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Original Article

The secret sex lives of sage-grouse: multiple paternity and intraspecific nest parasitism revealed through genetic analysis

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In lek-based mating systems only a few males are expected to obtain the majority of matings in a single breeding season and multiple mating is believed to be rare. We used 13 microsatellites to genotype greater sage-grouse (*Centrocercus urophasianus*) samples from 604 adults and 1206 offspring from 191 clutches (1999–2006) from Alberta, Canada, to determine paternity and polygamy (males and females mating with multiple individuals). We 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 only one brood, very few males fathered multiple broods, and the proportion of all sampled males in the population fathering offspring averaged 45.9%, suggesting that more males breed in Alberta than previously reported for the species. Twenty-six eggs (2.2%) could be traced to intraspecific nest parasitism and 15 of 191 clutches (7.9%) had multiple fathers. These new insights have important implications on what we know about sexual selection and the mating structure of lekking species. *Key words:* lek, multiple paternity, nest parasitism, paternity, polygyny, sage-grouse. *[Behav Ecol]*

INTRODUCTION

In lek mating systems, males congregate on communal display grounds, and females only visit to mate and then independently raise the young. In this form of polygynous mating system, female choice is generally unconstrained (Wiley 1973; Gibson and Bradbury 1986; Gibson et al. 1991) resulting in 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). However, patterns of genetic paternity often differ from observed copulations, revealing the potential for multiple mating by females. A variety of factors can affect the accuracy of paternity assessment based on field observations of lekking species, including incomplete coverage of known lekking sites in time or space, the existence of unknown lekking sites, or undocumented matings away from lekking sites (Wilmer et al. 1999; Gemmell et al. 2001; Semple et al. 2001).

Multiple paternity within clutches or litters is expected to be rare in all lekking species because females are believed to mate once per clutch or litter (Wiley 1973; Alatalo et al. 1996). Despite early predictions of low rates of multiple paternity for lekking species, substantial rates of multiple paternity have been documented in many species: black grouse (*Tetrao tetrix*; 4%; Lebigre et al. 2007), buff-breasted sandpiper (*Tryngites subruficollis*; 40%; Lanctot et al. 1997), cock-of-the-rock (*Rupicola rupicola*; 25%; Trail 1985), great snipe (*Gallinago media*; 12%; Fiske and Kålås 1995), peafowl (*Pavo cristatus*; 53%; Petrie et al. 1992), ruff (*Philomachus pugnax*; 50%; Lank et al. 2002), Houbara bustard (*Chlamydotis undulata undulata*; 60%; Lesobre et al. 2010), blue-crowned manakins (*Lepidothrix coronata*; 5%; Durães et al. 2009), wire-tailed manakin (*Pipra filicauda*; 18%; Ryder et al. 2009), lance-tailed manakins (*Chiroxiphia lanceolata*; 4.8%, DuVal and Kempenaers 2008), and greater sage-grouse (*Centrocercus urophasianus*; 20%; Semple et al. 2001). Polyandry (a female mating with multiple males) 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 even 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, elevated predation risk (Gibson and Bachman 1992), increased risk of sexually transmitted diseases (Petrie and Kempenaers 1998), or obtaining bad genes for their offspring, females are expected to mate with multiple males only when the benefits outweigh the costs.

Greater sage-grouse (hereafter sage-grouse) are a good model for studying mating patterns in lekking species because they are well studied and easy to sample. Research indicates 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 may 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 follow females off-lek (Gibson 1996). Males have been reported displaying to females away from leks (Dunn and Braun 1985) and yearling males (males

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hatched the previous spring that are physiologically capable of reproducing, but are assumed not to breed based on reduced testis size; Eng 1963) have been seen accompanying one or more females onto leks (Bush 2009). Furthermore, most visiting females are never observed to mate, even when leks are intensively monitored (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, whereas 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.

We used polymorphic microsatellites to study parentage and to test hypotheses regarding reproductive behavior in a lek breeding species using 8 years of paternity data from an endangered population of sage-grouse. First, by using genetic-based paternity analysis of 1206 samples from offspring and 604 samples from adults, we tested the hypothesis that multiple paternity is rare in lekking species because females are believed to mate only once and with a single male. We predicted that multiple paternity does occur at low levels in sage-grouse based on evidence from other lekking species (Lanctot et al. 1997; Semple et al. 2001; Lank et al. 2002; Lebigre et al. 2007). We also predicted this pattern because (1) reproductive behavior is typically monitored only on leks, which fails to record mating outside of this arena or at other times of day, and (2) Males are the sex intensively observed not females, therefore accurate information on female behavior is lacking. Multiple paternity levels are not expected to be high because multiple mating in the lek system is expected to be costly to females (Gibson and Bachman 1992), but may offer benefits that outweigh the costs if inbreeding, reduced genetic diversity, or infertility are issues (Wetton and Parkin 1991; Birkhead and Møller 1992; Sheldon 1994; Kempenaers et al. 1999). The second hypothesis examined was that intraspecific nest parasitism (a female laying an egg(s) in a nest incubated by another female) is a rare behavior in lekking species. We predicted that intraspecific nest parasitism is rare in sage-grouse because few reports exist in the literature and this behavior offers few benefits in a species where females invest heavily in incubating their own nests.

MATERIALS AND METHODS

Study location and sample collection

This study was conducted on sage-grouse from multiple leks in southeastern Alberta, Canada near Manyberries (Figure 1; 4000 km^2; Aldridge and Brigham 2001). 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 and 2006. Vehicular and predator mortalities were opportunistically sampled and feathers were collected on leks from 2003 to 2007. These feathers consisted of naturally molted feathers and feathers pulled during male conflict. All captured birds were aged following Eng (1955). "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 approximately every other day (Aldridge and Brigham 2002) to determine the date of nest initiation and nest fate (hatch/abandonment/predated). After fate was determined, clutches were sampled as hatched eggshell membranes, predated eggshell membranes, intact eggs, or dead chicks. Collected clutches (n = 191) contained 1–14 eggs (mean = 6.3±2.7). This is consistent with an observed average clutch size of 7.8 eggs in Alberta (Aldridge and Brigham 2001). All eggs were stored as described in Bush et al. (2005). We use the term "offspring" for samples from all eggs and chicks regardless of hatching success and "hatched offspring" for chicks that hatched. Survivorship after hatch was not known for the majority of chicks.

Between 1998 and 2006, each captured adult was fitted with a numbered metal leg band and a year-specific colored plastic leg band to allow for identification of individual birds on recapture. These bands were not easily visible during behavioral observations in the field, therefore in 2005 and 2006, each captured male was fitted with a unique plastic leg band color combination. Behavioral observations were only conducted in 2005 and 2006 and even with color leg bands, the terrain and vegetation made bands difficult to see on some leks.

In total, we collected 1422 adult samples (327 from blood, plucked feathers, mouth swabs, and road kills and 1095 from feathers collected on leks); 1391 of these samples were from the nine known active leks in Alberta and 31 samples were collected off-lek. We collected 1420 offspring samples (from 95 known mothers and nine unknown mothers) from 191 broods. Annual lek counts, the maximum number of males counted on a lek in a morning for each breeding season when leks were active, averaged 11.6 males and ranged from 1 to 35 (lek 1/9 = 3.3 [1–5], lek 2/24 = 5.8 [1–11], lek 10/11 = 8.5 [4–20], lek 16 = 27.4 [21–34], lek 22 = 10.1 [7–14], lek 30 = 18.5 [10–29], lek 31 = 16.6 [9–24], lek 34 = 8.6 [7–11], lek 35 = 5.8 [4–8]). Leks were named by the province in the order that they were discovered and leks that are designated by two numbers (e.g. 10/11) were the result of two neighboring leks merging (Alberta Fish and Wildlife, unpublished data).

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; Figure 1) every morning during the lekking period. This method likely 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 on a daily or yearly basis.

Microsatellite genotyping

DNA was extracted using Qiagen DNeasy® Tissue and QIAamp® DNA Micro kits and samples were DNA sexed using methods described in Bush et al. (2005). We used the 13 microsatellite loci and associated protocols described in Bush et al. (2010). PCR was performed 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, 2010). 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, Foster City, CA). Duplicate samples were determined using the procedure in Bush et al. (2010).

Paternity analysis

We tested for deviations from Hardy–Weinberg and linkage equilibrium in GENEPOP, version 3.4 (Raymond and

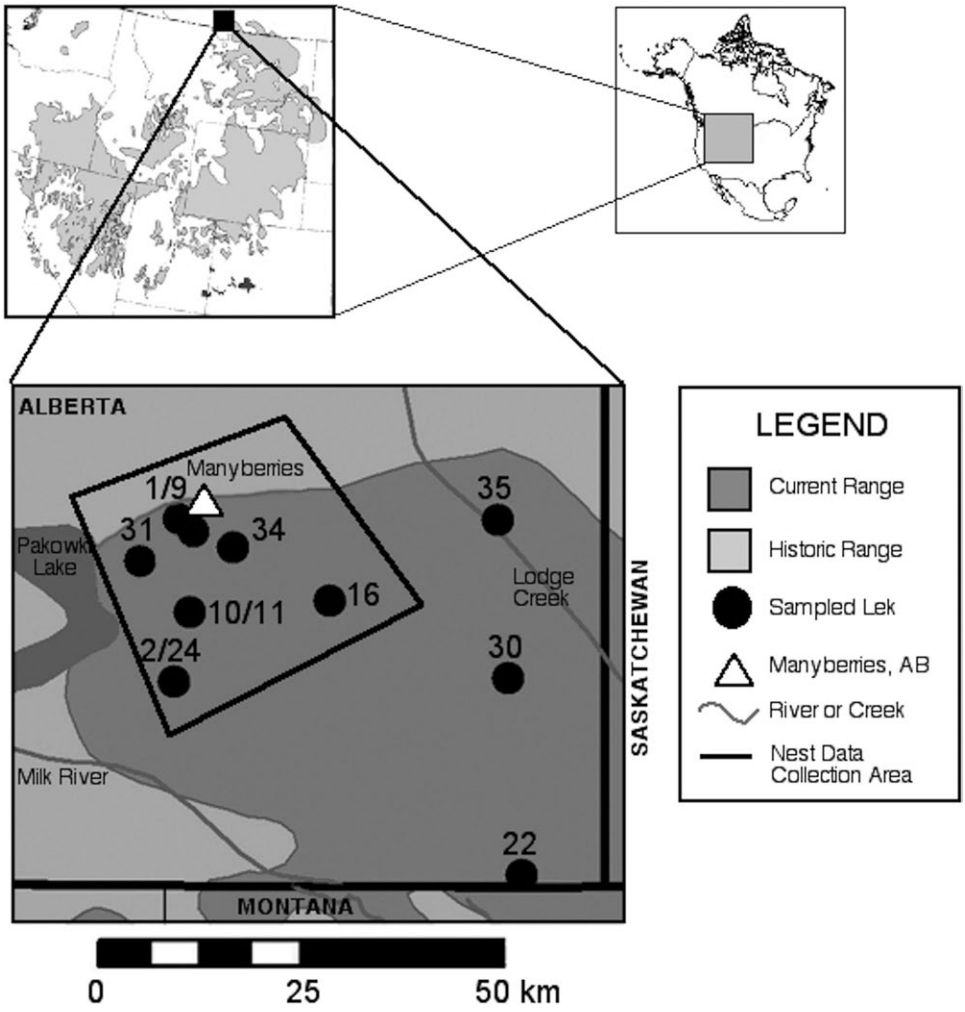

Figure 1
Location of the Alberta Sage-Grouse study area (bottom) in relation to the current distribution of sage-grouse in North America (upper left). All leks were sampled in Alberta and eggs were collected from focal area in the box in the northwest.

Rousset 1995). After correction for multiple tests, no loci at the lek level were in Hardy–Weinberg or linkage disequilibrium (Bush et al. 2010, 2011). We 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 two runs). Offspring matching a female at ≥12 of 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. Screening of interspecific hybrids was conducted using species-specific alleles and allele frequencies for sharp-tailed grouse (*Tympanuchus phasianellus jamesi*), the only other grouse species in the area (Bush 2009).

We 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 designated to the lek(s) on which they were sampled because only three adult males that were sampled ever switched leks (Bush 2009). Individual males were also designated years that we had sampled eggs (1999–2006), during which they were capable of reproducing (yearling and older). If males were not known to have died, they were conservatively assumed to be capable of fathering offspring until the end of the study (2006) because sampled males fathered offspring up to 7 years after they were first sampled and 71.7% of sampled males that were eventually credited paternity did not father offspring in the year in which they were actually sampled (data not shown). Although this assumption inflated the number of known males that could reproduce in any given year, it prevented the exclusion of true fathers simply because of a lack of observational data and does not inflate the number of true fathers. We could not estimate lifespan of males attending leks because leks were intensively observed only in 2005 and 2006.

Paternity analyses were 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 the year of interest. Both steps 1 and 2 were done for all offspring to ensure that offspring were assigned to the most likely fathers. The purpose of step 1 was to narrow down potential fathers if multiple candidates were present. Offspring assigning to a male at >80% confidence in steps 1 or 2 never assigned to another male with a greater confidence at a downstream step. Therefore 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" section) 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, lek counts, and the assumption that lek counts underestimate population size (Walsh et al. 2010) suggested that between 20% (1999–2002) and 90% (2005–2006) of known males were sampled genetically in a given year. The proportion of candidate males sampled in the simulations was set to the estimated value for a given year to conservatively estimate low male detection, less than 100% male attendance at leks, and the possibility of both unknown leks and off-lek mating.

Paternal assignments were accepted if there was ≤1 mismatch between the genotypes of the candidate male and the offspring (given the mother's genotype) and a significant ΔLOD at either >95% or >80% confidence. Although one offspring may perfectly match multiple candidate fathers, multiple siblings did not perfectly match greater than one male, the putative father. All offspring within the same clutch were compared with the male(s) considered the first and second most likely candidate fathers to each offspring within the clutch. Male(s) were then assigned to an entire clutch (single paternity) or part of a clutch (multiple paternity) provided that the male had 0–1 mismatches with his offspring and he assigned to all of his offspring at 80% or 95% confidence. In all cases, no other male was as good or equal of match as the putative father (i.e. all other males had a greater number of mismatches and assigned at a lower level of confidence).

Offspring of unassigned paternity were assumed to have unsampled fathers. We addressed the existence of unsampled fathers in three 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 observed heterozygosity of sampled males (0.68) was not greatly different than reconstructed males (0.64) suggesting excess homozygosity was not an issue. The reconstructed paternal genotypes were then compared against one another in GENALEX (Peakall and Smouse 2001) to see whether any unsampled fathers sired more than one brood and whether any of the reconstructed genotypes closely matched sampled males (at ≥11 of 13 loci). (2) The above-mentioned method may underestimate the actual number of fathers because it assumes no allele sharing among fathers or between the mother and the fathers, so we used GERUD 2.0 (Jones 2005) to determine the minimum number of fathers for a clutch and the number of offspring per father (Johnson and Yund 2007; Bos et al. 2009; Mobley and Jones 2009; Sefc et al. 2009; Wilson 2009; Borkowska and Ratkiewicz 2010; Yue and Chang 2010). (3) We used COLONY 2.0 (Wang and Santure 2009) to identify full-sib families in clutches of unknown paternity and to infer the genotypes of the unknown parents. We 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: (A) we included only sampled males to verify CERVUS paternity assignments and to identify multiple clutches fathered by single unsampled males. Separate analyses were performed on (i) each individual female including all clutches and (ii) year across leks in COLONY. (B) We 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 genetically characterized, we calculated mean annual and overall paternity success (total number of offspring fathered in a given year/number of males) for all sampled males (fathers and non-fathers), sampled fathers, and all fathers (sampled and unsampled males).

We used a combination of three methods to determine multiple paternity: (1) we 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 ≥1 locus) paternity, (2) we used CERVUS to identify clutches that had one or more fathers, and (3) we 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).

RESULTS

Duplicate samples

Of the 1095 feather samples collected on leks, 1093 (99.8%) contained enough DNA to amplify 7–13 loci in triplicate. Of the 1422 adult samples, 604 were unique and 495 (82%) of these samples were genotyped at all 13 loci (Bush et al. 2010). Probability of identity of 0.001 was achieved for non-relatives and for siblings at four and seven loci, respectively.

In years where molted feathers were intensively collected two times or more in a given breeding season, seven of eight leks showed that more males were genetically sampled on these leks than counted using traditional lek censuses (Table 1).

All 1420 offspring samples contained enough DNA to successfully amplify 7–13 loci and 1208 samples were unique. Only predated eggshell membrane fragments yielded duplicate samples from single individuals. Offspring samples had a drop out rate of 0.06. Combined with the higher drop out rate for molted feathers (1.68), we set a universal error rate of 1.0% for all analyses requiring a genotyping error estimate.

Paternity analysis

We found all offspring matched their putative mothers with the exception of 26 eggs spread among 10 clutches. Based on the number of mismatches, these eggs were deemed the result of intraspecific nest parasitism. There were up to six parasite eggs per clutch (Figure 2) with six (60.0%) parasitized clutches containing more than two non-maternal eggs. Within nests, the father(s) of the maternal eggs never matched the father(s) of the parasite eggs.

Table 1

Number of male sage-grouse counted genetically and with traditional lek count methods on leks where both methods were utilized

Lek	Year	Lek count (males only)	Number of males genetically detected	Effort (number of times feathers were collected)
1/9	2004	3	5	1
2/24	2005	3	4	1
10/11	2005	7	13	4
10/11	2006	12	26	6
16	2005	31	37	3
16	2006	25	37	6
22	2003	14	9	1
22	2004	13	6	1
22	2005	9	4	1
22	2006	7	15	3
30	2003	19	7	1
30	2005	18	20	1
30	2006	18	23	2
31	2004	12	3	1
31	2005	10	8	1
31	2006	9	9	1
34	2005	8	15	4
34	2006	11	19	7
35	2003	5	3	1
35	2004	8	11	1
35	2005	8	7	1
35	2006[a]	6	2	2

Males were counted genetically using captured male samples, molted feathers, and samples taken from males found dead on leks.
[a]A raptor kill on the lek made it difficult to collect feathers because most were likely from the killed male sage-grouse.

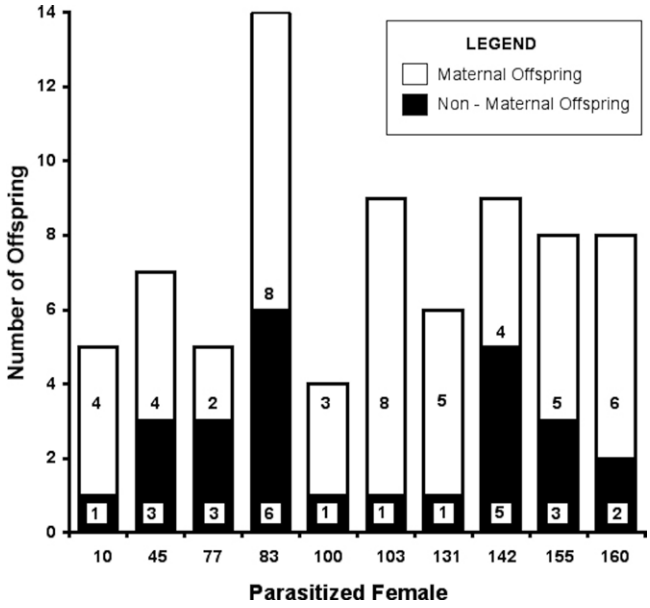

Figure 2
Ten incidences of intraspecific nest parasitism in sage-grouse in Alberta (1999–2006) showing number of both maternal (white) and non-maternal (black) offspring in each clutch.

Paternity was assigned to 443 sage-grouse offspring (36.7%) of known maternity (Table 2) 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. The latter two males were aged 2 and 3 when they fathered offspring; Table 2). 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 3 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 were yearlings. Of the 34 males with known lek affiliations, nine sired offspring of females never radio-tracked to that male's lek. In two of these instances, females were observed on the closest neighboring lek to the lek where the identified father was originally sampled, but in one case, the female was only observed on a lek 54 km away from the lek on which the father was sampled. Thirteen of 14 males that fathered multiple clutches mated with females that were observed attending the leks on which these males displayed.

If only offspring assigned with 95% confidence are considered, 268 less offspring are assigned. However, only seven fewer clutches are assigned, as most clutches contained offspring assigned at both 80% and 95% confidence. Four fewer fathers are identified (three captured males and one male sampled via molted feathers) resulting in 32 males fathering completely, or in part, 56 (29.5%) of the sampled clutches. The most clutches fathered in a given year was three (n = 4 males versus 5 males at 80% confidence). Nine unsampled males fathered more than one clutch, which was no change from 80% confidence. Seven of the 32 males with known lek affiliations fathered offspring with females never radio-tracked to their lek. At a level of 95% confidence, the observation of a female mating with a male only observed on a lek 54 km away remains valid. As these results do not vary greatly from 80% confidence, we use the 80% confidence results in all subsequent interpretations.

Across all years, observed paternity for individuals, in terms of number of offspring produced, ranged from 0 to 44, with a maximum of 24 offspring (in three clutches) fathered by an individual male in a single year. Sampled males that fathered multiple single paternity clutches were not likely to father multiple paternity clutches as well, as only 2 of these males fathered 2 of the 15 multiple paternity nests. 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 2). This is likely a low estimate based on our conservative methodology of identifying unsampled fathers and the low proportion of sampled clutches in Alberta. The conservative approach also likely underestimates the number of clutches fathered by individual unsampled males. If we assume a similar distribution to sampled fathers, we expect that unsampled fathers should father 10.1–23.9% fewer single or partial (multiple paternity) clutches than observed and 0–15.6% more multiple clutches. Of the 191 total 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 parasite eggs (single paternity in both clutches), one (0.5%) had two fathers of different species (sage-grouse and sharp-tailed grouse), and one (0.5%) was a mix of eggs belonging to the putative mother with two fathers and dumped eggs with single paternity (Figure 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% (Figure 4).

Of the 1206 eggs, 574 (47.6%) hatched. One-hundred and four females laid 191 clutches. Each female produced between

Table 2

Paternity assignment for sage-grouse offspring in Alberta, Canada (1999–2006)

Year	Number of offspring (number of sampled clutches)	Number of offspring with assigned paternity	Number of [a] sampled fathers[a]	Number of [a] unsampled fathers[a]	Number of fathers (sampled + unsampled)	Number of [a] sampled non-fathers[a]	Mean paternity success		
							All males (fathers + non-fathers)	Known fathers (sampled males)	All fathers (sampled + unsampled)
1999	84 (20)	32	4	16	20	52	1.62	8.00	4.20
2000	22 (8)	4	1	6	7	42	0.52	4.00	3.14
2001	138 (26)	41	8	19	27	61	2.26	5.13	5.11
2002	225 (34)	50	5	32	37	83	2.71	10.00	6.08
2003	242 (32)	96	6	21	27	82	2.95	16.00	8.96
2004	165 (24)	41	4	21	25	92	1.79	10.25	6.60
2005	196 (29)	91	9	21	30	148	1.32	10.11	6.53
2006	134 (18)	88	10	9	19	133	1.01	8.80	7.05
Overall	1206 (191)	443	36	138	174	379	3.18	12.31	6.93

[a]Does not sum to the total of all years combined as some males are sampled in multiple years.

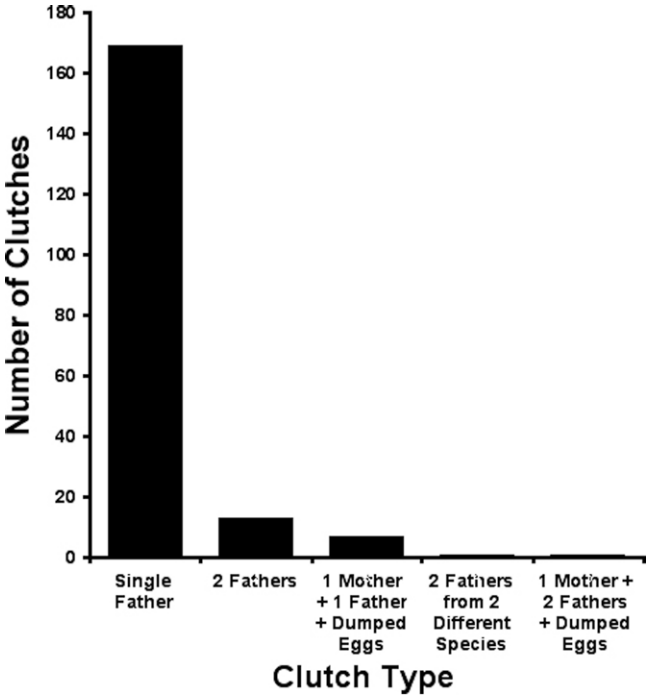

Figure 3
Distribution of clutches displaying different combinations of parentage based on patterns for 191 sage-grouse clutches in Alberta (1999–2006).

one and six clutches with a maximum of 44 offspring and 32 hatched offspring. Twenty-four females laid 2 sampled clutches in a single year; 8 (33.3%) of which had both clutches fathered by a single male, 14 (58.3%) had each clutch fathered by a different male, 1 (4.2%) had the 2 clutches fathered by 3 males (1 case of single paternity and 1 case of multiple paternity), and 1 (4.2%) had each clutch fathered by two different males (2 cases of multiple paternity with 2 different males). Thirty-seven females laid two or more clutches over their sampled lifetime (24 females within a single year and 13 females across years), 22 (59.5%) had single paternity by different males in all clutches, 7 (18.9%) had single paternity in all clutches and bred with the same male more than once, 5 (13.5%) had multiple paternity in 1 clutch, 2 (5.4%) had multiple paternity in all clutches, and 1 (2.7%) had multiple paternity in at least 1 clutch and bred with the same male for more than 1 clutch.

DISCUSSION

We used polymorphic microsatellites to study parentage and test hypotheses regarding reproductive behavior in a lek breeding species using 8 years of data from an endangered population of sage-grouse. Our prediction that multiple paternity does occur at low levels in sage-grouse was found to be true, with low levels of multiple paternity occurring within and across years. Our second prediction that intraspecific nest parasitism is rare in lekking species was found to be somewhat true, as it occurred at a higher frequency than expected. Here we discuss how these finding impact our knowledge of reproductive behavior in sage-grouse and lekking species.

Intraspecific nest parasitism

This study revealed the first evidence of intraspecific nest parasitism in sage-grouse and a rare glimpse into unusual female reproductive behavior in a lekking species. Ten of 104 (9.6%) females had their nests parasitized by other sage-grouse 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) with suspected occurrences in white-tailed ptarmigan (Choate 1963). No female was parasitized more than once during her sampled lifetime, but 60% of clutches with parasite eggs contained greater than two non-maternal eggs suggesting that most "parasite females" put multiple eggs into one parasitized nest instead of spreading eggs among nests. This is likely because sage-grouse are found at extremely low densities in Alberta and it is difficult to find sage-grouse nests because females do not nest together (Aldridge 2000). It is unknown whether parasitic females solely parasitize nests or if they tend nests of their own because none of the reconstructed genotypes of parasitic females matched any known female. Behaviorally, it is not known if nest parasitism occurs at a low frequency in all lekking avian species or if it is possibly an adaptation to low population sizes or high predation rates. The costs and benefits to nest parasitism in lekking species is also not known, but

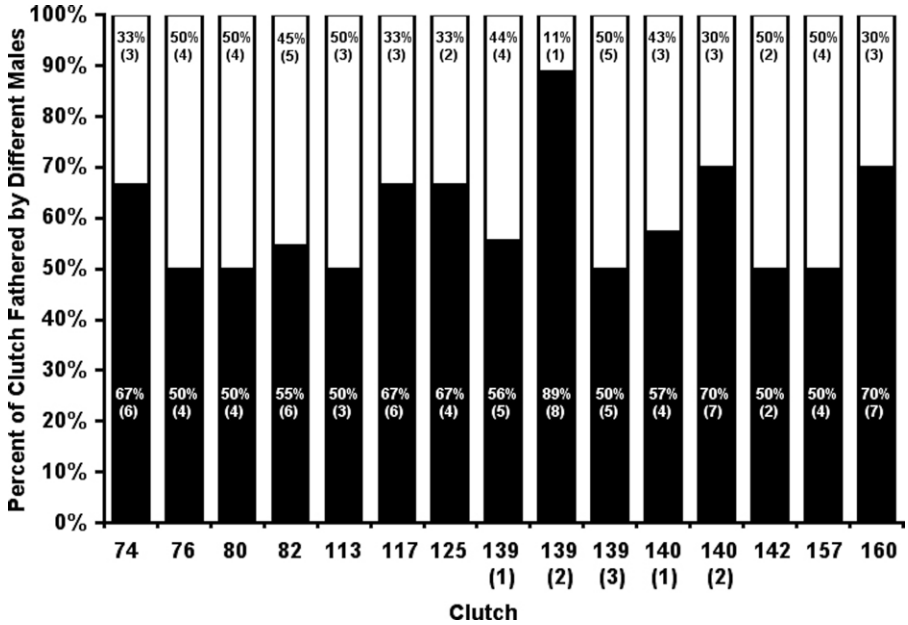

Figure 4
Distribution of paternity for clutches with two fathers. Black represents the more successful male and white represents the less successful male measured in terms of fathering offspring in the clutch. Numbers on the x-axis represent the identification number of individual females and numbers in parentheses indicate first, second, or third clutch when individual females had more than one clutch with multiple fathers.

42.3% of parasite eggs hatched providing a potential method for increasing a female's reproductive output.

Paternity

Our results suggest a considerably less-biased male reproductive output in sage-grouse than reported for other lekking species based on behavioral data. Very few males in Alberta fathered more than one clutch in a given year (with a maximum of three clutches within a year) or more than one clutch across years (between two to seven in a sampled lifetime) and approximately half of the sampled male population successfully reproduced. Males that were more successful (those that fathered more than one complete clutch) were unlikely to father offspring in nests exhibiting multiple paternity. In lekking species, males successful at copulating with females that choose to mate with a single male are expected to be successful at copulating with females that choose to mate with multiple males as well. This was not the case in sage-grouse and suggests that although multiple mating creates an opportunity for sexual selection (selection acting on differences in reproductive success among individuals caused by variation in mating success; Andersson 1994) on male traits (Byers et al 2004; Schlicht and Kempenaers 2011), it weakens sexual selection instead of strengthening it in sage-grouse. More simply, if individual males were exceptionally successful at fathering offspring, their traits would be selected for, but because success is more uniformly distributed, specific male traits do not appear to be selected for by sage-grouse females.

We found that a large proportion of the fathers in our population were unsampled males and that they had lower mean success than sampled fathers (Table 1). To investigate this, we determined the approximate bias in mating success due to our conservative methodological approach versus the likelihood that unsampled males represent subordinate males that do not frequently attend leks, males that move between leks, and/or transient males that do not breed on leks and truly have lower mean mating success. Based on the observed number of clutches sired by sampled and unsampled males and the likelihood that some males actually have lower mean siring success due to the reasons stated above, we estimated the number of unsampled males that should father two or more clutches, one clutch due to their on-lek mating behavior and/or strategy (i.e. hold a territory, but are unsuccessful at obtaining multiple copulations), and one clutch due to their off-lek mating behavior and/or strategy (i.e. subordinate males that do not frequently attend leks, males that move between leks, and/or transient males that do not breed on leks). We determined that approximately 4–10% of unsampled fathers should sire two or more clutches, 55–71% should sire one clutch due to their on-lek mating behavior and/or strategy, and 25–35% should sire one clutch as a function of decreased success due to their off-lek mating behavior and/or strategy. These latter males are not expected to breed at all based on conventional sage-grouse theories (Scott 1942; Wiley 1973), but because they do, there is a greater diversity and proportion of males fathering young in the population, which also has the potential to decrease the intensity of sexual selection (Schlicht and Kempenaers 2011).

The large proportion of the sampled males in Alberta successfully breeding also indicates that a few males are not responsible for the majority of matings in sage-grouse, and possibly, in most lekking species. Wiley (1973) observed individual sage-grouse males on single leks in a given year obtaining up to 50% of copulations while the maximum number of clutches that a male in Alberta could have fathered across years is seven. Although we could not accurately measure reproductive skew and compare it to other studies based on how we collected our data (Bush 2009), we can still look at the distribution of male reproductive success. The fact that paternity was more evenly distributed and was not monopolized by individual males across years suggests that either male quality (i.e. secondary sexual characteristics, display, disease resistance, etc.) or female preference for male traits varied between years and/or individuals. However, limited sample sizes may have obscured any observable pattern.

The large number of successful fathers has important implications for the genetic health of the population and the

determination of effective size (N_e). Polygynous mating systems affect N_e by reducing the number of breeding males and by biasing the proportional representation of male ancestors in the gene pool of future generations (Wright 1931; Kimura and Crow 1963; Leberg 2005). Reproductive success and the sex ratio of breeding adults also contribute to the rate of genetic drift when populations maintain a constant size (Wright 1938; Nunney 1993). Therefore, an increase in the proportion of males breeding in a population decreases variance among breeders, 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 a larger effective population size reduces the potential for inbreeding. However, years with poor productivity may have fewer breeders leading to lower values of N_e. This could have negative ramifications if the population goes into greater decline, as it has from 2007 to 2012. If our estimates of paternity are accurate, more birds are breeding than predicted for a typical lekking system with few males successfully mating (Wiley 1973; Höglund and Alatalo 1995), which could reflect the use of alternative mating strategies by both sexes.

Our data support the existence of inter-lek movement of females and off-lek mating in Alberta. Nine males mated with females not documented through telemetry to attend their lek suggesting that more inter-lek movement is taking place in Alberta than detected based on telemetry alone (Aldridge 2005). Although distance between leks in Alberta ranged from 5.4 km to 61.3 km, rapid long-distance movements have been documented for this population with birds moving approximately 50 km in less than 2 days during the winter (Carpenter et al. 2010). Females in Alberta are also known to nest up to 33.4 km (average of 12.2 km) from the lek that they were observed to attend (Aldridge CL, unpublished data), suggesting that females are physically capable of visiting multiple leks and mating at leks great distances apart. Radiomarked females were also observed to make seasonal movements of 40–50 km between summer and winter habitat (Carpenter et al. 2010), so it is possible that the female that mated with a male 54 km from her known lek, did so before she left her wintering habitat or mated along her or the male's migration path.

Despite extensive sampling of feathers, we found that unsampled males fathered the majority of offspring. We did not sample every male on every lek, but in 2005 and 2006, we intensively collected feathers on all known leks. In these years we genetically identified 53 (2005) and 43 (2006) more males (Bird KL, unpublished data) than were enumerated during lek counts (based on the maximum number of males attending a lek on a single morning during the lekking season; Alberta Fish and Wildlife, unpublished data). This, and intensive sampling of feathers on leks throughout the lekking season, suggests that we did genetically sample most lekking males on our focal leks in the last two years of the study and that some males did not attend leks regularly throughout the breeding season. Also, despite intensive sampling in 2005 and 2006, we 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 partially 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 (Sexton 1979; Dunn and Braun 1985; Lank and Smith 1987; Pruett-Jones 1988; Gibson 1996; Lanctot et al. 1997; Semple et al 2001; Eliassen and Wegge 2007; Lesobre et al. 2010). Some females may prefer these off-lek encounters due to decreased intra-sexual competition for males and reduced harassment by males.

Most broods had single paternity, but some 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 periphery of the species' range (southwest versus our study site at the northeast periphery), and were working with a small and isolated population. Our annual multiple paternity levels varied among years, ranging from 0% (1999 and 2000) to 16.7% (2004) suggesting that its occurrence is variable. We also had 51 single fathered clutches with four or fewer eggs, leaving the possibility that we 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. In black grouse, 25% of females were observed to mate with more than one male, but only 9% of females had clutches with multiple paternity (Lebigre et al. 2007). This suggests that multiple mating occurs more frequently than multiple paternity and that some males may have low fertility resulting in the inability to father young. This may reflect a trade-off between extravagant sexual displays early in life and fertility later in life (Preston et al. 2011). Another possibility is that female grouse utilize some form of post-copulation mechanism, such as sperm competition (Birkhead 1998) or sperm choice (Birkhead et al. 2004; Thuman and Griffith 2005), to bias insemination from all partners. Because fertility of clutches was high across all years (99.2%) and genetic diversity was high across leks (Bush et al. 2010, 2011), infertility of males is likely not an issue and sperm competition may represent a more likely mechanism behind multiple mating, yet single paternity. 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 and there was sperm storage from the first breeding attempt. We found no evidence of this scenario, as all cases of multiple paternity in the second clutch involved different males from the father of the first clutch. Levels of multiple paternity were actually lower for second nests (2/24) as compared with first nests (13/24) based on 24 females that laid two nests in a single season. Taken together, multiple paternity in sage-grouse is likely due to a multiple mating strategy followed by sperm competition and is not the result of re-mating in response to nest loss combined with sperm storage.

CONCLUSION

Despite their small numbers and restricted habitat, sage-grouse in Alberta exhibit a high level of genetic diversity and connectivity within Alberta (Bush et al. 2010) and high levels of gene flow from other parts of the northern Montana population (Bush et al. 2011), which is likely facilitated by a high proportion of the males in the population breeding (this study). However, since the time of this study, sage-grouse in Alberta have continued to decline, perhaps to the point of no return, with less than 100 birds estimated to remain. Therefore, genetic diversity alone is not enough to save

sage-grouse in Alberta and habitat related issues need to be addressed because the habitat available is limited in area and of poor quality (Aldridge and Boyce 2007), due to both natural and anthropogenic fragmentation (Alberta Sage Grouse Recovery Action Group 2005; Bush 2009). Sage-grouse in Alberta currently exhibit gene flow, but if dispersal from the rest of the population stops or usable habitat is further reduced, sage-grouse will not be able to sustain current levels of genetic diversity. 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) and if females actually breed on single or multiple leks in a given year. Leks are the primary focus of current sage-grouse conservation because they are closely associated with breeding and nesting activities for the species (Connelly et al., 2004). However, 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.

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