Attachment 2.

LANDSCAPE INFLUENCE ON GENE FLOW IN GREATER SAGE-GROUSE: CONSERVATION ACTIONS THROUGH CORES AND CORRIDORS

SUMMARY

Habitat and population fragmentation were among the primary factors contributing to the recent US Fish and Wildlife Service decision that listing greater sage-grouse (*Centrocercus urophasianus*, hereafter sage-grouse) was warranted but currently precluded by higher priority actions. Increasingly, current management is focused on core or priority areas containing the highest densities of breeding birds with little regard to understanding connectivity within and among areas. The most fundamental objective of species conservation is to first identify and subsequently maintain a set of viable and connected populations. Therefore, if management emphasis on core areas is to be successful for long-term conservation, it is important to know (1) the spatial delineation of breeding populations across the range-wide distribution of sage-grouse, (2) how primary populations located in high quality habitat are interconnected across regions of lower population densities and less suitable habitat, and (3) the spatial scale and relative importance of landscape features that influence gene flow.

The working model for spatial organization of the range-wide distribution of sage-grouse likely consists primarily of numerous small populations in the periphery surrounding large core populations. In this study, we will use non-invasive genetic data obtained from feathers collected at leks (breeding locations) to test this model and to understand the processes that underlie the spatial pattern of populations. Genetic data provide unique information on individual movements or dispersals and subsequent mating, as expressed through gene flow. Barriers to dispersal can fragment large populations, restrict exchange among small populations, and limit the ability of populations to respond to environmental stressors or changes in sagebrush (*Artemisia* spp.) land cover. Therefore, understanding how individuals disperse within and among breeding populations is important for maintaining genetic diversity, to sustain populations that are experiencing declining trends, or to recolonize extirpated populations when suitable habitat remains.

We first will use information on genetic relatedness derived from individual-, lek-, and population-based analyses to better delineate the range-wide network of breeding populations. We then will combine genetic data with landscape models of cost-surfaces to identify characteristics of barriers, including geographic distance, topographic features, and anthropogenic land uses that influence dispersal and genetic exchange. These results are important for incorporating landscape and genetic connectivity into conservation planning to delineate core or priority populations, and to reduce population fragmentation, isolation and subsequent risk of extirpation.

CONSERVATION SIGNIFICANCE

BACKGROUND

Delineating the spatial structure and connectivity of populations across a species' range is a critical requirement for conservation. Size of individual populations, their spatial arrangement, and ability of individuals to move through a landscape or regional matrix of environmental features are major influences on a species' response to changes in its environment (Gilpin and Hanski 1991, Wiens et al. 1993, Hanski 1998). Identifying priority population regions and connecting corridors also can help focus limited conservation resources where benefits will be greatest (Morrison and Reynolds 2006).

Greater sage-grouse are widely distributed across 11 states and 2 Canadian provinces. However, this range has been reduced to approximately half of the historical distribution (Schroeder et al. 2004). A recent model of the range-wide spatial structure of sage-grouse based on mapped distribution of leks delineated numerous small populations interspersed between a few large populations and around the periphery of the range (Fig. 1) (Knick and Hanser 2011). Current sagebrush habitats were relatively intact within the large populations. Nonetheless, additional habitat loss caused by natural or human disturbance could fragment and divide these large populations as well as further isolate small populations whose viability may depend on dispersal from neighboring populations. Habitat and population fragmentation were among the top factors contributing to the recent U.S. Fish and Wildlife Service (2010) decision that listing greater sage-grouse was warranted but currently precluded due to higher priority actions.

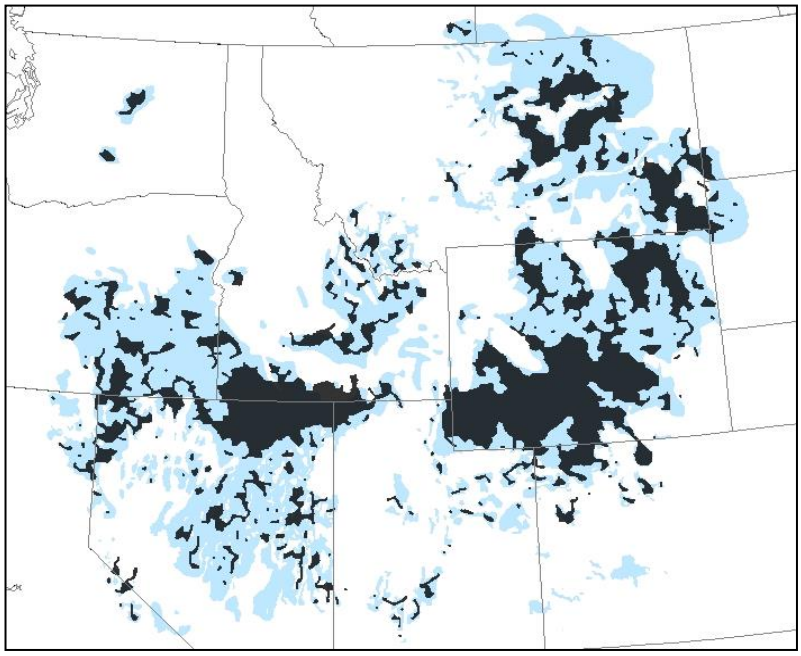

Fig. 1. Greater sage-grouse population structure (black) and current species range (blue). Population structure was delineated by clustering known leks that were interconnected by an 18-km dispersal distance.

We have limited understanding of what landscape features present barriers to sage-grouse dispersal that are significant enough to fragment or isolate populations. For several grouse species, patches of unsuitable habitat above a particular size threshold can prevent successful movement of individuals between populations (Fedy et al. 2008, Piertney et al. 1998). Distance, topography, or large blocks of unsuitable habitat all potentially influence dispersal at local and regional scales. Few studies using conventional radio-telemetry techniques or recaptures of marked individuals have documented either dispersal distances. Similarly, radio-telemetry data seldom are collected at a spatial and temporal resolution necessary to provide insights into the influence that landscape features have on dispersal movements (Connelly et al. 2011). Rather than applying these results obtained from a few study areas to broader regions, we might better assess the spatial organization of populations across the entire sage-grouse range by identifying the genetic structure relative to environmental characteristics that could influence dispersal rates and gene flow. Ultimately, this information will facilitate assessment of population vulnerability to

stochastic or environmental risks and will help focus conservation efforts on populations that may be more vulnerable due to potential lack of connectivity between other populations.

Dispersal and gene flow were sufficient at a broad scale to maintain genetic diversity across the sage-grouse range although populations in Washington state and the Bi-State Distinct Population Segment (Mono Basin region straddling California and Nevada) show evidence of isolation (Oyler-McCance et al. 2005, Oyler-McCance and Quinn 2011). However, the processes that maintain range-wide genetic diversity may be insufficient to sustain individual populations in landscapes undergoing rapid change or that become isolated due to habitat change and a loss of a connecting corridor. If so, then short-term and local-scale dynamics rather than range-wide processes may be more important in viability of individual sage-grouse populations.

We will use fine-scale genetic analyses based on the spatial distribution of genotypes to delineate the structure of sage-grouse populations and to identify patterns of gene flow. Spatial organization of populations and movements are important characteristics influencing long-term persistence. If the range-wide structure of sage-grouse functions in a metapopulation model (Gilpin and Hanski 1991, Hanski 1998), long-term persistence could be maintained through a long-term dynamic of colonization and extinction among populations that otherwise have independent dynamics and stressors. More likely, a source-sink model (Pulliam 1988) might be more accurate: peripheral populations that have low inherent viability are maintained by individuals dispersing from larger populations in more productive, least threatened regions. Management for core populations also suggests that gene flow is not omnidirectional but that larger populations within designated priority regions can be maintained to supply dispersals to regions delineated as general habitat. Under either model, exchange of individuals among populations is an important conservation concern.

More recent approaches can now identify the amount of exchange within and among delineated population components. Because small population size and isolation can have serious negative impacts on population persistence (Höglund et al. 2002, Briskie and Mackintosh 2004, Frankham 2006), our analyses also will seek to identify landscape characteristics that can prevent successful movement of individuals between populations. Genetic relatedness coupled with cost-surface models will be used to determine how isolation-by-distance, geographic, or environmental barriers influence metapopulation dynamics of sage-grouse and likelihood of long-term persistence for populations (Manel et al. 2003, McKelvey et al. 2010). This understanding can inform conservation planning about long-term effects of habitat loss due to anthropogenic or natural disturbance that potentially disrupt dispersal movements between populations. These spatial dynamics of population size and isolation can help assess viability when coupled with additional information on population trend and rate of environmental change.

MANAGEMENT RELEVANCE

State and federal agencies are designing management actions for greater sage-grouse based on core or priority areas containing the highest densities of breeding birds and their seasonal and annual habitats. This approach is intended to reduce threats to habitat and focus limited conservation resources in regions that have the greatest potential to benefit the largest proportion of sage-grouse (Doherty et al. 2011). As a trade-off, energy and other development is permitted

on public lands outside of core areas under normal controls, thus impacting a smaller percentage of the sage grouse in those regions. However, redistributing development from core areas into surrounding regions with fewer and less stringent requirements could fragment or increase the amount of unsuitable habitat and have the unintended consequence of isolating sage-grouse populations within those core areas. Therefore, it is important to understand how sage-grouse populations are structured, the relationship of breeding populations to delineated core areas, and how landscape features influence dispersal among core areas.

The concepts of structural and functional connectivity are critical components for guiding conservation actions emphasizing core areas coupled with identifying and maintaining corridors to facilitate gene flow. Structural connectivity, the spatial arrangement of habitat and environmental variables, is an important first step and is the foundation for delineating core habitat or population areas. Recent range-wide (Connelly et al. 2004, Knick and Connelly 2011) and ecoregional assessments conducted by the U.S. Bureau of Land Management have provided extensive spatial information on habitats, threats, and conservation actions that is necessary for understanding the structural connectivity of habitats (Tischendorf and Fahrig 2000). These data help delineate spatial patterns or "where things are." In this study based on genetic and landscape data, we are attempting to better understand functional connectivity, which is based on interpreting the spatial arrangement of habitats from a species' perspective (Wiens 2002). Functional connectivity is far more challenging but provides information on the processes underlying the patterns or "how things work". State and federal agencies have the opportunity to influence the future form and function of sagebrush landscapes across broad regions. These concepts provide keys to developing conservation strategies based population strongholds and movement corridors that facilitate dispersal and gene flow that are important sustain population viability.

This study provides an approach to delineate population structure in sage-grouse populations, estimate the degree of genetic connectivity and population isolation, and identify the underlying landscape components that influence connectivity. Other methods have been used on individual study areas to delineate sage-grouse movements. Considerable money and effort has been spent tracking the movement of animals using radio-telemetry and band recoveries for sage-grouse. Although these methods are effective, they are limited in the scale (both space and time) of the questions they can address. Genetic methods are less costly, allow for investigations over broader spatial extents, and measure the actual breeding consequences of animal movement (Segelbacher et al. 2010). In addition to defining populations and quantifying connectivity, genetic approaches also address many other relevant questions including the conservation of genetic diversity, the impacts of inbreeding, and the relationship of gene flow to landscape, geographic, and land cover features (Holderegger and Wagner 2008). In this study, we will use genetic techniques to gain a better understanding of greater sage-grouse population boundaries, connectivity, and environmental influences on gene flow.

OBJECTIVES

1. To delineate the genetic structure of sage-grouse populations. Achieving this objective will help identify biologically meaningful boundaries of greater sage-grouse populations for conservation planning.

2. To identify genetic connectivity among populations, estimate rates and direction of genetic exchange, and delineate corridors important for dispersal and gene flow. Population isolation is a primary factor that influences long-term species persistence. By delineating corridors and potential stepping stones for dispersal among population, we can develop conservation plans to minimize disturbance or focus restoration in key areas not contained within core or priority habitat regions. We also might identify source-sink dynamics relative to priority and general habitat regions from rates of genetic exchange.

3. To identify geographical, topographic, and environmental characteristics that facilitate and impede gene flow. Understanding landscape characteristics that influence dispersal and gene flow are critical to assessing the potential impact of habitat change resulting from natural or anthropogenic disturbance. Genetic monitoring tools could also be used to monitor the success of existing or established corridors and evaluate the success of conservation actions.

CONCEPTUAL MODEL OF META-POPULATION DYNAMICS

The spatial organization of populations across a species' range is an important factor influencing its long-term viability. Species that have multiple interconnected populations are more likely to persist because the risk of extirpation caused by regional events is confined to local populations; connectivity among populations ensures that recolonization can occur following extirpation assuming that sufficient suitable habitat remains (Gilpin and Hanski 1991, Thomas 1994, Hanski 1998). Thus, movement by individuals within this spatial network that becomes expressed through gene flow is one of the most critical, yet least understood, processes governing species persistence.

The range-wide extent of almost all species is orders of magnitude larger than the dispersal distance of any single individual. In addition, heterogeneity in habitat quantity, configuration, and quality creates spatial discontinuities in population densities. Consequently, species distributions do not consist of a single panmictic population but instead can be best described by a meta-population structure having hierarchical levels of connectivity (Fig. 2) (Wiens et al. 1993). At larger ecological scales, less frequent but longer movements by individuals between populations influences range-wide connectivity and are essential for population persistence. The probability that an individual will move from one population to another is influenced by the species life history strategies, relative densities among populations and the cost to movement. At smaller ecological scales, short dispersals characteristic of most individuals result in the majority of breeding occurring within a relatively distinct and confined area characterized by extensive internal connectivity. Identification of these demographically independent populations and defining their boundaries is a fundamental component to

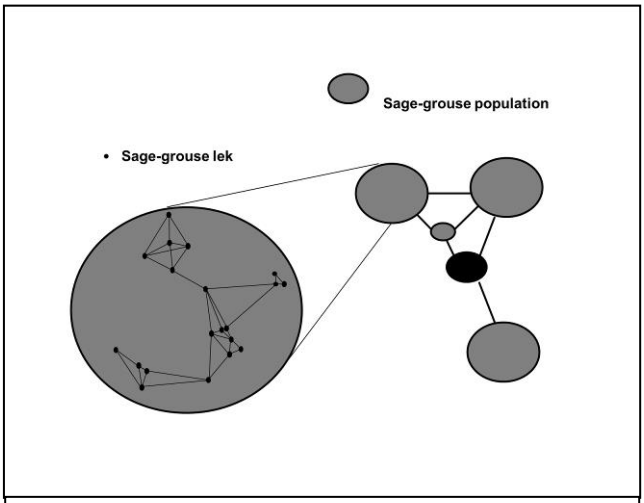

Fig. 2. Hierarchical organization of sage-grouse connectivity among leks and populations.

managing any wildlife species.

STUDY METHODS

We will combine genetic markers and landscape analyses to define the spatial structure of sage-grouse populations and evaluate environmental influences on dispersal. Genetic information will be obtained from sage-grouse feathers lost from birds displaying on breeding grounds (leks). The use of field-collected feathers for genetic analyses can be a reliable non-invasive sampling strategy when feathers are recently-collected, sufficient sample numbers are collected, and laboratory methods are employed to reduce errors inherent in tissues containing low DNA content (Segelbacher 2002). By collecting feathers from multiple sage-grouse at a large representative sample of the known leks, we will estimate genetic relatedness among breeding locations to delineate population structure within the sage-grouse range. Genetic relatedness coupled with spatial analyses then will be used to better understand how geographic distance, topographic characteristics and natural variation in land cover, or anthropogenic land uses influence dispersal and gene flow.

The multiple objectives on this project each require a different approach to derive statistically valid inferences (Cushman et al. 2006, Storfer et al. 2007). It is not possible to sample all of the >5,000 known leks in the sage-grouse range. Therefore, the sampling design will need to efficiently target collection efforts at a subsample of available leks and also respond to the ecological structure of sage-grouse and sagebrush systems. We will need to address variation within and among multiple scales (Schwartz and McKelvey 2009), including individual, lek, region, and range-wide organizational levels. We also will need to adequately sample across the entire sage-grouse range to delineate the range-wide population structure. It also is unlikely that we will have sufficient spatial information to develop range-wide cost-surface models for identifying effective barriers to gene flow. Consequently, we will find suitable replicates of representative landscape components across the range for our analyses (Short Bull et al. 2011).

We will employ an adaptive sampling strategy that addresses trade-offs among number of samples that can be collected, funding limits to the number of samples that can be analyzed in the laboratories, and a variable collection effort among years and across the spatial distribution of sage-grouse. Recent advancements in both landscape genetics as well as optimal design of monitoring studies have enabled a much more efficient collection of ecological data using fewer resources (Hooten et al. 2009, Hanks et al. 2010, Hooten et al. 2012). The basic premise is to fuse the landscape genetics modeling with the spatial structure in the monitoring design to allow the model to suggest modifications to the design in future years based on various design criteria (such as minimizing allele prediction error or maximizing the information pertaining to the identification of barriers to gene flow). We will collect >20 feathers from as many leks as possible in the first collection year in order to collect baseline information for initial modeling in the first collection year. The optimal monitoring design results based on those feathers then will then be used to refine the sampling to better focus on specific regions or types of landscape features.

MOLECULAR MARKERS

We will use neutral microsatellite markers in our genetic assessment of population structure and geneflow. Microsatellites are a useful and ubiquitous tool for addressing important questions in population and landscape genetics (Oyler-Mccance, et al. 2005, Fedy et al. 2008. Schwartz et al. 2009). In addition, rapidly advancing molecular technologies are making important contributions to conservation genetics and have the capacity to expand our understanding of wildlife and their interactions with the environment (Storfer et al. 2010). Additional markers, known as single nucleotide polymorphisms (SNPs) can augment more traditional population genetic markers such as microsatellites.

The combination of SNP and microsatellite markers allows for concurrent investigation of individuals using tens to hundreds of molecular markers including both neutral and potentially adaptive (under selection) markers across a landscape. The integration of data from neutral genetic markers, which can be used to infer gene flow and genetic drift, with data from markers under selection, which can be used to identify selective gradients and adaptive differences, will ultimately allow for a much more comprehensive understanding of how the landscape and environment influence natural populations and how those populations may change in response to future stressors such as climate change and energy development. We propose to explore the use of both types of markers in this study. The microsatellites (Supplemental Material) are well developed and optimized for this study. The development of a SNP panel for sage-grouse is underway yet not completed.

POPULATION STRUCTURE AND LANDSCAPE INFLUENCES

We will use a tiered approach to our analysis of sage-grouse population genetic structure. Each successive tier will increase in complexity and data requirements but also in the resolution at which we can delineate sage-grouse population structure and identify landscape and anthropogenic influences on gene flow. Within this approach, we first will identify biologically meaningful populations, then delineate the spatial distribution of populations, and finally, determine the natural and anthropogenic landscape factors that are most influential in shaping population and genetic structure (Table 1).

Table 1. Sequence of steps used to define and delineate populations, followed by analyses to identify landscape influences in gene flow.

Analysis Step	Objective
Clustering model	Initial approach to estimate number of populations in the data and assign individuals to populations
Delineate populations within a structurally-neutral landscape	Adds spatial coordinates to individuals and permits delineation of population boundaries
Landscape genetics	Identify landscape characteristics that influence gene flow

We will start with an initial clustering model, inputting solely individual genotypes, without identifying information on spatial location for each individual. This step will allow us to estimate number of populations and assign each individual to a population based on genotype (Pritchard et al. 2000). This model, based on ordination methods, assumes that there is population substructure but no underlying spatial distribution of the data. In the next step,

location and individual genotypes will be input and boundaries will be delineated for populations defined in the cluster analysis. The landscape is considered to be structurally-neutral or homogeneous across the study area. These results provide a spatial context that can be related to (sub)population boundaries defined in prior research (Connelly et al. 2004, Garton et al. 2011) or to current management focused on core or priority habitat regions (Doherty et al. 2011). Finally, we will use a landscape genetic approach on the georeferenced genetic data to estimate the influence of distance, environmental, and geographic characteristics (Schwartz and McKelvey 2009, Segelbacher et al. 2010) on gene flow within and among sage-grouse populations.

Identification of barriers to gene flow is one of the primary objectives of landscape genetics (Guillot et al. 2009, Storfer et al. 2010). There are two primary objectives: (1) to identify the natural and anthropogenic landscape features that correlate with discontinuities in the genetic data, and (2) use the relative influence of these landscape features to create a resistance-surface model that most parsimoniously explains patterns of gene flow across the sage-grouse range. From this resistance-surface, we can estimate relative isolation and connectivity for sage-grouse populations and gain insights into the metapopulation dynamics of sage-grouse and the likelihood of persistence for each population. Determining the spatial and temporal dynamics of colonization, based on rate of genetic exchange and barriers to gene flow, are important for predicting population viability relative to the rapidity of environmental changes.

We will use standard population-based (e.g., pairwise F_{ST}, Nei's D) and individual-based genetic distance analyses based on maximum likelihood and Bayesian approaches (Pritchard et al. 2000, Castric et al. 2002, Storfer et al. 2010). Mantel tests will be used to test for correlations between resistance surface cost-distance and genetic distance (Mantel 1967). Measures of genetic diversity and inbreeding will also be assessed using standard population-based estimates (e.g. H_O, H_E, F_{IS}) and individual based estimates (Belkhir et al. 2002, Murphy et al. 2010, Storfer et al. 2010). Barriers to gene flow will be identified using boundary detection techniques and model-identified barriers will be cross-examined by comparison to existing sagebrush distributional maps.

SPATIAL DATA

Availability of accurate spatial data that is consistent across the sage-grouse range will be one of the primary limitations for this study. We have some understanding of how sage-grouse respond to different landscape features (Aldridge and Boyce 2007, Johnson et al. 2011). Potential barriers that most likely limit sage-grouse movements and that will be most easily tested include linear features (e.g., interstate highways and roads, major rivers), topographic characteristics (e.g., mountain ranges), and large blocks of unsuitable land cover (e.g., agriculture and human development, forest). We have little knowledge of how sage-grouse use or move through heterogeneous landscapes composed of both suitable and unsuitable regions. Sage-grouse populations were not likely to persist in landscapes having <25% sagebrush land cover or >25% agriculture (Aldridge et al. 2008, Wisdom et al. 2011). However, the spatial scale and extent to which movement and gene flow is limited by these landscapes is unknown.

The only existing range-wide digital map of land cover is the Landfire Existing Vegetation Type (EVT) (http://www.landfire.gov), first derived from satellite imagery taken in 2001 and updated

in 2008 to reflect changes due to management activities and natural disturbance. The EVT includes agriculture and urban areas as part of the land cover classification. Other data layers considered in our analyses include digital elevation models (U.S. Geological Survey 1993), local topographic index (an index that combines landform and elevation model to model local topography; Leu et al. 2008), and maps delineating human activities (human footprint, Leu et al. 2008; individual anthropogenic development, Knick et al. 2011).

FEATHER COLLECTION

This project, one of the largest and most intensive terrestrial effort ever attempted, will be based on genetic data derived from greater sage-grouse feathers collected at breeding locations (leks) throughout the entire range spanning 11 western states and 2 provinces (Schroeder et al. 2004). Feathers will be collected during the breeding season (March-May) of each year. Standard protocols will be used by all field personnel for collecting feathers, entering field data into a web-based information system, and sending feathers to laboratories for processing (Supplemental Material).

PROJECT SCOPE AND RELATIONSHIP TO CURRENT RESEARCH

This major effort to examine gene flow across the range-wide distribution of greater sage-grouse is possible because of the collaboration among the U.S. Natural Resources Conservation Service through the Sage-Grouse Initiative, the Western Association of Fish and Wildlife Agencies, U.S. Bureau of Land Management, U.S. Forest Service, U.S. Department of Interior Northwest Climate Science Center, and the U.S. Geological Survey. Current studies of genetic connectivity already are being conducted in multiple states, including California, Montana, North Dakota, South Dakota, Washington, and Wyoming. This project will involve collaboration among states as well as with existing efforts.

PROJECT SCHEDULE

2012	February	2-page fact sheet, distributed to state and federal collaborators
	March	Feather collection procedures; distributed to states
	March-May	Feather collection[1]
	July	Study plan completed and distributed to state and federal collaborators
2013	Feb	Sampling design developed from initial collection results
2013	March-May	Feather collection. The 2013 sample will represent the largest effort
	May – Dec	Lab analysis and data compilation; preliminary reports prepared
2014	March-May	Feather collection. Locations sampled in 2014 will be targeted to fill in additional needs not met with the 2013 sample

	May – Dec	Lab analysis and data compilation; preliminary reports prepared
2015-		Product development: reports and publications; presentations as symposia

[1]/Number of feathers collected and leks sampled in 2012 were: Idaho (268/unknown); Montana (898/137); Nevada (62/unknown); North Dakota (92/7); Oregon (618/39); South Dakota (163/13); Utah (500 est./200). Feather samples from previous years are available from California, Washington, and Wyoming.

PRODUCTS

This project will provide an understanding of what constitutes a unique sage-grouse population and will identify the factors that influence connectivity among populations. This information is fundamental to understanding impacts and managing populations and will be provided to managers in multiple forms. New and integrated maps showing connectivity among core areas for sage-grouse will be produced and distributed in hard copy and digital form to states and federal management agencies. Maps will include inferences on the locations of barriers to sage-grouse dispersal and estimates of population boundaries. Maps will inform ongoing and future conservation decisions across the sage-grouse range by identifying areas important for connectivity of sage-grouse populations. These findings will generate high interest in management and policy arenas.

We also will make significant contributions to the scientific understanding of genetic and landscape ecology. Potential publications from this project include advances in laboratory analysis and techniques, statistical design for multi-scale sampling, relationship between structural and functional connectivity, multi-scale population and genetic structure in sage-grouse, and identification of barriers to gene flow in sage-grouse. Each of these topics has both scientific and management relevance.

CURRENT FUNDING[1]

Source	Amount	Purpose
USDA Natural Resources Conservation Service	$800,000	Lab analysis of genetic samples at USGS Molecular Ecology Lab (Ft. Collins) and USDA Forest Service Genetics Lab (Missoula)
US Geological Survey	$200,000 $ 10,000	1. Landscape genetic analyses 2. Develop statistical design for sampling feathers and leks 3. Lab analysis of 2012 feathers
US Fish and Wildlife Service Landscape Conservation Cooperative	$ 49,000	Develop SNPs panel
US DOI Northwest Climate Science Center	$ 30,000	Develop range-wide study plan; lab analysis of feather samples
US Geological Survey National Climate Change and Wildlife Science Center	$ 30,000	Develop project proposal; conduct workshop Denver, CO
State Wildlife Agencies	in-kind	Feather collection at leks

[1]/Represents funding received specific for the range-wide genetics project. Previous work has been funded by individual state agencies and the US Bureau of Land Management

COLLABORATORS

Federal Agencies
- Bureau of Land Management
- Fish and Wildlife Service
- Forest Service
- Geological Survey
- Natural Resources Conservation Service
- Northwest Climate Science Center

State Agencies
- California Department of Fish and Game
- Colorado Parks and Wildlife
- Idaho Department of Fish and Game
- Montana Fish, Wildlife, and Parks
- Nevada Department of Wildlife
- North Dakota Game and Fish Department
- Oregon Department of Fish and Wildlife
- Utah Division of Wildlife Resources
- South Dakota Game, Fish, and Parks
- Washington Department of Fish and Wildlife
- Wyoming Game and Fish Department

Universities
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SUPPLEMENTAL MATERIAL

LABORATORY METHODS

DNA extracted from feathers and a suite of polymorphic microsatellites markers (Segelbacher et al. 2000, Piertney and Höglund 2001, Taylor et al. 2003, Oyler-McCance and St. John 2010, Castoe et al. 2012) will be used to investigate genetic patterns at a fine scale across the landscape. We have tested and developed numerous microsatellite primers (Caizergues et al. 2001, 2003; Piertney and Höglund 2001, Taylor et al. 2003, Oyler-McCance et al. 2005, Oyler-McCance and St. John 2010, Castoe et al. 2012) that work on a variety of grouse species and have pioneered techniques to extract genetic information from feather samples.

DNA will be extracted from feathers and blood using the DNeasy Blood and Tissue Kit (Qiagen) following the manufacturers recommendations. Samples will be amplified across 15 grouse-specific microsatellite loci (MSP11, SGMS06.6, MSP18, SGMS06.8; Oyler-McCance and St. John 2010, reSGCA5, reSGCA11, SGCTAT1; Taylor et al 2003, TUT3; Segelbacher et al 2000, BG6: Piertney and Höglund 2001, SG21, SG24, SG28, SG36, SG29, SG39, unpublished). Amplifications will be performed in 10 μL PCRs consisting of 20 ng of template DNA, 0.2 mM of each dNTP, 0.25 μM dye-labeled forward primer, 0.25 μM reverse primer, 0.625 U GoTaq Flexi DNA polymerase (Promega), 2.25 mM $MgCl_2$ and 1X GoTaq Flexi Buffer (Promega). The amplification conditions for all loci will be as follows: 94^{o}C for 2 min, then 94^{o}C for 30 s, appropriate annealing temperature for 30 s, 72^{o}C for 30 s for 40 cycles, then 72^{o}C for 10 min and a final extension at 60^{o}C for 45 min. The sex of each sample will be determined by amplifying a region of the CDH gene using the primers 1237L (GAGAAACTGTGCAAAACAG) and 1272H (TCCAGAATATCTTCTGCTCC; Kahn et al. 1998). Amplification of the sexing marker will be the same as for the microsatellites with the exception of a 56^{o}C annealing temperature. PCR products will be multi-loaded based on product size and primer label, combined with GeneScan LIZ 600 internal lane size standard (Applied Biosystems), and electrophoresed through a capillary gel matrix using an AB3500 Automated DNA Sequencer (Applied Biosystems). Allele sizes will be determined for each locus using GeneMapper v4.1 software (Applied Biosystems).

SUPPLEMENTAL MATERIAL

FEATHER COLLECTION

Non-Invasive Feather Collection: When possible, please collect at least 20 different feathers from each lek site you visit. The goal is to obtain feathers from as many *different* individuals on a lek as possible. Therefore, it is best if you do not pick up all feathers from the *exact same* location, as they are likely to come from the same one or two individuals. Larger, less weathered feathers are preferable, although small feathers may also yield quality DNA. If feathers are collected on a lek, this counts as one location and only one label needs to be filled out for that location on that day. If a feather is collected from a single individual off the lek, then that feather needs to be placed in a small #10 envelope and a label needs to be associated with that unique location. The small envelope containing the sample needs to be placed in a large envelope with a sticky label on it.

1. Use one #10 size paper envelope per feather.
2. Do not handle the tip (quill) of the feather (this is where the cells containing the DNA are found—handling can remove these cells). A sound method is to pick the feather up by the end opposite the quill and place it in the envelope (if the tip of the feather is missing, the feather is not worth collecting).
3. Place only *one* feather into each small paper envelope.
4. Put all the envelopes from each lek into one large paper envelope.
5. Record all required information on label and attach it to the large envelope.
6. Store samples in a cool, dry, dark location until sending to the lab (freezers work well).

Labeling Samples: Exact location information is key to this analysis. We ask collectors to fill out a sticky label that will be put on a large envelope for every location. Please complete all fields of the label while in the field and adhere it to the large envelope for each collection location each collection day. This information should also be entered into the form on this website.

The following data should be recorded:

1. Collection date
2. Sage-grouse Management Zone
3. State and County
4. Lek Name
5. Alternate lek name (if applicable)
6. Collection location (NAD 83, UTM, including Zone)

7. Number of feathers/samples (if applicable)

8. Lead collector's email address

Following collection, samples should be stored in a cool, dry, dark location, as UV and elevated temperature will degrade DNA. Samples can be shipped at room temperature in boxes or large envelopes using regular mail.

Samples should be shipped to the following locations based on the state or province in which they were collected. All samples collected in Washington, Oregon, Nevada, California, Utah, Colorado, Alberta, and Saskatchewan should be sent to Sara Oyler-McCance (address below). Samples collected in Montana, North Dakota, South Dakota, and Idaho should be sent to Todd Cross (address below).

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