathered only one brood, very few males fathered multiple broods, and a greater proportion of males in the population fathered offspring than expected suggesting that more males breed in Alberta than previously reported for the species. Twenty-six eggs (2.2%) could be traced to intra-specific nest parasitism and 15 of 191 clutches (7.9%) had multiple fathers. Reproductive variance, measured as the opportunity for selection, was higher among males than females, lower than expected if only a small proportion of the male population mates, and within the range exhibited by other lekking species.

In chapter 5, I evaluated the genetic diversity and structure of Canadian Sage-Grouse from 1895 – 2007 using both historic (museum and private) and contemporary samples to determine if genetic diversity or structure had declined or changed over time. I found high genetic diversity across all time periods and no decline over time. Genetic structure did not change and there was no evidence of a genetic bottleneck. Effective population size decreased with time and was estimated at 46.8 – 93.6 for the most recent time periods (1965 – 1991 to 1998 – 2007). The number of effective breeders in Canada was estimated to be at least 440 birds suggesting that Canada was part of a larger, genetically diverse, panmictic population

My research revealed that Sage-Grouse in Canada appear to be maintaining genetic diversity and a lack of genetic structure through gene flow from the rest of the northern Montana population, despite demographic declines and loss of habitat. However, there is no way of predicting how long this will last. We do not know if there is a time lag between anthropogenic disturbance (e.g., loss of fragmentation of habitat) and the ability to detect its effects in the genetics of the population. While there still appears to be enough birds dispersing to maintain genetic diversity, increased fragmentation will likely only exacerbate demographic declines and loss of leks. We are currently seeing a significant population decline, therefore we need to manage Sage-Grouse in Canada quickly and efficiently to ensure their long-term survival.

2. Management Recommendations

The following are a list of recommendations for both the northern Montana population and Canada based on a compilation of the genetic data in my thesis and data previously gathered on Sage-Grouse in Canada (Lumsden 1968; Aldridge 1998, 2000a, 2000b, 2001, 2005; Aldridge et al. 2001, 2004; Aldridge and Brigham 2001, 2002, 2003; Harris et al. 2001; Braun et al. 2003; McAdam 2003; McNeil and Sawyer 2003; Watters et al. 2004; Alberta Sage-Grouse Recovery Action Group 2005; Chandler 2005; Thorpe et al. 2005; Aldridge and Boyce 2006, 2007; Carpenter 2007; McNeil et al. 2007; Lungle and Pruss 2008; McNeil 2009) and northern Montana (Moynahan 2004; Naugle et al. 2004, 2005; Schroeder et al. 2004; Montana Sage Grouse Working Group 2005; Moynahan et al. 2006; Sauls 2006).

2.1. Recommendations for the northern Montana population

1. Attach GPS collars on juvenile Sage-Grouse throughout the northern Montana population, particularly the subpopulation north of the Milk River, in the late summer to determine areas through which juveniles disperse. Based on the data presented in this thesis, gene flow is occurring, but genetics and conventional telemetry cannot identify whether birds are using corridors, discontinuous habitat

patches, or flying "non-stop" over large areas of unsuitable habitat to disperse to new locations. Habitat that dispersing Sage-Grouse use should be protected to ensure sustainability of the species in both Canada and Montana.

2. Perform intensive searches to find unknown leks (leks not previously identified or located) north of the Milk River. Based on how known leks are scattered sparsely across the landscape and the high genetic diversity exhibited within the majority of leks, it is likely there are numerous unknown leks connecting regions by acting as stepping stones. Finding these leks will identify areas of suitable habitat and potential corridors for bird movement. Much of this region has been converted to agriculture so detecting areas used by Sage-Grouse will provide valuable information on the connectivity between Canada and the United States. Because Sage-Grouse in Canada are located at the northern periphery of the range, in sparse silver sagebrush habitat, and have suffered substantial demographic declines in conjunction with the destruction of sagebrush habitat in northern Montana, it is possible that Canada has always been a sink that was previously sustained by migrants from Montana. Identifying how Sage-Grouse are using the matrix of highly fragmented habitat in northern Montana will help to put my findings on gene flow into context and steer identification and management of these key habitats.

3. Foster cross-border management and cooperation and initiate on-the-ground projects. All birds north of the Missouri River in Montana form a single genetic population and do not recognize political boundaries. Currently, the Northern Sagebrush Steppe Initiative exists, which is a partnership between Alberta, Saskatchewan, and northern Montana for cooperation on the conservation of sagebrush-obligate species, including Sage-Grouse, However, apart from lek counts, little on the ground management is consistently occurring throughout the population and virtually none of it involves interagency cooperation. The primary goal of all agencies, with regard to Sage-Grouse, should be to preserve and protect sagebrush habitat because without habitat, there are no birds. Once habitat

is protected, remediation efforts can occur to increase connectivity between habitat patches, and once connection is accomplished, gene flow within the population should increase as Sage-Grouse are capable of long-distance dispersal (chapter 2). Also, with habitat protection and restoration, Sage-Grouse may naturally recolonize regions from which they are currently extirpated and translocations and reintroductions may not be required.

4. If recommendations 1 – 3 are either not adopted or are performed too late and the severity of the population decline increases, it may be necessary to simulate gene flow and augment population numbers via translocations of birds into Canada from other parts of the northern Montana population. Ideally, this would be performed prior to regional and/or lek extirpation, as it has been shown that re-establishing Sage-Grouse in an area where they have been extirpated is difficult and normally unsuccessful (Musil et al. 1993; Musil et al. 1994; Reese and Connelly 1997; Connelly et al. 2000; Baxter 2003; Baxter 2008).

2.2. Recommendations for Alberta and Saskatchewan

1. Perform intensive searches to find all unknown leks in both provinces to allow more accurate estimates of population size. Both paternity analyses (chapter 3) and genetic estimates based on molted feathers (K. L. Bush, unpubl. data) indicate that more males are present in Alberta than are being counted on known active leks. Determining the location of unknown leks will also provide valuable information on habitat use for the species.

2. Continue genetic surveys in Canada so that any signs of genetic deterioration (e.g., decline in genetic diversity, inbreeding, etc.) can be detected immediately.

3. Combine genetic, ecological, and habitat mapping data to detect locations where gene flow/dispersal is occurring and to identify landscape features that are acting as barriers to Sage-Grouse. This will pinpoint habitat that should be protected (if currently important) or restored (if currently a barrier to movement).

4. Reduce the footprint of oil and gas development in Alberta, as it has been found to impact Sage-Grouse ecologically in Alberta (Aldridge and Boyce 2007) and both genetically (K. L. Bush, unpubl. data) and ecologically (Lyon and Anderson 2003; Holloran 2006; Kaiser 2006; Holloran et al. 2007; Walker et al. 2007; Doherty 2008; Doherty et al. 2008; Walker 2008) in Wyoming. This could involve decommissioning old wells sites and roads, restoring habitat on these sites, using directional drilling from current well pads instead of creating new ones, and creating underground pipelines instead of storing oil in tanks on site, which requires daily tanker truck traffic.

5. Start a captive breeding program for birds from north of the Milk River. It is possible that these birds have adaptive physiological or genetic advantages for living at the northern periphery of the range and/or for living in silver sagebrush habitat. Maintaining a separate gene pool in captivity will ensure that these adaptive advantages are not lost if the region requires supplementation or complete reintroduction. It would also provide a safe guard against the possibility of a natural disaster (e.g., disease, weather, etc.) decimating the small number of birds left in the wild.

6. Consider the translocation of birds from stable (i.e., non-declining or slightly declining) parts of the population if habitat loss continues, genetic isolation increases, and detrimental genetic effects occur (e.g., declining genetic diversity or inbreeding). This may be required to simulate gene flow and potentially sustain birds in Canada if habitat south of the border is not restored.

7. Use the available ecological and genetic data to concentrate on immediate management concerns instead of long-term goals. Aiming for > 865 males on leks and 46 active leks by 2026 (Lungle and Pruss 2008) is unrealistic based on the 50% population decline seen in Canada since the first National Recovery plan was written in 2001 (Harris et al. 2001). With only 109 enumerated males left in

Canada as of spring 2009, management should concentrate on immediate concerns, such as protecting remaining sagebrush habitat from further degradation of any kind, maintaining connectivity between blocks of sagebrush habitat, and restoration of habitat that is no longer used by Sage-Grouse to promote gene flow and possible population growth. This could be accomplished by encouraging increased stewardship by private landowners and those leasing public lands, purchase of essential habitat if privately owned, and alteration to leasing agreements (i.e., lower stocking rates, stocking only during certain parts of the year, etc.) for critical habitat.

3. Suggested Integration into the Provincial and Federal Recovery Plans

The following are management suggestions that should be integrated into both the Alberta and Federal recovery plans (or be used to revise future recovery plans) to help gather needed information on Sage-Grouse, disseminate correct scientific information needed to properly manage Sage-Grouse in Canada, and to ensure the long-term survival of the species.

1. Continue to collect molted feathers from all active leks in Canada for genetic monitoring. Sample all birds deliberately (field studies) or opportunistically (vehicular or predator mortalities, feathers off lek, eggshells, etc.) encountered to ensure the widest range of birds are sampled.

2. Adopt a standardized method of conducting lek counts for the population so that trends can be easily discerned and leks can be consistently sampled.

3. Refer to Sage-Grouse in Canada as belonging to a subpopulation of the northern Montana population that occurs north of the Milk River instead of belonging to multiple populations based on geographic location (see Lungle and Pruss 2008, page 3). This infers that these regions are physically and genetically isolated, which they are not, and promotes regional or lek-based management

instead of population-based management, which is direly needed to ensure future connectivity across Canada and northern Montana.

5. Literature Cited

Alberta Sage-Grouse Recovery Action Group (2005) Alberta Greater Sage-Grouse Recovery Plan. Alberta Sustainable Resource Development, Fish and Wildlife Division, Alberta Species at Risk Recovery Plan No. 8. Edmonton, Alberta.

Aldridge CL, Boyce MS (2007) Linking occurrence and fitness to persistence: habitat-based approach for endangered greater sage-grouse. Ecological Applications 17: 508 – 526.

Aldridge CL, Boyce MS (2006) Silver sagebrush community associations in southeastern Alberta, Canada. Rangeland Ecology and Management 58: 400 – 405.

Aldridge CL (2005) Identifying habitats for persistence of greater sage-grouse (*Centrocercus urophasianus*) in Alberta, Canada. Ph.D. Dissertation, University of Alberta, Edmonton, Alberta.

Aldridge CL, Boyce MS, Baydack RK (2004) Adaptive management of prairie grouse: how do we get there? Wildlife Society Bulletin 32: 92 - 103.

Aldridge CL, Brigham RM (2003) Distribution, abundance, and status of the greater sage-grouse, *Centrocercus urophasianus*, in Canada. Canadian Field Naturalist 117: 25-34.

Aldridge CL, Brigham RM (2002) Sage-Grouse nesting and brood habitat use in southern Canada. Journal of Wildlife Management 66: 433 – 444.

Aldridge CL (2001) Do Sage-Grouse have a future in Canada? Population dynamics and management suggestions. Proceedings of the 6th Prairie Conservation and Endangered Species Conference, Winnipeg, Manitoba, February 22 - 25, 2001. 11pp.

Aldridge CL, Brigham RM (2001) Nesting and reproductive activities of greater sage-grouse in a declining northern fringe population. Condor 103: 537 - 543.

Aldridge CL, Oyler-McCance SJ, Brigham RM (2001) Occurrence of greater sage-grouse X sharp-tailed grouse hybrids in Alberta. Condor 103: 657 – 660.

Aldridge CL (2000a) Reproduction and habitat use by sage grouse (*Centrocercus urophasianus*) in a northern fringe population. Msc. Thesis, University of Regina, Regina, Saskatchewan.

Aldridge CL (2000b) Assessing chick survival of Sage Grouse in Canada. Alberta Sustainable Resource Development, Fish and Wildlife Service, Alberta Species at Risk Report No. 19. Edmonton, Alberta. 25pp.

Aldridge CL (1998) Status of the Sage Grouse (*Centrocercus urophasianus urophasianus*) in Alberta. Alberta Environmental Protection, Wildlife Management Division, and Alberta Conservation Association, Wildlife Status Report No. 13, Edmonton, AB. 23pp.

Baxter RJ, Flinders JT, Mitchell DL (2008) Survival, movements, and reproduction of translocated greater sage-grouse in Strawberry Valley, Utah. Journal of Wildlife Management 72: 179 – 186.

Baxter RJ (2003) Greater sage-grouse brood and non-reproductive female habitat selection and population dynamics in Strawberry Valley, Utah. MSc. Thesis, Brigham Young University, Provo, Utah.

Bouzat JL, Johnson K (2004) Genetic structure among closely spaced leks in a peripheral population of lesser prairie-chickens. Molecular Ecology 13: 499 - 505.

Boyce MS (1990) The Red Queen visits sage grouse leks. American Zoologist 30: 263 – 270.

Boyko AR, Gibson RM, Lucas JR (2004) How predation risk affects the temporal dynamics of avian leks: greater sage grouse versus golden eagles. American Naturalist 163: 154 - 165.

Braun CE, Oedekoven OO, Aldridge CL (2003) Oil and gas development in western North America: effects on sagebrush steppe avifauna with particular emphasis on Sage Grouse. Transactions of the 67th North American Wildlife and Natural Resources Conference. 337-349.

Carpenter JE (2007) West Nile virus and parasites in greater sage-grouse (*Centrocercus urophasianus*) populations. Msc. Thesis, University of Alberta, Edmonton, Alberta.

Chandler JL (2005) Collaborative and adaptive resource management planning for endangered species recovery. Evaluation of Sage Grouse recovery planning in Alberta. MSc. Thesis, University of Calgary, Calgary, Alberta.

Connelly JW, Knick ST, Schroeder MA, Stiver SJ (2004) Conservation Assessment of Greater Sage-grouse and Sagebrush Habitats. Western Association of Fish and Wildlife Agencies. Unpublished Report. Cheyenne, Wyoming.

Connelly JW, Schroeder MA, Sands AR, Braun CE (2000) Guidelines to manage sage grouse populations and their habitats. Wildlife Society Bulletin 28: 967 – 985.

Doherty KE, Naugle DE, Walker BL (2008) Sage-grouse winter habitat selection and energy development. Journal of Wildlife Management 72: 187 – 195.

Doherty KE (2008) Sage-grouse and energy development: integrating science with conservation planning to reduce impacts. Ph.D. Thesis, University of Montana, Missoula, Montana.

Dunn PO, Braun CE (1986) Late summer-sprig movements of juvenile sage grouse. Wilson Bulletin 98: 83 – 92.

Dunn PO, Braun CE (1985) Natal dispersal and lek fidelity of sage grouse. Auk 102: 621 – 627.

Gibson RM, Pires D, Delaney KS, Wayne RK (2005) Microsatellite DNA analysis shows that greater sage grouse leks are not kin groups. Molecular Ecology 14: 4453 - 4459.

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. Animal Behavior 28: 1140 – 1162.

Harris W, Lungle K, Bristol B, Dickinson D, Eslinger D, Fargey P, Kroshus J, Livingston T. Lunn S, McAdam S, Michaud I, Milner D, Poirier T, Scobie D, Veitch L (2001) Canadian Sage Grouse Recovery Strategy. The Canadian Sage Grouse Recovery Team. 55pp.

Höglund J, Alatalo RV (1995) Leks. Princeton University Press, Princeton, New Jersey.

Holloran MJ, Kaiser RC, Hubert WA (2007) Yearling greater sage-grouse population response to the infrastructure of natural gas fields in southwestern Wyoming. Unpublished Government Report. Laramie, Wyoming.

Holloran MJ (2006) Greater sage-grouse (*Centrocercus urophasianus*) population response to natural gas field development in western Wyoming. Ph.D. Dissertation, University of Wyoming, Laramie, Wyoming.

Kaiser RC (2006) Recruitment by greater sage-grouse in association with natural gas development in western Wyoming. MSc. Thesis, University of Wyoming, Laramie, Wyoming.

Kokko H, Lindström J (1996) Kin selection and the evolution of leks: whose success do young males maximize? Proceedings of the Royal Society of London Series B 263: 919 - 923.

Kokko H (1997) The lekking game: can female choice explain aggregated male displays? Journal of Theoretical Biology 187: 57 - 64.

Lumsden HG (1968) The displays of the sage grouse. Ontario Department of Lands and Forests Research Report (Wildlife) no. 83.

Lungle K, Pruss S (2008) Recovery strategy for the greater sage-grouse (*Centrocercus urophasianus urophasianus*) in Canada. *In* Species at Risk Act Recovery Strategy Series. Parks Canada Agency. Ottawa. vii + 43 pp.

Lyon AG, Anderson SH (2003) Potential gas development impacts on sage grouse nest initiation and movement. Wildlife Society Bulletin 31: 486 – 491.

McAdam S (2003) Lek occupancy by greater Sage-Grouse in relation to habitat in southwestern Saskatchewan. M.Sc. Thesis. Royal Roads University, Victoria, British Columbia.

McNeil R (2009) Strategies to maintain or enhance silver sagebrush habitats for greater sage grouse in southwestern Saskatchewan. Prepared by LandWise Inc. for Interdepartmental Recovery Fund (IRF), Prairie Farm Rehabilitation Administration, Agriculture and Agri-Food Canada (PFRA), Grasslands National Park, Parks Canada (GNP), and Saskatchewan Watershed Authority (SWA) 182 pp.

McNeil J, Yin H, McNeil R (2007) Analysis of the climate in the Greater Sage Grouse range of southwestern Saskatchewan and southeastern Alberta. Prepared for PFRA, Grasslands National Park, Sask. Watershed Authority and Interdepartmental Recovery Fund (IRF), by LandWise Inc., Lethbridge, Alberta. 43 pp.

McNeil RL, Sawyer BJ (2003) Effects of water management practices and precipitation events on sagebrush habitat in southeastern Alberta. Prepared for Alberta Conservation Association and Alberta Sustainable Resource Development, by LandWise Inc., Lethbridge, Alberta. 36 pp.

Montana Sage Grouse Working Group (2005) Management plan and conservation strategies for sage grouse in Montana – Final. Unpublished government report. http://www.fwp.mt.gov/content/getItem.aspx?id=31187

Moynahan BJ, Lindberg MS, Thomas JW (2006) Factors contributing to process variance in annual survival of female greater sage-grouse in Montana. Ecological Applications 16: 1529 – 1538.

Moynahan BJ (2004) Landscape-scale factors affecting population dynamics of Greater Sage-Grouse (*Centrocercus urophasianus*) in north-central Montana, 2001-2004. Ph.D. Dissertation. University of Montana, Missoula, Montana.

Musil DD, Reese KP, Connelly JW (1994) Nesting and summer habitat use by translocated sage grouse (*Centrocercus urophasianus*) in central Idaho. Great Basin Naturalist 4: 228 – 233.

Musil DD, Connelly JW, Reese KP (1993) Movements, survival, and reproduction of sage grouse translocated into central Idaho. Journal of Wildlife Management 57: 85 – 91.

Naugle DE, Aldridge CL, Walker BL, Doherty KE, Matchett MR, McIntosh J, Cornish TE, Boyce MS (2005) West Nile virus and sage-grouse: what more have we learned? Wildlife Society Bulletin 33: 616 – 623.

Naugle DE, Aldridge CL, Walker BL, Cornish TE, Moynahan BJ, Holloran MJ, Brown K, Johnson GD, Schmidtmann ET, Mayer RT, Kato CY, Matchett MR, Christiansen TJ, Cook WE, Creekmore T, Falise RD, Rinkes ET, Boyce MS (2004) West Nile virus: pending crisis for greater sage-grouse. Ecology Letters 7: 704 – 713.

Patterson RL (1952) The sage grouse in Wyoming. Sage Books, Inc. Denver, Colorado.

Reese KP and Connelly JW (1997) Translocations of sage grouse *Centrocercus urophasianus* in North America. Wildlife Biology 3/4: 235 – 241.

Sauls HS (2006) The role of selective foraging and cecal microflora in sage-grouse nutritional ecology. MSc. Thesis. University of Montana, Missoula, Montana.

Schroeder MA, Aldridge CL, Apa AD, Bohne JR, Braun CE, Bunnell SD, Connelly JW, Diebert PA, Gardner SC, Hilliard MA, Kobriger GD, McAdam SM, McCarthy CW, McCarthy JJ, Mitchell DL, Rickerson EV, Stiver SJ. (2004) Distribution of sage-grouse in North America. Condor 106: 363 - 376.

Segelbacher G, Wegge P, Sivkov AV, Höglund J (2007) Kin groups in closely spaced capercaillie leks. Journal of Ornithology 148:79 - 84.

Semple K, Wayne RK, Gibson RM (2001) Microsatellite analysis of female mating behaviour in lek-breeding sage grouse. Molecular Ecology 10: 2043-2048.

Sherman PW (1999) Birds of a feather lek together. Nature 401: 119 - 120.

Thorpe J, Godwin B, McAdam S (2005) Sage-Grouse habitat in southwestern Saskatchewan: Differences between active and abandoned leks. SRC Publication No. 11837-1E05. Saskatchewan Research Council, Saskatoon, Saskatchewan. 39 pp.

Walker BL (2008) Greater sage-grouse response to coal-bed natural gas development and West Nile virus in the Powder River Basin, Montana and Wyoming, USA. Ph.D. Dissertation, University of Montana, Missoula, Montana.

Walker BL, Naugle DE, Doherty KE (2007) Greater sage-grouse population response to energy development and habitat loss. Journal of Wildlife Management 71: 2644 – 2654.

Watters M, McMaster G, Springer G (2004) Site plans for Sage-Grouse (*Centrocercus urophasianus*) in southern Saskatchewan. Unpublished Report, Saskatchewan Watershed Authority, Regina, Saskatchewan.

Wiley RH (1973) Territoriality and non-random mating in the sage grouse, *Centrocercus urophasianus*. Animal Behaviour Monographs 6:87 - 169