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Population Genetics of Greater Sage-Grouse in Strawberry Valley, Utah

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ABSTRACT

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This study examined population genetics of greater sage-grouse (*Centrocercus urophasianus*) in Strawberry Valley, Utah located in the north-central part of the state. The Strawberry Valley population of sage-grouse experienced a severe population decline with estimates of abundance in 1998 less than 5% (~150 individuals) of similar estimates from the 1930s (>3,000 individuals). Given the population decline and reduced genetic diversity, recovery team partners translocated sage-grouse from four different populations into Strawberry Valley over 6 years (2003-2008). Translocations have been used as a strategy to increase both population size and genetic diversity in wildlife populations. We assessed whether genetic diversity increased following the translocation of sage-grouse into Strawberry Valley by looking at both nuclear and mitochondrial DNA indices. We observed an overall increase of 16 microsatellite alleles across the 15 loci studied ($\overline{x}$ =1.04 alleles per locus increase, SE ± 0.25). Haplotype diversity increased from 4 to 5. Levels of genetic diversity increased for both nuclear and mitochondrial DNA (16% and 25% increases for allelic richness and haplotype diversity, respectively). These results show that translocations of greater sage grouse into a wild population can be an effective tool to increase not only population size but also genetic diversity.

Second, we studied fitness-related traits and related them to genetic diversity indices in a population of greater sage-grouse in Strawberry Valley, Utah from 2005 to 2013. We captured 93 sage-grouse in Strawberry Valley and fitted them with a radio collar and drew and preserved blood. We monitored sage-grouse weekly, throughout each year. From blood, we extracted and amplified DNA with 15 microsatellite loci. We determined genetic diversity as multilocus heterozygosity and mean d^2. To determine if there was a relationship between genetic diversity and survival, we used known-fate models in Program MARK. We also determined if there was a relationship between genetic diversity measures and nest initiation, nest success, clutch size, and number of eggs hatched using generalized linear models where reproductive measures were modeled as a function of genetic diversity. We found no significant relationship between mean d^2 and microsatellite heterozygosity with measures of survival or reproductive fitness. Overall, these results suggest that the often-reported strong heterozygosity-fitness correlations detected in small, inbred populations do not reflect a general phenomenon of increasing individual survival and reproductive fitness with increasing heterozygosity.

Keywords: *Centrocercus urophasianus*, greater sage-grouse, genetic diversity, translocation, heterozygosity-fitness correlation, genetic diversity, microsatellites, mean d^2

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CHAPTER 1

INFLUENCE OF TRANSLOCATION ON GREATER SAGE-GROUSE IN STRAWBERRY VALLEY, UTAH: HAS GENETIC DIVERSITY INCREASED FOLLOWING AUGMENTATION?

ABSTRACT

Conserving genetic diversity is a priority for wildlife managers because low diversity is often correlated with relatively low fitness of individuals. Translocation of individuals has been used as a strategy to increase both population size and genetic diversity in wildlife populations. In central Utah, the Strawberry Valley population of greater sage-grouse (*Centrocercus urophasianus*) experienced one of the most severe declines reported for this species with estimates of abundance in 1998 less than 5% (~150 individuals) of similar estimates from the 1930s (> 3,000 individuals). Genetic analysis of microsatellite and mitochondrial DNA indicated that this population had low genetic diversity compared to sage-grouse from other areas. Given the population decline and reduced genetic diversity, recovery team partners translocated sage-grouse from four different populations into Strawberry Valley over a 6 year period (2003-2008). Our objective was to assess whether genetic diversity increased following translocation by examining indices of diversity for both nuclear and mitochondrial DNA. Translocations resulted in an overall increase of 16 microsatellite alleles across the 15 loci studied ($\overline{x}$ =1.04 alleles per locus, SE ± 0.25). Haplotype diversity increased from 4 to 5 suggesting that at least one independent maternal line was successfully introduced into the population. Levels of genetic diversity increased for both nuclear and mitochondrial DNA (16% and 25% increases for allelic richness and haplotype diversity, respectively). Our results suggest

that translocations of greater sage grouse into a wild population can be an effective tool to increase not only population size but also genetic diversity.

INTRODUCTION

Maintaining genetic variation is a major concern for wildlife managers since low levels of heterozygosity often correlate with low fitness (Reed and Frankham 2003). Small populations inevitably lose genetic diversity over time due to the effect of genetic drift (Lacy 1997, Bellinger et al. 2003). Small populations experience increased inbreeding, which in turn results in increased homozygosity, which is correlated with lower viability and fecundity (Ralls and Ballou 1983, Lacy 1987, Falconer and Mackay 1996, Lacy 1997). For example, there is a correlation between heterozygosity and individual fitness-related traits such as survival (Da Silva et al. 2006, Kretzmann et al. 2006, Da Silva et al. 2009), reproductive success (Hoglund et al. 2002, Olano-Marin et al. 2011), body condition (Herdegen et al. 2013, Monceau et al. 2013), and parasite resistance (Isomursu et al. 2012). These reductions in fitness can create a negative feedback that, over time, is associated with decreases in evolutionary flexibility that can have significant and long-term adaptation consequences (Selander 1983).

The introduction or translocation of new individuals into existing populations has been used as a strategy to increase size and genetic diversity in wildlife populations (Storfer 1999, Bouzat et al. 2009, Weeks et al. 2011). The degree of success for translocation programs can be assessed by monitoring genetic diversity before and after a translocation event (Arrendal et al. 2004). Translocation of genetically diverse individuals into existing populations can slow, halt, or even reverse the loss of genetic variation (Lacy 1997). Genetic diversity, for example, was restored in a population of Rocky Mountain bighorn sheep (*Ovis canadensis*) after introduction

of individuals from two outside populations (Hogg et al. 2006). Genetic diversity and population size were also recovered by the arrival of a single immigrant into a Scandinavian population of grey wolves (*Canis lupus*; Vila et al. 2003).

Interestingly, examples also exist where translocation of individuals have not resulted in increased genetic diversity. Release of Eurasian otters (*Lutra lutra*) into two Swedish populations resulted in increased genetic diversity in only one of the populations (Arrendal et al. 2004). Understanding why translocation efforts fail to increase genetic diversity is complicated because most translocations efforts lack long-term follow-up studies monitoring the changes in genetic diversity (Bouzat et al. 2009, Weeks et al. 2011). Several factors can contribute to the failure of a translocation program, including inadequate control of predators, release of too few individuals, inadequate habitat, and inadequate post-release monitoring (Sigg et al. 2005, Bouzat et al. 2009). Mating systems can also contribute to the success of translocation projects. For example, the reproductive success of male bridled nailtail wallabies (*Onychogalea fraenata*) was measured after translocation. The bridled nailtail wallaby has a polygynous mating system and larger translocated males had higher reproductive success than those with lower weight, indicating that translocations of polygynous species should include a greater proportion of females and only release males of high breeding potential to maximize increases in genetic diversity (Sigg et al. 2005).

Greater sage-grouse (*Centrocercus urophasianus*; hereafter referred to as sage-grouse) are lek-breeding, polygynous birds of conservation concern (Wiley 1973, U. S. Fish and Wildlife Service 2012). Sage-grouse numbers have declined across much of their historic range due to the loss, fragmentation, and degradation of sagebrush (*Artemisia* spp.) habitats (Braun 1998). Estimates of decline in size of breeding population vary by region, but range from 17% to 47%

(Connelly and Braun 1997). Overall, sage-grouse occupy approximately 56% of pre-settlement range with some localized populations experiencing even greater range contractions (Schroeder et al. 2004). In a recent review, the United States Fish and Wildlife Service (USFWS) found listing sage-grouse as threatened or endangered under the Endangered Species Act of 1973 was warranted, but precluded by higher priority species at greater risk of extinction (U. S. Fish and Wildlife Service 2012). Consequently, sage-grouse are currently a candidate species and a listing decision will be made after a status review by USFWS in the fall on 2015 (U. S. Fish and Wildlife Service 2012).

In central Utah, the Strawberry Valley population of sage-grouse experienced one of the most severe declines reported for an extant population. Estimates of abundance in 1998 were less than 5% (~150 individuals) of similar estimates from the 1930s (> 3,000 individuals) (Bunnell 2000). Genetic analysis of microsatellite markers and mitochondrial DNA indicated that this population, pre-translocation, had significantly lower levels of genetic diversity than populations of sage-grouse from other areas (Oyler-McCance et al. 2005). Given the declining population size and low levels of genetic diversity, recovery team partners translocated 395 sage-grouse, from four different populations over 6 years (2003-2008), into Strawberry Valley, Utah (Baxter et al. 2013).

The objective of this study was to determine if the translocation of sage-grouse into Strawberry Valley increased genetic diversity. Our specific objectives were to 1) determine microsatellite allelic frequency in the pre- and post-translocation populations, 2) determine haplotype diversity in the pre- and post-translocation populations, and 3) compare the genetic structure of the Strawberry Valley population (pre- and post-translocation) with four source populations. Because translocated sage-grouse survived reasonably well and demonstrated

evidence of reproduction (Baxter et al. 2013), we predicted an increase in genetic diversity following translocation.

MATERIALS AND METHODS

Study Areas

Our study area was located in Strawberry Valley, Utah, USA (NAD 83 Zone 12T; UTM 0492078/4445216; Figure 1). Strawberry Valley is characterized as montane sagebrush steppe with mountain big sage-brush (*A. tridentata*) as the dominant shrub and silver sagebrush (*A. cana*) occurring at lower densities in wet meadows and riparian areas. The valley is approximately 24 km long and 9 km wide (Baxter et al. 2013).

In an effort to increase population size and genetic diversity, 395 sage-grouse were translocated from four different source populations: Deseret Land and Livestock, Diamond Mountain, Parker Mountain, and western Box Elder County (Figure 1; Baxter et al. 2013). The source populations were originally chosen because of their large size, proximity to Strawberry Valley, and behavioral and genetic similarity to the Strawberry Valley population (Oyler-McCance et al. 2005, Baxter et al. 2013). Translocation source sites were between 122 and 275 km away from the release site and varied in elevation, terrain, sagebrush type, and precipitation (Baxter et al. 2013). A more detailed description of the study area and translocation source sites can be found in Baxter et al. (2013).

Blood Collection and DNA Extraction

As part of a long term project, we captured male and female sage-grouse on and around leks from March 1 to May 31 (1998-2013) using the original and a modified spotlight method (Giesen et al. 1982, Wakkinen et al. 1992). Sage-grouse were also trapped sporadically during

the fall using the same methods. We determined the age (yearling or adult) of each captured sage-grouse based on feather characteristics (Crunden 1963). We obtained blood samples by clipping a toe nail and collecting 2-3 drops in a microfuge tube. From the blood samples, we extracted DNA using the DNEasy® Blood and Tissue Kit according to the manufacturer's protocol (QIAGEN®, Valencia, CA).

Microsatellite Fragment Analysis and Mitochondrial Sequencing

We performed microsatellite fragment analysis on 15 nuclear microsatellite loci (Table 1) using a multiplex PCR procedure. We dye-labeled forward primers for each microsatellite marker and electrophoretically resolved amplified products on an AB3500 Genetic Analyzer (Applied Biosystems®) at the FORT Molecular Ecology Laboratory in Fort Collins, Colorado, USA. In addition to microsatellite analysis, we amplified a 380 bp DNA fragment of the mitochondrial control region I according to the methods described by Oyler-McCance et al. (2005) and Kahn et al. (1999). We sequenced the mitochondrial DNA fragment using a standard dye terminator cycle sequencing reaction at the BYU DNA Sequencing Center on the Applied Biosystems 3730xl DNA Analyzer (Life Technologies, Foster City, California, USA).

Data Analysis

We calculated the total number of microsatellite alleles per locus, mean number of alleles for each population, and deviations from Hardy-Weinberg equilibrium (HWE; Guo and Thompson 1992) using the computer program GenAlEx version 6.5 (Peakall and Smouse 2006, Peakall and Smouse 2012). We corrected for sample size differences between pre- and post-translocation populations using the computer program FSTAT version 2.9.3 (Goudet 1995).

We edited and trimmed mtDNA sequences to 141 bp and aligned sequences using SEQUENCHER® version 5.1 (Sequence Analysis Software, Gene Codes Corporation, Ann

Arbor, MI USA). We used this 141 bp region because it was previously shown to contain the majority (92%) of the variable sites in the larger 380 bp spanning control region (Kahn et al. 1999). We calculated gene diversity and molecular diversity indices using ARLEQUIN version 3.5.1.3 (Excoffier et al. 2005). We identified haplotypes by comparing Strawberry Valley post-translocation data with previously identified sage-grouse haplotypes.

We examined genetic structure of the population using the computer program STRUCTURE version 2.3.4 (Pritchard et al. 2000). For this procedure, individuals are grouped into genetic clusters without regard to assigned populations using a model-based clustering analysis. For the source populations, the number of unique genetic clusters (K) was estimated by conducting 10 independent runs each of K = 1-10 with 1,000,000 Markov Chain Monte Carlo (MCMC) repetitions with a 100,000 burnin period using the model with admixture, correlated allele frequencies, and no prior information. We further analyzed the STRUCTURE results using STRUCTURE HARVESTER version 0.6.93 (Earl and Vonholdt 2012) to determine the appropriate value of K using the second-order rate of change in log likelihood for each K (Evanno et al. 2005). Once we determined that all four source populations and the Strawberry Valley pre-translocation population differed genetically, we added the Strawberry Valley post-translocation data into the STRUCTURE analysis.

RESULTS

Microsatellite and Mitochondrial Haplotype Analysis

Results from this study are based on the genetic analysis of 168 samples collected from Strawberry Valley pre- and post-translocation populations. Samples from the pre-translocation population (n = 23; 2000) were collected previously by Oyler-McCance et al. (2005). Post-

translocation samples were collected from 2003 to 2013 (n = 145). We observed an increase in allelic richness. Prior to translocation, the average number of alleles per locus was 6.62 (SE ± 0.46; Figure 2). The mean number of alleles across all fifteen loci following translocation was 7.66 (SE ± 0.52). We observed an overall increase of 16 alleles across the 15 loci studied ($\overline{x}$ =1.04 alleles per locus, SE ± 0.25). Results of a paired t-test showed a significant difference in the number of alleles per locus before and after sage-grouse were translocated into Strawberry Valley (t-ratio = 4.22, $p < 0.001$). Alleles per locus increased for all loci except reSGCA11 and SG39. The four source populations had allelic diversity calculated as follows: Box Elder 9.6 (SE ± 0.77), Deseret Land and Livestock 10.2 (SE ± 0.73), Parker Mountain 7.2 (SE ± 0.58), and Diamond Mountain 8.00 (SE ± 0.52). All loci studied were in HWE before sage-grouse were translocated into Strawberry Valley. After translocation, four loci were not in HWE (reSGCA5, SGCTAT1, SG39, and SG21; Table 3).

We sequenced a 141 bp portion of the mitochondrial control region I in 25 individuals (samples collected in 2012 and 2013), adding to the 23 individuals previously sequenced from the Strawberry Valley pre-translocation population (Oyler-McCance et al. 2005). Of the 25 individuals sequenced post-translocation, we identified 5 unique haplotypes, DR, B, W, C, and DT (Table 4). All haplotypes were previously described by Kahn et al. (1999), Benedict et al. (2003), and Oyler-McCance et al. (2005). The Strawberry Valley post-translocation population had an overall increase of 1 haplotype, specifically the DT haplotype. The DR haplotype decreased in frequency from 0.65 (pre-translocation) to 0.36 (post-translocation) and haplotype B increased from 0.13 (pre-translocation) to 0.52 (post-translocation). The Strawberry Valley pre-translocation mtDNA gene diversity was 0.55 (S.E. ± 0.02), while the post-translocation gene diversity was 0.62 (S.E. ± 0.01).

Population Structure Analysis

For analysis of genetic structure of the population, we included 145 pre-translocation and 23 post-translocation samples from Strawberry Valley and an additional 117 samples from the four translocation source populations. We used 31 samples from Deseret Land and Livestock, 27 from Diamond Mountain, 28 from Parker Mountain, and 31 from Box Elder County. The STRUCTURE analysis estimated the most likely number of unique genetic clusters (K) given the data set and then assigned each individual a probability of belonging to each genetic clusters. STRUCTURE HARVESTER identified six genetic clusters that clearly identified all four source populations as distinct genetic clusters (Figure 3). Individuals from Strawberry Valley pre- and post-translocation populations were assigned largely to a mixture of two genetic clusters, showing significant levels of admixture. The post-translocation population also exhibited admixture from the four source populations.

DISCUSSION

Our results represent the first documented increase in genetic diversity of a sage-grouse population as a result of translocation. There was an increase in genetic diversity based on indices of both nuclear and mitochondrial DNA (16% and 25% increases for allelic richness and haplotype diversity, respectively). Interestingly, our results are very similar to those reported for translocation of greater prairie-chickens (*Tympanuchus cupido pinnatus*), where allelic richness increased by 16% and haplotype diversity increased by 22% (Bouzat et al. 2009). For prairie chickens, the translocation of new individuals effectively removed detrimental variation associated with inbreeding depression and restored neutral genetic variation to historical levels (Bouzat et al. 2009).

The genetic analysis of microsatellite and mtDNA indicated that the pre-translocation population of Strawberry Valley sage-grouse had low genetic diversity, consistent with a large population decline (Oyler-McCance et al. 2005). The pre-translocation sage-grouse population averaged only 3.86 alleles per locus, nearly two-thirds the range-wide average of 5.86 (Oyler-McCance et al. 2005). The haplotype diversity of the pre-translocation population was also low, exhibiting only 4 haplotypes, while the range-wide average was 7. These decreases in genetic diversity in the Strawberry Valley population are consistent with evidence of a recent population bottleneck suggested by Oyler-McCance et al. (2005).

Generally, we would expect that translocations of individuals from a diverse gene pool into an existing population with low genetic diversity would improve genetic diversity, but there are concerns about the detrimental effects associated with loss of local adaptation in the extant population (Edmands 2007, Bouzat et al. 2009). Managers should try to maintain the genetic integrity of the original population and avoid replacement of the entire population by translocated individuals. The STRUCTURE analysis revealed that pre-translocation samples consistently clustered into two genetic groups and the four source populations were each represented by a distinct genetic group. Strawberry Valley post-translocation appeared to cluster into the same two groups as the pre-translocation population but with some additional clustering from the other four populations. The number of mtDNA haplotypes post-translocation increased from 4 to 5. This finding suggests that some independent maternal lines have been successfully introduced into Strawberry Valley from other areas. The retention of genetic clusters and haplotypes from the pre-translocation population suggests that the overall increase in genetic diversity resulted from genetic admixture between individuals from the focal and source populations and not from the genetic replacement by the translocated birds. A study on survival

of resident and translocated sage-grouse in Strawberry Valley also revealed that resident sage-grouse had higher survival rates than translocated sage-grouse during their first year, however, not statistically significant (Baxter et al. 2013). This further suggests that resident birds were not completely replaced by translocated birds allowing for genetic information from the pre-translocation population persist.

Haplotype diversity did not increase as much as we expected. The average number of haplotypes per population for sage-grouse across their range was 6.9 (Oyler-McCance et al. 2005). Haplotype A was found in all four source populations and was the most abundant haplotype in two of the four source populations. This haplotype, however, was not found in Strawberry Valley samples following translocation. We note that only a limited number of individuals were included in the haplotype analysis (23 pre-translocation, and 25 post-translocation birds) and that we could have potentially missed other haplotypes found in the population. Sampling additional individuals may provide a better estimate of haplotype diversity, although our comparisons would suffer from unequal sample sizes.

Interestingly, four of the 15 microsatellite loci studied were not in HWE. Reasons for deviations from HWE include non-random mating, mutation, selection, migration and small population size (Allendorf et al. 2012). While any of these could explain the deviation from HWE for our microsatellite loci, it is suggestive that since sage-grouse follow a lekking system, relatively few males from the population breed each year (Scott 1942, Patterson 1952, Wiley 1973). Scott (1942), for example, observed a population of greater-sage grouse and found that of 355 males, only a few performed 74% of all recorded copulations. New genetic information, however, suggests that more males in sage-grouse populations mate than previously thought with approximately half of males sampled successfully reproducing (Bird et al. 2012). The low

percentage of reproducing males may disrupt the HWE assumption of random mating and be one reason that some loci in the population are not in HWE.

Sage-grouse are an important game bird and an indicator of healthy sagebrush ecosystems (Reese and Connelly 1997). With landscape-level declines in population size, significant range contraction during the past century, and fragmentation of sagebrush habitats becoming more common, movements and subsequent exchange of genetic information between populations is increasingly important to preserve population-level and range-wide genetic diversity. While previous efforts to translocate sage-grouse have had little success (Reese and Connelly 1997), the results of this study suggest that even in a small isolated population with fragmented habitat, translocations can be an effective strategy to increase genetic diversity.

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Table 1-1. Primers used for microsatellite fragment analysis from Oyler-McCance et al. (2005) of greater sage-grouse from Strawberry Valley, Utah (2003-2012). Primer name, primer sequence, annealing temperature (AT), size of band, reference, and dye are reported.

Primer Name	Primer Sequence (5'-3')	Optimal AT (°C)	Allele size range	Reference	Dye
MSP18	F: CAATGACAGTATTTCCCAGATTA R: GAATGGTAATATACTAAGCACAGG	55	98-119	Oyler-McCance and St. John 2010	PET
BG6	F: AAAGAGGCAAGCACTCACAATG R: CCCTTGGAATATCCTTTAACAAAAC	57	248-304	Piertney and Hoglund 2001	NED
Sexing	F: GAGAAACTGTGCAAAACAG R:TCCAGAATATCTTCTGCTCC	55	224-252	Kahn et al. 1998	6FAM
reSGCA5	F: CGGACAGGTACATCCTGGAA R: GGGAAAAGATGTCAGAATCTACAAA	55	122-140	Taylor et al. 2003	VIC
TUT3	F: CAGGAGGCCTCAACTAATCACC R: CGATGCTGGACAGAAGTGAC	60	144-164	Segelbacher et al. 2000	6FAM
reSGCA11	F: GCAGTAAAGAAAATTTGGAAGCA R: TCTTGAACTGATGTTGGATTTG	58	177-197	Taylor et al. 2003	VIC
SGMS06.6	F: CAAACAACTGTCTTCCAGTAAGAC R: AGAGCCTTCATTTCTGGCAG	58	128-176	Oyler-McCance and St. John 2010	PET
SGCTAT1	F: GCGACATGCTCCCACCT R: GAAAGGTTGTAAGAGGTCGT	60	90-112	Taylor et al. 2003	6FAM
MSP11	F: CACACCTAGATGGTGGTG R: CATTGTCAGCTTGCAGAC	52	206-258	Oyler-McCance and St. John 2010	6FAM
SGMS06.8	F: GCAAAATCAATAGAAGTAGAGAGG R: CAGTAGCAGCTTTGTTTGG	52	115-147	Oyler-McCance and St. John 2010	NED

SG28	F: ACAGGGGAAGGACAGACTGG R: ACCTCTGCTTTTCCATTGCC	60	128-172	Oyler-McCance 2014 unpublished, manuscript in prep	NED
SG39	F: GAAAGTCTGAATGCTGGAGAACC R: AAGCGTACTGTTTGCTCCCC	60	170-197	Oyler-McCance 2014 unpublished, manuscript in prep	PET
SG36	F: TTCCAGACATTTTGGGAGCC R: CACATGTCCATCCAACCACC	60	222-262	Oyler-McCance 2014 unpublished, manuscript in prep	NED
SG21	F: AGGCAAAACAGTCACACATGC R: ATCACAAGCAGAGTGCAGGC	60	205-241	Oyler-McCance 2014 unpublished, manuscript in prep	VIC
SG24	F: GAGCCTTCATTTCTGGCAGC R: GCTCTTTATTTCAAACAACTGTCTTCC	60	155-202	Oyler-McCance 2014 unpublished, manuscript in prep	6FAM
SG29	F: AAGGGGCTTAGGGTTTTAATGG R: AGTTAACTAAGTTGGGCAGGGG	60	137-159	Oyler-McCance 2014 unpublished, manuscript in prep	VIC

Table 1-2. Allelic richness for 15 microsatellite loci for greater sage-grouse from Strawberry Valley before and after translocation. Post-translocation data was corrected for sample size difference in program FSTAT. *N*=21 for each population.

Locus	Pre-Translocation	Post-Translocation	Difference
MSP18	7.82	8.05	0.23
reSGCA5	5.91	6.09	0.18
WYBG6	7.91	9.08	1.17
MSP11	7.91	8.53	0.62
SGCTAT	3.00	5.04	2.04
SGMS06.6	8.00	10.24	2.24
SGMS06.8	8.91	9.79	0.88
TUT3	4.00	4.20	0.20
reSGCA11	6.00	5.56	-0.44
SG28	6.95	9.46	2.51
SG36	4.00	5.82	1.82
SG39	6.91	6.84	-0.08
SG21	8.00	9.88	1.88
SG24	7.95	9.91	1.95
SG29	6.00	6.44	0.44
Mean	6.62	7.66	1.04
S.E.	0.46	0.53	0.25

Table 1-3. Summary of chi-square tests of Hardy-Weinberg equilibrium for 15 microsatellite loci studied for the Strawberry Valley population of greater sage-grouse. Post-translocation (2004-2013) *N*=145, pre-translocation (2000) *N*=23. Significant departures from HWE marked with an *.

	Pre-Translocation			Post-Translocation		
Locus	DF	ChiSq	Prob	DF	ChiSq	Prob
MSP18	28	20.19	0.86	36	33.37	0.59
reSGCA5	15	12.58	0.63	28	138.89	<0.001*
WYBG6	28	28.40	0.44	66	55.90	0.81
MSP11	28	30.98	0.32	78	72.02	0.67
SGCTAT1	3	7.55	0.06	36	297.39	<0.001*
SGMS06.6	28	21.20	0.82	91	84.07	0.68
SGMS06.8	36	29.71	0.76	55	53.85	0.52
TUT3	6	2.67	0.85	21	10.68	0.97
reSGCA11	15	21.70	0.12	15	16.65	0.34
SG28	21	11.55	0.95	91	109.55	0.09
SG36	6	5.52	0.48	28	31.34	0.30
SG39	21	12.18	0.94	36	51.33	0.047*
SG21	28	22.30	0.77	120	270.90	<0.001*
SG24	28	23.21	0.72	78	77.28	0.50
SG29	15	10.00	0.82	36	20.00	0.99

Table 1-4. Haplotype frequencies for greater sage-grouse in Strawberry Valley pre-translocation, Strawberry Valley post-translocation, and translocation source populations. Pre-translocation and source population samples were collected in 2000. Post-translocation samples were collected 2004-2013.

Population	*N*	Number of haplotypes	DR	DT	AA	AG	EC	A	AC	FA	FB	B	W	C	EX	EF	T	S
S.V. Pre	23	4	15									3	1	4				
S.V. Post	25	5	9	1								13	1	1				
Box Elder	28	7		2	1	1	1	10				12			1			
Diamond Mountain	26	9		1	1			9	2			6		2		1	1	3
Parker Mountain	25	8		4	1			6	1			7	4		1			1
Deseret Land Livestock	28	11		5	1		1	3	1	1	1	6	4	4				1

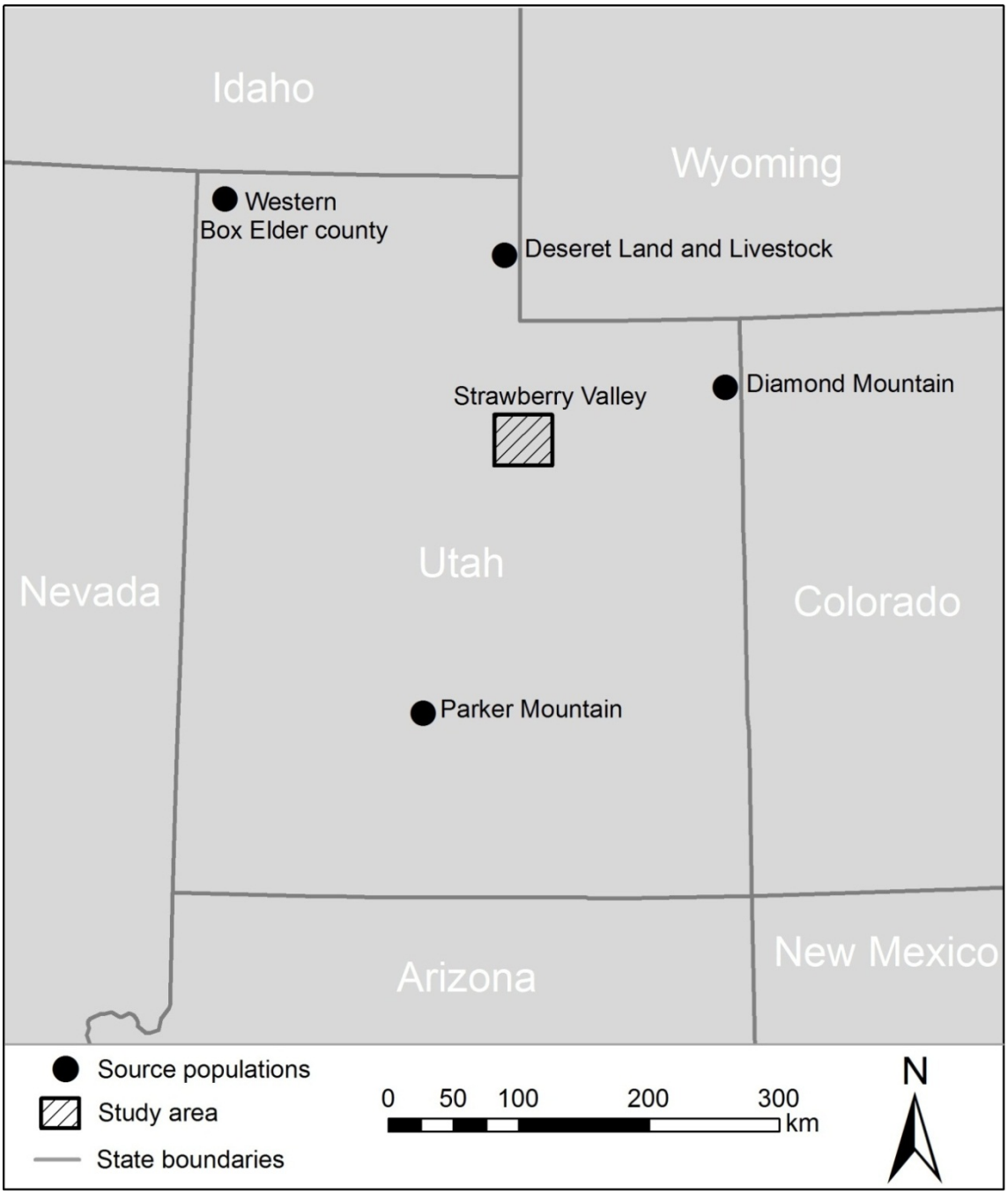

Figure 1-1. Strawberry Valley study area and greater sage-grouse (*Centrocercus urophasianus*) translocation source sites from which blood samples were collected (2004 to 2013) for fragment analysis and mitochondrial DNA sequencing. Pre-translocation samples were collected from all five sites in 2000 (Oyler-McCance et al. 2005).

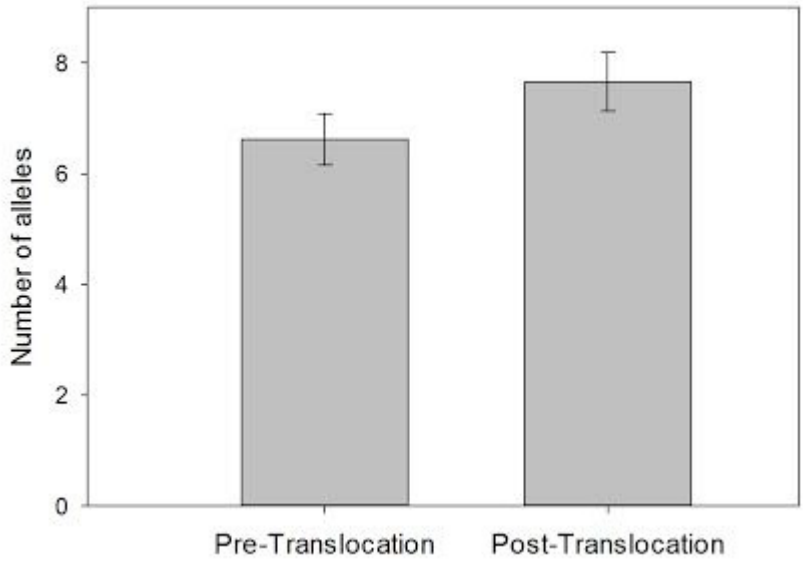

Figure 1-2. Average number of alleles per locus (± SE) for greater sage-grouse in Strawberry Valley, Utah before (Pre-translocation) and after (Post-translocation) translocation. Fifteen microsatellite loci were included in analysis. Pre-translocation data was corrected for sample size differences in program FSTAT. *N*=21 for each population.

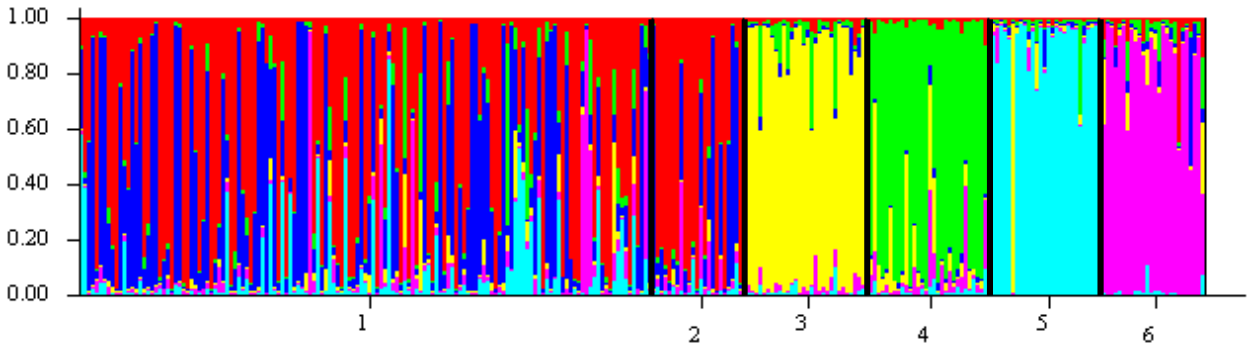

Figure 1-3. Results of program STRUCTURE showing sampled greater sage-grouse best fit into 6 distinct clusters (represented by 6 unique colors) with populations: (1) Strawberry Valley post-translocation, (2) Strawberry Valley pre-translocation, (3) Box Elder, (4) Deseret Land and Livestock, (5) Parker Mountain, and (6) Diamond Mountain. Each bar represents an individual sage-grouse. Different colors of each bar represent the probability of belonging to a certain cluster.

CHAPTER 2

GENETIC DIVERSITY AND FITNESS: DOES INCREASED HETEROZYGOSITY LEAD TO INCREASED SURVIVAL AND REPRODUCTIVE FITNESS?

ABSTRACT

The relationship between genetic diversity and individual fitness is a key concern for species conservation because low genetic diversity is often associated with low fitness. We determined if fitness-related traits were related to genetic diversity in a population of greater sage-grouse (*Centrocercus urophasianus*) in Strawberry Valley, Utah from 2005 to 2013. After capture, we fitted sage-grouse in Strawberry Valley with a radio collar and drew and preserved blood. We monitored 93 sage-grouse using a 4-element Yagi antenna and an R-1000 digital radio receiver. We monitored all nesting females two to four times a week. From blood, we extracted and amplified DNA with 15 microsatellite loci. We measured genetic diversity as multilocus heterozygosity and mean d^2. We analyzed the relationship between survival and indices of genetic diversity using known-fate models in Program MARK. We also analyzed the relationship between nest initiation, nest success, clutch size, and number of eggs hatched and measures of genetic diversity using generalized linear models where reproductive measures were modeled as a function of genetic diversity. We found no evidence for a relationship between microsatellite heterozygosity and mean d^2 with measures of survival or reproductive success. Overall, these results suggest that the often-reported strong heterozygosity-fitness correlations detected in small, inbred populations do not reflect a general phenomenon of increasing individual survival and reproductive fitness with increasing heterozygosity.

INTRODUCTION

The relationship between genetic diversity and individual fitness is a key concern for species conservation. Decreased genetic diversity in wildlife populations can be associated with decreased fitness (Lacy 1997, Reed and Frankham 2003, Vandewoestijne et al. 2008, Markert et al. 2010, Kojo et al. 2012). Relationships between genetic diversity and fitness are increasingly important to conservation because the amount of habitat available for wildlife has decreased and become more fragmented. Habitat fragmentation can create small insular populations which are more prone to loss of genetic diversity over time due to the effect of genetic drift (Lacy 1997). Natural populations are finite in size and mating sometimes occurs between related individuals (Duarte et al. 2003). When populations are small, mating between related individuals is more likely to occur which can have negative genetic consequences. Inbreeding leads to less heterozygous genotypes. Reductions in fitness occur when harmful genes are revealed in homozygous individuals which can impact population persistence (Lacy 1993, Hansson and Westerberg 2002, Isomursu et al. 2012).

Genetic diversity may be associated with several key life-history traits in wildlife populations. There is evidence, for example, of a positive influence of genetic diversity on juvenile survival in European Alpine marmot (*Marmota marmot*; Da Silva et al. 2006), roe deer (*Capreolus capreolus*; Da Silva et al. 2009), and harp seals (*Phoca groenlandica*; Kretzmann et al. 2006). Similarly, reproductive success was positively correlated with mean d^2 (a measure of inbreeding where higher values indicate reduced inbreeding) in male black grouse (*Tetrao tetrix*; Hoglund et al. 2002). Furthermore, clutch size, number of eggs sired by males, and number of recruits produced by males and females was positively correlated with individual heterozygosity in blue tit (*Cyanistes caeruleus*) implying a link to reproductive output (Olano-Marin et al.

2011). Finally, increased body size was predicted by increased heterozygosity in crested newt (*Triturus cristatus*; Herdegen et al. 2013).

Although many positive examples exist, the strength and universality of heterozygosity-fitness correlations (HFCs) for many taxa remains unclear (David 1998, Da Silva et al. 2006). This debate results from examples where genetic diversity was not correlated with fitness (Rowe and Beebee 2001, Duarte et al. 2003, Chapman and Sheldon 2011). Moreover, negative examples may be underrepresented because of bias to publish positive results (Da Silva et al. 2006). A few well documented cases showed the absence of depression in inbred populations (Gibbs and Grant 1989, Keane et al. 1996). In a population of ground finches (*Geospiza fortis*), there was no detected effect of inbreeding on reproductive success (Gibbs and Grant 1989). Similarly, close inbreeding observed in a population of dwarf mongoose (*Helogale parvula*) had no measurable effect on offspring production or adult survival (Keane et al. 1996). A significant negative correlation was found between body condition and heterozygosity in juvenile zenaida doves (*Zenaida aurita*), indicative of outbreeding depression (Monceau et al. 2013). In order to detect fitness correlations, samples sizes must be large and the population structure must allow inbreeding (Isomursu et al. 2012). The observed relationship between heterozygosity and fitness should be interpreted with caution when only a small number of microsatellite loci are used.

We examined the relationship between genetic diversity and fitness in a lekking bird, greater sage-grouse (*Centrocercus urophasianus*), of conservation concern in the western United States. We compared levels of genetic diversity to life history traits known to be associated with fitness including survival, nest initiation, nest success, clutch size, and number of eggs hatched. We estimated the level of genetic diversity using microsatellite heterozygosity and mean d^2. We

predicted that individuals with higher genetic diversity would have higher survival, nest initiation, nest success, and larger clutch sizes.

MATERIALS AND METHODS

Study Area

The study area was located in Strawberry Valley, Utah, USA (NAD 83 Zone 12T; UTM 0492078/4445216; Figure 1). This area was characterized as montane sagebrush steppe with mountain big sage brush (*Artemisia tridentata*) as the dominant shrub and silver sagebrush (*A. cana*) occurring at lower densities in wet meadows and riparian areas. The valley is approximately 24 km long and 9 km wide. A more detailed description of the study area can be found in Baxter et al. (2013).

Captures, Blood Collection and DNA Extraction

We captured resident sage-grouse on and around leks from March 1 to May 31 from 2005 to 2013 using the original and modified spotlight method (Giesen et al. 1982, Wakkinen et al. 1992). We also trapped sage-grouse sporadically during the fall using the same methods. We assigned each sage-grouse an age class after capture based on feather characteristics (yearling or adult) as described by Crunden (1963) and fitted each grouse with a 22-g necklace-style radio transmitter (Advanced Telemetry Systems, Inc. ® Isanti, MI; 19 hour duty cycle, 45 ppm, with mortality after 8 hours and maximum battery life of 30 months). We obtained blood samples by clipping a toe nail and collecting two to three drops of blood in a microfuge tube. All sage-grouse captured were handled in accordance with protocol approved by the Utah Division of Wildlife Resources and Brigham Young University's Institutional Animal Care and Use Committee (IACUC approval #05-0301, #08-0402, and #11-0301). From these samples, we

extracted DNA using the DNEasy Blood and Tissue Kit according to the manufacturer's protocol (QIAGEN, Valencia, CA).

Monitoring

We monitored sage-grouse weekly, throughout each year, using a 4-element Yagi antenna (Telonics Incorporated®, Mesa, Arizona) and an R-1000 digital radio receiver (Communication Specialists Incorporated®, Orange, California). When a radio was not heard for several weeks, we used fixed-wing aircraft to assist with relocation (approximately 6 flights/year). We listened for signals from radio-marked sage-grouse within an 80 km radius of the lek sites. Once relocated, we resumed monitoring from the ground and located birds visually to confirm fate (alive or dead).

After locating a nest, we obtained a GPS location. Thereafter, we monitored all nesting females two to four times a week, from a distance of ~20 m, to minimize disturbance. Nest fate for each female was assessed after she was no longer detected at the nest site. A nest was considered successful if at least one egg hatched. Egg shells with a detached membrane (Klebenow 1969) and/or visual observation of a female with a brood were used to determine nest success. A nest was considered depredated if no eggs hatched and at least one egg was found punctured, crushed, or missing or if the female was found dead on or near the nest. Nest desertion was assumed if a female did not return to a formerly incubated nest after ≥ 24 h.

Microsatellite Fragment Analysis

After DNA extraction, we performed microsatellite fragment analysis on 15 nuclear microsatellite loci using a multiplex PCR procedure. We dye-labeled forward primers for each microsatellite marker and electrophoretically resolved amplified products on an AB3500 Genetic Analyzer (Applied Biosystems®) at the FORT Molecular Ecology Laboratory in Fort Collins,

Colorado, USA. Primer information can be found in Kahn et al. (1998), Oyler-McCance and St John (2010), Piertney and Hoglund (2001), Segelbacher et al. (2000), and Taylor et al. (2003).

Data analysis

We estimated the level of genetic diversity for each individual as the multilocus heterozygosity and mean d^2 over 15 microsatellite loci. We calculated multilocus heterozygosity as the proportion of typed loci for which an individual was heterozygous. We calculated mean d^2 as the squared difference in repeat units between two alleles at a locus averaged over all loci at which an individual was scored (Coulson et al. 1998).

We analyzed the relationship between genetic diversity indices and survival using known-fate models in Program MARK version 5.1 (White and Burnham 1999) and used model selection (Burnham and Anderson 2002) to evaluate hypotheses about survival across the study period. We formatted an encounter history into months beginning 1 April and ending 31 March. We coded each encounter (month) for each bird as live, dead, or censored. We formatted our input file with year as a group. We then estimated annual survival rates as derived parameters (complete with appropriate SEs and CIs estimated using the delta method) within Program MARK 5.1 (White and Burnham 1999). We included age, sex, nest initiation, nest success, mean d^2, and multilocus heterozygosity as individual covariates potentially influencing survival rates.

We followed standard model selection protocol and built a list of *a priori* candidate models (Burnham and Anderson 2002) by using models previously selected by Baxter et al. (2013). To limit the number of potential models, we used a 2-stage approach where we first identified the best model for time (month, seasonal, year, and interactions) and then assessed the influence of individual covariates assuming the time structure identified in stage 1. This 2-stage

approach was an attempted balance between under and over-fitting our data given the large number of conceivable models and unknown problems of an all-possible-models approach (Burnham and Anderson 2002).

Our candidate models for stage 2 included each time structure from stage 1 with a model weight > 10%; the two genetic diversity indices; individual covariates and their interactions; and combinations of time, genetic diversity indices, and individual covariates (Table 1). Individual covariates included age, sex, nest initiation, and nest success. We also looked at the interaction between age and sex; sex and nest initiation; and sex, age, nest initiation, and nest success. Individual covariate interactions were chosen because they came out as supported models for sage-grouse survival in Strawberry Valley (Baxter et al. 2013). The two genetic diversity indices were also combined with each combination of time structure and individual covariate and interaction. We based model selection on the minimization of Akaike's Information Criterion (Akaike 1973) corrected for small sample size (AICc; Lebreton et al. 1992) and AICc weights (w_i; Buckland et al. 1997, Burnham and Anderson 2002). In the face of model uncertainty, we obtained model-averaged estimates.

We also analyzed the relationship between estimates of genetic diversity and nest initiation, nest success, clutch size, and number of eggs hatched using generalized linear models (Zar 2010) where we modeled reproductive measures as a function of genetic diversity. We set the α level to 0.05 for all statistical tests. All generalized linear model analyses were performed using program R (R Core Team 2013).

RESULTS

We genotyped a total of 93 individual sage-grouse at an average of 14.12 (SE ± 0.16) microsatellite loci. We characterized a total of 1313 genotypes. The overall mean heterozygosity of the population was 0.75 (SE ± 0.01) and ranged from 0.47 to 1.00. The overall mean d^2 of the population was 186.05 (SE ± 9.69).

The first stage of our modeling approach identified five time models with AICc weight > 10% (Table 2). The top model held survival constant through all time periods and received 34% of AIC_c weight. The year model split time up by year across the study period and received 20% of AIC_c weight. The two season model split the year into breading and nesting (March-April) and the rest of the year (July-February) and received 19% of AIC_c weight. The three season model split the year into breeding-nesting (April and May), summer (June-August), and fall-winter (September-March) and received 14% of AIC_c weight. The four season model split time up into four seasons: March and April, May and June, July-October, and November-February and received 11% of AIC_c weight. The top time model showed no variation in survival through time.

In the next stage of analysis, we added the individual covariates to our best time models (Table 4). The most supported model from this stage of analysis included constant time and no individual covariates and accounted for 4.5% of overall AICc weight. The second most supported model accounted for 3.7% of AICc weight. Given model uncertainty, we obtained model averaged estimates for all effects of interest (Table 3). The β estimate for sex (male=1) was negative (β = -0.63, 95% CI = -1.54 – 0.28) suggesting that males had lower survival than females; however, the confidence interval for sex slightly overlapped zero. The β estimate for age was positive but demonstrated significant uncertainty (β = 0.24, 95% CI = -0.73 – 1.21).

The β estimate for nest initiation (initiated = 1) was positive (β = 0.98, 95% CI = -0.52 – 2.30) whereas the β estimate for nest success was near zero (β = -0.23, 95% CI = -1.61 – 1.14).

Estimates of genetic diversity did not affect survival. Multilocus heterozygosity and mean d^2 did not show up in any of the top models. The β estimate for multilocus heterozygosity was not different from zero (β = -1.30, 95 % CI = -5.02 to 2.42). The β estimate for mean d^2 was also not different from zero (β = -9.32×10^{-4}, 95% CI = $-5.76 \times 10^{-3} – 3.90 \times 10^{-3}$).

Indices of genetic diversity poorly explained reproductive success (Table 5). Mean d^2 and heterozygosity were not predictors of nest initiation, nest success, clutch size, and number of eggs hatched as none of these predictors differed from zero ($P > 0.05$). Based on samples with both genetic and fitness information, 85% of females initiated a nest and 35% of females had a successful nest. Mean clutch size was 5.65 (SE ± 0.26), and mean number of eggs hatched was 2.33 (SE ± 0.49) over all nests, including depredated, abandoned, and successful nests.

DISCUSSION

Neutral marker heterozygosity was not correlated with measures of survival or fitness in this population of greater sage-grouse. Our results were similar to those for great tits (*Parus major*); there was no evidence for either multilocus or single-locus HFCs (Chapman and Sheldon 2011). Similarly, no evidence of positive HFCs were found in a population of zenaida dove (Monceau et al. 2013). However, positive correlations between genetic diversity and fitness-related traits have been recognized in organisms as diverse as plants (Leimu et al. 2006), mammals (Da Silva et al. 2006, Amos et al. 2001, Charpentier et al. 2005), birds (Amos et al. 2001), and fish (Fessehaye et al. 2009).

Several possibilities could explain our lack of HFCs. First, the lack of significant HFCs could be a function of a small effect size coupled with modest sample sizes. Our sample sizes for life-history traits ranged from 40 to 93 (survival N=93; nest initiation and nest success N=55; clutch size and number of eggs hatched N=40) depending on the life-history trait evaluated. The sample sizes used in this study compare to previous studies that also did not find significant HFCs (Chapman et al. 2001). The range of heterozygosity observed in individuals was also small. The overall mean population heterozygosity was 0.75 (SE ± 0.01) and ranged from 0.47 to 1.00. Most individuals studied had > 50% heterozygosity. To detect a relationship between genetic diversity and fitness, a wider range of observed multilocus heterozygosity may be needed.

A second possible explanation for the lack of significant HFCs is that the effects of inbreeding depression may have already been purged from the population at early life-history stages. Natural selection may actually favor inbreeding when costs are low (Duarte et al. 2003). Outbreeding may eliminate local adaptations in wild populations. Mating between inbred individuals can bring direct benefits to males (and inclusive benefits to females through increased reproductive output of related males), as long as they do not forfeit other breeding opportunities (Duarte et al. 2003).

Although microsatellite markers are widely used to detect HFCs in wildlife populations, some debate still exists about their use. Reviews on empirical studies have shown that HFCs are on average positive, but small, so studies lack the power to confidently estimate effect size (Forstmeier et al. 2012, Coltman and Slate 2003, Chapman et al. 2009). Existence of significant HFCs assume that genetic diversity at marker loci reflects genome-wide genetic diversity (Chapman et al. 2009). The microsatellite markers we used may not accurately reflect genetic

diversity at loci that affect trait variation or we may need to include different microsatellite markers in our study to find positive heterozygosity-fitness correlations.

Many examples exist where multilocus heterozygosity was not significantly correlated with fitness traits but mean d^2 was and vice versa (Kretzmann et al. 2006, Amos et al. 2001, Hoglund et al. 2002, Hedrick et al 2001). In one population of harp seals (*Phoca groenlandica*), heterozygosity was not significantly different between survivors and nonsurvivors, but mean d^2 was higher in survivors (Kretzmann et al. 2006). Similarly, mean d^2 was positively correlated with reproductive success in male black grouse, but the relationship between microsatellite heterozygosity and fitness was not significant (Hoglund et al. 2002). Mean d^2 was a substantially less effective measure of parental similarity when comparing parental relatedness with reproductive success in two marine mammals and three species of albatross (Amos et al. 2001). Mean d^2 was also shown to be a poor predictor of both inbreeding and fitness in a captive wolf (*Canis lupus*) population (Hedrick et al. 2001).

Genetic diversity may be a poor predictor of reproductive success because nest predation is the main cause of nest failure in sage-grouse populations. We calculated that 85% of females initiate a nest and 35% of nests are successful. Nest predation is the primary cause of sage-grouse nest failure and accounts for an average of 94% of nest loss (Moynahan et al. 2007, Coates et al. 2008). A high percentage of females in Strawberry Valley initiate a nest, similar to sage-grouse in other areas (Lyon and Anderson 2003). Because most females initiate a nest and nest failure is primarily due to predation, and not factors the bird can control, perhaps other life-history traits would be a better indication of fitness in this population.

A positive relationship exists between male lekking performance and genetic diversity in a population of black grouse (Hoglund et al. 2002). Relatively few males from sage-grouse

populations breed each year (Scott 1942, Patterson 1952, Wiley 1973). In a population of greater-sage grouse, of 355 males, only a few performed 74% of all recorded copulations (Scott 1942). New genetic information, however, suggests that more males in sage-grouse populations mate than previously thought with approximately half of males sampled successfully reproducing (Bird et al. 2013). There may be a relationship between sage-grouse lekking performance and genetic diversity.

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Table 2-1. *A priori* models used to determine the influence of group and individual covariates on survival of sage-grouse in Strawberry Valley, Utah from 2005 to 2013. Each time model was evaluated with each group and individual covariate and each genetic diversity estimate.

Model Structure	Hypothesis Description
Time Models	
S.	Survival did not vary by time
S(year)	Survival varied yearly
S(3season)	Survival varied by a 3 season year
S(2season)	Survival varied by a 2 season year
S(4season)	Survival varied by a 4 season year
Genetic Diversity Estimates	
d^2	Survival was influenced by mean d^2
hetero	Survival was influenced by multilocus heterozygosity
Group and Individual Covariates	
age	Survival was influenced by individual covariate age
sex	Survival was influenced by individual covariate sex
InNest	Survival was influenced by individual covariate nest initiation
NestSuc	Survival was influenced by individual covariate nest success
sex + age	Survival was influenced by individual covariates sex and age
sex + InNest	Survival was influenced by individual covariates sex and nest initiation
sex + age + InNest + NestSuc	Survival was influenced by individual covariate sex, age, nest initiation, and nest success

Table 2-2. Models from the first stage of analysis (time varying) for survival of greater sage-grouse in Strawberry Valley, Utah (2005-2013) showing model structure, Akaike's Information Criterion adjusted for small sample sizes (AICc), change in AICc from the most supported model (ΔAICc), model weight (w_i), model likelihood, number of parameters (K), and model deviance.

Model	AICc	ΔAICc	w_i	Model Likelihood	K	Deviance
S.	164.49	0.00	0.37	1	1	162.47
S(year)	165.76	1.27	0.20	0.53	9	147.21
S(3season)	165.81	1.32	0.19	0.52	3	159.74
S(2season)	166.48	1.99	0.14	0.37	2	162.44
S(4season)	166.96	2.48	0.11	0.29	4	158.84
S(3season x year)	179.56	15.07	0.00	0.00	27	136.89
S(month)	179.58	15.09	0.00	0.00	12	154.61
S(2season x year)	183.88	19.39	0.00	0.00	19	143.47
S(4season x year)	210.61	46.12	0.00	0.00	37	127.17
S(t)	437.97	273.49	0.00	0.00	108	118.25

Table 2-3. Model-averaged parameters and descriptive statistics of covariates included in all models of survival of greater sage-grouse in Strawberry Valley, Utah (2005-2013). Lower and upper 95% CI derived by Program MARK. Covariate names match those from Table 1.

Covariate	w_i	β	Lower 95% CI	Upper 95% CI
d2	0.23	0.00	-5.76E-03	3.90E-03
hetero	0.24	-1.30	-5.02	2.42
sex	0.44	-0.63	-1.54	0.28
age	0.19	0.24	-0.73	1.21
InNest	0.32	0.98	-0.53	2.49
NestSuc	0.12	-0.23	-1.61	1.14

Table 2-4. Supported ($w_i > 0.01$) models from the second stage of analysis for greater sage-grouse survival in Strawberry Valley, Utah (2005-2013). Showing model structure, Akaike's Information Criterion adjusted for small sample sizes (AICc), change in AICc from the most supported model (ΔAICc), model weight (w_i), model likelihood, number of parameters (K), and model deviance.

Model	AICc	ΔAICc	w_i	Model Likelihood	K	Deviance
s.	164.49	0.00	0.04	1.00	1	162.47
s(sex)	164.83	0.35	0.04	0.84	2	160.80
s(InNest)	165.20	0.72	0.03	0.70	2	161.17
s(d2)	165.41	0.93	0.03	0.63	2	161.38
s(sex + InNest)	165.41	0.93	0.03	0.63	3	159.34
s(year)	165.76	1.27	0.02	0.53	9	147.21
s(3season)	165.81	1.32	0.02	0.52	3	159.74
s(3season + sex)	165.89	1.40	0.02	0.50	4	157.77
s(hetero)	166.00	1.51	0.02	0.47	2	161.96
s(NestSuc)	166.34	1.86	0.02	0.39	2	162.31
s(sex + age)	166.37	1.89	0.02	0.39	3	160.30
s(age)	166.43	1.95	0.02	0.38	2	162.40
s(3season + sex + InNest)	166.46	1.97	0.02	0.37	5	156.28
s(2season)	166.48	1.99	0.02	0.37	2	162.44
s(sex + hetero)	166.51	2.03	0.02	0.36	3	160.44
s(3season + innest)	166.55	2.07	0.02	0.36	4	158.43
s(sex + d2)	166.58	2.09	0.02	0.35	3	160.51
s(innest + hetero)	166.64	2.15	0.02	0.34	3	160.56
s(year + sex)	166.79	2.31	0.01	0.32	10	146.11
s(4season + sex)	166.82	2.34	0.01	0.31	5	156.64
s(year + InNest)	166.83	2.34	0.01	0.31	10	146.15
s(2season + sex)	166.84	2.35	0.01	0.31	3	160.76
s(innest + d2)	166.96	2.47	0.01	0.29	3	160.89
s(4season)	166.96	2.48	0.01	0.29	4	158.84
s(sex + InNest + Hetero)	167.04	2.56	0.01	0.28	4	158.92
s(2season + InNest	167.20	2.71	0.01	0.26	3	161.12
s(sex + InNest +d2	167.23	2.74	0.01	0.25	4	159.11
s(3season + hetero)	167.25	2.77	0.01	0.25	4	159.13
s(3season + d2)	167.31	2.83	0.01	0.24	4	159.19
s(4season + sex + InNest	167.32	2.83	0.01	0.24	6	155.06

s(2season + sex + InNest)	167.42	2.93	0.01	0.23	4	159.30
s(3season + sex + d2)	167.42	2.94	0.01	0.23	5	157.24

Table 2-5. β estimates and p-values for generalized linear modeling. Nest initiation, nest success, clutch size, and number of eggs hatched were each estimated by mean d^2 and multilocus heterozygosity for greater sage-grouse in Strawberry Valley (2005-2013).

Covariate	B	SE	*P*
nest initiation			
intercept	1.78	2.23	0.42
heterozygosity	-0.21	2.98	0.95
intercept	2.28	0.84	0.01
d2	-3.49E-03	3.91E-03	0.37
nest success			
intercept	-1.15	1.79	0.52
heterozygosity	0.70	2.39	0.77
intercept	-1.29	0.65	0.05
d2	3.55E-03	3.13E-03	0.26
clutch size			
intercept	4.45	1.60	0.01
heterozygosity	1.64	2.16	0.45
intercept	5.91	0.55	3.83E-13
d2	-1.58E-03	2.93E-03	0.59
number of eggs hatched			
intercept	-1.08	2.97	0.72
heterozygosity	4.66	4.01	0.25
intercept	1.68	1.02	0.11
d2	3.94E-03	5.47E-03	0.48

Figure 2-1. Study area at Strawberry Valley, Utah where we obtained greater sage-grouse blood samples for microsatellite fragment analysis, 2005-2013.