University of Alberta

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

by

Krista Lee Bush

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Examining Committee

Cindy Paszkowski, Biological Sciences

David Coltman, Biological Sciences

Mark Boyce, Biological Sciences

Frank Robinson, Agricultural, Food and Nutritional Science

Colleen Cassady St. Clair, Biological Sciences

Robert Gibson, University of Nebraska - Lincoln

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