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RESEARCH ARTICLE

Genetic recapture identifies long-distance breeding dispersal in Greater Sage-Grouse (*Centrocercus urophasianus*)

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ABSTRACT

Dispersal can strongly influence the demographic and evolutionary trajectory of populations. For many species, little is known about dispersal, despite its importance to conservation. The Greater Sage-Grouse (*Centrocercus urophasianus*) is a species of conservation concern that ranges across 11 western U.S. states and 2 Canadian provinces. To investigate dispersal patterns among spring breeding congregations, we examined a 21-locus microsatellite DNA dataset of 3,244 Greater Sage-Grouse sampled from 763 leks throughout Idaho, Montana, North Dakota, and South Dakota, USA, across 7 yr. We recaptured ~2% of individuals, documenting 41 instances of breeding dispersal, with 7 dispersal events of >50 km, including 1 of 194 km. We identified 39 recaptures on the same lek up to 5 yr apart, which supports the long-held paradigm of philopatry in lekking species. We found no difference between the sexes in breeding dispersal distances or in the tendency to disperse vs. remain philopatric. We also documented movements within and among state-delineated priority areas of conservation importance, further supporting the need to identify movement corridors among these reserves. Our results can be used to better inform the assumptions of count-based population models and the dispersal thresholds used to model population connectivity.

Keywords: Centrocercus urophasianus, Greater Sage-Grouse, mark–recapture, microsatellite, molecular genetics, long-distance dispersal, philopatry

La recaptura genética identifica dispersión reproductiva de larga distancia en *Centrocercus urophasianus*

RESUMEN

La dispersión puede influenciar fuertemente la demografía y la trayectoria evolutiva de las poblaciones. Para muchas especies, poco se sabe sobre la dispersión, a pesar de su importancia para la conservación. *Centrocercus urophasianus* es una especie de preocupación para la conservación que se distribuye a través de 11 estados del oeste y de 2 provincias canadienses. Para investigar los patrones de dispersión entre las congregaciones reproductivas de primavera, examinamos una gran base de datos de 21 loci de microsatélites de ADN de 3,244 individuos de *C. urophasianus* colectados en 763 cortejos reproductivos a través de Idaho, Montana, Dakota del Norte y Dakota de Sur, a lo largo de 7 años. Recapturamos ~2% de individuos, documentando 41 eventos de dispersión reproductiva con 7 eventos de dispersión >50 km incluyendo 1 evento de 194 km. Tuvimos 39 recapturas en el mismo cortejo hasta con 5 años de diferencia, lo que apoya el paradigma de larga data de filopatría en las especies que forman cortejos reproductivos. No encontramos diferencia entre los sexos en las distancias de dispersión reproductiva o en la tendencia a dispersarse versus la permanencia filopátrica. Nuestros resultados pueden ser usados para establecer mejor las suposiciones de los modelos poblacionales basados en conteos y los umbrales de dispersión usados para modelar la conectividad poblacional. Otro punto importante para la conservación es que documentamos movimientos al interior y entre áreas prioritarias designadas por los estados como islas de importancia especial de conservación, reforzando la necesidad de identificar corredores de movimiento entre estas reservas de conservación.

Palabras clave: Centrocercus urophasianus, dispersión de larga distancia, filopatría, genética molecular, marca-recaptura, micro-satélite

INTRODUCTION

Dispersal is crucial to maintaining population connectivity. It is the precursor to gene flow, influencing evolutionary processes such as local adaptation and speciation, and demographic processes such as population growth and persistence (Ronce 2007, Ellstrand and Rieseberg 2016). Rates of dispersal are influenced by intrinsic factors such

as population density and access to mates, and by extrinsic, or environmental, factors such as habitat quality and resource availability (Clobert et al. 2009). Offspring often disperse away from parents at a breeding site (natal dispersal) due to kin competition and inbreeding avoidance (Gandon 1999, Platt and Bever 2009). Individuals may also disperse among breeding sites following attempts at reproduction (breeding dispersal) to enhance mating opportunities and increase lifetime reproductive success (Johnson and Gaines 1990).

Long-distance dispersal may be important for population persistence of wide-ranging species (Bohrer et al. 2005). This is especially true in naturally fragmented or human-altered landscapes (Bohrer et al. 2005), where individuals must disperse through unsuitable habitat with limited available resources along the way. The main limitation to connectivity is the distance between populations that individuals are capable of dispersing; a single successful disperser per generation can transport genes across the landscape, eliminating inbreeding depression and increasing population fitness, survival, and viability (Mills and Allendorf 1996, Schwartz and Mills 2005, Whiteley et al. 2015). Therefore, the occurrence of long-distance dispersal can be vital to some species' persistence.

Long-distance dispersal is well documented for plant species (Nathan 2006). However, due to the difficulty of documenting long movements, it is less well known in animals (but see Lowe 2009, Moriarty et al. 2009, Hawley et al. 2016). The frequency and extent of emigrating individuals is often underestimated because the quantification of long-distance dispersal is not the primary purpose of many studies (compared with, e.g., fine-scale habitat assessment), because sample sizes are too small to capture rare long-distance dispersal events, and because individuals dispersing long distances may leave the study area and be lost from detection (Koenig et al. 1996, Hassal and Thompson 2012). Furthermore, in studies designed expressly to quantify long-distance movements by tracking individuals using global positioning technology or geologgers, cost can be prohibitive (Bridge et al. 2013, Earl et al. 2016). Noninvasive genetic approaches can help to fill this knowledge gap. Genetic recapture can be used to estimate dispersal frequency and distance when the focus of such studies is the spatial redistribution of large numbers of marked animals across large areas. However, events between capture and recapture go undetected (Nathan et al. 2003). Such approaches allow landscape-scale sampling of great numbers of individuals at relatively low cost per sample, and the collection of data that can additionally be used to plan biodiversity conservation.

The Greater Sage-Grouse (*Centrocercus urophasianus*; hereafter, sage-grouse) is a lekking gallinaceous bird. Every spring, between March and May, individuals congregate on leks across the western United States and southern Canada. Lek locations are highly stable over generations, such that, following natal dispersal, most individuals are thought to exhibit philopatry, returning to the same lek every spring throughout their lifetime (Patterson 1952, Dalke et al. 1963, Emmons and Braun 1984, Dunn and Braun 1985). Natal dispersal of females is reported to be greater than that of males (median 8.8 vs. 7.4 km). However this oft-cited dispersal distance is based on a study in which the maximum distance between monitored leks was 13.1 km (Dunn and Braun 1985), and genetic data from 1 northern California population suggest that distances traveled by females may be underestimated (Davis et al. 2015).

On leks, a few territorial males may command the vast majority of mating, while nonterritorial adult and second-year males occupy the fringes (Semple et al. 2001), leading to high variation in breeding success (Payne 1984). Subdominant males may find mating opportunities by displaying and mating off the lek (Dunn and Braun 1986), or by visiting multiple leks within one breeding season to increase their chances of displacing dominant males or of finding females off the lek (Semple et al. 2001). Females are also known to visit multiple leks in a breeding season (Dunn and Braun 1985, Semple et al. 2001), occasionally visiting multiple leks within a week (Semple et al. 2001). Breeding dispersal and mate selection may occur multiple times with multiple mates during a single breeding season. However, distances traveled during breeding dispersal are unknown.

Field data show that sage-grouse are capable of long-distance movements. Among seasons, migratory sage-grouse may move 20 km (Tack et al. 2012), 30 km (Dunn and Braun 1986), or even 80 km (Connelly et al. 1988, Leonard et al. 2000) depending on habitat availability. Annual, obligate migrations of 122 km and 240 km have been documented by telemetry studies (Tack 2009, Tack et al. 2012). Most migratory movements are made in stepping-stone fashion (Tack 2009), but abrupt singular movements are possible when suitable habitat is lacking (Dunn and Braun 1986).

Research has documented sage-grouse natal dispersal distances, seasonal migration distances, and breeding behavior, but little is known about breeding dispersal distances. Furthermore, most studies of lek-site philopatry have been limited in geographic extent and sample size. New dispersal information would come at a critical time for sage-grouse, an imperiled species added to the federal Endangered Species Act (ESA) candidate list in 2010 following several petitions for protection (U.S. Fish and Wildlife Service 2010). A U.S. Fish and Wildlife Service determination in September 2015 found current efforts by state and federal agencies and other partners adequate to obviate the need for listing, but significant conservation

challenges remain and the species' status will again be reviewed in 2020 (U.S. Fish and Wildlife Service 2015).

Understanding breeding dispersal is a critical step toward comprehending the relationship between the distribution and abundance of extant populations in fragmented landscapes. State wildlife management agencies across the range of sage-grouse in the western U.S. have collectively delineated Priority Areas for Conservation (PACs) to conserve population strongholds. This conservation strategy is directed at conservation management within individual PACs and planning for connectivity among PACs to prevent isolation and divergence of existing populations in the future (Finch et al. 2016). Understanding the distance and frequency of breeding dispersal vs. philopatry is critical to ongoing conservation planning (Crist et al. 2017).

As part of a larger study examining genetic substructure and how it relates to PAC delineation (Cross et al. 2016), we used molecular genetics tools to analyze thousands of sage-grouse feathers collected from hundreds of leks scattered across Idaho, Montana, North Dakota, and South Dakota, USA, to quantify breeding season dispersal. Our 4 primary objectives were: (1) to evaluate patterns of sage-grouse lek-site philopatry; (2) to quantify distances, frequency, and patterns of breeding season dispersal among leks; (3) to assess differences in breeding season dispersal characteristics between the sexes; and (4) to examine the relative cost of breeding season dispersal using known mortalities from a subset of dispersing individuals.

METHODS

Study Area and Sampling

For our analyses we used 7,629 spatially referenced sage-grouse feather ($n = 7{,}399$) and blood ($n = 230$) samples from across the northeastern extent of the species' range in Idaho, Montana, and North and South Dakota. Feather samples were collected noninvasively (Segelbacher 2002, Bush et al. 2005) from leks, mostly during the months of March through May. These samples were supplemented by blood and feather samples collected from sage-grouse trapped on leks as part of a radio-telemetry project in central Montana. Samples were collected from 835 leks (median: 9 samples per lek, IQR: 3–10) of 2,292 known active leks from 2007 to 2013 by field biologists and technicians with the Bureau of Land Management, Montana Fish, Wildlife and Parks, and Montana Audubon (see Cross et al. 2016 for detailed methods).

Laboratory Analysis

DNA extraction. Feather DNA was extracted from the quill (calamus) using the DNeasy Blood and Tissue Kit (QIAGEN, Valencia, California, USA) and the user-developed protocol for purification of total DNA from nails, hair, or feathers (QIAGEN). We modified the protocol by incubating samples for a minimum of 8 hr after addition of Proteinase K (QIAGEN) and by eluting DNA with 100 μL of Buffer AE (QIAGEN). Feather samples were extracted in a lab used only for noninvasive DNA extraction to avoid potential contamination from samples with greater DNA concentrations. Blood samples were extracted using QIAGEN's DNeasy Blood and Tissue Kit and protocol for nucleated blood.

Microsatellite DNA amplification and electrophoresis. We amplified 21 variable microsatellite loci and 1 sex-diagnostic locus in 8 multiplex polymerase chain reactions (PCR). We used procedures detailed in Cross et al. (2016), with the addition of the following loci: SG21, SG28, SG29, SG36, and SG39 (Appendix Table 3). Primers and locus-specific reaction mixes, annealing temperatures, and thermal cycler profiles are presented in Appendix Table 3, 4, and 5.

Genotyping and Identification of Recaptures

To ensure correct genotyping from low-quality and low-quantity feather DNA samples, each sample was PCR-amplified across the 22 loci to screen for allele dropout, stutter artifacts, and false alleles (DeWoody et al. 2006). To minimize genotyping error, each sample was scored by 2 observers. If any locus failed to amplify in either replicate, or if there was a discrepancy between locus genotypes as scored by the 2 observers, PCR-amplification and genotyping was repeated twice more. If a genotype was confirmed by this repeat analysis then it was retained. If a genotype failed again, the sample was assigned a missing score at the failed locus.

To screen samples for quality control, we removed from analysis any individual for which amplification failed at >⅓ of the loci (i.e. 7 loci). After removal of poor-quality samples, genotypes were screened to ensure consistency between allele length and length of the microsatellite repeat motif. To screen for and correct genotyping error, we used DROPOUT 2.3 (McKelvey and Schwartz 2005 as implemented in Schwartz et al. 2006) and package ALLELEMATCH 2.5 (Galpern et al. 2012) in R 3.3.0 (R Core Team 2016). In ALLELEMATCH, we used the amUnique function to generate a list of all potentially matched sample genotypes, using an alleleMismatch setting of 6 (as calculated using the amUniqueProfile function). The alleleMismatch setting is approximately equivalent to matching samples with up to 6 pairwise mismatched loci. We used the list of potentially matched samples as a basis for reexamination and repeat analysis to confirm the genotype scores.

We reviewed all potentially matching samples, confirming a recapture only when we found no mismatch in genotype across the 22-locus panel. Detection of philopa-

TABLE 1. Summary of genetic capture of Greater Sage-Grouse in Idaho, Montana, North Dakota, and South Dakota, USA, including year of genetic sample collection (Year), total number of individuals genotyped each year (*N*), total number of captures (n_c) and recaptures (n_r) each year, other collection years in which captured individuals were recaptured at the same lek as their lek of initial capture and how many were recaptured in each year (Recaptured same lek (*n*)), and collection years in which captured individuals were recaptured at a different lek from their lek of initial capture and how many were recaptured in each year (Recaptured different lek (*n*)).

Year	*N*	n_c	n_r	Recaptured same lek (*n*)	Recaptured different lek (*n*)
2007	85	2	0	(0)	(0)
2008	25	1	0	(0)	(0)
2009	590	14	3	2007 (1), 2008 (1)	2009 (1)
2010	276	9	9	2009 (5)	2009 (3), 2010 (1)
2011	269	9	9	2009 (3), 2010 (2)	2009 (1), 2010 (1), 2011 (2)
2012	1,045	35	31	2007 (1), 2010 (5), 2011 (6)	2012 (19)
2013	954	8	28	2009 (1), 2011 (1), 2012 (12)	2012 (5), 2013 (9)
Total	3,244	78	80	38	42

try required that the same individual attend the same sampled lek in 2 different sampling years, shedding a feather in both years that was then both collected and successfully genotyped in the lab. Detection of a dispersal event required that an individual successfully emigrate from a sampled lek of first capture and successfully immigrate to a sampled lek of recapture, shedding a feather at each lek that was both collected and successfully genotyped in the lab. We calculated great-circle distance between all confirmed pairs of recaptures using the coordinates for each sample collection location with the spDistsN1 function in the sp package in R 3.3.0 (R Core Team 2016).

For all individuals, across all 22 loci, we quantified the power of our microsatellite locus panel to discern individuals by calculating probability identity (P_{ID}; Evett and Weir 1998)—the probability that 2 individuals drawn at random from the population could have the same genotype across all loci—using DROPOUT 2.3. Because we knowingly sampled from multiple populations (Cross et al. 2016), we did not test for deviation from Hardy-Weinberg proportions (~~HWP~~) or gametic disequilibrium (~~GD~~) among loci.

Sex-biased Breeding Season Dispersal

Because bias in natal dispersal has been documented (Dunn and Braun 1986), we tested whether the frequency of breeding season dispersal differed between the sexes by performing a Fisher's exact test for count data. To test whether males or females dispersed farther we performed a Mann-Whitney *U*-test, and to evaluate whether breeding season dispersal distances differed between the sexes within or among years we performed a Kruskal-Wallis rank sum test. All tests were performed in R (R Core Team 2016).

Mortality and Breeding Season Dispersal

Dispersal can be costly to individuals. Therefore, the cost of dispersal combined with varied reproductive success in different habitats may affect the propensity of individuals to disperse (Leturque and Rousset 2002). In lek breeding systems, reproductive success varies greatly for individuals (Payne 1984). Therefore, individuals may hazard long-distance movements to improve breeding opportunity by visiting distant leks. To test whether breeding season dispersal distances differed between individuals recaptured as known mortalities and all other recaptures, we performed a Mann-Whitney *U*-test in R (R Core Team 2016). All values reported in the Results are means ± SD.

RESULTS

Genotyping

After removing samples of inferior quality (those that failed at ≥7 loci; $n = 1{,}782$, ~23%) and recaptures of the same sage-grouse at the same lek on either the same or different day within the same year ($n = 2{,}603$), we retained 3,244 of 7,629 samples analyzed (~43%). The 3,244 high-quality genotypes from feathers ($n = 3{,}017$) and blood samples ($n = 227$) were from 763 leks, with an average of 4.11 ± 3.99 samples per lek (range: 1–62 samples per lek). We determined sex for 3,212 (99%) of the final individual genotypes: 600 females (~19%) and 2,612 males (~81%). Using our 22-locus panel (21 autosomal loci and 1 sex-linked locus), P_{ID} was 2.20×10^{-29}, providing substantial power to discern individuals, given a suggested P_{ID} of 0.001–0.0001 for law enforcement forensic applications in natural populations (Waits et al. 2001).

Identification of Recaptures

We recaptured ~2% of captured individuals, with 80 recaptures of 78 individuals from 3,244 total captures of 3,164 individuals. Recaptures matched initial capture genotypes across all 22 loci. Of 78 recaptured individuals, 9 were females (~12%) and 69 were males (~88%); ~2% of the 582 females and ~3% of the 2,472 males were genotyped.

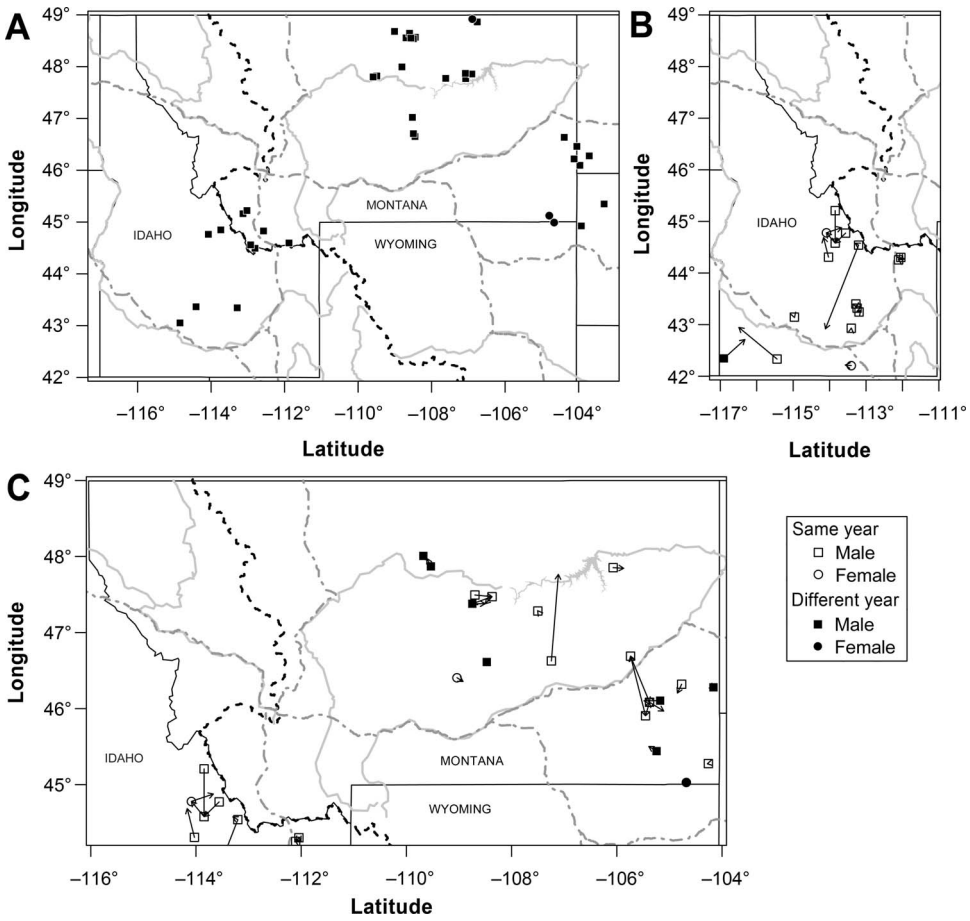

FIGURE 1. Greater Sage-Grouse recapture locations based on feather genotypes at (**A**) the same lek in different years (philopatry), and (**B**) in the same or different years at different leks in Idaho, USA, and (**C**) Montana, USA, 2007–2013. Arrows show breeding season dispersal between capture (tail) and recapture (head) locations. The dotted black line represents the North American continental divide, solid black lines represent state boundaries, solid light gray lines represent major rivers, and dashed dark gray lines represent major highways.

Individuals were either recaptured in different years at the same lek (35 males and 3 females; Table 1, Figure 1A), in the same year at different leks (26 males and 5 females; Figures 1B, 1C, 2A), or in different years at different leks (10 males and 1 female; Figures 1B, 1C, 2A). Two males were recaptured twice; all other individuals were recaptured just once. One of the males was captured twice in the same year (2012) at different leks (14 km apart) and once 1 yr later (2013) at a different lek 30 km away. We captured the second male 3 times in the same year (2013) at 3 different leks that were 21 km, 73 km, and 90 km apart. Five years was the longest time between capture and recapture at the same lek, but we also recaptured 3 individuals 3 yr apart, and another 9 individuals 2 yr apart. Time between capture and recapture averaged 300 ± 322 days (range: 0–1,809 days, $n = 80$).

Thirty-three breeding season dispersal movements were within, among, out of, or into a PAC (Table 2). Breeding season dispersal events within sage-grouse PACs was greater than double the number of movements documented outside PACs. Twenty-four movements occurred within PAC boundaries and another 10 occurred outside PAC boundaries. Three movements occurred among PACs, all in Idaho, in which 3 different sage-grouse made 31 km, 35 km, and 70 km movements. Another 6 individuals dispersed into or out of PACs. Two individuals moved into PACs, both from a distance of 62 km. One other individual was first captured within a PAC and then recaptured outside that PAC, moving 13 km. Three individuals moved into or out of PACs, traveling 14 km, 127 km, and 194 km in a single season.

Sex-biased Breeding Season Dispersal

The frequency of breeding season dispersal was similar between the sexes (Fisher's exact test for count data: $P = 0.49$, 95% CI = 0.35–12.16, odds ratio = 1.83, 2-tailed). Among dispersing individuals, the distance moved across all years was similar between the sexes (Mann-Whitney *U*-

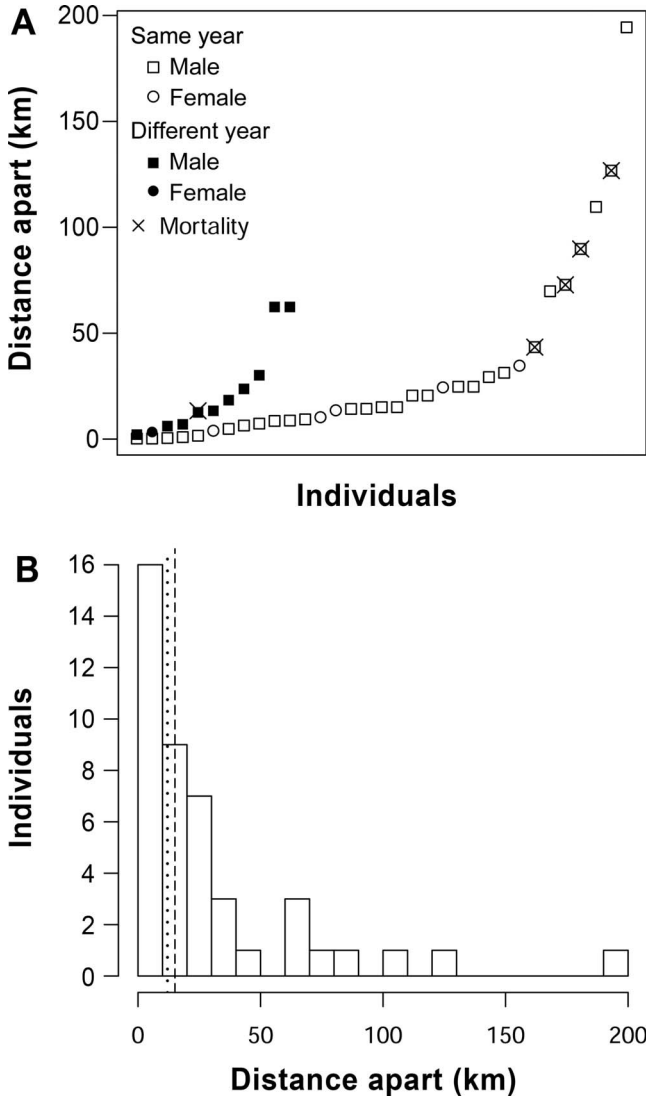

FIGURE 2. (**A**) Individual distances between capture and recapture locations and (**B**) distribution of distances travelled by Greater Sage-Grouse in Idaho, Montana, North Dakota, and South Dakota, USA, 2007–2013. In (**A**), points represent individuals plotted in order of increasing dispersal distance. In (**B**), the dotted line indicates the median dispersal distance for females (12.02 km), and the dashed line indicates the median dispersal distance for males (15.08 km). Philopatry is not plotted.

test: $W = 133$, $P = 0.53$, 2-tailed), as was distance moved within and among years (Kruskal-Wallis rank sum test: $\chi^2 = 1.54$, df = 3, $P = 0.67$). Dispersing females moved 15 ± 12 km (range: 3–35 km, $n = 6$) and dispersing males moved 32 ± 41 km (range: 0–194 km, $n = 38$; Figures 2A, 2B).

Mortality and Breeding Season Dispersal

Feathers from 5 recaptured sage-grouse (all male) were collected from known mortalities found on or near active leks. Four of the carcasses showed evidence of predation. Two of these were recaptured in the same year: one was 127 km from its lek of origin, and the second had been captured previously live at 2 different leks 73 km and 90 km away. The other 2 sage-grouse were recaptured a year after initial capture: one near the same lek and the second 13 km from its original capture site. The 5th known mortality had struck a powerline 43 km away from initial capture. Individuals recaptured as mortalities dispersed significantly farther than did all other recaptures (Mann-Whitney *U*-test: $W = 31$, $P = 0.01$, 2-tailed). Individuals recaptured as mortalities moved 69 ± 43 km (range: 13–127 km, $n = 5$), whereas all other recaptured individuals moved 25 ± 36 km (range: 0–194 km, $n = 39$; Figure 2A).

DISCUSSION

Collectively, our findings support the long-held paradigm of lek philopatry in sage-grouse, yet we also identified highly mobile segments of breeding populations that readily dispersed farther than previously known. Long-distance dispersal events are certainly more common than we were able to detect. Individuals showed strong philopatry to leks across the 4-state study region, both within and between years, with evidence of recapture at the same site 5 yr apart. The lek selected during natal dispersal (Dunn and Braun 1985) likely establishes the lek to which most sage-grouse remain philopatric (Schroeder and Robb 2003). However, breeding season dispersal also shapes populations. Our genetic approach is the first of its kind in sample size and geographic scope, and is novel for capturing long-distance exchanges in sage-grouse populations, documenting 7 movements of >50 km, 6 of which occurred within a single lekking season. Our estimates of

TABLE 2. The number of Greater Sage-Grouse breeding season dispersal movements among, entering (incoming) or leaving (outgoing), outside, or within priority areas for conservation (PACs) in Idaho, Montana, North Dakota, and South Dakota, USA, 2007–2013. Also shown are summary statistics for distances in each direction of movement.

		Distance (km)			
Direction	Number of movements	Minimum	Median	Mean ± SD	Maximum
Among	3	31.30	34.73	45.28 ± 21.31	69.80
Incoming or outgoing	6	13.37	62.40	67.64 ± 59.04	194.39
Outside	10	0.27	17.40	26.60 ± 30.34	89.77
Within	24	0.26	9.05	16.62 ± 22.65	109.61

philopatry and dispersal are biased low, given the events required to successfully capture and recapture an individual using genetic sampling. Furthermore, the number of dispersers recaptured is more biased than the number of philopatric individuals recaptured as we sampled only 36% of known active leks ($n = 835$, $N = 2{,}292$), thereby missing any dispersal from or to unsampled leks.

Sage-grouse genetic structure is governed by the process of isolation by distance, whereby the cumulative effects of many short- and fewer long-distance dispersals shape patterns of relatedness (Oyler-McCance et al. 2005, Bush et al. 2011, Schulwitz et al. 2014, Davis et al. 2015, Cross et al. 2016). Lek-based mating systems should result in inbreeding depression, but dispersal as documented here may alleviate such a deleterious effect (Tallmon et al. 2004, Whiteley et al. 2015) and may also function to extend the neighborhood of advantageous adaptations (Richardson et al. 2014).

Breeding season dispersal may present additional mating opportunities as grouse visit multiple leks, although further study is needed because breeding outcomes for dispersing sage-grouse are unknown. Dominant males may simply be maximizing reproductive opportunity, their subdominant counterparts may be displaced by dominant males, or males may disperse following unsuccessful mating attempts. Females may disperse following nest failure near their first attended lek, or may disperse to mate with males at other leks, which could result in multiple-paternity broods (Bush et al. 2010). Regardless of mechanism, we could not detect a difference in breeding season dispersal behavior between the sexes, likely due to low statistical power. Male-biased sampling is an artifact of the greater amount of time that males spend on leks compared with females, and of energetic male display and fighting on leks compared with relatively quiescent female behavior; male behaviors are more likely to result in a great number of dropped feathers, which can be collected. Widely assumed, but undocumented until now, however, is a potential tradeoff for dispersing to increase mating opportunities: based on a small sample of mortalities, heightened risk appears to accompany long-distance breeding season dispersal.

Greater breeding season dispersal distances within years compared with across years is likely not an underlying biological driver but instead an artifact of higher sampling intensity in later years (Hassall and Thompson 2012). We may have misclassified year of capture or recapture because feathers can persist for multiple years on a lek. However, feathers weather poorly, and we avoided extracting DNA from feathers which appeared aged (feathers that were dirty, physically damaged, or for which the calamus had become opaque from extended UV exposure). In harsh sagebrush environments, high UV radiation and freeze–thaw cycles should rapidly degrade and shear DNA, rendering most old samples incapable of producing a viable genotype (Segelbacher 2002). Regardless, we likely underestimated the frequency and maximum distance of breeding season dispersal events, estimates of both of which will increase with continued feather collection (Hassall and Thompson 2012). Still, our estimates are valuable for parameterizing individual-based models (Wood et al. 2015) and for seeding scenarios that evaluate connectivity under variable dispersal rates and distances (Knick and Hanser 2011, Knick et al. 2013, Crist et al. 2017). Furthermore, population trends are monitored by counting males annually on hundreds of leks across the 11-state, 2-province sage-grouse range, and our dispersal estimates may be incorporated into trend or density modeling to account for breeding season dispersal among leks and the reality that some individuals are counted more than once (McCaffery et al. 2016).

Having documented breeding season dispersal within, between, and outside PACs, we recommend that future connectivity research focus on resistance surface–based modeling (Wade et al. 2015) to identify low-cost paths that facilitate continued movement. To date, conservation planning for imperiled sage-grouse has relied on the findings of a few localized dispersal and migratory behavior studies as a surrogate for understanding long-distance dispersal. Localized dispersal studies have revealed short-distance movements—mostly <10 km, with few >20 km—and have drawn lek fidelity into question (Dalke et al. 1963, Wallestad and Schladweiler 1974, Dunn and Braun 1985, Hanf et al. 1994, Schroeder and Robb 2003). Research into sage-grouse migratory behavior has revealed that some individuals move in stepwise fashion among stepping-stones of intact habitat, and are capable of 250-km round trips (Smith 2012, Tack et al. 2012). Now, in addition to the understanding that behavioral research has provided, it is possible to parameterize connectivity models using dispersal distances and genetic data gained from this large-scale, high-sampling-resolution research. Resultant findings will allow managers to quantify connectivity and prioritize leks for conservation according to those that contribute the most to gene flow (Jacoby and Freeman 2016).

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Ethics statement: Handling of sage-grouse and collection of feathers was conducted by our partners, under appropriate required permitting. For our laboratory research and analysis, no permitting was required.

Author contributions: T.B.C. conceived the idea, collected the data, analyzed the data, and conducted the research. T.B.C. and M.K.S. developed and designed methods. J.C.C. oversaw sample collection and contributed substantial materials, resources, and funding. T.B.C., D.E.N., and M.K.S. wrote the paper or substantially edited the paper.

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APPENDIX TABLE 3. Forward and reverse primer sequences used to genotype Greater Sage-Grouse in Idaho, Montana, North Dakota, and South Dakota, USA, 2007–2013. Repeat motif, size range, number of alleles (# alleles), probability identity (P_{ID}), probability identity sibling (P_{IDsib}), and sources for the primer sequence are shown for the 21 variable microsatellite loci and 1 diagnostic sex locus (1237) used in this study. Primers that required redesign to increase efficacy with noninvasive samples are indicated with an asterisk.

Locus	Forward Primer	Reverse Primer	Repeat motif	Size range	# alleles	P_{ID}	P_{IDsib}	Source
1237	GAGAAACTGTGCAAAACAG	TAAAGCTGATCTGGAATTTCA*	Bi-allelic	224 & 252	2	0.7020	0.8401	a
BG6	AAAGAGGCAAGCACTCACAATG	CCCTTGGAATATCCTTTAACAAAAC	$(GATA)_{15}$	199–311	18	0.0172	0.3034	b
BG16	GTCATTAGTGCTGTCTGTCTATCT	TGCTAGGTAGGGTAAAAATGG	$(CTAT)_{15}$	125–185	12	0.0698	0.3712	b
BG18	CCATAACTTAACTTGCACTTTC	CTGATACAAAGATGCCTACAA	$(CTAT)_{17}$	135–171	10	0.6538	0.8166	b
MS06.4	CCTGGAGCAACTTGAGG	GTGACATTICCCCCCAC	$(GATA)_2(GGTA)_6(GATA)_5$	118–178	14	0.0602	0.3604	c
MS06.6	CAAACAACTGTCTTCCAGTAAGAC	AGAGCCTICATTTCTGGCAG	$(CAT)_{16}$	122–185	22	0.0275	0.3208	c
MS06.8	GCAAAATCAATAGAAGTAGAGAGG	CAGTAGCAGCTTTGTTTGG	$(GATA)_{17}$	103–159	15	0.0336	0.3276	c
MSP11	GGTGAAAAGTGTGGCAACTG*	CATTGTCAGCTTGCAGAC	$(CA)_{22}$	206–280	35	0.0207	0.3091	c
MSP18	CAATGACAGTATTTCCCAGATTA	GAATGGTAATATACTAAGCACAGG	$(CA)_{14}$	96–120	13	0.0333	0.3294	c
SG21	AGGCAAAACAGTCACACATGC	ATCACAAGCAGAGTGCAGGC	$(TC)_{54}$	180–438	67	0.0128	0.2956	d
SG28	ACAGGGGAAGGACAGACTGG	ACCTCTGCTTTTCCATTGCC	$(AC)_{50}$	113–169	29	0.0114	0.2926	d
SG29	AAGGGGCTTAGGGTTTTAATGG	AGTTAACTAAGTTGGGCAGGGG	$(AC)_{50}$	110–154	21	0.0207	0.3086	d
SG36	TTCCAGACATTTTGGGAGCC	CACATGTCCATCCAACCACC	$(ATGG)_{52}$	103–251	14	0.0794	0.3816	d
SG39	GAAAGTCTGAATGCTGGAGAACC	AAGCGTACTGTTTGCTCCCC	$(ATC)_{45}$	148–184	12	0.0441	0.3414	d
SGCA5	CGGACAGGTACATCCTGGAA*	GGGAAAAGATGTCAGAATCTACAAA*	$(CA)_{12}$	120–144	13	0.0604	0.3612	e
SGCA11	GCAGTAAAGAAAATTTGGAAGCA*	TCTTGAACTGATGTTGGATTTG*	$(AC)_{14}(AG)_2(CA)_5$	181–203	11	0.0759	0.3806	e
SGCTAT1	GCGACACTGCTCCCACCT	GAAAGGTTGTAAGAGGTCGT	$(CTAT)_{11}$	93–133	11	0.0607	0.3587	e
TTD6	GGACTGCTTGTGATACTTGCT	CATGCAGATGACTTTCAGCA	$(CA)_{17}$	107–153	20	0.0443	0.3410	f
TTT3	TAGCAAACGAACCAGCCAAC	GCTCTGAATCTGCCCATCTCT	$(CATC)_n$	192–228	10	0.1243	0.4290	g
TUD3	TCCAAGGGGAAAATATGTGTG	TTCTTCCAGCCCTAGCTTTG	$(TG)_{12}$	154–222	29	0.0108	0.2909	h
TUT3	CAGGAGGCCTCAACTAATCACC	CGATGCTGGACAGAAGTGAC	$(TATC)_{11}$	140–172	9	0.1431	0.4462	h
TUT4	GGAGCATCTCCCAGAGTCAG*	TCAGCTGTGAACCAGCAATC*	$(TATC)_8$	163–203	11	0.0412	0.3369	h

[a] Kahn et al. (1998)
[b] Piertney and Höglund (2001)
[c] Oyler-McCance and St. John (2010)
[d] S. Oyler-McCance personal communication
[e] Taylor et al. (2003)
[f] Caizergues et al. (2001)
[g] Caizergues et al. (2003)
[h] Segelbacher et al. (2000)

APPENDIX TABLE 4. Microsatellite locus multiplexes, primer annealing temperatures, and reagent mixes used in polymerase chain reactions (PCR) to genotype Greater Sage-Grouse samples from Idaho, Montana, North Dakota, and South Dakota, USA, 2007–2013. Columns 3–14 (F1, F2, F3, R1, R2, R3, Taq, 10× buffer, dNTP, $MgCl_2$, BSA, and H_2O) are measured in μL. F1–F3 indicate the amount of forward primer added to the reactions, and R1–R3 indicate the amount of reverse primer added to the reactions. All reactions used 1 μM IDT Custom DNA Oligos Forward Primer (Integrated DNA Technolgies, Coralville, Iowa, USA), 10 μM Eurofins MWG Operon Custom DNA Oligos Reverse Primer (Eurofins Scientific, Lancaster, Pennsylvania, USA), Invitrogen 5 U μL^{-1} AmpliTaq Gold DNA Taq Polymerase (Thermo Fisher Scientific, Waltham, Massachusetts, USA), Invitrogen GeneAmp 10× PCR Buffer II (100 mM Tris-HCl, 1.5 mL pH 8.3, 500 mM KCl; Thermo Fisher Scientific), New England Biolabs Deoxynucleotide Set (25 μmol 100 mM ultrapure dATP, dCTP, dGTP, dTTP)—dNTP (New England Biolabs, Ipswich, Massachusetts, USA), Invitrogen 25 mM $MgCl_2$ (Thermo Fisher Scientific), bovine serum albumen (~66 kDA, used to stabilize enzymes during digestion of DNA—to prevent adhesion of the enzyme to reaction tubes, to inactivate contaminating nucleases and proteases, to stabilize nucleic acid modifying enzymes, as a blocking agent to minimize background, and to increase PCR yield from low purity templates; Thermo Fisher Scientific), and nuclease-free water (Thermo Fisher Scientific).

Primer multiplex	Annealing temperature (°C)	F1	F2	F3	R1	R2	R3	Taq	10× buffer	dNTP	$MgCl_2$	BSA	H_2O
1237 / BG18 / MSP18	54	0.10	0.10	—	0.20	0.20	—	0.26	1.00	1.00	0.80	0.10	4.24
BG16 / MS06.8 / MSP11	52	0.10	0.07	0.10	0.20	0.20	0.20	0.26	1.00	1.00	0.80	0.10	3.97
BG6 / MS06.4 / SGCA5	54	0.10	0.20	0.07	0.20	0.20	0.20	0.26	1.00	1.00	1.20	0.10	3.47
MS06.6 / SG21 / SG28	60	0.10	0.10	0.10	0.20	0.20	0.20	0.26	1.00	1.00	0.80	0.10	3.94
SG29 / SG36 / SG39	60	0.10	0.07	0.20	0.20	0.20	0.20	0.26	1.00	1.00	0.80	0.10	3.87
SGCA11 / SGCTAT1 / TUT4	60	0.10	0.10	0.13	0.20	0.20	0.20	0.26	1.00	1.00	0.80	0.10	4.17
TTD6 / TUT3	56	0.13	0.20	—	0.20	0.20	—	0.26	1.00	1.00	1.20	0.10	3.71
TTT3 / TUD3	55	0.20	0.10	—	0.20	0.20	—	0.26	1.00	1.00	0.80	0.10	4.14

APPENDIX TABLE 5. Polymerase chain reaction (PCR) thermocycler phase, temperature, time, and number of cycles used to amplify microsatellite loci for Greater Sage-Grouse samples from Idaho, Montana, North Dakota, and South Dakota, USA, 2007–2013. Specific loci multiplexes and primer annealing temperatures are given in Appendix Table 4.

Phase	Temperature (°C)	Time (min)	Cycles
Initial denaturation	94	11	1
Denaturation	94	1	44
Primer annealing	see Appendix Table 4	1	44
Extension	72	1	44
Holding	12	∞	1