Executive Summary
The greater sage-grouse (*Centrocercus urophasianus*) is one of the most intensively studied upland game bird in North America. Ongoing research continues to improve our understanding of genetic diversity across the species' range including the degree to which observed differences represent distinct or isolated populations. Techniques and metrics used to evaluate and describe genetic isolation, divergence, and diversity have also evolved and improved since previous genetic studies were published in 2005 and summarized in 2011 (Oyler-McCance et al. 2005; Oyler-McCance and Quinn 2011). Since then, new research on the genetics of sage-grouse has been completed while other studies are now in progress.

In 2010, the U.S. Fish and Wildlife Service (Service) determined the greater sage-grouse warranted protection under the Endangered Species Act (ESA or Act) but that listing was precluded by higher priorities. Habitat loss and fragmentation due to a variety of factors were cited as the primary threats to the species. Fragmenting habitats can isolate or reduce the size of populations, which may be more vulnerable to population declines, reduced genetic diversity, and potentially extinction. We used this workshop to better understand the latest science regarding sage-grouse genetics and any potential risks associated with isolation and small populations.

The Service is currently assessing the status of the greater sage-grouse and is gathering the best available scientific and commercial data to inform a status determination by September 30, 2015. While a rider to the Fiscal Year 2015 Omnibus Appropriations Bill will prevent the Service from writing or publishing a proposed rule, it does not relieve the Service from completing a status review to determine whether the species still warrants protection under the Act.

As the Service gathers the best available information on sage-grouse genetics, the Service partnered with the U.S. Geological Survey (USGS) to organize a workshop to elicit information from scientific experts about recent developments in the field of sage-grouse genetics. The workshop explored the characteristics of gene flow across the range, including potential impediments, or barriers, to gene flow and any implications of genetic divergence across the range of the greater sage-grouse. The workshop also explored potential interactions between threats and genetic processes, and provided an opportunity for scientific experts to discuss ongoing and upcoming research on sage-grouse genetics. The workshop was structured to elicit information from the scientific experts that could later help the Service identify genetic differentiation across the range, populations with low genetic diversity, or populations with unique genetic characteristics.
The following is a brief summary of the key issues identified by individual experts during the workshop. Group consensus was not asked for or provided during the workshop, so this summary reflects individual perspectives shared by experts:

- Currently, the published genetic data is not sufficient to determine the amount of landscape-level gene flow between large, eastern and western portions of the overall range, but upcoming research will investigate further. Some degree of gene flow likely occurs from east-to-west across the range.
- Natural and human-caused features that impede the movement of individual birds, such as mountains, large rivers, deserts, forests, large reservoirs, agricultural fields, electrical power lines, highways, and energy development, may generally act as barriers to gene flow for the greater sage-grouse.
- Available genetic data indicates that the Bi-State, Columbia Basin, and Jackson Hole populations are isolated from other populations and have low levels of gene flow with other sage-grouse populations. The Columbia Basin and Jackson Hole populations are also small, isolated, and less genetically diverse than the Bi-State population. The Bi-State population has likely been isolated for thousands of years, but it is currently unclear how long the Columbia Basin and Jackson Hole populations have been isolated.
- The Missouri River Valley likely acts as a barrier to gene flow between populations located to the north and south.
- The Strawberry Valley population in Utah has low genetic diversity likely due to predation, but is still connected to other nearby populations.
- Small, isolated populations are more vulnerable to extirpation or extinction from demographic processes and stochastic events. Threats that fragment sagebrush habitats potentially increase the number and magnitude of barriers to gene flow and amplify the risks of small, isolated populations.
- Populations at the periphery of the range may have low genetic diversity as a result of low numbers or isolation, but may also be uniquely adapted to specific environmental conditions. These unique adaptations may increase the adaptive potential of sage-grouse so that the species has a higher probability of persistence during potential future changes to habitat or climate.
- Upcoming research, particularly a range-wide landscape connectivity study, will provide more information about the role that barriers play in moderating gene flow across the species’ range.
- Available research has not evaluated the genetic makeup of all sage-grouse populations. Upcoming research may also identify additional isolated populations.

This workshop was one component of the Service’s information gathering process for the status review. Information gathered during the workshop will be used by the Service in conjunction with other published literature or information submitted by interested parties, to evaluate the status of the species. The Service is committed to using the best available scientific and commercial information, and will incorporate new information as it becomes available.
Introduction
The following report summarizes the workshop’s organization and proceedings, with supporting materials attached in appendices.

We begin with the organization and meeting preparation, then a summary of the workshop exercises and discussion. In the Appendices, we have some of the specific materials used in preparation, slides shared with the group, and Notes from the meeting.

This workshop was one component of the Service’s information-gathering process for the status review. Information gathered during the workshop will be used by the Service in conjunction with other published literature or information submitted by interested parties, to evaluate the status of the species. The Service is committed to using the best available scientific and commercial information, and will incorporate new information as it becomes available.
# Table of Contents

Executive Summary .......................................................................................................................... i
Introduction .......................................................................................................................................... iii
Table of Contents ............................................................................................................................... iv

## Organizing the Workshop

- Identifying and Inviting Experts ................................................................................................ 1
- Preparing Experts for the Workshop ........................................................................................... 1
- Workshop Facilitation .................................................................................................................. 2
- Workshop Agenda ...................................................................................................................... 2

## Summary of Workshop Exercises and Discussions

- Day 1: Wednesday, October 22, 2015 ......................................................................................... 2
  - Part 1: State of the science: Genetics and the greater sage-grouse ........................................ 2
  - Part 2: Barriers to gene flow and genetic divergence in sage-grouse .................................... 3
- Day 2: Thursday, October 23, 2014 8:00 am – 12:00 pm ....................................................... 13
  - Part 3 and Part 4: Genetic divergence and persistence ............................................................ 13
  - Part 5: Reflection, new studies, and wrap up ........................................................................ 15

Literature Cited .................................................................................................................................. 17

## Appendix 1: Workshop Planning Team and Agenda

- Planning Team ............................................................................................................................. 19
- Workshop Agenda: ...................................................................................................................... 21
  - Wednesday October 22, 2014: 8:00 AM – 5:00 PM and .......................................................... 21
  - Thursday October 23, 2014: 8:00 am – 12:00pm ................................................................. 22

## Appendix 2: Criteria to Identify and Invite Experts

- Criteria to ensure that participants are scientific experts ......................................................... 23
- Criteria to ensure that participants are experts familiar with the workshop topic ................ 23
- Criteria to ensure that the group of invited participants represent the diversity of expert judgment within the scientific community ................................................................. 23

## Appendix 3: Expert Identification and Invitation List

- .................................................................................................................................................. 24

## Appendix 4: Pre-Workshop Webinars and Workshop Ground Rules

- .................................................................................................................................................. 26

## Appendix 5: Workshop Bibliography

- .................................................................................................................................................. 35

## Appendix 6: Presentations given by Dr. Sara Oyler-McCance

- .................................................................................................................................................. 51

## Appendix 7: Workshop Notes

- .................................................................................................................................................. 102
<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purpose of the Workshop Notes</td>
<td>102</td>
</tr>
<tr>
<td>Day 1: Wednesday, Oct. 22, 2014</td>
<td>102</td>
</tr>
<tr>
<td>PART 1: INTRODUCTION</td>
<td>102</td>
</tr>
<tr>
<td>PART 2: BARRIERS TO GENE FLOW AND GENETIC DIVERGENCE IN SAGE-GROUSE</td>
<td>105</td>
</tr>
<tr>
<td>PART 3: GENETIC DIVERGENCE</td>
<td>113</td>
</tr>
<tr>
<td>Day 2: Thursday, Oct. 23, 2014</td>
<td>115</td>
</tr>
<tr>
<td>Thursday Exercise 1:</td>
<td>115</td>
</tr>
<tr>
<td>Thursday Exercise 2:</td>
<td>118</td>
</tr>
<tr>
<td>Thursday Exercise 3:</td>
<td>119</td>
</tr>
<tr>
<td>Thursday Exercise 4:</td>
<td>121</td>
</tr>
<tr>
<td>Wrap-up:</td>
<td>124</td>
</tr>
</tbody>
</table>
Organizing the Workshop

The workshop brought together a group of scientific experts to discuss genetic techniques, conservation genetics, and the greater sage-grouse. A workshop planning team with members from the Service and USGS:

- Identified, invited, and prepared the experts;
- Developed an agenda; and
- Facilitated the meeting.

We summarize these organizational steps below. Appendix 1 provides the list of the planning team members and the workshop’s agenda.

**Identifying and Inviting Experts**

The planning team reviewed publications on sage-grouse genetics to identify experts who had authored studies or participated in research relevant to the workshop topic. Then, the planning team used selection criteria, including a candidate’s professional credentials, position, area of expertise, and experience with the greater sage-grouse, to develop a list of potential invitees (Appendix 2). These criteria helped ensure that invitations to participate were made only to scientific experts familiar with the topic and that the selections were transparent, unbiased, and captured a broad diversity of expertise and professional judgments related to the topic.

The planning team identified experts based solely on their scientific qualifications, rather than their affiliation with a particular organization or interested party. State partners from the Western Association of Fish and Wildlife Agencies (WAFWA) reviewed the list and suggested additional experts. The workshop planning team then invited experts that met the selection criteria. Appendix 3 provides the invitation list. The USGS facilitators emailed the invitations and served as the primary points of contact for the experts. If an expert declined the invitation, the facilitators invited a replacement from the planning team’s list.

Invited experts represented a diversity of professional backgrounds and experience, including scientists with sage-grouse expertise, expert knowledge of related species, and broad expertise in the field of conservation genetics. The facilitators limited the meeting size to nine experts in order to maximize open, productive discussion between all participants. Further, in order to maintain an open, intimate meeting environment, only members of the workshop planning team were invited to observe the workshop.

**Preparing Experts for the Workshop**

Before the workshop, the planning team hosted two informational webinars attended by all the experts. The webinars explained the workshop’s purpose, agenda, and ground rules (Appendix 4). The planning team also provided a bibliography of background references (Appendix 5). Before the workshop, each expert completed screening forms to identify any potential conflicts of interest. None of the invited experts identified any conflict of interest.
**Workshop Facilitation**

During the workshop, trained facilitators led by USGS used best practices for expert elicitation to engage the experts in facilitated discussion (EPA 2011; Drescher 2013). The facilitators used formal elicitation techniques on specific technical questions regarding genetics and the greater sage-grouse. Throughout the workshop, the facilitators asked experts for their individual, professional knowledge on specific topics and did not seek or obtain any group consensus from the participants. The workshop obtained facts and information only, and if needed to address uncertainty, professional judgment from each individual expert. To address uncertainty, the facilitators asked each expert to explain a hypothesis and identify any studies underway or that could be devised to test the hypothesis.

**Workshop Agenda**

The workshop’s agenda guided discussion on the following general topics regarding the genetics of the greater sage-grouse:

- Part 1: The latest techniques and metrics used to evaluate genetic divergence;
- Part 2: Potential barriers to gene flow and the areas with evidence of genetic divergence;
- Part 3: Potential implications of genetic divergence;
- Part 4: Potential interactions between threats and areas with low genetic diversity; and
- Part 5: Ongoing and upcoming research and research ideas.

As summarized below, the workshop proceedings generally adhered to the agenda.

**Summary of Workshop Exercises and Discussions**

All exercises were conducted to maximize opportunities for individuals to express their viewpoints. At no time did the Service ask for consensus during the workshop. In notes and documentation, the conversations are summarized, but this should not be interpreted as a unified perspective from the group.

**Day 1: Wednesday, October 22, 2015**

**Part 1: State of the science: Genetics and the greater sage-grouse**

To provide background information and context, Dr. Sara Oyler-McCance gave two presentations at the beginning of the workshop (Appendix 6). The first presentation provided an overview of the laboratory techniques and metrics used by geneticists to measure gene flow and genetic divergence. Background information important to later discussion included:

- DNA is found in cell nucleus and in mitochondria
- Mitochondrial (mtDNA) and nuclear DNA are two types of DNA that have different properties (mode of inheritance, size):
  - **MtDNA**
    - MtDNA is maternally inherited.
    - MtDNA haplotypes are assigned based on DNA sequence (different sequences are different haplotypes)
Haplotypes can differ by only one substitution or many, which determines how divergent they are.

Comparing the distribution of haplotypes between populations tells you something about how much gene flow there is between populations.

Reciprocally monophyletic refers to the situation wherein two groups, haplotypes within the group are more closely related to each other than they are to haplotypes outside the group.

Some species definitions require two groups to be reciprocally monophyletic in order to be considered a good species.

Since the haplotypes of Gunnison sage-grouse are not reciprocally monophyletic with the haplotypes of greater sage-grouse, these two species would not be recognized as different species using this definition.

**Nuclear DNA**

Nuclear DNA is bi-parentally (from both parents) inherited.

Geneticists usually target nuclear DNA that evolves quickly (e.g. microsatellites).

Can compare microsatellite allele frequencies between populations to make inferences about levels of gene flow.

Single Nucleotide Polymorphisms (SNPs) are another type of nuclear DNA marker.

- Microsatellites are repetitive regions that do not code for proteins (thus we consider them to be neutral markers – not under selection, reflect neutral processes like gene flow and changes in population size).
- SNPs occur throughout the genome in non-coding as well as coding regions so they can potentially be used to measure both neutral processes and measure selection and adaptation.

Dr. Oyler-McCance’s second presentation provided a summary of the available literature, papers in review, and research in progress on sage-grouse genetics ([Appendix 6](#)).

**Part 2: Barriers to gene flow and genetic divergence in sage-grouse**

**Exercise 1 - Gene flow between eastern and western portions of the range**

- **Topic:** Evaluate landscape-scale, recent gene flow between the eastern and western portions of the greater sage-grouse’s range.

**Exercise Purpose:**

The purpose of the first topic of discussion was to evaluate gene flow between the eastern and western portions of the greater sage-grouse's range (Figure 1). One or several north-to-south oriented barriers could limit gene flow between the eastern and western portions of the range, potentially resulting in genetic differences from east to west.
Exercise Discussion:

To begin, the experts were asked to define categories of gene flow. The experts’ discussion led to the following operational definitions for categories of gene flow based on migrants per generation (Mills et al. 2003; Lowe and Allendorf 2010). For the purposes of this workshop, the experts described the following five (5) categories of gene flow:

- No gene flow;
- Low gene flow corresponded to greater than 0 to 1 migrants per generation;
- Moderate gene flow corresponded to 2 to 10 migrants per generation;
- Substantial gene flow corresponded to greater than 10 migrants per generation; and
- Complete mixing indicative of unrestricted gene flow.

With these operational definitions, the facilitators then asked the experts:
Range-wide, does the genetic evidence currently available suggest that there is an east-to-west barrier to sage-grouse gene flow acting over recent time scales and across a large spatial scale?

The experts debated the term barrier and agreed that a barrier reduces gene flow, but is not necessarily impermeable. Then, the facilitators asked each expert to answer the question by distributing 100 points across the previously developed five categories of gene flow to score the likelihood that a category accurately described the barrier, if one exists, to east-to-west gene flow. Initially, the experts shared a strong reluctance to speculate or score a numerical response to this question for the following reasons:

- The available genetic evidence does not specifically address genetic flow or differences between large, eastern and western portions of the range;
- This question could be answered with greater certainty following a reanalysis of the 2005 data (Oyler-McCance et al. 2005) for this purpose (see Table 3 below, “An east-to-west split”); and
- Upcoming results from a range-wide genetics assessment expected to be published in the fall of 2015 (see Table 3 below, “Range-wide landscape connectivity”) should help address this question with greater certainty.

Although the experts expressed reluctance to answer and score the question without scientific evidence, they participated as a preliminary exercise, but stressed that there was little scientific validity to their scores (Figure 2). The resulting discussion regarding the value of quantifying their opinions prompted the facilitators to rework all of the workshop questions to allow the experts to characterize information they provided as either information supported by genetic evidence in the available scientific literature or a currently untested hypothesis based on professional opinion.
Figure 2. Exercise 1 asked each expert to rank a barrier to gene flow between the eastern and western portions of the range. The experts stressed that there was little scientific validity to their scores due to the lack of scientific evidence in the available literature. The experts discussed that they felt that there is some degree of gene flow from east-to-west across the overall range of the greater sage-grouse, but there is not complete mixing or a complete barrier (Figure 2). Gene flow from east-to-west could be best described as a cline, or gradient, with more genetic divergence between the easternmost and westernmost extremes of the range, such as a pattern of isolation by distance as previously described (Oyler-McCance et al. 2005). Additionally, smaller, more isolated populations likely experience more genetic divergence due to genetic drift. Reanalyzing data from Oyler-McCance et al. (2005) could provide measures of gene flow between the eastern and western portions of the range (Table 3 below, “An east-to-west split”). In addition, the upcoming range-wide connectivity study should specifically address this question (Table 3 below, “Range-wide landscape connectivity”).

To summarize Exercise 1, the available genetic data do not specifically address landscape-level gene flow between large, eastern and western portions of the range. There is likely a gradient of genetic divergence from east to west, with the greatest genetic difference between the eastern and western extremes of the range. Existing data will be reanalyzed and new data will soon be available that should more specifically investigate genetic divergence between the eastern and western portions of the range. The Service is committed to using the best available information. We will consider relevant information as it becomes available prior to our deadline for making a status determination.

### Table: GRSG gene flow across E-W barrier

<table>
<thead>
<tr>
<th>Definition of categories: based on operational definitions (cf Mills et al 2000 book chapter, Lowe and Allendorf) using migrants per generation:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Low gene flow means: 0-1 migrants per generation</td>
</tr>
<tr>
<td>• Moderate gene flow means: 2 to 10 migrants per generation</td>
</tr>
<tr>
<td>• Substantial gene flow means: &gt;10 migrants per generation</td>
</tr>
</tbody>
</table>

---

**Exercise 1:**

**GRSG gene flow across E-W barrier**

- A. No gene flow
- B. Low gene flow
- C. Moderate gene flow
- D. Substantial gene flow
- E. Complete mixing (no barrier to gene flow)

**Expert Panel Responses**

<table>
<thead>
<tr>
<th>Expert</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. No gene flow</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>B. Low gene flow</td>
<td>50</td>
<td>60</td>
<td>33.3333</td>
<td>20</td>
<td>50</td>
<td>50</td>
<td>33</td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td>C. Moderate gene flow</td>
<td>50</td>
<td>30</td>
<td>33.3333</td>
<td>40</td>
<td>50</td>
<td>50</td>
<td>34</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>D. Substantial gene flow</td>
<td>0</td>
<td>0</td>
<td>33.3333</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>33</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>E. Complete mixing (no barrier to gene flow)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sum Check</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

---

Greater Sage-Grouse 2015 Status Review, Genetics Workshop Summary Report
Summary of Workshop Exercises and Discussions
Page 6
Exercise 2 - Potential barriers to gene flow across the range

- Topic: Identify potential barriers to sage-grouse gene flow, if any.

Exercise Purpose:
The purpose of the second topic of discussion was to identify and characterize potential barriers to sage-grouse gene flow, if any exist.

Exercise Discussion:
The facilitators asked the experts to characterize potential barriers to gene flow across the overall range of the greater sage-grouse. Specifically, the facilitators asked:

- Are there barriers that may restrict or prevent gene-flow between populations or groups of populations of the greater sage-grouse?
- If yes, identify each barrier and describe its location, source, age, mechanism, and magnitude.

To complete this exercise, the facilitators organized the experts into breakout groups of three. The facilitators asked each group to draw lines that represent potential barriers to gene flow. Each group characterized a line as either:

- A real barrier supported by existing, data-driven, genetic evidence (Figure 3); or
- A hypothesized barrier based on qualitative information, physical features on the landscape, or other data not yet evaluated by the scientific community (Figure 4).

The experts stressed that the barriers they identified are not definitive (Figure 3); there are likely other barriers and areas on the landscape with reduced levels of gene flow that have not been examined. Further, ongoing research in Wyoming (Table 3 below, “Landscape genetics in Wyoming”) and the range-wide connectivity study (Table 3 below, “Range-wide landscape connectivity”) should specifically address the question. The Service will consider relevant information as it becomes available prior to our deadline.
Figure 3. Barriers to gene flow supported by existing genetic evidence discussed by the expert groups in Exercises 2 and 3. Blue line is river, Brown is mountains/forest, Green is cropland, Orange/Yellow is desert, Purple is high capacity power-lines. Some locations were given labels to improve communication during the workshop, but the labels do not necessarily correspond directly to an otherwise mapped feature. Lines are not definitive, and the absence of a line does not imply unrestricted gene flow between any areas, but that genetic data has not yet been examined.
Figure 4. Hypothesized barriers to gene flow discussed by the experts in Exercises 2 and 3. Blue is river, Brown is mountains/forest, Green is cropland, Orange/Yellow is desert Black is interstate highway, Gray area is energy development. Some locations were given labels to improve communication during the workshop, but the labels do not necessarily correspond directly to an otherwise mapped feature. Lines are not definitive, and the absence of a line does not imply unrestricted gene flow between any areas, but that genetic data has not yet been examined.
While reviewing their maps, the experts discussed the following natural features that may generally act as barriers to gene flow for the greater sage-grouse:

- Mountains, such as the Wind River Range in western Wyoming, the Big Horn Mountains in northern Wyoming and southern Montana, the Grand Tetons in western Wyoming, and the Rocky Mountains in northern Colorado.
- Large rivers and water bodies, such as the Columbia River, the Snake River, and the Missouri River.
- Low-elevation, hot deserts, such as those in southern Nevada and Utah.
- High-elevation coniferous forests, such as those in Colorado, Montana, Idaho, and Wyoming.

The experts discussed the Snake River Plain in Idaho as a potential barrier to gene flow, with agriculture and expanding human populations along the river corridor contributing to the barrier and further reducing gene flow. However, sage-grouse may still be able to cross the Snake River Plain, and the available genetic data do not indicate that there are significant genetic differences on either side of the Snake River Plain. Further, elevation itself may not be a barrier, but the habitat features associated with elevation extremes may act as a barrier to gene flow. Populations that were large in the past, but have become isolated may be more vulnerable than historically small populations that have been isolated.

The experts also discussed the following anthropogenic (human-caused) features that could act as barriers to gene flow for the greater sage-grouse in some circumstances:

- Large reservoirs, such as those along the Missouri River.
- Areas with extensive agricultural development.
- Electrical transmission lines (e.g., Washington State).
- Interstate highways such as Interstate 80 (I-80) in southern Wyoming.
- Areas of extensive energy development and its associated infrastructure (e.g., NE Wyoming).

Exercise 3 – Review the evidence and hypotheses for limited gene flow across the range

The facilitators revised this exercise in order to continue the discussion from Exercise 2.

- Original Topic: Evaluate the relative gene flow across the barriers identified in Exercise 2.
- Revised Topic: Continue discussing the evidence-based barriers and the hypothesized barriers to gene flow identified in Exercise 2.

Exercise Purpose:

The third topic of discussion reviewed the genetic evidence or supporting hypotheses discussed by experts in the workshop for barriers and areas with potentially limited gene flow that the experts identified in Exercise 2 (Figure 3 and Figure 4). The facilitators asked:
Have we captured the places where limited gene flow (no or low gene flow) has been identified by data-driven genetic evidence and the places where limited gene flow has been hypothesized?

Exercise Discussion:
The experts again stressed that the list of identified barriers and areas with limited gene flow likely is not definitive or comprehensive. Other barriers and places on the landscape that have restricted gene flow likely exist, but they have not been identified or analyzed. There may not be distinct breaks, but instead a variation or gradient across populations. More research, such as the upcoming range-wide connectivity study (Table 3 below, “Range-wide landscape connectivity”, could result in identification of additional barriers to gene flow.

As summarized in Table 1, the experts identified four areas with limited gene flow that based on available genetic evidence.

Table 1. Areas with limited gene flow based on the available genetic evidence.

<table>
<thead>
<tr>
<th>AREA OR POPULATION WITH LIMITED GENE FLOW BASED ON THE AVAILABLE GENETIC EVIDENCE</th>
<th>STATE(S)</th>
<th>SUMMARY OF DISCUSSION BY THE EXPERTS</th>
</tr>
</thead>
</table>
| Bi-State                                                                          | California and Nevada | • The Bi-State population has a large proportion of unique haplotypes that are found nowhere else.  
• The genetic evidence indicates that the Bi-State population may have been isolated for thousands to tens of thousands of years.  
• During the last glaciacion (the Pleistocene), Lake Lahontan may have acted as a barrier.  
  • The Bi-State has haplotypes from both clades and the haplotypes in the Bi-state are not reciprocally monophyletic compared to haplotypes outside the Bi-state.  
  This means that they haven’t been separated long enough to experience lineage sorting.  
• The haplotype composition of this group is very different than all other populations of greater sage-grouse and does suggest that it is unique and has been isolated for a long time. |
<table>
<thead>
<tr>
<th>AREA OR POPULATION WITH LIMITED GENE FLOW BASED ON THE AVAILABLE GENETIC EVIDENCE</th>
<th>STATE(S)</th>
<th>SUMMARY OF DISCUSSION BY THE EXPERTS</th>
</tr>
</thead>
</table>
| Columbia Basin                                                                  | Washington | • The Columbia Basin population is small and isolated.  
• Large areas of non-habitat separate the Columbia Basin from other populations, so the lack of gene flow is likely due to the isolation by non-habitat.  
• The Columbia Basin population shares some common haplotypes with the rest of the range, but it has low genetic diversity, indicating that it is isolated from the rest of the range and experienced a population decline with no or very little emigration (migration into the population from outside).  
• The Columbia Basin population has likely experienced genetic drift.  
• The genetic isolation of the Columbia Basin likely occurred more recently than the isolation of the Bi-State population, because prior to conversion to croplands, sagebrush habitats connected Oregon and Washington.  
• Additional analysis of the mtDNA data from museum specimens collected prior to extensive agricultural development would help establish when the Columbia Basin population became isolated (Table 3 below, “Historical connectivity of the Columbia Basin”).  
• Two populations in eastern Washington have received translocated sage-grouse from Oregon and Nevada, which could complicate an analysis of contemporary gene flow. |
| Jackson Hole                                                                     | Wyoming | • The Jackson Hole population is small, isolated, and declining.  
• The surrounding forested mountains isolated the population, but the time of isolation is unclear.  
• Additional analysis of the mtDNA data would help establish when the population became isolated. |
| Missouri River Valley                                                            | Montana | • The Missouri River Valley could inhibit sage-grouse movement, which may have resulted in genetic differentiation on either side of the river.  
• The Missouri River is not necessarily a complete barrier; there is likely limited gene flow across the river. |

To summarize Exercises 2 and 3, the experts identified evidence-based and hypothesized barriers to gene flow across the range of the greater-sage grouse. Hypothesized barriers not yet considered that could reduce gene flow include natural and anthropogenic features, such as forested mountains, transmission lines, and busy interstate highways. The available genetic evidence indicates that greater sage-grouse populations in the Bi-State, Columbia Basin, Jackson Hole, and Missouri River areas...
experience reduced gene flow as a result of isolation or physical barriers to movement. The Columbia Basin and Jackson Hole populations are small and isolated and, as a result, are less genetically diverse. Additional research would be needed to evaluate the time of isolation for the Jackson Hole and the Columbia Basin populations and the impact of the hypothesized barrier to gene flow caused by I-80 in Wyoming and the Missouri River Valley.

**Day 2: Thursday, October 23, 2014 8:00 am – 12:00 pm**

*Part 3 and Part 4: Genetic divergence and persistence*

**Exercise 4 - Areas with low genetic diversity across the range and areas where genetic processes and threats might interact to affect species persistence**

- Topic: Evaluate how the potential loss of a particular area of the range may decrease overall genetic diversity. Also identify areas, if any, where low genetic diversity or inbreeding depression may affect persistence.

**Exercise Purpose:**

The fourth topic of discussion asked each expert to identify mechanisms that cause low genetic diversity. The facilitators then asked the experts to identify areas within the greater sage-grouse’s overall range where low genetic diversity or inbreeding depression could affect greater sage-grouse persistence. The facilitators also asked:

- Are there areas within the range of the greater sage-grouse where genetic processes might exacerbate the effect of a threat?

**Exercise Discussion:**

The experts identified the following mechanisms that decrease the genetic diversity of a population:

- Isolation and the loss of gene flow.
- Reduced population size and genetic drift within the isolated population.

The potential consequences, or “genetic risks” associated with decreased genetic diversity include:

- Inbreeding depression.
- The rapid propagation of deleterious traits due to genetic drift.
- Increased loss of genetic diversity.

Table 2 summarizes the discussion regarding small, isolated populations of greater sage-grouse with low genetic diversity. The experts stressed that results from the upcoming range-wide connectivity study (see Table 3 below, “Range-wide landscape connectivity”) should help evaluate the genetic

---

1 This conversation was initiated on Day 1 and resumed on Day 2. For the sake of coherence, this report combines the two discussions.
diversity in these areas, and could reveal more areas of concern with low genetic diversity. Additionally, the range-wide connectivity study should help identify important stepping stones, or hubs to gene flow, that are crucial to maintaining genetic connectivity across the range.

The experts stressed that if the overall range becomes increasingly fragmented, populations will become more isolated, thereby increasing the risk of losing genetic diversity across the range. Further, small populations are more susceptible to genetic drift. Small populations, particularly those at the periphery of the range, may also possess unique, adaptive genetic traits that not only provide diversity to the species as whole, but may be important in facilitating the species’ adaptation to changing environmental conditions. Therefore, from a geneticist’s perspective, threats that fragment habitats and isolate small populations would be the most important for the maintenance of genetic diversity.

Table 2. Areas and populations with low genetic diversity discussed by the experts.

<table>
<thead>
<tr>
<th>AREA(S) OR POPULATION(S) THAT MAY BE LESS GENETICALLY DIVERSE AS A RESULT OF SMALL POPULATION SIZE &amp; ISOLATION</th>
<th>STATE(S)</th>
<th>HYPOTHESESIZED OR BASED ON EXISTING GENETIC EVIDENCE?</th>
<th>SUMMARY OF DISCUSSION BY THE EXPERTS</th>
</tr>
</thead>
</table>
| Columbia Basin     | Washington | Based on existing genetic evidence | • Populations in the Columbia Basin are small and isolated, and have low genetic diversity.  
• They likely have low genetic diversity because they are isolated and populations have declined to low numbers.  
• Additional research is required to evaluate the time of isolation (Table 3 below, “Historical connectivity of the Columbia Basin”).  
• The existing data does not provide insight into adaptive variation of the Columbia Basin. |
| Jackson Hole       | Wyoming   | Based on existing genetic evidence | • The Jackson Hole population is small and isolated, with low genetic diversity. |
| Strawberry Valley  | Utah      | Based on existing genetic evidence | • The population in the Strawberry Valley has reduced genetic diversity, largely the result of a high concentration of predators coupled with a small sage-grouse population, but the population does not necessarily have low gene flow.  
• Translocations could complicate genetic analyses. |
### Part 5: Reflection, new studies, and wrap up

**Ongoing and upcoming genetic studies and results**

- Topic: Discuss the potential impact of upcoming research on the outcomes of this workshop.

**Exercise Purpose:**
This topic provided an opportunity for the experts to discuss recently completed research, ongoing studies, research ideas, and their implications for the current understanding of sage-grouse genetics.

**Exercise Discussion:**
As summarized in Table 3, several upcoming or recently completed genetic studies should provide additional scientific information regarding gene flow, barriers, and genetic diversity across the range of the greater sage-grouse.

<table>
<thead>
<tr>
<th>AREA(S) OR POPULATION(S) THAT MAY BE LESS GENETICALLY DIVERSE AS A RESULT OF SMALL POPULATION SIZE &amp; ISOLATION</th>
<th>STATE(S)</th>
<th>HYPOTHESESIZED OR BASED ON EXISTING GENETIC EVIDENCE?</th>
<th>SUMMARY OF DISCUSSION BY THE EXPERTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klamath area</td>
<td>Oregon</td>
<td>Hypothesized</td>
<td>• Isolated pocket of occupied habitat.</td>
</tr>
<tr>
<td>Weiser</td>
<td>Idaho</td>
<td>Hypothesized</td>
<td>• Isolated pocket of occupied habitat.</td>
</tr>
<tr>
<td>Belt Mountains</td>
<td>Montana</td>
<td>Hypothesized</td>
<td>• Isolated pocket of occupied habitat.</td>
</tr>
</tbody>
</table>
| Populations at the periphery of the range | S. Utah  
N. Colorado  
N. Montana  
S. Idaho | Hypothesized | • Populations at the periphery of the range are increasingly fragmented and isolated, and as a result are likely more susceptible to inbreeding depression and genetic drift and loss of genetic diversity.  
• However, peripheral populations may be uniquely adapted to specific environmental conditions, expressed by unique genetic adaptive markers. Unique markers provide genetic diversity and may be important in the future as the species adapts to changing environmental conditions. |
Table 3. Recently published studies, ongoing research, and research ideas regarding the genetics of the greater sage-grouse discussed by the experts.

<table>
<thead>
<tr>
<th>PROJECT NAME</th>
<th>CITATION OR RESEARCHERS</th>
<th>LOCATION</th>
<th>PROGRESS</th>
<th>SUMMARY OF DISCUSSION BY THE EXPERTS</th>
</tr>
</thead>
</table>
| Low neutral genetic diversity in isolated Greater Sage-Grouse (*Centrocercus urophasianus*) populations in northwest Wyoming | Schulwitz *et al.* 2014 | Jackson Hole, Wyoming | Recently completed and published | • The Jackson Hole population in Wyoming is isolated and has a significantly different genetic makeup than surrounding populations, due largely to the surrounding forested mountains.  
• But it is unclear how long the population has been isolated. |
| Genetic differentiation using SNPs                                           | Oyler-McCance           | Range-wide      | Currently in review    | • This study will assess differentiation of the greater sage-grouse, the Bi-State population, and the Gunnison sage-grouse using SNPs. |
| Landscape genetics in Wyoming                                               | Row *et al.*             | Wyoming          | Currently in review    | • This study will assess landscape genetics of sage-grouse in Wyoming, focusing on the importance of seasonal habitats to drive landscape connectivity. |
| Range-wide landscape connectivity                                          | Knick *et al.*           | Range-wide      | Ongoing                | • Study investigating range-wide landscape connectivity analysis using microsatellites and SNPs.  
• Previous analyses looked at 44 populations across the overall range using 7 mtDNA and 14 microsatellite loci.  
• This new research uses 14 microsatellite loci and is developing SNPs with over 8,000 samples from across the species range.  
• Completion timeline is unclear: Ideally available by spring 2015, but not likely available before fall 2015. SNP panels will not be complete before fall 2015. |
| Impact of translocations in Washington                                      | Schroeder                | Washington      | Ongoing                | • Study investigating the impact of translocations on sage-grouse genetics in Washington.  
• Availability timeframe unknown. |
<table>
<thead>
<tr>
<th>PROJECT NAME</th>
<th>CITATION OR RESEARCHERS</th>
<th>LOCATION</th>
<th>PROGRESS</th>
<th>SUMMARY OF DISCUSSION BY THE EXPERTS</th>
</tr>
</thead>
</table>
| Impact of translocations in Strawberry Valley   | Dunken et al.           | Utah       | Ongoing  | • Study investigating the impact of translocations on sage-grouse genetics in the Strawberry Valley.  
• Availability timeframe unknown.                                                                                                                                  |
| Connectivity across the Missouri River           | Schwartz et al.         | Montana    | Ongoing  | • Study investigating genetic difference between sage-grouse populations located to the north and south of the Missouri River.  
• Preliminary results indicate that there are genetic differences and that the Missouri River is a barrier to dispersal.  
• Available in a few months.                                                                                                                                           |
| An east-to-west split                            | Oyler-McCance           | Range-wide | Ongoing  | • Reevaluate data collected in 2005 to investigate the cline of genetic difference from east-to-west across the range.  
• Available in a few months.                                                                                                                                                                                                     |
| Highway I-80 as a barrier to gene flow           | Schroeder and Oyler-McCance | Wyoming    | Ongoing  | • Investigate I-80 as a potential barrier to gene flow using available data.  
• Available in a few months.                                                                                                                                                                                                         |
| Historical connectivity of the Columbia Basin    | Oyler-McCance and Fedy  | Washington | Unfunded research idea | • Use mtDNA from museum specimens to evaluate the historical connectivity of sage-grouse in the Columbia Basin to the rest of the overall range.  
• Could complete before fall 2015.                                                                                                                                                                                                     |
| Time of isolation for the Jackson Hole population | Unknown                 | Wyoming    | Unfunded research idea | • Evaluate the time of isolation for the Jackson Hole population.                                                                                                                                                                           |

**Literature Cited**


http://dx.doi.org/10.1890/ES12-00415.1


Appendix 1: Workshop Planning Team and Agenda

The workshop planning team (Table 1) developed the workshop’s purpose, invitation list, and agenda. Twelve of the 19 planning team members observed the meeting. Meeting observers did not participate as experts and outside observers were not invited to participate in order to ease facilitation. After the meeting, the workshop planning team drafted this summary report. The draft report and workshop notes were provided to the expert participants for their review.

Planning Team

Table 4. Members of the workshop planning team, their organizational affiliations, and primary role on the team.

<table>
<thead>
<tr>
<th>PLANNING TEAM MEMBER</th>
<th>ORGANIZATION</th>
<th>WORKSHOP ROLE</th>
<th>OBSERVED THE WORKSHOP?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angela Burgess</td>
<td>U.S. Fish and Wildlife Service</td>
<td>Planning</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Region 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carey Galst</td>
<td>U.S. Fish and Wildlife Service</td>
<td>Planning</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Headquarters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Craig Hansen</td>
<td>U.S. Fish and Wildlife Service</td>
<td>Workshop coordinator and planning</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Region 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>David Smith</td>
<td>U.S. Geological Survey</td>
<td>Facilitator</td>
<td>Yes</td>
</tr>
<tr>
<td>Dawn Davis</td>
<td>U.S. Fish and Wildlife Service</td>
<td>Planning</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Region 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genevieve Skora</td>
<td>U.S. Fish and Wildlife Service</td>
<td>Planning</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Region 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Holly Freifeld</td>
<td>U.S. Fish and Wildlife Service</td>
<td>Planning</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Region 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jeff Everett</td>
<td>U.S. Fish and Wildlife Service</td>
<td>Planning</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Region 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jesse D’Elia</td>
<td>U.S. Fish and Wildlife Service</td>
<td>Planning</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Region 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jonathan Cummings</td>
<td>U.S. Geological Survey</td>
<td>Facilitator</td>
<td>Yes</td>
</tr>
<tr>
<td>PLANNING TEAM MEMBER</td>
<td>ORGANIZATION</td>
<td>WORKSHOP ROLE</td>
<td>OBSERVED THE WORKSHOP?</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------------------------------------------</td>
<td>---------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Kate Norman</td>
<td>U.S. Fish and Wildlife Service Region 6</td>
<td>Planning</td>
<td>Yes</td>
</tr>
<tr>
<td>Kris Tita</td>
<td>U.S. DOI Office of the Solicitor</td>
<td>Planning</td>
<td>No</td>
</tr>
<tr>
<td>Lief Wiechman</td>
<td>U.S. Fish and Wildlife Service Region 6</td>
<td>Planning</td>
<td>Yes</td>
</tr>
<tr>
<td>Mary Grim</td>
<td>U.S. Fish and Wildlife Service Region 8</td>
<td>Planning</td>
<td>Yes</td>
</tr>
<tr>
<td>Pat Deibert</td>
<td>U.S. Fish and Wildlife Service Region 6</td>
<td>Planning</td>
<td>Yes</td>
</tr>
<tr>
<td>Sarah Converse</td>
<td>U.S. Geological Survey</td>
<td>Planning</td>
<td>No</td>
</tr>
<tr>
<td>Steve Abele</td>
<td>U.S. Fish and Wildlife Service Region 8</td>
<td>Planning</td>
<td>No</td>
</tr>
<tr>
<td>Steve Morey</td>
<td>U.S. Fish and Wildlife Service Region 1</td>
<td>Facilitator</td>
<td>Yes</td>
</tr>
<tr>
<td>Theo Stein</td>
<td>U.S. Fish and Wildlife Service Region 6</td>
<td>External Affairs</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Workshop Agenda:

**Expert Elicitation Workshop on the Genetics of the Greater Sage-Grouse**

*Wednesday October 22, 2014: 8:00 AM – 5:00 PM and*

*Fort Collins, Colorado*

<table>
<thead>
<tr>
<th>Day/time</th>
<th>Topic</th>
<th>Lead</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PART 1: INTRODUCTION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>WEDNESDAY</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8:00 am</td>
<td>Welcome, logistics, and introductions</td>
<td>Craig Hansen</td>
</tr>
<tr>
<td></td>
<td>Purpose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Description of information packets</td>
<td></td>
</tr>
<tr>
<td>8:30 am</td>
<td>Methods and metrics used to evaluate greater sage-grouse barriers to gene flow and genetic divergence.</td>
<td>Sara Oyler-McCance</td>
</tr>
<tr>
<td></td>
<td>Overview of greater sage-grouse genetics: state of the science</td>
<td></td>
</tr>
<tr>
<td>10:00 am</td>
<td>Break</td>
<td></td>
</tr>
<tr>
<td><strong>PART 2: BARRIERS TO GENE FLOW AND GENETIC DIVERGENCE IN SAGE-GROUSE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:15 am</td>
<td>Barriers to Gene Flow (Exercise 1): EVALUATE GENE FLOW BETWEEN THE EASTERN AND WESTERN PORTIONS OF THE GREATER SAGE-GROUSE’S RANGE.</td>
<td>Dave Smith</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Steve Morey</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jonathan Cummings</td>
</tr>
<tr>
<td>noon</td>
<td>Lunch</td>
<td></td>
</tr>
<tr>
<td>1:00 pm</td>
<td>Barriers to Gene Flow (Exercise 2): IDENTIFY POTENTIAL BARRIERS TO SAGE-GROUSE GENE FLOW, IF ANY</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barriers to Gene Flow (Exercise 3): EVALUATE THE RELATIVE GENE FLOW ACROSS THE POTENTIAL BARRIERS</td>
<td></td>
</tr>
<tr>
<td><strong>PART 3: GENETIC DIVERGENCE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4:00-5:00 pm</td>
<td>Genetic Divergence (Exercise 4): EVALUATE HOW THE LOSS OF PARTICULAR AREAS OF THE RANGE RESULT IN LOSS OF THE GENETIC DIVERSITY OF THE SPECIES? (E-W PORTIONS)</td>
<td></td>
</tr>
<tr>
<td>Day/time</td>
<td>Topic</td>
<td>Lead</td>
</tr>
<tr>
<td>----------</td>
<td>----------------------------------------------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>8:00 am</td>
<td>Logistics and check in</td>
<td>Craig Hansen</td>
</tr>
<tr>
<td></td>
<td>Genetic Divergence (continuation of Exercise 4, if needed)</td>
<td>Dave Smith</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Steve Morey</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jonathan Cummings</td>
</tr>
<tr>
<td></td>
<td><strong>PART 4: GENETIC DIVERGENCE AND PERSISTENCE</strong></td>
<td></td>
</tr>
<tr>
<td>10:00 am</td>
<td>Identify areas within greater sage-grouse range, if any, where low</td>
<td></td>
</tr>
<tr>
<td></td>
<td>genetic diversity or inbreeding depression is likely to affect</td>
<td></td>
</tr>
<tr>
<td></td>
<td>greater sage-grouse persistence.</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>PART 5: REFLECTION AND NEW STUDIES AND WRAP UP</strong></td>
<td></td>
</tr>
<tr>
<td>11:15 am</td>
<td>DISCUSS THE POTENTIAL IMPACT OF UPCOMING RESEARCH RESULT ON THE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OUTCOMES OF THIS WORKSHOP.</td>
<td></td>
</tr>
<tr>
<td>11:50 am</td>
<td>CLOSING</td>
<td>Craig Hansen</td>
</tr>
</tbody>
</table>
Appendix 2: Criteria to Identify and Invite Experts

The workshop planning team followed accepted best practices for eliciting information from experts to identify and invite experts (Burgman 2005). To ensure a transparent, unbiased capture of a broad diversity of expertise and judgments related to the topic selection process, the workshop planning team used the following criteria to identify and select experts:

Criteria to ensure that participants are scientific experts
Potential participants must meet all of the following criteria 1-3:

1. Participant holds a graduate degree in wildlife biology, zoology, genetics, or a related field.
2. Participant holds a research position in government, academia, or in the nonprofit research sector; or participant holds a governmental management agency position with responsibility for sage-grouse.
3. Participant has expertise in wildlife genetics or ecology of sage-grouse or lekking grouse species, demonstrated by recent (within the past 10 years) peer-reviewed publications and related types of professional scientific expression.
   - The science and techniques of genetics have advanced rapidly within the last decade, so we define recent as the past 10 years to ensure that participants are qualified and proficient in the latest genetic research and applications.

Criteria to ensure that participants are experts familiar with the workshop topic
Each participant must also meet one or more of the following criteria 4-6:

4. Participant is directly engaged in analysis of connectivity of sage-grouse populations or sage grouse habitat.
5. Participant is directly engaged in the study of sage-grouse population genetic structure.
6. Participant is a government or academic research scientist with expertise in conservation, population or landscape genetics, demonstrated by recent (within the past 10 years) peer-reviewed publications and related types of professional scientific expression.

Criteria to ensure that the group of invited participants represent the diversity of expert judgment within the scientific community
Consistent with best practices for an effective expert elicitation workshop, we recommend selecting nine or fewer participants. The basis for selection would be to ensure diverse and representative scientific judgment. We recommend expert selection be structured by stratifying on affiliation type (academia, government, NGO), specialty (population genetics, conservation or landscape genetics, evolutionary genetics), and taxa experience (GSG, related species, non-GSG). Then select participants to represent each strata.
# Appendix 3: Expert Identification and Invitation List

Table of experts, invitees, and their responses to the Genetics Expert Elicitation workshop.  
**GREEN** = RSVP Yes  **RED** = RSVP No  **WHITE** = No RSVP or Invitation

<table>
<thead>
<tr>
<th>EXPERT NAME</th>
<th>ORGANIZATION</th>
<th>CATEGORY</th>
<th>RSVP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mevin Hooten</td>
<td>Colorado Cooperative Fish and Wildlife Research Unit</td>
<td>1</td>
<td>Yes</td>
</tr>
<tr>
<td>Michael Schwartz</td>
<td>USFS</td>
<td>1</td>
<td>Yes</td>
</tr>
<tr>
<td>Sara Oyler-McCance</td>
<td>USGS</td>
<td>1</td>
<td>Yes</td>
</tr>
<tr>
<td>Mike Schroeder</td>
<td>Washington State Department of Fish and Wildlife</td>
<td>1</td>
<td>Yes</td>
</tr>
<tr>
<td>Christin Pruett</td>
<td>Florida Institute of Technology</td>
<td>2</td>
<td>Yes</td>
</tr>
<tr>
<td>Jesus Maldonado</td>
<td>Smithsonian Center for Conservation and Evolutionary Genetics</td>
<td>3</td>
<td>Yes</td>
</tr>
<tr>
<td>Chris Funk</td>
<td>Colorado State University</td>
<td>3</td>
<td>Yes</td>
</tr>
<tr>
<td>Robert Gibson</td>
<td>University of Nebraska – Lincoln</td>
<td>2</td>
<td>Yes</td>
</tr>
<tr>
<td>Jeffrey Olsen</td>
<td>USFWS Conservation Genetics Laboratory Alaska</td>
<td>3</td>
<td>Yes</td>
</tr>
<tr>
<td>Robert Zink</td>
<td>University of Minnesota</td>
<td>3</td>
<td>Initially agreed to participate, but later withdrew due to prior commitments</td>
</tr>
<tr>
<td>Steve Knick</td>
<td>USGS</td>
<td>1</td>
<td>No</td>
</tr>
<tr>
<td>Brad Fedy</td>
<td>Univ. of Waterloo</td>
<td>1</td>
<td>No</td>
</tr>
<tr>
<td>Lisette Waits</td>
<td>Univ. of Idaho</td>
<td>3</td>
<td>No</td>
</tr>
<tr>
<td>EXPERT NAME</td>
<td>ORGANIZATION</td>
<td>CATEGORY</td>
<td>RSVP</td>
</tr>
<tr>
<td>----------------</td>
<td>------------------------------------------------------------------------------</td>
<td>----------</td>
<td>------</td>
</tr>
<tr>
<td>J.L. Bouzat</td>
<td>University of Illinois at Urbana-Champaign</td>
<td>2</td>
<td>No</td>
</tr>
<tr>
<td>Andrew Martin</td>
<td>University of Colorado – Boulder</td>
<td>3</td>
<td>No</td>
</tr>
<tr>
<td>Rob Fleischer</td>
<td>Smithsonian Center for Conservation and Evolutionary Genetics</td>
<td>3</td>
<td>No</td>
</tr>
<tr>
<td>Melanie Murphy</td>
<td>University of Wyoming</td>
<td>2 or 3</td>
<td></td>
</tr>
<tr>
<td>Ephraim Hanks</td>
<td>Penn State</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Todd Cross</td>
<td>University of Montana</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sergei Drovetski</td>
<td>University of Alaska</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Tom Quinn</td>
<td>University of Denver</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Krissy Bird (Bush)</td>
<td>University of Alberta</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Christian Hagen</td>
<td>Oregon State University</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Sue Haig</td>
<td>Oregon State University</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Ron Van Den Bussche</td>
<td>Oklahoma State University</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
Appendix 4: Pre-Workshop Webinars and Workshop Ground Rules
The following slides were used during webinars to prepare the experts before the workshop.

Workshop on the Genetics of the Greater Sage-Grouse

October 22-23, 2014
Fort Collins, CO

Pre-workshop webinar

Call in: 866-704-5568 (9453-5433#)

- USGS Fort Collins Science Center
  - Wednesday October 22, 8:00 AM – 5:00 PM and Thursday October 23, 8:00 AM – 12:00 PM
- Experts
  - Chris Funk, Colorado State University
  - Robert Gibson, Univ of Nebraska – Lincoln
  - Mevin Hooten, USGS CO CF&WRU
  - Jesus Maldonado, Smithsonian Center
  - Jeff Olsen, US Fish and Wildlife Service
  - Sara Oyler-McCance, USGS FC Science Center
  - Christin Pruett, Florida Institute of Technology
  - Mike Schroeder, Washington Dept of Fish and Wildlife
  - Mike Schwartz, Univ of Montana and USFS
  - Robert Zink, Univ of Minnesota
- Organizers: Craig Hansen and FWS Team
- Facilitators: Dave Smith (USGS) and Steve Morey (FWS)
Purpose

- Genetic differences exist among individuals and populations across the range of the greater sage-grouse; however, the degree to which these genetic differences create distinct or markedly isolated conservation units is largely unclear.
- Additionally, the techniques and metrics used to analyze genetic isolation or divergence may have changed or improved since previous studies were published in 2005.
- Therefore, this workshop will investigate questions about potential patterns in and impediments to gene flow across the species’ range, and about the magnitude and source of such impediments, or potential barriers to gene flow.
- Consequently, the workshop will also help the Service identify potential conservation units within the range of the sage-grouse.

Approach

- The expert elicitation workshop will bring together a diverse body of scientific experts to share and discuss their individual opinions regarding genetics, conservation genetics, and the greater sage-grouse.
- The elicitation will involve facilitated discussion and formal elicitation on specific technical questions.
- The Service is not seeking consensus from this workshop. The workshop will obtain facts and information, and if needed, advice and opinion from each individual expert.
- Information will be discussed and exchanged, and individual opinions informed by professional judgment will be discussed and compiled.
Draft Agenda

• Wednesday
  – Overview of greater sage-grouse genetics (Sara Oyler-McCance)
  – Discussion topics
    • Methods and metrics for evaluating evidence of genetic divergence
    • Evidence of genetic divergence, or lack thereof, for each sage-grouse population or population group in question using the appropriate criteria or metrics. Include assessment of uncertainty.
    • Evidence for barriers to gene flow within and across the range of greater sage-grouse: Source, type (natural or anthropogenic), age, and permeability of each identified barrier that may restrict or prevent gene flow. Include assessment of uncertainty.

Draft Agenda

• Thursday
  – Discussion topics
    • Continue discussion of genetic divergence and barriers to gene flow
    • Areas within greater sage-grouse range, if any, where low genetic diversity or inbreeding depression would affect persistence. Include assessment of uncertainty.
    • Genetic mechanisms or processes that would cause threats to have an increased or magnified effect on persistence.
Ground Rules  
(based on ESA, FACA, and APA)

- The Service is seeking the best available scientific information. The agenda will be designed to focus the discussion on scientific and technical questions.
- Participants were invited to share their scientific expertise as an individual. Group debate is encouraged, but the Service does not seek consensus or group advice.
- The Service may also ask participants to complete a questionnaire to evaluate and document any potential conflicts of interest.
- The discussion will not involve the determinations that the Service is charged with making under the ESA.
- Participants should follow standard norms of behavior for effective meetings. We will facilitate an open, inclusive, and science-based discussion characterized by respectful, orderly dialogue.

Products

- Information provided by meeting participants through the course of expert meetings will be documented in meeting notes and a written summary report by the Service including results of any specific elicitations.
- Notes and summary will be circulated for review and comment.
- The notes and corresponding report will become one part of the administrative record for use in agency decision-making.
- The meeting documentation will summarize the biological and other scientific information gained during the meeting.
Background

• Bibliography with genetics-themed citations for the greater sage-grouse
  — *Bibliography will be distributed*
  — Annotated to note scale, metrics, classification, variables, and criteria

• Participants were invited based on existing knowledge. The bibliography is provided for preparation as time and interest permits.

Logistics

• Latest update on travel support...

• The meeting will be held at the USGS Fort Collins Science Center, 2150 Centre Avenue, Building C, Fort Collins. See detailed directions at: [https://www.fort.usgs.gov/about/directions](https://www.fort.usgs.gov/about/directions). You will need to see the receptionist on arrival to sign in. Please bring a photo ID.

• To get to Fort Collins from DIA:

• Hotel (0.6 mile north of Fort Collins Science Center):
  — The hotel block has expired, but you can still call to check availability
  — Hilton Fort Collins, 425 West Prospect Road, Fort Collins

• Jason’s Deli will deliver lunch on Wednesday and Thursday. Lunch delivery is optional. Order online. Instructions in the Travel Information Sheet.
Q & A
Introduction

The U.S. Fish and Wildlife Service (Service) and the U.S. Geological Survey (USGS) invite you to participate in a workshop for scientific experts to address the genetics of the greater sage-grouse. This meeting, scheduled for October 22 and 23, 2014, at the Fort Collins Science Center in Fort Collins, Colorado, is part of the Service’s process for collecting the best available scientific information for its status review of the greater sage-grouse across its range. This status review will inform the Service’s decision on whether to propose the species for listing under the Endangered Species Act of 1973 (ESA), as amended (16 U.S.C. 1531 et seq.).

An important aspect of this meeting is ensuring that it complies with the Federal Advisory Committee Act (FACA) and the Administrative Procedure Act (APA). The ground rules stated below are intended to serve this purpose. These ground rules are also intended to ensure that the meeting focuses on providing the Service with the best available scientific information for its status review and listing decision under the ESA.

Principles Underlying These Ground Rules

**ESA:** Under the ESA, the Service is required to determine whether to list a species based consideration of the five factors set out in the statute, based solely on the best scientific and commercial data available to it. As described above, this meeting is part of the Service’s effort to collect the best available scientific information for this listing determination. Thus, the Service is not asking for input from participants on the determinations that are the Service’s responsibility under the ESA. Instead, the Service is only seeking expert, unbiased input on the questions the Service has provided you in order to collect scientific information that Service biologists will use in assessing the species’ status and formulating recommended determinations under the ESA. The information the Service receives through the expert meeting will be one source of information, among other sources, that it Service will consider its listing determination under the ESA.

**FACA:** The purpose of the Federal Advisory Committee Act (FACA) is to ensure that advice provided to the Executive Branch of the Federal government by advisory committees is

---

2 This status review does not include the Bi-State Distinct Population of the Greater Sage-Grouse, which is the subject of a separate status review and listing process. See generally [78 FR 64357](http://www.gpo.gov/fdsys/pkg/FR-2013-01-24/pdf/2013-04934.pdf).
transparent and accessible to the public. To achieve this purpose, FACA mandates a process for establishing, operating, overseeing, and terminating advisory bodies that are covered by the Act Groups that are assembled to provide individual advice to a Federal agency, rather than advice from the group as a whole, are not covered by FACA and hence are not required to comply with FACA’s procedural and other requirements. The same is true of groups assembled to exchange facts and information with a Federal agency. The meeting you will be attending will be structured and conducted to meet both of these criteria: The primary purpose of the meeting will be to exchange facts and information and, to the extent any advice may be sought on biological or other questions relevant to the Service’s status review, that advice will only be sought on an individual basis, not from the group as a whole. Maintaining these principles is critical to ensuring that the meeting does not violate FACA’s requirements for advisory committees.

APA: The APA requires Federal agencies to maintain and make available to the public for review all information used by them in developing a proposed rule, including a proposed rule to list a species under the ESA. The APA also mandates that judicial review of any final Federal decision be based on the administrative record that includes all records presented to or used by the Federal agency in making the challenged decision. As a result of both of these APA requirements, the Service must maintain open, public records to document its decision-making process regarding the greater sage-grouse. Therefore, information gained via this meeting will be documented and will be subject to public disclosure through the processes just described. This documentation will also be subject to release upon request under the Freedom of Information Act (FOIA).

Ground Rules

1. The Service is only seeking the best available scientific information from the experts participating in this meeting.

2. The Service has developed a meeting agenda that, among other things, identifies the scientific questions on which the Service seeks the scientific expertise of the meeting participants. The meeting participants should focus their participation in the meeting to providing information that is responsive to these questions.

3. The Service invited the individuals participating in this expert meetings based solely on their scientific qualifications, rather than as a representative of a particular organization or interested party. Accordingly, the participants should share their scientific expertise during the meeting and not seek to represent any particular position of an agency, their employer, or other interested party.

4. Participants should fully disclose to the Service and all other participants any potential conflict of interest (such as a commercial stake) in the Service’s listing determination regarding the greater sage-grouse. The Service may also ask participants to complete a questionnaire to evaluate and document any potential conflicts of interest.
5. The Service is not asking for and will not accept input on the determinations it is charged with making under the ESA. The Service retains full control over the ESA determination and no pre-decisional assumptions of those determinations will be discussed.

6. Participants should follow standard norms of behavior for effective meetings so that the Service can receive the information it seeks from all participants. The Service encourages an open, inclusive, and science-based discussion characterized by respectful, orderly dialogue.

7. Any opinion from any participants in this meeting will be provided on an individual basis and not from the group as a whole. Group debate is encouraged, but the Service does not seek consensus or group advice on issues to be discussed, and such consensus or group advice should not be provided.

8. Information provided by meeting participants through the course of expert meetings will be documented in meeting notes and a written summary report by the Service. The notes and corresponding report will become part of the administrative record for use in agency decision-making. The meeting documentation will summarize the biological and other scientific information gained during the meeting. Consistent with the preceding ground rule limiting advice provided to individual advice only, this report will document any advice provided by a meeting participant on an individual basis.

9. Conference calls or other communications between the Service and meeting participants may occur after the meeting to clarify, for documentation purposes, the information discussed at the meeting. Any such communications will also be subject to and follow these ground rules.
Appendix 5: Workshop Bibliography

Background References – Greater Sage-grouse Genetics Workshop
October 22 and 23, 2014
USGS Fort Collins Science Center
Fort Collins, Colorado
UPDATED OCTOBER 14, 2014


Abstract

Population sex ratio is an important metric for wildlife management and conservation, but estimates can be difficult to obtain, particularly for sexually monomorphic species or for species that differ in detection probability between the sexes. Noninvasive genetic sampling (NGS) using polymerase chain reaction (PCR) has become a common method for identifying sex from sources such as hair, feathers or faeces, and is a potential source for estimating sex ratio. If, however, PCR success is sex-biased, naively using NGS could lead to a biased sex ratio estimator. We measured PCR success rates and error rates for amplifying the W and Z chromosomes from greater sage-grouse (Centrocercus urophasianus) faecal samples, examined how success and error rates for sex identification changed in response to faecal sample exposure time, and used simulation models to evaluate precision and bias of three sex assignment criteria for estimating population sex ratio with variable sample sizes and levels of PCR replication. We found PCR success rates were higher for females than males and that choice of sex assignment criteria influenced the bias and precision of corresponding sex ratio estimates. Our simulations demonstrate the importance of considering the interplay between the sex bias of PCR success, number of genotyping replicates, sample size, true population sex ratio and accuracy of assignment rules for designing future studies. Our results suggest that using faecal DNA for estimating the sex ratio of sage-grouse populations has great potential and, with minor adaptations and similar marker evaluations, should be applicable to numerous species.

Abstract

The status of Sage-grouse (*Centrocercus urophasianus*) is of increasing concern, as populations throughout its range have contracted as a result of habitat loss and degradation. Historically, Sage-grouse were classified into two subspecies: eastern (*C. u. urophasianus*) and western Sage-grouse (*C. u. phaios*) based on slight differences in coloration noted among eight individuals sampled from Washington, Oregon, and California. We sequenced a rapidly evolving portion of the mitochondrial control region in 332 birds from 16 populations. Although our sampling area covers the proposed boundary between the eastern and western subspecies, no genetic evidence to support the delineation of these subspecies was found. However, a population straddling southwestern Nevada and eastern California was found to contain an unusually high proportion of unique haplotypes, consistent with its genetic isolation from other Sage-grouse populations. Of additional interest was the lack of diversity in the two populations sampled from Washington, one of which contained only a single haplotype. We suggest that multiple lines of evidence are valuable for the formulation of conservation strategies and hence the southwestern Nevada/eastern California population merits further morphological, behavioral, and molecular investigation.


http://beheco.oxfordjournals.org/content/24/1/29.full.pdf+html

Abstract

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

Population declines of Greater Sage-Grouse (*Centrocercus urophasianus*) throughout the western United States have been attributed to the loss, degradation, and fragmentation of sagebrush (*Artemisia* spp.) habitats. Increased energy development may further fragment sagebrush habitat, isolating sage-grouse populations and resulting in genetic drift, inbreeding, local extinction, or rapid divergence. We conducted a genetic survey of 3 remote sagegrouse populations in northeastern Utah to assess mitochondrial diversity relative to other portions of the species’ range. We did not detect any unusual haplotype compositions in these populations. However, haplotype composition of the Anthro Mountain and Strawberry Valley reference populations differed from haplotype compositions of other northeastern Utah populations. These populations are spatially separated by Desolation Canyon of the Green River. This canyon may constitute a geographic barrier to gene flow in this area, given low population densities and reduced dispersal potentials. This potential barrier will be an important consideration in future conservation efforts such as translocations. The halotype composition of the Anthro Mountain and Strawberry Valley reference populations may be altered by translocations subsequent to our sampling effort. The effect of these translocations on the reference halotypes and population vital rates is currently under study.

Abstract

Range-edge dynamics and anthropogenic fragmentation are expected to impact patterns of genetic diversity, and understanding the influence of both factors is important for effective conservation of threatened wildlife species. To examine these factors, we sampled greater sage-grouse (*Centrocercus urophasianus*) from a declining, fragmented region at the northern periphery of the species’ range and from a stable, contiguous core region. We genotyped 2,519 individuals at 13 microsatellite loci from 104 leks in Alberta, Saskatchewan, Montana, and Wyoming. Birds from northern Montana, Alberta, and Saskatchewan were identified as a single population that exhibited significant isolation by distance, with the Milk River demarcating two subpopulations. Both subpopulations exhibited high genetic diversity with no evidence that...
peripheral regions were genetically depauperate or highly structured. However, river valleys and a large agricultural region were significant barriers to dispersal. Leks were also composed primarily of non-kin, rejecting the idea that leks form because of male kin association. Northern Montana sage-grouse are maintaining genetic connectivity in fragmented and northern peripheral habitats via dispersal through and around various forms of fragmentation.


Abstract

Endangered species are sensitive to the genetic effects of fragmentation, small population size, and inbreeding, so effective management requires a thorough understanding of their breeding systems and genetic diversity. The Greater Sage-Grouse (Centrocercus urophasianus) is a lekking species that has declined by 66–92% during the past 35 years in Alberta. Our goals were to assess the genetic diversity of Greater Sage-Grouse in Alberta and to determine the degree of sex-specific relatedness within and among leks. Six hundred and four individuals sampled in 1998–2007 were genotyped at 13 microsatellite loci. Levels of genetic diversity were high, with the exception of one recently founded lek, and did not change over time. Overall, we did not observe isolation-by-distance among leks, and most leks were not differentiated from one another, which suggests that gene flow occurs across the study area. Males and females exhibited similar patterns of isolation-by-distance, so dispersal was not sex-specific. Overall relatedness was close to zero for both sexes at the level of the province, lek, and year, which suggests that neither sex forms strong kin associations. However, we found relatedness within leks at the year level to be greater than zero, which indicates interannual variation. We also found no evidence that Greater Sage-Grouse follow the typical avian pattern of male philopatry. Although the species is endangered in Alberta and occurs in fragmented habitat, it has maintained genetic diversity and connectivity.


[No abstract]

[Contact author for a .pdf: dawn_davis@fws.gov]

Abstract (excerpt)

To evaluate the population genetic structure of greater sage-grouse a total of 167 blood samples collected from birds captured on 13 known, active leks and 20 blood samples from birds captured off-lek during the autumn were genotyped at 19 microsatellite loci. Although greater sage-grouse populations are declining and have lost a significant portion of their range in northeastern California, the species in my study exhibited high genetic diversity. I observed no population structure and despite population declines and habitat loss, leks in northeastern California were not differentiated. My results showed significant isolation-by-distance among males, suggesting that male greater sage-grouse are the more philopatric sex and females have a greater predisposition to disperse. Furthermore, spatial autocorrelation analysis revealed stronger spatial structuring for males than for females. The results from the corrected Assignment Index (AIC) also confirmed female-biased dispersal, although difference between genders was not significant. Although greater sage-grouse in northeastern California have maintained gene flow across the sampled region and have tolerated some degree of habitat fragmentation and deterioration without losing genetic diversity, continued habitat loss could result in small, isolated greater sage-grouse populations at risk of losing genetic variation. Thus, improving habitat quality and connectivity of greater sage-grouse habitats in northeastern California is critical for maintaining gene flow and is important for the long-term persistence of greater sage-grouse occurring on the geographic periphery of the species’ distribution.


Abstract

The spectacular social courtship displays of lekking birds are thought to evolve via sexual selection, but this view does not easily explain the participation of many males that apparently fail to mate. One of several proposed solutions to this ‘lek skew paradox’ is that kin selection favours low-ranking males joining leks to increase the fitness of closely related breeders. We investigated the potential for kin selection to operate in leks of the greater sage grouse, Centrocercus urophasianus, by estimating relatedness between lekking males using microsatellite DNA markers. We also calibrated these estimates using data from known families. Mean relatedness within leks was statistically indistinguishable from zero. We also found no
evidence for local clustering of kin during lek display, although males tended to range closer to kin when off the lek. These results make kin selection an unlikely solution to the lek skew paradox in sage grouse. Together with other recent studies, they also raise the question of why kin selection apparently promotes social courtship in some lekking species, but not in others.


**Abstract**

Greater sage-grouse (*Centrocercus urophasianus*) within the Bi-State Management Zone (area along the border between Nevada and California) are geographically isolated on the southwestern edge of the species’ range. Previous research demonstrated that this population is genetically unique, with a high proportion of unique mitochondrial DNA (mtDNA) haplotypes and with significant differences in microsatellite allele frequencies compared to populations across the species’ range. As a result, this population was considered a distinct population segment (DPS) and was recently proposed for listing as threatened under the U.S. Endangered Species Act. A more comprehensive understanding of the boundaries of this genetically unique population (where the Bi-State population begins) and an examination of genetic structure within the Bi-State is needed to help guide effective management decisions. We collected DNA from eight sampling locales within the Bi-State (*N* = 181) and compared those samples to previously collected DNA from the two most proximal populations outside of the Bi-State DPS, generating mtDNA sequence data and amplifying 15 nuclear microsatellites. Both mtDNA and microsatellite analyses support the idea that the Bi-State DPS represents a genetically unique population, which has likely been separated for thousands of years. Seven mtDNA haplotypes were found exclusively in the Bi-State population and represented 73% of individuals, while three haplotypes were shared with neighboring populations. In the microsatellite analyses both STRUCTURE and FCA separate the Bi-State from the neighboring populations. We also found genetic structure within the Bi-State as both types of data revealed differences between the northern and southern part of the Bi-State and there was evidence of isolation-by-distance. STRUCTURE revealed three subpopulations within the Bi-State consisting of the northern Pine Nut Mountains (PNa), mid Bi-State, and White Mountains (WM) following a north–south gradient. This genetic subdivision within the Bi-State is likely the result of habitat loss and fragmentation that has been exacerbated by recent human activities and the encroachment of singleleaf pinyon (*Pinus monophylla*) and juniper (*Juniperus* spp.) trees. While genetic concerns may be only one of many priorities for the conservation and management of the Bi-State greater sage-grouse, we believe that they warrant attention along with other issues (e.g., quality of sagebrush habitat, preventing future loss of habitat). Management actions that promote genetic connectivity, especially with respect to WM and PNa, may be critical to the long-term viability of the Bi-State DPS.


Abstract

Recent research on Greater Sage-Grouse (*Centrocercus urophasianus*) genetics has revealed some important findings. First, multiple paternity in broods is more prevalent than previously thought, and leks do not comprise kin groups. Second, the Greater Sage-Grouse is genetically distinct from the congeneric Gunnison Sage Grouse (*C. minimus*). Third, the Lyon-Mono population in the Mono Basin, spanning the border between Nevada and California, has unique genetic characteristics. Fourth, the previous delineation of western (*C. u. phaios*) and eastern Greater Sage-Grouse (*C. u. urophasianus*) is not supported genetically. Fifth, two isolated populations in Washington show indications that genetic diversity has been lost due to population declines and isolation.


Abstract

The distribution and abundance of the greater sage-grouse (*Centrocercus urophasianus*) have declined dramatically, and as a result the species has become the focus of conservation efforts. We conducted a range-wide genetic survey of the species which included 46 populations and over 1000 individuals using both mitochondrial sequence data and data from seven nuclear microsatellites. Nested clade and analyses revealed that, in general, the greater sage-grouse populations follow an isolation-by-distance model of restricted gene flow. This suggests that movements of the greater sage-grouse are typically among neighbouring populations and not across the species, range. This may have important implications if management is considering translocations as they should involve neighbouring rather than distant populations to preserve any effects of local adaptation. We identified two populations in Washington with low levels of genetic variation that reflect severe habitat loss and dramatic population decline. Managers of these populations may consider augmentation from geographically close populations. One population (Lyon/Mono) on the southwestern edge of the species’ range appears to have been isolated from all other greater sage-grouse populations. This population is sufficiently genetically distinct that it warrants protection and management as a separate unit. The genetic data
presented here, in conjunction with large-scale demographic and habitat data, will provide an integrated approach to conservation efforts for the greater sage-grouse.


http://www.jstor.org/stable/3803733

Abstract

The newly described Gunnison sage-grouse (*Centrocercus minimus*) is a species of concern for management because of marked declines in distribution and abundance due to the loss and fragmentation of sagebrush habitat. This has caused remaining populations to be unusually small and isolated. We utilized mitochondrial DNA sequence data and data from 8 nuclear microsatellites to assess the extent of population subdivision among Gunnison sage-grouse populations in southwestern Colorado and southeastern Utah, USA. We found a high degree of population structure and low amounts of gene flow among all pairs of populations except the geographically adjacent Gunnison and Curecanti populations. Population structure for Gunnison sage-grouse was significantly higher than has been reported for greater sage-grouse (*C. urophasianus*). Further, we documented low levels of genetic diversity in some populations (particularly Dove Creek/Monticello and Pifion Mesa with an average of only 3.00 and 2.13 alleles per locus respectively) indicating that translocations from larger, more genetically diverse populations may be warranted. Bayesian analysis identified 3 potential migrants (involving San Miguel, Dove Creek/Monticello, Crawford, and Curecanti). Further, this analysis showed that 4 individuals from Cerro/Cimarron were more closely related to birds from San Miguel than to its geographically closer neighbors Gunnison and Curecanti. This suggests the Cerro/Cimarron area may act as a stepping stone for gene flow between San Miguel and Gunnison and that habitat restoration and protection in areas between these 2 basins should be a priority in an attempt to facilitate natural movement among these populations. Conservation plans should include monitoring and maintaining genetic diversity, preventing future habitat loss and fragmentation, enhancing existing habitat, and restoring converted sagebrush communities.


Abstract only:

Sage grouse *Centrocercus urophasianus* are a classic example of a lek-mating species, and are becoming yet another example of a species encountering dramatic human-induced changes to its environment. A study of the patterns of genetic variation across the range of both subspecies
and including the unique small-bodied birds in the Gunnison Basin, Colorado was done by sequencing 141 bp or more within region I of the mitochondrial control region. Within Colorado, the same haplotype was present in 31/32 (97%) of the small-bodied birds surveyed in the Gunnison Basin whereas a wider variety of haplotypes was found at each of the five surveyed locales within the range of the large-bodied birds (132 individuals). The predominant haplotype within the Gunnison Basin is at a frequency of 97%, but elsewhere that haplotype is at a frequency of less than 20%. This, and the observation that other haplotypes which predominate in large-bodied birds are not present in the Gunnison Basin provides evidence there has been virtually no recent gene flow into the Gunnison population from large-bodied sage grouse found elsewhere in Colorado. Preliminary sampling from across the western USA revealed similarities across the range among large-bodied birds in that three haplotypes are shared throughout, but there was also a subset of haplotypes that was more localized in distribution and, hence, potentially more informative for defining population subdivision.

Schroeder, M.A. 2008. Variation in greater sage-grouse morphology by region and population. Washington Department of Fish and Wildlife, Bridgeport, WA.  

Abstract

The greater sage-grouse (Centrocercus urophasianus) is wildlife species with important conservation and management considerations in western North America. Research on sage-grouse has established their historical and current distribution (Schroeder et al. 2004) as well as providing an assessment of their populations and habitats (Connelly et al. 2004). This research helped define the presence of 41 distinct populations (Fig. 1) based on the Berryman’s (2002) definition of a population; “as a group of individuals of the same species that live together in an area of sufficient size to permit normal dispersal and/or migration behavior and in which numerical changes are largely determined by birth and death processes.

In addition to defining populations, research has provided an evaluation of the rangewide genetics of sage-grouse with an assessment of potential issues associated with population size, population connectivity, and sub-specific variation (Young 1994; Young et al. 1994; Oyler-McCance et al. 1999, 2005). Although research helped establish the Gunnison sage-grouse as a behaviorally and morphologically distinct species (Young et al. 1994), similar research within the remaining populations of greater sage-grouse has been somewhat limited (Oyler-McCance et al. 2005, Taylor and Young 2006). Oyler-McCance et al. (2005) showed that sage-grouse displayed relatively integrated genetics across their range with notable exceptions being the Moses Coulee, N Mono Basin, and S Mono Basin populations.

The initial objective of this research was to examine the availability of previously collected morphological and behavioral data from both published and unpublished sources. Although the collection of additional data would certainly aid in this process, this was not an objective for this initial phase of research. The overall goal of the research was to acquire and examine data with reference to variation associated with region, population, and/or previously established genetic
characteristics. Because of the nature of this type of data (rarely published), it was believed that this initial report effort would be ‘preliminary’ and that analysis would continue as additional data was collected and/or acquired.


http://www.eenews.net/assets/2012/03/28/document_gw_01.pdf

Executive Summary

Impacts from energy development to sage-grouse (*Centrocercus urophasianus*) present a challenge to public land managers tasked with maintaining large and intact landscapes that support viable populations. We provide decision support to Bureau of Land Management (BLM) managers tasked with maintaining sage-grouse populations in the oil and gas (energy) fields of northeast Wyoming by assessing four aspects of energy development as they relate to sage-grouse. Findings reflect the status of a small remaining sage-grouse population that has already experienced an 82% decline within the expansive energy fields (Walker et al. 2007a), a level of impact that has severely reduced options for delineating core areas that are large enough and in high enough quality habitats to sustain populations.

1) We identified the spatial scale at which energy development most influences sage-grouse populations, as indexed by counts of males at leks. Ignoring state boundaries to include counts from unimpacted leks in Montana was critical to identifying the far reaching impacts of development on grouse in Wyoming. Using a statistical technique (AIC, Burnham and Anderson 1998) that is akin to using a dial to tune a radio to pick up the strongest signal, we detected that development had the greatest influence on male counts within 12.4 mi (20 km) surrounding a lek. The signal is much stronger at the 12.4-mi radius than any of the smaller radii we tested, encompassing a large spatial scale that covers an area of 483 1,257 km².

2) We evaluated the current viability of sage-grouse populations. We linked lek count data to energy development and West Nile virus (WNv) by associating the density of producing wells within the 12.4-mi (20-km) radius to each lek count, and the occurrence of widespread WNv outbreaks in the year preceding the count. Predictions of resulting male lek counts were consistently within 0 to 1% of the observed counts, underscoring the success of our approach (Table 2).

Declines in number of active leks and number of attending males indicate that both energy development and WNv outbreaks reduce sage-grouse populations. At current well spacing (328 ac spacing, 0.75 wells/km²) and without accounting for WNv outbreaks, our predicted number of males on leks is 3,648 (95% CIs = 3,147, 4,204, Table 3). Absent an outbreak year, the lower 95% confidence limit on the count is 3,147 males, suggesting that immediate extirpation of the northeast Wyoming population is unlikely if all environmental conditions for sage-grouse other than energy development remain favorable.

Wyoming’s core area policy will be most effective where implemented in advance of extensive energy development, and in southwest portions of the state where high elevation populations are less susceptible to WNv impacts. But in northeast Wyoming, WNv outbreak years are the
wild card in core area management, and predictions made without accounting for WNv are
optimistically high. The effect of a WNv outbreak year alone can more than cut a population in
half, which is similar to drilling an undeveloped landscape at 4-8 wells/mi² (1.5-3.1 wells/km²,
Table 3). When we include impacts of an outbreak year at all leks, we predict 1,473 males given
current well densities (Table 3). With outbreak years as part of the equation, the lower
confidence limit on our prediction is 65 males, which, if reached, would indicate functional
extinction. Incorporating outbreaks into analyses suggests that even with no additional energy
development many local populations may be one bad WNv year away from extirpation.
3) We formulated and simulated potential, realistic future management scenarios for sage-
grouse populations, using the models we developed above to evaluate viability. Our results
suggest that if development continues, future viability of the already small sage-grouse
populations in northeast Wyoming will be compromised. Small populations are vulnerable to
extirpation by chance events (Soule and Mills 1998), and WNv outbreaks are an excellent
example of this type of catastrophic event. Despite impacts, the potential may still exist to
maintain a population inside core areas, but further drilling in and around cores will compromise
their remaining value. Notably, core areas in northeast Wyoming were delineated after
widespread development had already occurred, leaving few options for conserving populations.
Our findings do not negate the benefits of core areas, in general. However, to achieve maximum
effectiveness, core areas must be constructed proactively by conserving high quality habitat, not
reactively by drawing borders around planned and existing development.
4) We provide recommendations for evaluating the future viability of sage-grouse populations if
restoration efforts begin as the energy play subsides. First and foremost we recommend that
BLM commit to monitoring outcomes of restoration as measured by the distribution and number
of sage-grouse in northeast Wyoming. Focusing restoration where plugged and abandoned
wells are clustered would increase the size of habitats available to birds, thus enhancing the
chance of increasing their abundance and distribution. Leaving energy infrastructure such as
roads, power lines, and water impoundments on the landscape for other purposes is an
unintended impact of development that will compromise restoration success. Appropriate
monitoring of leks as wells are removed from production is imperative to allow for a rigorous
analysis of restoration success, which cannot be supported by currently available data. Lek
counting needs to be conducted at least across the area of northeast Wyoming, and preferably
across the entire study region including eastern Montana (Fig 1).
Genetic connectivity is the glue that holds populations together, and remaining core areas,
though impacted, may help maintain connectivity among populations further south in Wyoming
and those in Montana. Until genetic studies currently underway delineate the degree to which
sage-grouse populations are connected, we recommend maintaining the potential areas of
connectivity outlined in the Wyoming Governor’s Executive Order as undeveloped, contiguous
habitat.

microsatellite loci in Greater Sage-Grouse (Centrocercus urophasianus). Molecular Ecology
Notes 3(2):262-264.
Abstract

Primers for five polymorphic microsatellite loci were developed for Greater Sage-Grouse (Centrocercus urophasianus) using an enrichment/detection protocol. The high level of polymorphism (nine to 33 alleles) suggests that these loci will be applicable for investigating mating systems and paternity analysis as well as population genetics. Cross-species amplification was successful for each locus in at least two other galliform species.


[This link likely not available outside of DOI]

https://fishnet.fws.doi.net/projects/home/NSGC/Literature/Tebbenkamp,%20JM.%202014,%20Greater%20sage-grouse%20in%20the%20bi-state%20DPS-%20an%20evaluation.pdf

Abstract

Because isolation may imperil populations, maintaining demographic and genetic connectivity is a high priority for conservation and management. One population of conservation concern due to its high degree of isolation is the Bi-State Distinct Population Segment (DPS) of greater sage-grouse (Centrocercus urophasianus). Located on the periphery of their range, along the California-Nevada border, the Bi-State DPS is genetically isolated from other populations. Further, telemetry data suggest there is additional subdivision within the Bi-State DPS. Here, we combine telemetry and genetic data to investigate both demographic and genetic structuring within the Mono County, California portion of the Bi-State DPS. From 2007-2012, we radio-marked and monitored 122 greater sage-grouse and collected genetic data at 17 microsatellite loci for 334 individuals. Pairwise FST estimates (mean = 0.146, range = 0.090-0.205) along with 2 Bayesian clustering methods provided evidence for 5 genetic populations. We did not document any dispersal events between populations using radio-, however, using 4 genetic assignment methods found 10 individuals were likely recent dispersers. Combined, these data show that there is both demographic and genetic subdivision within the Bi-State DPS, and while demographic support between populations is unlikely due to the low number of dispersers, these infrequent dispersal events are capable of preventing genetic isolation. Thus, effective conservation of the Bi-State DPS will require maintaining genetic connectivity while also attending to demographic processes of each population.
ABSTRACT

The greater sage-grouse (Centrocercus urophasianus; hereafter sage-grouse) has undergone dramatic population declines over the last 25 years as a result of loss, fragmentation, and degradation of sagebrush (Artemisia tridentata spp.) habitats on which it depends. Because of these declines and the subsequent loss of habitat, knowledge concerning the juvenile ecology of sage-grouse, including natal dispersal patterns and abilities and its influences on population persistence, colonization, and connectivity are critical for the conservation planning and management of this species. The focus of this dissertation was two-fold: first, to assess the feasibility of actively collecting and hatching sage-grouse eggs from wild radiomarked sage-grouse and rearing subsequent domestically-hatched (DH) chicks from 1-10 days of age before augmenting wild sage-grouse broods (Chapter 2), and second to investigate natal dispersal in greater sage-grouse through both demographic (radio telemetry) and genetic methods. In Chapter 3, I monitored survival and causes of mortality in wild-hatched chicks (n = 431) in wild broods (n = 115) from hatch to 16 weeks of age in the Axial Basin and Cold Springs Mountain study areas in northwestern Colorado, 2005-2007 and evaluated potentially important predictors of brood and chick survival. In addition, I monitored survival from hatch to 16 weeks of age for a cohort of DH chicks raised to 1-10 days of age in captivity (n = 116) and introduced into a subset of wild broods during this same time period. Model averaged estimates of brood and chick survival indicated that survival varied both temporally and spatially. In Chapter 4, I captured, radiomarked, and monitored survival and recruitment of 183 transmitter-equipped juveniles (from Chapter 3) from 1 September – 31 March. Survival from September through March was similar for all juveniles, but varied by month, study area, and gender. Median dispersal distance was greater for juvenile males compared to females (M: 3.84 + 1.26 km; F: 2.68 + 0.30 km), as well as the proportion dispersing > 5 km (M: 31.6%; F: 15.5%). In Chapter 5, I examined the patterns of dispersal, gene flow, and genetic structure at 15 leks in 6 population management zones (PMZs). Genetic analyses were largely congruent and suggested that gene flow followed an isolation-by-distance pattern, and supported male-biased dispersal findings based on demographic data (Chapter 4). Finally, in Chapter 6, I investigated how coarse-grained landscape characteristics influenced dispersal and settlement patterns. Landscape metrics primarily differed between study areas rather than genders, and among pre-dispersal, winter, and post-dispersal landscapes. Effect of extent upon analyses depended upon the specific metric and landscape.

Abstract

The greater sage-grouse (Centrocercus urophasianus) was once widespread in western North America but its range has contracted by an uncertain degree owing to anthropogenic and natural causes. Concern over population declines has led to its proposed listing as threatened under the U.S. Endangered Species Act. Detailed genetic and demographic analyses of this species throughout its range are available but heretofore have not been compared. Reduced genetic variability is often taken as a proxy for declining populations, but rarely are there quantitative population estimates with which to compare. I compared published mitochondrial DNA (mtDNA) control region sequences, microsatellite allele frequencies at seven loci, and estimates of numbers of males per lek, number of active leks, percent decline in the best population models, and the probability (P) of Ne < 50 in 30 years and P(Ne < 500) in 100 years, at two spatial scales, 45 local population samples and 16 larger aggregates of samples. When excluding the populations from the Columbia Basin, which show little genetic diversity and are statistical outliers, there were no consistent relationships between estimates of genetic variation and demographic trends across the remainder of the range at either spatial scale. A measure of inbreeding derived from microsatellite data was also not related to population trends. Thus, despite habitat reduction and range fragmentation, the greater sage-grouse does not exhibit expected genetic signatures of declining populations. Possibly, the mtDNA and microsatellite data are insufficiently sensitive to detect population declines that have occurred over the span of a half century. Alternatively, only when populations are reduced to the levels seen in the Columbia Basin will genetic effects be seen, suggesting that the bulk of the range of the greater sage-grouse is not currently in genetic peril.

Additions October 14, 2014


http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1224&context=bioscifacpub&sei-redir=1&referer=http%3A%2F%2Fscholar.google.com%2Fscholar%3Fhl%3Den%26q%3DStiver%262008%26Polygyny%2626female%2626Breeding%26Failure%26Reduce%26Effective%262Bpopulation%262BSizes%262Bin%262BThe%262BLekking%262BGunnison%262Bsage%2626grouse%2626Female%2626Breeding%26Failure%26Reduce%26Effective%262Bpopulation%262Bsize%2620lekking%262BGunnison%262Bsage-grouse%22

Abstract

Populations with small effective sizes are at risk for inbreeding depression and loss of adaptive potential. Variance in reproductive success is one of several factors reducing effective
population size ($Ne$) below the actual population size ($N$). Here, we investigate the effects of polygynous (skewed) mating and variation in female breeding success on the effective size of a small population of the Gunnison sage-grouse ($Centrocercus minimus$), a ground nesting bird with a lek mating system. During a two-year field study, we recorded attendance of marked birds at leks, male mating success, the reproductive success of radio-tagged females, and annual survival. We developed simulations to estimate the distribution of male reproductive success. Using these data, we estimated population size ($N^*$) and effective population size $Ne$ for the study population. We also simulated the effects of population size, skewed vs. random mating, and female breeding failure on $Ne$. In our study population, the standardized variance in seasonal reproductive success was almost as high in females as in males, primarily due to a high rate of nest failure (73%). Estimated $Ne$ (42) was 19% of $N^*$ in our population, below the level at which inbreeding depression is observed in captive breeding studies. A high hatching failure rate (28%) was also consistent with ongoing inbreeding depression. In the simulations, $Ne$ was reduced by skewed male mating success, especially at larger population sizes, and by female breeding failure. Extrapolation of our results suggests that six of the seven extant populations of this species may have effective sizes low enough to induce inbreeding depression and hence that translocations may be needed to supplement genetic diversity.


http://search.proquest.com/docview/304119371

Abstract

I studied the mating behavior, ecology, and genetics of an isolated population of sage grouse ($Centrocercus urophasianus$) in the Gunnison Basin, Colorado. Sage grouse have a lek mating system in which only a small percentage of males mate. Sexual selection in such a mating system can lead to rapid evolution of sexual dimorphism in size, plumage characteristics and mating behavior as well as associated female preferences for such male traits. As a result, I predicted that sexual selection could increase population divergence as well as reduce population persistence. Field observations indicated that traits important to male mating success, such as mating vocalizations, had diverged in the Gunnison population relative to other sage grouse populations. To determine the effect of this divergence in male mating vocalizations on female behavior, I conducted reciprocal field playback experiments at two leks, one in Gunnison and the other in a nearby, but allopatric, northern Colorado population. Females in each population avoided male vocalizations from the other population, suggesting the existence of a pre-mating barrier. While female mating behavior differed between populations, my investigation of female nesting ecology and summer habitat use showed that Gunnison females are ecologically similar to females in other sage grouse populations. Females in Gunnison (a) chose nest sites with more sagebrush density and structure than random sites, (b) had higher nesting success in areas with greater shrub density and forb and grass cover,
and (c) used flat, mesic areas with extensive grass and forb components when rearing broods. Genetic analyses of four sage grouse populations revealed moderately higher band-sharing and \( F_{st} \) values compared to non-lekking bird species. In addition, some genetic differentiation exists among the four populations and between leks in Gunnison. My results demonstrate the Gunnison population is distinct in secondary sexual traits, but not female ecology. These results are consistent with the view that sexual selection can have a direct role in the initial stages of population divergence leading to speciation. My results also suggest that lek mating species may have reduced genetic variation relative to non-lekking species and subsequently are more vulnerable to environmental changes.
INTRODUCTION TO GENETICS: THE BASICS
Sara Oyler-McCance
USGS Fort Collins Science Center
Slide 3

**THE BASICS**

DNA (the genetic material) is coiled and folded into chromosomes which are located in the nucleus of the cell.

Slide 4

**THE BASICS**

- **Gene** – sequence of DNA that codes for some gene product (protein or RNA)
- Not all DNA codes for gene products
- The location where a gene or non-coding sequence is found in the genome is called a **locus**
### Slide 5

**Genotypes**

- Pairs of genes or non-coding sequences at a locus
- Homozygous – both sequences are the same
- Heterozygous – the two sequences are different

### Slide 6

**Sources of DNA**

Any biological tissue or fluid:
- Blood, saliva, urine, semen, feces (scat)
- Muscle, liver, tongue, skin, etc.
- Hair, feather, scale, claw, nail
- Bone, tooth, ivory
- Study skin, taxidermy mount
POLYMERASE CHAIN REACTION
Amplification of specific regions (markers) of DNA

MOLECULAR MARKERS

- Some molecular markers are coding sequences (non neutral)
  - Proteins
  - RNA

- Others are non-coding sequences (neutral)
  - Introns
  - Microsatellites
MITOCHONDRIAL DNA

- Maternally Inherited
- Small, circular genome (~16,000 bp in birds)
- Evolves more quickly than nuclear genome
MITOCHONDRIAL DNA

MT DNA SEQUENCING

...CTGAAAGGCT... Haplotype A
...CTGTAAGGGT... Haplotype B

Base substitution
Slide 13

[Diagram showing haplotypes A, B, C, D, E, F, and G, connected by lines indicating genetic relationships.]

Slide 14

[Haplotype sequences and substitutions:

- Haplotype A: `....AATCGCTATTGCAACTGAG.....`
- Haplotype B: `....AATCGCTATTGCAACATGCAACTGAG.....`
- Haplotype C: `....AATCGCTATTGCAACATGCAACTGAG.....`
- 1 substitution
- 8 substitutions]
Phylogenetic Relationship Among Haplotypes

Haplotype A
Haplotype B
Haplotype C

Greater Sage-Grouse 2015 Status Review, Genetics Workshop Summary Report
Appendix 6: Presentations given by Dr. Sara Oyler-McCance
Page 58
NUCLEAR DNA

- Large
- Typically evolves slowly
- Some regions evolve fast
- Inherited from both parents
Slide 19

**MICROSATELLITES**

Allele A: ..ATAGATAGATAGATAGATAG.......................... (ATAG)_5
Allele B: ..ATAGATAGATAGATAGATAGATAG............. (ATAG)_6
Allele C: ..ATAGATAGATAGATAGATAGATAGATAG........ (ATAG)_7

one repeat deleted

one repeat added

Slide 20

**SINGLE NUCLEOTIDE POLYMORPHISMS**

- A region in the genome where individuals vary by a single nucleotide
- Most common type of genetic variant
- SNPs can occur in coding regions or in non-coding regions
**Slide 21**

**SINGLE NUCLEOTIDE POLYMORPHISMS**

Individual 1 (A) ATGC\text{GCGCGATTGCCATGGGTA}
Individual 2 (A) ATGC\text{GCGCGATTGCCATGGGTA}
Individual 3 (A) ATGC\text{GCGCGATTGCCATGGGTA}
Individual 4 (B) ATGC\text{GCGCGATTGCCATGGGTA}
Individual 5 (B) ATGC\text{GCGCGATTGCCATGGGTA}
Individual 6 (B) ATGC\text{GCGCGATTGCCATGGGTA}

**SNP**

**Slide 22**

**MICROSATELLITE GENOTYPING**

Individual 1
A: 159/163
B: 181/185
C: 224/226

Individual 2
A: 159/159
B: 185/185
C: 224/226

Individual 3
A: 159/163
B: 181/185
C: 224/226
Slide 23

SNP Genotyping

Slide 24

Microsatellite data

<table>
<thead>
<tr>
<th>Individual</th>
<th>Pop</th>
<th>SNP1</th>
<th>SNP2</th>
<th>SNP3</th>
<th>SNP4</th>
<th>SNP5</th>
<th>SNP6</th>
</tr>
</thead>
<tbody>
<tr>
<td>k_33_60</td>
<td>1</td>
<td>179</td>
<td>130</td>
<td>120</td>
<td>139</td>
<td>109</td>
<td>170</td>
</tr>
<tr>
<td>k_33_62</td>
<td>1</td>
<td>179</td>
<td>130</td>
<td>120</td>
<td>139</td>
<td>109</td>
<td>170</td>
</tr>
<tr>
<td>k_33_63</td>
<td>1</td>
<td>179</td>
<td>130</td>
<td>120</td>
<td>139</td>
<td>109</td>
<td>170</td>
</tr>
<tr>
<td>k_33_64</td>
<td>1</td>
<td>179</td>
<td>130</td>
<td>120</td>
<td>139</td>
<td>109</td>
<td>170</td>
</tr>
<tr>
<td>k_33_65</td>
<td>1</td>
<td>179</td>
<td>130</td>
<td>120</td>
<td>139</td>
<td>109</td>
<td>170</td>
</tr>
<tr>
<td>k_33_66</td>
<td>1</td>
<td>179</td>
<td>130</td>
<td>120</td>
<td>139</td>
<td>109</td>
<td>170</td>
</tr>
<tr>
<td>k_33_67</td>
<td>1</td>
<td>179</td>
<td>130</td>
<td>120</td>
<td>139</td>
<td>109</td>
<td>170</td>
</tr>
<tr>
<td>k_33_68</td>
<td>1</td>
<td>179</td>
<td>130</td>
<td>120</td>
<td>139</td>
<td>109</td>
<td>170</td>
</tr>
<tr>
<td>k_33_69</td>
<td>1</td>
<td>179</td>
<td>130</td>
<td>120</td>
<td>139</td>
<td>109</td>
<td>170</td>
</tr>
<tr>
<td>k_33_70</td>
<td>1</td>
<td>179</td>
<td>130</td>
<td>120</td>
<td>139</td>
<td>109</td>
<td>170</td>
</tr>
</tbody>
</table>

Alleles represent fragment sizes

SNP data

<table>
<thead>
<tr>
<th>Individual</th>
<th>Pop</th>
<th>SNP1</th>
<th>SNP2</th>
<th>SNP3</th>
<th>SNP4</th>
<th>SNP5</th>
<th>SNP6</th>
</tr>
</thead>
<tbody>
<tr>
<td>BH32A</td>
<td>A</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>LV18A</td>
<td>A</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>NV91B</td>
<td>B</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>EG9B</td>
<td>B</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>CU29C</td>
<td>C</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>DVCC</td>
<td>C</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Alleles are different nucleotides (1 = A, 2 = G, 3 = C, 4 = T)
### Assessment of Parentage

<table>
<thead>
<tr>
<th>Individual</th>
<th>SG1</th>
<th>SG2</th>
<th>SG3</th>
<th>SG4</th>
<th>SG5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chick 1</td>
<td>173/181</td>
<td>152/154</td>
<td>156/160</td>
<td>157/161</td>
<td>156/156</td>
</tr>
<tr>
<td>Chick 2</td>
<td>173/181</td>
<td>154/172</td>
<td>156/160</td>
<td>155/161</td>
<td>152/154</td>
</tr>
<tr>
<td>Hen</td>
<td>173/175</td>
<td>154/166</td>
<td>160/160</td>
<td>157/161</td>
<td>152/158</td>
</tr>
</tbody>
</table>

Which chick does not belong to the hen?
**Slide 27**

**How connected are populations?**

---

**Slide 28**

**Microsatellite Primer 8**

- Cold Springs
- Blue Mountain
- Dry Creek
- Dove Creek
- Eagle
- Middle Park
- North Park
- Crawford
- Gunnison Basin
**GENETIC DISTANCES BETWEEN POPULATIONS**

<table>
<thead>
<tr>
<th></th>
<th>Gunnison</th>
<th>Curecanti</th>
<th>Crawford</th>
<th>Cimmaron</th>
<th>San Miguel Basin</th>
<th>Dove Creek/Monticello</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gunnison Basin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curecanti</td>
<td>0.01976</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crawford</td>
<td>0.16961</td>
<td>0.18145</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cimmaron</td>
<td>0.20025</td>
<td>0.21331</td>
<td>0.16078</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>San Miguel Basin</td>
<td>0.09165</td>
<td>0.11175</td>
<td>0.14317</td>
<td>0.08954</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dove Creek/Monticello</td>
<td>0.27581</td>
<td>0.32011</td>
<td>0.38536</td>
<td>0.27149</td>
<td>0.19017</td>
<td></td>
</tr>
<tr>
<td>Pinon Mesa</td>
<td>0.45559</td>
<td>0.44904</td>
<td>0.44846</td>
<td>0.21331</td>
<td>0.29601</td>
<td>0.43082</td>
</tr>
</tbody>
</table>

**STRUCTURE ANALYSIS – INDIVIDUAL BASED METHODS**
GENETIC DIVERSITY

<table>
<thead>
<tr>
<th>Population</th>
<th>Mean sample size (SD)</th>
<th>Allelic Richness per population (SD)</th>
<th>% of polymorphic loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gunnison</td>
<td>83.13 (4.45)</td>
<td>5.00 (3.85)</td>
<td>100</td>
</tr>
<tr>
<td>Curecanti</td>
<td>25.00 (1.46)</td>
<td>3.88 (1.25)</td>
<td>88</td>
</tr>
<tr>
<td>Crawford</td>
<td>22.50 (0.76)</td>
<td>3.00 (1.41)</td>
<td>88</td>
</tr>
<tr>
<td>San Miguel</td>
<td>56.75 (2.55)</td>
<td>3.25 (1.98)</td>
<td>75</td>
</tr>
<tr>
<td>Dove</td>
<td>42.38 (2.26)</td>
<td>3.00 (1.77)</td>
<td>75</td>
</tr>
<tr>
<td>Creek/Monticello</td>
<td>19.50 (0.93)</td>
<td>2.13 (1.55)</td>
<td>50</td>
</tr>
<tr>
<td>Piñon Mesa</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LANDSCAPE GENETICS

- Population Genetics
- Spatial Statistics
- Landscape Ecology
- Remote Sensing
LANDSCAPE GENETICS

Moving from “population” genetics....

- Populations defined ahead of time
- Genetic distance calculated between all pairs of "populations"
- Coarse scale

LANDSCAPE GENETICS

to “landscape” genetics.

- Much finer scale
- Many more individuals spread more evenly across the landscape
- Calculate genetic distance between pairs of individuals
- Let the data describe biologically meaningful populations
- Assess levels of connectivity and identify barriers to gene flow
LANDSCAPE GENETICS

- Shift from gene flow in theoretical space characterized by geographical distances only
- To gene flow in fragmented landscapes providing estimates of functional connectivity

Slide 36

Random
Isolation by distance
Isolation by barrier
Isolation by distance with a barrier
Isolation by resistance
QUESTIONS?
OVERVIEW OF GREATER SAGE-GROUSE GENETICS

Sara Oyler-McCance
USGS Fort Collins Science Center

Slide 2

OVERVIEW OF GREATER SAGE-GROUSE GENETICS

• BACKGROUND
• PUBLISHED STUDIES
  – Investigating the lek mating system
  – Reassessing taxonomic boundaries
  – Population genetics
  – Miscellaneous studies
• PAPERS IN REVIEW
  – Taxonomy
  – Population/landscape genetic studies
• ONGOING RESEARCH
  – Impacts of translocations
  – Population/landscape genetic studies
  – Rangewide Connectivity
  – Development of genomic markers
Slide 3

Figure from Connelly et al., 2004, adapted from Schroeder et al. 2004.

Slide 4

- Regional population declines in some areas (17% – 47%)
- Likely linked to loss, fragmentation, and degradation of sagebrush habitat
MOVEMENTS

- Seasonal movements are variable
- Some populations are described as migratory moving over 80 km
- Movements and connectivity among populations remain largely unknown

MATING SYSTEM

- Polygynous
- Males defend territories on leks and conduct an elaborate mating display
- Females are attracted to dominant males
- Not clear what proportion of males actually breed
**Molecular Genetic Methods**

- Parentage/Mating System
- Taxonomy
- Population structure
  - Gene Flow
  - Connectivity
  - Genetic Diversity

---

**Parentage**

Microsatellite analysis of female mating behaviour in lek-breeding sage grouse

Objective: Investigate the mating system by comparing behavioral data and genetic data

Markers: Microsatellites

Location: California
**Parentage**

- 20% of broods had multiple fathers
- In all cases where they had data, the genetic data matched the observational data
- Multiple paternity may be more prevalent than once thought

---

**Slide 12**

**Parentage**

**Objective:** Determine paternity and polygamy

**Markers:** Microsatellites

**Location:** Alberta
**Parentage**

- Most clutches had a single mother and father but there was evidence of multiple paternity and intraspecific nest parasitism
- Nest parasitism occurred in 2.2% of eggs
- Multiple paternity occurred 7.9% of clutches
- The proportion of all sampled males who fathered offspring was 45.9% (from 1999-2006)

**Lek Formation**

**Objective:** Test whether lekking behavior evolved in GRSG because kin selection favored subordinate males joining leks of close relatives in order to increase their inclusive fitness

**Marker:** Microsatellites

**Location:** California
LEK FORMATION

- Mean relatedness values on leks were not different from zero
- Males on leks are not closely related
- Mean relatedness values were significantly lower than relatedness values calculated among known family members
- No evidence for local clustering of related individuals while on leks, yet they did find that related individuals tended to cluster together when off the lek
- Leks consist of groups of largely unrelated males with little if any spatial association among kin either within or between leks

Slide 16

LEK FORMATION

Objectives: Assess the genetic diversity of GRSG in Alberta and to determine the degree of sex-specific relatedness within and among leks

Marker: Microsatellites

Location: Alberta
**LEK FORMATION**

- Levels of genetic diversity were high and did not change over the 9 years of the study.
- Did not observe isolation-by-distance among leks.
- Leks were not differentiated from each other, suggesting substantial gene flow across the study area.
- Overall relatedness was ~ 0 for both sexes at the level of province, lek and year suggesting neither sex forms strong kin associations.
- No evidence of male philopatry.

**TAXONOMY**

- Large-bodied
- Small-bodied
**TAXONOMY**

**Objectives:**
Determine whether genetic differences exist between the large and small-bodied forms of Sage Grouse and to estimate a crude time frame involved in the morphological and behavioral differences between the forms.

**Marker:** mtDNA (control region)

**Location:** Colorado

---

**TAXONOMY**

- Haplotypes fell into 2 divergent clades likely representing an ancestral isolation into two distinct populations, that have since extensively intermixed.
- Estimated that the two clades began diverging at least 850,000 years ago.
- Haplotype frequencies in the small-bodied population were different and less diverse than the large-bodied populations.
- Absence of gene flow between the two groups.
**TAXONOMY**

A population genetic comparison of large- and small-bodied sage grouse in Colorado using microsatellite and mitochondrial DNA markers

**Objectives:** To determine whether there was gene flow between the large- and small-bodied sage grouse in Colorado

**Markers:** mtDNA control region, microsatellites

**Location:** Colorado

**Slide 22**

**TAXONOMY**

- Significant differences between large- and small-bodied birds using both marker types
- Genetic distances and neighbor-joining trees using allele frequencies showed a distinct separation between groups, yet cladistic analysis of mtDNA haplotypes showed a lack of reciprocal monophyly
- Results further support recognition of small-bodied birds as a distinct species based on the Biological Species Concept
Slide 23

**Taxonomy**

- Gunnison Sage-grouse recognized as a new species (*Centrocercus minimus*)
- All other Sage-grouse renamed Greater Sage-grouse (*C. urophasianus*)

Slide 24

**Taxonomy**

Objectives: Evaluate the validity of the eastern and western subspecies of Sage-grouse

Marker: mtDNA control region

Location: Western portion of the range
TAXONOMY

• No genetic evidence was found to support the delineation of these subspecies
• Population straddling the border between California and Nevada (Bi-State) had an unusually high proportion of unique haplotypes, consistent with its genetic isolation from other populations
• This population may have been isolated for thousands or tens of thousands of years
• Also found low genetic diversity in the two populations from Washington

POPULATION GENETICS

• Objective: Assess the extent of population subdivision among Gunnison Sage-grouse populations
• Markers: mtDNA control region, microsatellites
• Location: Colorado, Utah
**Population Genetics**

- High degree of population structure and low amounts of gene flow among populations (except between Gunnison Basin and Curecanti)
- Population structure for GUSG was higher than had been reported for GRSG
- Low levels of diversity in Dove Creek/Monticello and Pinion Mesa
- Identified 3 potential migrants
- Cerro/Cimarron/Sims may act as a stepping stone for gene flow between San Miguel Basin and Gunnison

**Objectives:** To examine the distribution of genetic variation across the entire range

**Markers:** mtDNA control region, microsatellites

**Location:** Rangewide
**POPULATION GENETICS**

- Populations follow an isolation-by-distance model of restricted gene flow
- Identified two populations in Washington with low levels of genetic variation that reflect severe habitat loss and dramatic population decline
- Another population in Strawberry Valley, UT also had low diversity
- The Lyon/Mono (Bi-state) population is isolated and genetically distinct

---

**Slide 30**

**POPULATION GENETICS**

- **Objectives:** Examining range-edge dynamics and impacts of fragmentation
- **Marker:** Microsatellites
- **Location:** Northeast portion of the range
POPULATION GENETICS

- Birds from northern MT, Alberta and Saskatchewan were identified as a single population that exhibited isolation-by-distance with the Milk River demarcating two subpopulations
- Both subpopulations had high genetic diversity
- River valleys and a large agricultural region were significant barriers to dispersal
- Leks were primarily made up of non-kin
- Northern Montana sage-grouse are maintaining genetic connectivity in fragmented habitats

Objective: To assess mtDNA diversity of 3 remote populations relative to other portions of the species range

Marker: mtDNA control region

Location: Utah
**Population Genetics**

- No unusual haplotype compositions in the three remote populations
- Haplotype composition of the Anthro Mountain and Strawberry Valley reference populations differed from other northeastern Utah populations
- Desolation Canyon of Green River may constitute a barrier to gene flow
- Anthro Mountain and Strawberry Valley may be altered by translocations subsequent to this study

---

**Objective:** Compare published genetic and demographic data to determine whether populations estimated to be on downward trajectories also showed reductions in levels of genetic diversity

**Markers:** mtDNA control region, microsatellites

**Location:** Rangewide
**Population Genetics**

- With the exception of the Washington populations, no consistent relationships were found between estimates of genetic variation and demographic trends across the range at either of two spatial scales
- A measure of inbreeding derived from microsatellite data was not related to population trends
- GRSG does not exhibit expected genetic signatures of declining populations

---

**Objectives:**
- Gaining a better understanding of the boundaries of the Bi-State population and examining genetic structure within it

**Markers:** mtDNA control region, microsatellites

**Location:** Bi-State (NV and CA)
Both mtDNA and microsatellite analyses support the idea that the Bi-State represents a genetically unique population.

There was genetic structure within the Bi-State with differences between the northern and southern part of the Bi-State.

Evidence for 3 subpopulations following a north-south gradient.

Objective: To assess genetic structure among isolated populations (Jackson Hole and Gros Ventre) in parts of Wyoming and Southeast Montana.

Marker: Microsatellites

Location: Parts of WY, MT
Found four genetic clusters with Pinedale and Casper forming one cluster, Powder River Basin and southeast MT forming a second cluster, and both Jackson Hole and Gros Ventre forming their own clusters.

All samples outside of JH and GV showed isolation-by-distance.

JH and GV were separate but had asymmetric dispersal into GV.

Both JH and GV had reduced genetic diversity and low effective number of breeders.

Only a few alleles were unique to JH and GV suggesting some historical gene flow.

Objective: Examine patterns of dispersal, gene flow, and genetic structure in northwest Colorado.

Markers: Microsatellites

Location: Northwestern Colorado
**Population Genetics - Dissertation**

- All leks had high genetic diversity
- Moderate-high gene flow between neighboring PMZ
- PMZs showed isolation-by-distance with gene flow following a directional or two-dimensional stepping-stone pattern that was local and between neighboring leks, but also between neighboring PMZs
- Evidence of male-biased dispersal in both direct (radio-telemetry) and indirect (genetic) methods
- Higher levels of genetic structure in females
- Significant fine scale structuring for both males and females at distances < 15 km
- Dispersal was rare at distances > 40 – 60 km for both sexes

**Objective:** To assess patterns of dispersal among greater sage-grouse in northeastern California

**Marker:** Microsatellites

**Location:** Northeastern California
**POPULATION GENETICS - DISSERTATION**

- Significant isolation-by-distance among males, suggesting that males are more philopatric sex and that females have a greater predisposition to disperse
- Stronger spatial structuring for males than females
- Assignment Index confirmed female-biased dispersal, although differences between genders were not significant

**POPULATION GENETICS - THESIS**

**Chapter 2 - A multi-method evaluation of connectivity in a threatened DPS of Greater Sage-grouse**
Joel M. Tebbenkamp, University of Idaho (Kerry Reese and Lisette Waits, Co-Advisors)
Submitted to Conservation Genetics

**Objectives:** To evaluate population genetic substructure and dispersal

**Marker:** Microsatellites

**Location:** Bi-State
POPULATION GENETICS - THESIS

- Pairwise F_{ST} estimates and Bayesian clustering methods provided evidence for 5 genetic populations within the Bi-State.
- No documented dispersal events between populations using radio-telemetry, however, using 4 genetic assignment methods found 10 individuals were likely recent dispersers among populations.

MISCELLANEOUS – SEX DETERMINATION

A comparison of sample types varying in invasiveness for use in DNA sex determination in an endangered population of greater Sage-Grouse (Centrocercus urophasianus)

Krzysztof Stajny, Michael D. Vinsky, Carlton L. Aldridge, & Cynthia A. Pastewka

Department of Biological Sciences, Western University, Canada.

(Author for correspondence: E-mail: mvdv@uwg.edu; Fax: 519-866-3601; Website: www.biology.uwaterloo.ca)

Molecular Ecology

A method for estimating population sex ratio for sage-grouse using noninvasive genetic samples

J. A. Sprogis, F. C. Grapes, J. P. Hepp, H. C. McCann, & C. A. Pastewka

(Received in revised form 30 November 2015)

Greater Sage-Grouse 2015 Status Review, Genetics Workshop Summary Report
Appendix 6: Presentations given by Dr. Sara Oyler-McCance
Page 92
Slide 47

**MISCELLANEOUS – PRIMER NOTES**

**PRIMER NOTE:**
Isolation and characterization of microsatellite loci in Greater Sage-Grouse (*Centrocercus urophasianus*)

S. E. Takeuchi, L. D. Gill, M. L. W. McCracken & D. M. H. Gurney

*Department of Biological Sciences, Montana Technological University, Moscow, ID 83843, USA.*

**Characterization of small microsatellite loci for use in non-invasive sampling studies of Gunnison Sage-grouse (*Centrocercus minimus*)**

Sara E. Oyler-McCance & Judy N. John

Development of 17 microsatellites for Gunnison Sage-grouse (*Centrocercus minimus*) using next-generation shotgun sequencing and their utility in Greater Sage-grouse (*Centrocercus urophasianus*)

Jennifer D. Galvez & Sara E. Oyler-McCance

Slide 48

**MISCELLANEOUS – GENOMIC APPLICATIONS**

**Rapid Microsatellite Identification from Illumina Paired-End Genomic Sequencing in Two Birds and a Snake**

**DNA**

**informa**

**ORIGINAL ARTICLE**

Blood from a tissue biorepository of low-coverage shotgun sequencing libraries reflects recovery of microsatellite sequences

**Two Low-Coverage Bird Genomes and a Comparison of Reference-Guided versus De Novo Genome Assemblies**

Sara E. Oyler-McCance, David A. Schachner, Alejandro M. Gropp, Andrew H. Fowle, Joe W. Zeller, Paul A. Anderson, Andrew Shaffer, Kari Severson, and Donald R. Pardue
**PAPERS IN REVIEW**

**Re-examining genetic variation in Sage-grouse using genomic methods**
S. J. Oyler-McCance, R. S. Cornman, K. L. Jones, and J. A. Fike
Submitted to AUK: Ornithological Advances

**Objectives:** Examine differentiation between GRSG, GUSG and Bi-State using genomic approaches

**Marker:** Single Nucleotide Polymorphisms

**Location:** GUSG, Bi-State, Southern part of GRSG range

---

**PAPERS IN REVIEW**

**Fast divergence of Z-linked nucleotide polymorphisms in a genus of lekking birds**
S. J. Oyler-McCance, R. S. Cornman, K. L. Jones, and J. A. Fike
Submitted to Heredity

**Objectives:** Investigate the chromosomal location of nuclear genomic divergence between GUSG and GRSG

**Marker:** Single nucleotide polymorphisms

**Location:** GUSG and southern GRSG
**PAPERS IN REVIEW**

**Latent spatial models and sampling design for landscape genetics**  
Submitted to: *Biometrics*  
**Objective:** Develop a spatially explicit approach for modelling genetic variation across space and illustrate how this approach can be used to optimize spatial prediction and sampling design for landscape genetic data

**Marker:** Microsatellites  
**Location:** Rangewide

**PAPERS IN REVIEW**

**Integration of genetic and demographic data to assess relative population risk**  
B.C. Fedy, J. Row, and S.J. Oyler-McCance  
Submitted to *Ecological Applications*  
**Objectives:** To assess population structure and use those defined population boundaries to assess population trends

**Marker:** Microsatellites  
**Location:** Wyoming
PAPERS IN REVIEW
Landscape characteristics influencing the genetic structure of greater sage-grouse within the stronghold of their range: A holistic modeling approach
Submitted to Ecology and Evolution
Objective: Establish and compare the importance of seasonal habitat distribution and landscape components in driving functional connectivity
Marker: Microsatellites
Location: Wyoming

RESEARCH EFFORTS IN PROGRESS
• Assessing impacts of translocations in Washington
• Assessing impacts of translocations into Strawberry, Valley, UT
**Research Efforts in Progress**

**Landscape Genetics**

Intensive landscape genetic efforts in Wyoming and Montana and for Gunnison Sage-grouse in Colorado.
**Research Efforts in Progress**

**RangeWide Connectivity Assessment**

---

**Objectives**

- Delineate the genetic population structure of Greater Sage-grouse across their range
- Quantify genetic connectivity among populations
- Identify the spatial scale and relative importance of landscape features that influence gene flow
METHODS

• Collect genetic data across the range
  — Collect feathers at leks in all states and provinces
  — Process feathers in lab to obtain genetic information

• Use genetic data in combination with spatial analysis of landscape components
RESEARCH EFFORTS IN PROGRESS
DEVELOPMENT OF A HIGH RESOLUTION
GENOME-WIDE SNP ARRAY

GENOME WIDE MARKER DEVELOPMENT

Gallus 600k SNP Assay  Greater sage-grouse DNA Sequences  Microsatellites

Bioinformatics

Candidate SNPs

Tests for Target Accuracy & Selection/Neutrality

Adaptive SNPs  Neutral SNPs  Microsatellites
IDENTIFY CONSERVATION UNITS INFERRED FROM GENETIC VARIATION ACROSS THE LANDSCAPE

• Do CUs designated using microsatellites differ from CUs delineated using neutral SNPs?

• Is local adaptation found within or overlapping CUs?

QUESTIONS?
Appendix 7: Workshop Notes

Purpose of the Workshop Notes
The Workshop Notes presented here were collected for the purpose of drafting the formal genetics expert elicitation report (Report). This information is provided here in the interest of transparency. Notes to clarify the record are provided in brackets.

These notes are not exhaustive. They capture the highlights of conversations and the general flow of the meeting. These notes do not represent a complete transcript; statements are not attributed to individuals. We have tried to indicate which statements were provided by U.S. Fish and Wildlife Service (FWS), U.S. Geological Survey (USGS), or Experts.

These notes should not be used for citations.

Day 1: Wednesday, Oct. 22, 2014

PART 1: INTRODUCTION

FWS: Welcome, logistics, and introduction

Purpose: Gather information, published literature. One step to gather information to inform the Service’s decision. This meeting is strictly to gather information. All issues related to policy are the Service’s decision.

Civil, orderly, respectful discussion requested at the workshop. No group consensus. Individual judgments and opinions only.

Introductions:

- Dave Smith: USGS facilitating discussion. Appreciates expert participation in workshop.
- Jonathan Cummings: Post-doc with USGS, primarily providing computer support for workshop
- Steve Morey: USGS facilitating discussion.
- Jesse D’Elia: FWS, R1
- Kate Norman: FWS, R6
- Mary Grim: FWS, R1
- Theo Stein: FWS, R6 EA
- Holly Freifeld: FWS, R1
- Pat Diebert: FWS, R6
- Angela Burgess: FWS, R6
- Lief Wiechman: FWS, R6
- Jesus Maldonado- Smithsonian Center for Conservation and Evolutionary Genetics
- Jeff Olsen- FWS, Conservation Genetics Laboratory, Anchorage, Alaska
- Chris Funk – Colorado State University
- Michael Schroeder – Washington Dept. of Fish and Wildlife
Sara Oyler-McCance: Introduction to Genetics - The Basics

Dr. Oyler-McCance provided this presentation; it can be found above in Appendix 6.

Questions:

1) USGS: Reciprocal Monophyly: One set of cluster looked different from rest, is that what the “mono” in monophyly comes from?
   • Those that share same node on tree would be reciprocally monophyletic.
   • If they are reciprocally monophyletic, they may be considered by some to be separate species
   • Some may argue that’s not a good way to define a species because there may not have been enough time for speciation to occur.

2) Expert : SNPs - looking at microsatellite data, there are typically thousands, correct?
   • Yes, typically thousands of microsatellites are compared.
   • Would you look at the same part of the genome to look at SNPs that you look at microsatellite?
   • Expert A: Typically different regions, though SNPs may be adjacent to microsatellite.

3) USGS: Metrics of SNPs?
   • used to measure genetic diversity – heterozygosity; not allelic richness like in microsatellite metrics
   • Comparing microsatellites and SNPs: More heterozygosity in SNPs, though they would be correlated with each other. A single microsatellite loci has a lot of power per locus, but when you look at tens of thousands of SNPs, it could potentially be more powerful than 15 microsatellites.
   • Recent studies have compared how powerful SNPs are compared to microsatellites. Need about 300 SNPs to do that.
4) Expert: Primers used for microsatellites, same markers still being used?
   • Yes, some are still being used, in addition to some new ones

5) Expert: just because there is a lot of new information and excitement about that does not mean that microsatellite data is inferior.

Sarah Oyler-McCance: Overview of Greater Sage-Grouse Genetics

Dr. Oyler-McCance provided this presentation; it can be found above in Appendix 6.

Questions/Discussion:

• Expert: Currently don’t have good study for paternity – we know that other things happen outside of the lek that we don’t know about. Feathers collected in the Bush study on paternity from males were collected from leks, which may not be the best method to collect genetic information for paternity studies.

• Expert: a male in some leks can mate a lot of times in a morning - fertility may drop throughout the morning

• Expert: is lek the unit of sample?
  o Expert: Not all studies use lek information. For example, some studies use wings collected at hunter stations. Each lek is not considered its own population.
  o Expert: More of a hierarchical structure: individual, lek, etc. [Note: **individuals are found on leks, multiple leks may be part of a population.**]
  o Expert: Birds use different parts of landscape at different times of the year. In spring, males go to leks and females look for somewhere to nest. Leks tend to be near where females are nesting. Once mating is over, birds congregate in completely different areas based on food sources, so distribution on landscape is completely different. Therefore, notion of lek as population is likely not accurate. Leks are useful for sampling because breeding birds go there.

• Expert: non-migratory population – does not think there is really this type of population, unless it is so isolated they have nowhere else to go. More likely that all birds move around to different habitats depending on time of the year.

• Expert: What’s known about female decisions on where to nest in comparison to lek?
  o Some females move from one failed nest to re-nest 20 km away. We don’t know how a female response to an area will affect where they are and if fidelity to an area would outweigh moving to a new location.
  o Females will first visit area where they will eventually nest, and then go to lek, and go back to nest. Most females will go to peripheral areas around the lek to nest

• Expert: Across the range of the GRSG, there is probably a 25% variation in body size. Display behavior is pretty comparable. Gunnison SG, however, is recognizably different. Sometimes body size is a plastic characteristic, as can be seen in GRSG – birds in the northern part of the range are larger than birds farther south. May have implications to translocation. Body size alone is not enough to distinguish them as different species.

USGS: East/West (E/W) split – more info?
• Expert: Subspecies delineation – original paper was based on a very small number of individuals in museum specimens. Genetic study showed that there was no genetic delineation between east and west.
• Expert: Rangewide study of genetics could be re-examined with east/west split question in mind
• FWS: We were petitioned for E and W – nothing behaviorally or movement-wise that suggested the delineation. 2010 finding determined that FWS did not believe subspecies delineation was necessary.
• Expert: There could be a different east/west split than was previously analyzed – Green River – WY and UT
• Expert: Data in MT may show a split in W Montana – concern about ensuring that there are enough samples that could show potential connectivity.
• Expert: Something to keep in mind - in the 1940s and 50s, translocations related to crop-related to damage were extensive and birds were released all over WY and MT – thousands of birds. A lot of movements were not successful – moving birds from WA to NM – birds were not in great shape after a few days.
• Expert: What’s going on with landscape and habitat?
• Expert: Possibly expanding based on historic levels of sagebrush pollen – prior to latest contraction; could make the case that they expanded into Canada – don’t show up in literature until 1930s. Lewis and Clark did not see GRSG in E Montana – “Prior to latest contraction"- the population recently has been hammered in the last 30 years or so, but prior to 1930s, we just don’t know.

BREAK

PART 2: BARRIERS TO GENE FLOW AND GENETIC DIVERGENCE IN SAGE-GROUSE

Barriers to Gene Flow (Exercise 1): Evaluate gene flow between the eastern and western portions of the GRSG range

USGS: Goal is to have a much richer response having a discussion on genetics with a group of geneticists in the room (compared to just asking one geneticist). Different frames of reference based on background. Ask that everyone participate even if they don’t feel it’s their specific realm of expertise in an attempt to bring out further constructive discussion. No right or wrong answer, no pressure to conform.

Format based on information needs from FWS – 4 or 5 topics/questions that have been identified. Introduce topic, discuss/clarify any issues, ask formal question (usually) – will allow us to take narrative discussion and make it more specific and allow us to capture uncertainty in terms of judgment. It will also allow us to capture uncertainty between experts.

• Expert: Seems like there’s a lot of upcoming research coming out – how will FWS use the information that comes out subsequent to the meeting?
• USGS: FWS has deadline of Sept 2015 to make a specific decision, but they will look at all available information. We want to know uncertainties and rationale for thoughts
USGS: First question: Evaluating gene flow between the E and W portions of the GRSG range.

Low/High diversity, Low/moderate gene flow across a barrier previously discussed. We want to get a common understanding of what that means to each expert. We’ll use those definitions and standards in later exercises.

USGS: East/west split – just looking at the map from a naïve point of view, one could see within the map larger areas in the W and E with various blank areas in between. We’d like you to look at the whole range of the species and consider any types and amounts of gene flow.

Spread 100 points across categories of A) No Gene Flow; B) Low Gene Flow; C) Moderate Gene Flow; D) Substantial Gene Flow; E) Complete Mixing (No barrier to gene flow)

Discuss how you’re thinking about gene flow so we can build definitions for the above categories (Round 1), and then think about how to answer the questions again based on the definitions (Round 2).

- Expert: Barrier is a tough thing to define, are we stuck in that category?
- USGS: Yes, for now, we’d like to see where it takes us.
- Expert: Very uncomfortable with how the exercise is being laid out…to give opinions instead of data driven process
- USGS: If there’s a paper or data that answers the question, that can feed into your opinion; but sometimes decisions have to be made that don’t fit into the exact category, which is where expert opinion comes into play. If you know upcoming research will reduce uncertainty, we would like to know that as well. If you are completely uncertain on this issue, you can add 20 points to each of these. Regulatory processes have to march on regardless of timing of research.
- Expert: What do you define as E and W portions of the range? Seems like we have to have some idea of genetic groupings as assess flow between those groupings?
- USGS: What makes sense from your point of view on east/west barrier?
- Expert: Seems like it would have to be based on Oyler-McCance 2005 paper. Not super-distinct genetic groups.
- Expert: Point of the paper was not to specifically look at east/west gene flow, but there was a lot more information from that study that could be looked to attempt to look at that east/west issue
- Expert: Gene flow is tough to define- for example, what scale, temporally, are we looking at? Are we looking at short-term scale or long-term scale?
- USGS: We would like your opinion on what the best way to look at this…
- Expert: Important to define if it’s historical or current
- USGS: Would contemporary gene flow be a good starting point?
- Expert: Gene flow now is a complete guess [Note: Expert is responding to the question of analyzing gene flow happening at this point in time (currently)
Gene flow is analyzed based upon changes over time therefore this expert is stating that quantifying current levels of gene flow would not be reasonable. Experts went on to describe timeframes that would be reasonable

- Expert: What’s the shortest timeframe to look at?
- Expert: Haplotype would be long term timescale, Microsatellite would potentially more contemporary
- Expert: Simulations and empirical analyses can potentially look at genetic differences based on barriers, but just because you see a genetic difference, you can’t say exactly how long ago those differences formed. Effective population size - # of individuals contributing their genes to the populations – 80 generations for patterns to form?
- Expert: Could vary based on what’s happening on the landscape, hard to get at…
- USGS: Genetic evidence that has something significant to say about barriers from east/west, could be a wide ranging period
- Expert: Nuclear vs. Mitochondrial – Mitochondrial DNA doesn’t pick up any male patterns of dispersal.
- Expert: Male vs. Female dispersal – no consistent patterns in sage grouse
- FWS: may be helpful to look at other data sources other than genetics.
- Expert: Note, there is typically a conflict between behavioral analyses of dispersal vs. genetic analysis. Genetic analysis is much more reliable because of bias related to behavioral research.
- FWS: first question – line of east/west boundary – potentially based on lek location?
- Expert: Hypothesis – evidence does not support gene flow between east/west?

Exercise – use sticky notes to write your estimate.

Results of Exercise:

USGS: Some folks saw symmetric distribution, and some distributions showed a tail (rather than a normal bell-shape) near lower levels of gene flow.

Discussion:

- Expert: In order to answer this question, we have to consider spatial scale- if there’s 10 years apart, maybe a ton of gene flow. If we’re talking about long-term, there may not be quite as much.
- Expert: These birds move around frequently to make it throughout the year. If we were to take question about how it varies across distance X, it would vary significantly – seems to be an empirical question rather than professional estimate.
- USGS: How much gene flow there is between these 2 large east/west patches?
- Expert: Snake River, Idaho- higher human population, ag., becomes non-habitat for most purposes. However, that doesn’t necessarily mean that they don’t cross it – a bird travelling in Nov. may not have any issues crossing the river. Even so, that’s only representing changes in the last century and not historically across the whole range. Birds on the W. side of the Tetons connect to the rest of Idaho. Do the birds in Jackson
Hole get to the birds on the other side of the Tetons – not very far distance, but have the Tetons in the middle. Lean towards low gene flow from E to W. Thinking that “low gene flow” as a certain percent below where you would have unrestricted movement. Behaviorally, small populations show that movement is related to population size- area of occupancy. Density dependence may play a role along with a genetic component.

- USGS: Low vs. moderate gene flow - how are you thinking about it?
- Expert: Number of migrants from A to B per generation. 1 migrant per generation would cause pretty substantial difference in FST value for populations.
- Expert: Isn’t that a function of what size of pop. you’re going to or coming from?
- Expert: If you have a huge pop, you’re not getting much genetic drift, if you have a tiny population, genetic drift could be an issue.
- Expert: You can only really ask these questions if you know where we’re discussing these limitations for gene flow.
- Expert: We don’t even really know where the east/west split is – may be helpful for an expert who works on the ground would draw the line. To clarify, we are trying to think about if there’s any disconnect between the rest of the range of the GRSG?
- USGS: Yes
- Expert: Is another goal to assess if there’s DPS within the range?
- USGS: We’re just talking about evidence of genetic barriers, not to make policy decisions.
- Expert: Historical evidence (bones in caves) could give us more genetic information/evidence of location of GRSG and Gunnison – no evidence of a split for east side of the Rockies and west side of the Rockies that we’re aware of.
- Expert: If we focus on the barrier itself, it would be a completely different question. There’s not a single N-S oriented barrier – the question would be where and how many reproductive birds are going across.
- FWS: How are you looking at Oyler-McCance et al. 2005 when thinking about your answers to questions?
- Expert: Data could probably be used in different ways to try to get to better answer to the question.
- Expert: What was the thought process behind others previous answers – an attempt to better understand the question.
- Expert: Thinking of it in terms of a hypothetical barrier –
- Expert: We all need to be on same page with question, so how about we define the question: How much gene flow is there between east/west - divide into scales – ranking between large-scale gap between E and W
- USGS: Next exercise will help us define these questions
- Expert: Need to all be in agreement on how we’re discussing space and time – small, large scale; recent, historic time
Round 2 of Exercise 1: Re-evaluate your answers based on previous discussion.

Question: Large-scale, recent barrier (north-south) separating E and W – what is the likelihood of individuals to cross (as in what is the degree of gene flow) an imaginary north-south barrier between east and west sections of the range?

LUNCH BREAK

Barriers to Gene Flow (Exercise 2): Identify potential barriers to sage-grouse gene flow across potential barriers

Identify potential barriers to GRSG gene flow, if any.

Break out into 3 groups (30 minutes), use distribution map to identify no more than 5 barriers to GRSG gene flow (if any) in terms of location, source (why they’re barriers), how long they’ve been barriers, and to what degree they’re a barrier – in the scale of gene flow defined in last discussion.

Indicate the permeability and the spatial and temporal scale considered.

Discussion pre-exercise:

- Expert: You don’t want us to put hypothetical barriers, but rather actual barriers (assuming we know enough about it).
- USGS: Yes.
- Expert: these may not be impermeable barriers, but rather something that may be passable, but add difficulty, correct?
- USGS: Yes – something that might reduce natural levels of dispersal.
- Expert: Ongoing research in WY about potential barriers – current study in review?
- USGS: When groups come back in, we can get summaries and may discuss any thoughts. Opportunity tomorrow morning to discuss if there are analyses that can be done to inform genetics perspective

Discussion post-exercise:

Group 1 - 3 natural features and 3 anthropogenic features

**Natural Features**

- Mountains:
Wind River
Big Horn
Tetons
N. CO Mountains

Large Rivers:
- Columbia River – wide and deep valley
- Missouri River – anthropogenic factor – large reservoirs

Desert:
- Portion of S. Nevada (Shrub-Steppe habitat on ridge tops, have to cross desert to get to other habitat.)

Anthropogenic Features

- Cropland:
  - E. Washington

- Powerlines
  - Washington – connectivity issues in Columbia plateau (pub in review) related to transmission lines – Huge kV lines, 4 right next to each other

- Highways
  - Interstate 80 – 24 hour roads/365 days a year

Discussion:

- Expert: Tree impacts on GRSG?
- Expert: Trees may impact ability to fly. While they typically walk rather than fly, though can and will fly ridge to ridge
- Expert: Avoidance of areas with trees is likely from raptor/predator perches
- FWS: If you were strictly looking at genetic data, how different would the lines be?
- Expert: Clearly Bi-State area. Washington – primarily because of cropland issues. Rest of the range is a matter of degree- less movement associated with some feature, but does not completely limit movement – it would not suddenly show up but rather be a gradual effect. A barrier of 10-20 km may be detectible now; something around 5 km may be hard to detect.
- Expert: Website available that shows Washington info: waconnected.org
- USGS: How did highways impact those in WA?
- Expert: Powerlines were more important than highways

Group 2 –

Barriers:

- Low, hot desert
- High elevation, coniferous forests
- Large habitat gaps with anthropogenic shifts in habitat
- Roads
• Ag Fields?

Divisions:

• Bi-State
• Washington – division between Washington and Oregon (more recent)
• Eastern Montana/Western Montana – Jackson Hole unique
• Snake River- Looking at genetic data, it didn’t look like big differences on either side of the river

Discussion:

• Expert: Why is WA/OR separation considered more recent?
• Expert: Compared to Bi-State, considered more recent because there was sagebrush habitat there until recently
• Expert: Potential for a lake near Bi-State may have also been a reason for separation during Ice Age
• Expert: Barrier around Jackson Hole population drawn due to forested mountains
• FWS: Will new genetic information potentially available provide information related to barriers (SNPs)?
• Expert: May be able to compare birds on one side of highway compared to other side (I-80)
• Expert: Because highways are in some parts of the range are in locations with large rivers (valleys through mountainous regions), can we tell which feature would cause the barrier?
• Expert: That is not the case with I-80, which goes straight through GRSG habitat.

Group 3

Divisions - Looked at genetics to look for strong evidence of breaks:

• Bi-State
• Jackson Hole
• Strawberry Valley
• Anthro Mountain

Barriers:

• Trees
• Agriculture
• High Mountain Ranges
• Large Water Bodies

Discussion:
- Expert: Energy Development discussed, but it seemed like a small island of development rather than a large, unpassable barrier.
- USGS: Mechanism vs. Barrier, what’s the difference?
- Expert: Differences between mechanism on landscape – models were for sampling not for separations of populations
- Expert: Agreed, viewed more of a hypothesis that then you would test with data
- Expert: Jackson Hole population naturally isolated – is there a difference between how the long-term fragmented populations behave compared to those that have secondary impacts?
- Expert: SNP information can give us info about that duration of the bottleneck – areas that have been isolated and population size at isolation event
- Expert: Populations that have been historically large and become isolated may be more vulnerable than historically small populations that have been isolated
- Expert: Management context- for translocation, is it better to get birds from areas that are already isolated and used to isolation?
- Expert: Generally a bad idea – tends to be a failed strategy and safer to go with large populations
- Expert: Strawberry Valley – found in study that it has low genetic diversity – afterwards UT stated that there’s a large predator population
- FWS: Clarification – Are there any places in the range where energy development is so extensive that it could provide genetic barriers?
- Expert: Yes, when that development is put over a huge swath of area, that type of energy development would be lumped into similar effects of agricultural development. So much habitat is turned into non-habitat. Should have added some lines in WY with barriers, perhaps a “zone”
- Expert: What happens when you have a high density of leks on landscape, do you remove stepping stones with major effects on the landscape? What happens if we lose some of the leks due to energy development?
- Expert: Bird dispersal related to translocation- single biggest determining factor of if they stay or come back is related to if they find other birds. Social component to dispersal. Plays a bigger role in declining densities.
- FWS: Evidence for genetic barrier in MO River Valley?
- Expert: Yes, still in process of being analyzed and completed.
- USGS: Discussion of “stepping stones” – what would a discussion about that look like?
- Expert: Distance as a mechanism of separating genetics over space – Think of the core area – don’t want them completely separate, it would likely be worth having stepping stones. At what distance between areas do they not become stepping stones or allow dispersal/gene flow.

USGS: As discussions progress, hopeful that dialogue continues for all exercises and increase comfort level on all sides to provide useful information.

BREAK
**FWS:** Uncertainty is something we’re used to dealing with. This is just one piece of information for our listing decision. Any policy decisions will be made at a later time – we are not asking you to be involved in that decision. After we collect more information, we feel we will be more able to evaluate additional genetic information and as it relates to the species’ status.

- **Expert:** Decisions should be data-based as opposed to opinion-based, which is where some of the discomfort from this morning.
- **FWS:** The conversation from these exercises is key and provides a good foundation of information and background that will be used as a part of our status review - helpful to those of us in information analysis and decision-making positions.
- **Expert:** What’s uncomfortable about it is that they feel like they could have the data and information in a couple of years but may not have the exact data at this time.
- **Expert:** Putting lines on maps made me uncomfortable at first because I did not know what it was to be used for. As long as the maps are used in described ways, feel much better about this.
- **Expert:** Good to get FWS’s perspective and appreciate the work being done – level of discomfort comes from the level of controversy related to research done. We have much of the data that is being discussed and that information may be able to be provided.
- **Expert:** Interesting to look at map and look at the lines, thinking about potential hypotheses and research that could be conducted.
- **FWS:** 2 maps- one of genetic barriers and one of hypotheses
- **Expert:** That would be very beneficial, especially with further review following the meeting

**PART 3: GENETIC DIVERGENCE**

Genetic Divergence (Exercise 4): What analyses could be done to assess the loss of genetic diversity for the GRSG if any portion of the range was lost?

- What metric(s) would be useful, e.g., percent of haplotypes lost? Are there others?
- What can be done with existing data?
- What analyses/studies are ongoing related to this question?

**Discussion:**

- **Expert:** Pattern of loss is important, isolating the portion of the range may matter a lot.
- **Expert:** Mechanism may matter as well- if you have a lot of movement, effect is smaller pop size and greater risk for genetic drift – if there is genetic variation between subpopulation and there is a decrease in subpopulation, you’re not only losing numbers in the population, but also whatever is unique about that subpopulation.
- **Expert:** What are your units on the landscape? Tricky for GRSG because they are somewhat continuously distributed on landscape.
• Expert: Worked really hard to keep local genes on the landscape, even if they’re mal-adapted because of genetic drift. Looking at what they’ve done with Attwater’s prairie chicken – Pygmy rabbit and Atwater’s prairie chicken are good examples of this.

• Expert: Suggestion to table gene underselection. Neutral genes- want to maintain genetic variation. One way to allow genetic variation is to maintain gene flow. Long term splits, you probably don’t want gene flow [Note: in some instances, it is preferable not to mix materials from genetically isolated populations. In other cases, outside genetic material may be beneficial to the population], whereas areas with very recent loss of flow you would want to increase connectivity. Need to be very careful about genetic variation – sink populations could have genetic variation from genes coming in. Loss of genetic variation compared to surrounding areas, it’s a red flag [Note: “red flag” is used here to describe areas that could benefit from additional research into the cause of lost genetic variation] to try to determine what is going on there.

• USGS: Don’t want to lose any genetic diversity due to linkage of persistence over time.

• Expert: Red flags – some areas with lower genetic variation. Other component is temporal variation- data to see how things have changed over time. Need monitoring over time to determine temporal variation.

• Expert: Strawberry Valley and Washington – both showed red flags. [Note: “red flag” in this discussion refers to a difference in genetic diversity between these populations and surrounding populations]

• Expert: Phenotype issues – weight variation across range, but other things show up in certain populations – 1) Number of tail feathers (WA- fixed at 20), almost everywhere else it’s not fixed- either 18 or 20. Why is it mixed across the range except for WA? 2) Clutch size – 9 in WA, average across range is 7.5; 3) High rate of nesting and re-nesting in WA- not sure why it doesn’t show up in other areas. Probably other things as well, but we may not be paying attention, may not be measuring all birds when trapped. Curious how important those things are. Important in translocations because of unique characteristics.

• Expert: Phenotype data wouldn’t be picked up through genetic study

• Expert: Take into account phenotypic variation as well as the adaptive information. In terms of determining vulnerability of those phenotypes would be hard to determine.

• Expert: Wing measurement variation? Likely proportional to body size.

• Expert: In order to assess these issues you need more localized studies that focus on fitness and how those phenotypes affect local fitness.

• Expert: Translocation of birds to new areas can show if it’s a plastic trait or not.

• Expert: Don’t know if you can use a phenotypic trait to show if there’s a loss of genetic diversity.

• Expert: Temporal variation can be used infers to differences genetically or potentially phenotypes

• USGS: Has genetics been able to identify GRSG populations?

• Expert: Hard to do because of methods of collection
• Expert: Genetic modeling can lead to inferences of genetic variation over time – gene flow based using data we’ve been collecting.
• Expert: How do you separate any recent anthropogenic stuff that may be happening? What sort of data would that take to statistically show that information? Probably need to have temporally spaced data.
• Expert: Always an issue but if we know population size we can probably get some sort of inference.
• Expert: One approach to answer posed question is modeling- but that relies on assumption after assumption. Building uncertainty in it, it would likely to be reliable? [Note: discussion of modeling identifies the limitations of models. Models relying upon multiple assumptions with large uncertainties may be less useful]
• Expert: Looking at variation over time would be a way to do it.
• Expert: Developing a set of markers that could be a tool to allow for temporal comparison- how have things changed over time?
• Expert: SNPs should be able to be translated to whatever is used in the future
• Expert: Is the possibility of going back and looking at a very coarse scale- looking at wings from 2000 and looking at temporal variation in that amount of time to those being collected now.
• Expert: General test to estimate change over time and inference to some of the landscape metrics.
• USGS: Red flags- are there any others – any surprises that might come out for GRSG, or do you feel you have a good handle on it?
• Expert: I would be surprised if there are more surprises. Need to check on sensitivity and details in areas where there are currently no red flags.
• Expert: Seems like Steve Knick would be a good person to talk to about this last issue

Quick preview of tomorrow: Areas of low diversity that could have an effect on persistence? Revisit issue of things that could or will be done to address issues of genetics and importance of status review- what is coming down, are there things that could be done to help address some of these questions about loss of diversity and genetic persistence of sage grouse.

Day 2: Thursday, Oct. 23, 2014
Logistics and check in – USGS

Thursday Exercise 1:
Question 1: Have we captured the places where limited gene flow (no or low gene flow) have been found from data-driven genetic evidence and the places where limited gene flow have been hypothesized?

USGS: Places where there is low or no gene flow- not just limited, but “no” or “low” gene flow – there were a few identified yesterday – we want to check in – did we capture them all?

Washington, Jackson Hole, Strawberry Valley, Missouri River in Montana.
• **Expert:** Not sure Strawberry Valley has low gene flow. May have low genetic diversity due to predator problem, but not convinced that it has low gene flow. Bi-State also does not actually have lower genetic diversity – it is different genetics.

• **Expert:** Some of these are likely to have greater than “low” gene flow – it’s all gradations.

• **FWS:** When everyone discusses “low” gene flow, what do you mean? How big are the “holes in the colander”?

• **Expert:** Using adaptive markers to identify Conservation Units and Evolutionarily Significant Units [NOTE: Conservation Units and Evolutionarily Significant Units (ESUs) do not equate to the Service’s use of these terms or the term “Distinct Population Segment”] – akin to “subspecies” that have been isolated for long periods of time and may have adaptive differences between them and can be identified using various genetic methods. Use all loci – neutral loci that show historical and adaptive loci that show adaptation. Some ESUs identified show no emigration/immigration. So far we don’t have that data yet to identify ESUs. ESUs are units that have very little gene flow between them – may have evolutionary differences or adaptive differences – management units within ESU are demographic populations that exchange some level of gene flow- maybe moderate – don’t have evolutionary or adaptive differences.

• **USGS:** When would that information potentially be available

• **Expert:** Early spring?

• **Expert:** Management units are usually watershed based…fisheries are managed to achieve a certain number of spawning adults- ESUs are typically on a broader scale.

• **Expert:** Some populations may exhibit distinct trait that may limit gene flow temporarily or spatially.

• **Expert:** ESUs sometimes considered using microsatellites, but may also include behavior or habitat-based reasoning

• **Expert:** Reciprocal monophyly – previously defined for use in ESUs as NO gene flow in many generations, now considered too strict. Now it’s been accepted to look at adaptive genes

• **Expert:** Debate in the literature to confirm that something is actually adaptive

• **Expert:** Good examples of status revision based on adaptive genes in North Sea stocks, such as herring, cod, or Atlantic salmon – markers under selection were similar – looking at different classes of markers to determine if they’re actually adaptive or not.

• **Expert:** Our lab is developing tool to look at array of tools to look at adaptation

• **Expert:** There are likely to be more boundaries as more data – not safe to assume that just because we haven’t identified any additional boundaries, doesn’t mean they won’t be found in the future.

• **Expert:** If we had to identify where ESUs were now – other than the Bi-State, are there any other ESUs on the landscape

• **Expert:** Interesting to look at haplotypes for Jackson Hole- could compare to what we have on Bi-State. When WA analysis was done, there were some really common haplotypes but really low diversity – probably not going to have a bird move naturally from WA to OR.
• Expert: What does historical data look like related to WA pop?
• Expert: We have samples, but haven’t had the opportunity to do it yet. Samples came from N-Central WA.
• Expert: What is the recent translocation info in WA?
• Expert: Translocations in Yakima – tribe – also in the Yakima training center- haven’t analyzed the data to see if the birds are now part OR/part WA birds now. Weren’t overwhelmingly optimistic that we have gene flow – needs more research
• Expert: What we’ve looked at so far – the break shows up pretty strongly in SE Montana across Continental Divide – doesn’t go away even when you consider if it’s a sampling effect.
• Expert: You might hypothesize that here’s the range that has very different environments – that would make me have a hypothesis that those may constitute distinct ESUs and I would test for that. Most people wouldn’t consider something an ESU because it’s been divided by anthropogenic forces, as ESUs are considered large, long-term evolutionary divisions.
• Expert: In context of adaptive variation – in E there’s a lot of summer rainfall and ground cover, the farther W you go, the less ground cover you have – appears to be a totally different situation – no breaks but yet there is an incline in variation, how does that affect adaptive variation
• Expert: Maybe there aren’t distinct breaks or ESUs, but it may be important for managers to recognize that there is adaptive variation within the species through the gradient and maintaining it through the population.
• Expert: Difference between genetic change from drift (like WA) – short term, small population dynamics that’s causing the population to spread apart – not necessarily significant from the adaptive side (like Bi-State) – the data needs to be looked at in WA to compare historic connectivity. Could handle 10 samples in a very short period of time. Have to work with labs on historic DNA analysis. Pretty important to know historic genetic analysis to determine gene flow, as we might find out something different from what we expect currently.
• Expert: If you were going to do that – useful to compare to OR and MT. Could probably get something going very soon.
• USGS: Anything to add about Jackson Hole
• Expert: I would be really interested to get mitochondrial data from Jackson Hole- hard to say without collecting/analyzing additional data.
• Expert: Agreed, looking at mitochondrial DNA would be really important to confirm.
• Expert: Bi-State populations – most have haplotypes that don’t occur anywhere else – if you actually had all the haplotypes in the pie graph map, it would tell you a different story. Map shows that common haplotypes shift as you go across the range, but there’s more that could be looked at.
• USGS: Hypothesized barriers with low or no gene flow- are there any of the places identified yesterday (mountains, highways, deserts, rivers) – is there any existing data that can be analyzed to test these hypothesized barriers.
• Expert: Definitely I-80 – currently being looked at – current analysis of “paved roads” did not pop out as a barrier in existing data – but you could look specifically at SW WY data or I-80 to look more closely at that.

• Expert: Look at 50 genotypes N of I-80 and compare to 50 genotypes S of I-80. Model “100 highways” and compare those breaks to what’s happening in I-80.

• Expert: If it doesn’t show up in I-80, it likely wouldn’t show up in any other road in N. America. With the sample sizes available, the amount of traffic, the amount/type of habitat there – if it doesn’t show up there, it would probably suggest that there’s not too much to worry about. Isolation by distance model – Null Model.

• Expert: Would not want to bias samples by only comparing samples that also have east/west affects (some by Montana, some by Cheyenne)- would need variation across I-80 to have comparable samples.

• Expert: Yes, there is data available for that – quick look at where the samples are show that there’s a good variety of data points at leks around I-80

• USGS: Are there any other barriers (other than I-80) hypothesized that have data that could be tested?

• Expert: Yes, everything in the range-wide analysis could be looked at – a lot of the hypothesized barriers could be looked at.

• FWS: Threats appear very different on E and W side of the range – when looking at Management Units, would it make sense that there’s any disconnection between the east/west of the line?

• Expert: Would like to look at the data that went into previous genetic papers – don’t know how much of it is a gradient, might be able to tell you something in a couple of weeks. Have data to resolve it right now – Could be figured out…what’s the FWS timeframe for questions to be answered?

• FWS: Current timeline for recommendation is April – of course the FWS will use the best available science, so if new information comes available, we would incorporate that to the best of our abilities.

• Expert: Important to look at in broader population scale- becomes more of a management/long-term conservation question.

**Thursday Exercise 2:**

*Identify areas within the GRSG range where low genetic diversity or inbreeding depression is likely to affect GRSG persistence?*

**Question 1: What is the significance of these, or any/all combination of these areas to the persistence of GRSG? So what (for each/any/all combination) if we lost these areas?**

• Expert: A lot of literature of importance of peripheral populations- WA and Bi-State are margins of the species range and adapted to specific environmental conditions that may be “pre-adapted” to what the environment of the species as a whole would be in the future (climate change). These populations could be disproportionally important because they might be adapted to these marginal conditions that may be important in the future for the species as a whole.
• Expert: Study has shown that birds in Alberta are in a sink population maintained by birds in MT – not sure that these birds in Canada would be locally adapted – if they’re really MT birds. If there are peripheral parts of the range that are in fact sinks, perhaps those may not be locally adapted. May just be for that particular situation.

• Expert: Like thinking of it as a “red flag” – not a specific metric that can test for everything – still need ecology and people on the ground – don’t expect us to deliver a silver bullet.

• Expert: Example- Strawberry Valley – genetics looked like a red flag, but on the ground learned that it was related to predators.

Question 2: How much can be lost before we go from a minor “so what” to “oh darn it”?

• USGS: Is there a point where a loss of areas – from a genetic perspective- that changes the genetic integrity of the species at large –

• Expert: Exactly the network analysis- how connected are leks across a landscape, if you lose this one, what is the significance for the range as a whole- planned for analysis in the rangewide study. Stay tuned.

Thursday Exercise 3:

Question 1: Channeling a decision-makers perspective, are there areas where genetics exacerbate the effect of threats on species persistence?

Real-world manager objective – conceptual feedback, response

• Expert: Statistical comment – Is the question really - is there an interaction between genetic and non-genetic threats?

• Expert: The way we’re using genetics – looking at the genome to characterize what’s occurring on the landscape. Similar to taking DBH of trees – couldn’t ask if DBH is exacerbating the threats

• Expert: May make more sense to say “Do the effects of other threats exacerbate genetic issues related to the species”

• Expert: Could say that inbreeding depression could exacerbate the threats – 2 reasons why genetics are important for conservation – a tool to measure things AND they can affect things like genetic fitness – are there populations where genetic variation is so low or have so few individuals that it is exacerbating the issue. What I heard was that WA may be a candidate where I would try to assess the fitness.

• Expert: Could estimate effective population size of all the isolated areas – 50 rule- when genetics would potentially be a problem for inbreeding depression – population size less than 50 – could be entering into depression vortex – would potentially be a red flag.

• Expert: This type of issue has been studied with Gunnison SG – you could look at that information. One of the other layers of information should be population size, reproductive success, mortality rates

• Expert: Strawberry Valley population may be a good one to think about- if the manager is thinking of instances where genetic information is influenced by what’s going on “on the ground”
• Expert: Look at juvenile survival, or probably better - hatching success – which is a common place where you see inbreeding effects – might be a way to look at areas to alert geneticists about potential issues.

• Expert: Prairie chicken where it became genetically a problem- located it in the hatchability (hatching success) of eggs- normally 9.5 out of 10 would hatch successfully; what happened was that 4 out of 10 eggs weren’t hatching because they were infertile or not viable- brought in birds from outside areas and were able to bring it up. No examples known where anything has looked at the chick survival –

• Expert: Effective population size tends to be much smaller than the actual demographic population size is much smaller, so don’t wait until you have demographic size of 50 to determine effective population size. Has that been done for GRSG?

• Expert: Gunnison sage-grouse was looked at…female breeding success was 23%

• Expert: WA calculated it too but not with genetics – also have to make assumptions of how many males are actually breeding, which is hard information to get.

• Expert: You can estimate that information with some assumptions if you have markers.

• Expert: Reference – Maples/Waples 2013/2014

• Expert: History of population as well as all the other things that play into the sex ratio- If you do it demographically, you’d get “this is what’s happening right now”

• Expert: Demographic vs. genetic estimates would be different – demographic would likely be smaller because they are influence by current things happening on the ground.

• Expert: Because it’s a lekking species it would probably have a smaller effective population size than a species that’s monogamous

• Expert: In some cases it’s difficult to make these estimates in areas with contiguous populations (compared to distinct populations like WA)

• Expert: Still not sure exactly the way to respond to this type of question related to other threats- we know there are other threats but not the extent or how they interact with these issues. How do you tie genetic information into modelling threats?

• Expert: Example – could include genetic as a covariant in models- additive effect of inbreeding coefficient on a fitness measure. Theory and empirical data suggest that inbreeding can really be a problem in harsh years or with other threats/issues – drought, ex. Models can see if in those years there’s really a threat from inbreeding effects.

• Expert: Survival (population or individual) = threat + inbreeding + threat*inbreeding; examine the correlation between demography and inbreeding coarsely.

• Expert: Long-term genetic research needed for future monitoring.

• USGS: If a manager were to say – I need to know if there’s a place to focus my resources related to genetics, in addition to WA, which we’ve discussed

• Expert: Jackson Hole has been declining and has also had decreased genetic diversity, so that would likely be an area to explore further.

• Expert: There likely will be areas, but don’t want to identify that yet

• Expert: Weiser, Klamath area (down to 7 males a few years ago). Areas experiencing fragmentation that always catch my eye are the isolated areas in Utah, which have been declining for years. South-Central UT, areas in NE interior of UT are also isolated. Piceance also a naturally fragmented area but also really heavy in energy development
currently. This is speaking more on a demographic level vs. a genetic level – currently unknown at a genetic level. Belt Mountain, Wisdom, Red Rocks in E. Montana.

**Catch up on topics – anything thought of that didn’t get said yesterday?**

- Expert: Non-genetic issue about birds that find other birds stay and those that don’t go back – literature does exist about territorial dispersal – males looking for territory will look for other males and then stay on the peripheral – wide spread documented phenomenon that leads to clustering rather than widespread, even distribution that some might expect.
- Expert: 25 historic specimens – Puget Sound, Museum at Washington State- Pullman – also some potential from OR, which would make sense to also analyze for comparison. A lot of translocations to N OR in an attempt to re-establish populations, which didn’t work
- Expert: Mitochondrial samples from those specimens would be very beneficial
- Expert: Good to compare S. OR to those in WA to see if the genetic diversity differences are relatively recent.

**Thursday Exercise 4:**

**Question: Opportunity for the F&W Service members to ask unaddressed questions**

FWS: Central Idaho has a lot of threats that we get questions about – will the new data be able to tell us anything about this?

- Expert: New data definitely will. Currently working on it – hope to have it available by April.

FWS: Drift in WA – question previously discussed about is it actually drift or exacerbated by isolation – tying threat occurrence and intensity of threats and how they influence the threat of bottlenecking

- Expert: If your threats get to the point where you’re isolating a population and it’s becoming small, genetics will become an issue.
- Expert: The greatest risk of threats seem like they would occur in the large portion of the range as opposed to the outer areas – don’t forget about those.
- Expert: Could be related to PACs and creating individual pockets of isolated populations. Reason why some area is not a PAC is because energy developers are really interested in developing in those areas.

FWS: Background of PACs – Developed map of PACs – states indicated that they could manage for long term conservation if they had PACs. Over half of GRSG habitat is on Federal lands, which has multiple-use management. Question: We are asked – are there places where we can potentially allow further development in these PACs without affecting long-term conservation? Is there anything related to genetics (solely related to genetics) that would be related to this question?
Expert: Cumulative impacts analysis must be done to help with this. Every little thing needs to be evaluated against the whole.

FWS: We’re interested in the genetic component that can be put into that cumulative analysis. Are there key areas where genetics may be an integral part of cumulative analysis.

Expert: Yes, we are asking that type of a question in the rangewide study. What are the key areas that are acting as stepping stones or within the main areas? Looking for leks that are disproportionately affecting gene flows- looking for “hubs” for gene flow.

Expert: Specific scenario that would be interesting to look at: NE WY where there are only a few small areas identified as PACs – Can those areas persist if all other areas were developed? Same for Montana.

Expert: Some states tried to determine the smallest amount of areas that had the most number of birds.

FWS: Some states also took into account what areas had already been leased and therefore focused on areas where conservation actions would benefit the most number of birds.

Expert: I would love to have a map that shows “If you remove this area, this is what happens to the rest of the areas and overall connectivity”, but we don’t have that yet.

Expert: Has been produced in WA as centrality map- which leks are the most important – also ranks connections – Andrew Shirk – on WA website.

Expert: Any effort to think about corridors between PACs so you don’t isolate areas?

FWS: That was the recommendation, but typically outside the PACs, regulatory mechanisms are more relaxed. Connectivity map shows links.

FWS: On west side, other threats (such as fire and invasive) may relate to genetic implications. These may work together such that genetic implications are greater for those PACs than may otherwise be apparent when viewing genetic issues alone.

Expert: PAC map would appear very depressing if you pictured it as a range map-doesn’t look good. On the other hand, there are birds that live in naturally fragmented areas on the W side.

Expert: What is the risk of invasives?

FWS: Cheat grass and other vegetation encroachment

Expert: This issue is also being looked at in genetic rangewide study.

Expert: What about predation from Strawberry?

FWS: Related to recreation and human population in Strawberry Valley area – fox in areas that had not been seen before and capitalized on the predation situation.

Expert: Did they get rid of predators in Strawberry?

FWS: yes, they maintain very intensive predator control

Expert: Good to consider- if that changed, it could become a genetic issue after all.

FWS: Maintaining peripheral populations related to adaptation - Any information that would suggest that some areas are problematic (genetically) in terms of maintaining rangewide integrity?
• Expert: For one specific peripheral population, it’s likely it wouldn’t be a huge impact on entire population given the entire size – could look to see if any alleles are lost.
• Expert: Spatial analysis of diversity vs. individuals.
• Expert: See if genetic diversity is different if you took out some of the central individuals vs. peripheral populations and see if there is a difference in the inflection point. Could also compare current distribution vs. PAC.
• FWS: What does the inflection point actually mean?
• Expert: Inflection point is where rate of change changes. If you get to the point where there’s no gene flow over many generations, it would be too late.
• Expert: This does not take into account the point at which populations become isolated, really has to be spatial.
• FWS: We’d probably get asked, “So what if you lose the Columbian Basin, what does that mean for genetic diversity for GRSG? How much emphasis should be placed on protecting that genetic variation?” [The purpose of this question was to understand the biological importance of the Columbia Basin population to assist the Service with future decisions.]
• Expert: In non-genetic terms, you lose that organism on the landscape and the impact of the species on the ecosystem.
• Expert: For the neutral markers analysis, you could find something that’s completely unique compared to the rest of the range and change your thinking on how important the area is genetically.
• Expert: Depends on if it’s determined that it’s already in the “extinction vortex” and if it’s worth going to the extra effort to save it
• Expert: Could look at data and say “If we lose WA, what would happen?” Current data just indicates information about losing neutral variation.
• Expert: Amount of total genetic variation may not be related to the amount of area the species occurs in.
• Expert: Curious how the PAC map could change related to climate change, would some areas not even be habitat anymore, even with conservation?
• Expert: Sagebrush plants would presumably move N – are populations going to track that, or would those populations just disappear?
• FWS: Work underway on climate change modeling.
• Expert: Related to genetics, given that we predict shifting demographics and habitat, genetic diversity is important related to connectivity to allow birds to adapt and follow where sagebrush occurs. Warm-adapted populations may be very important for potentially warmer areas in the future.
• Expert: West Nile Virus (WNV), are there any individuals that have been seen to be more fit genetically to survive?
• Individuals that do get WNV die, the birds are susceptible to West Nile virus, but some survive. Difficult to study because you have to get the carcasses before they decay. There are mortalities every year; sometime more when conditions are right for the virus, - transmission issues and collection of mortalities – potential die offs that are easy to miss. Biggest die-offs from WNV are in areas with coal-bed methane and
surface water, such as the Powder River Basin, especially in late summer when hens bring broods to mesic habitats, which promotes transmission.

**Wrap-up:**
On behalf of everyone in FWS, THANKS!

We will circulate notes and appreciate any review possible to make sure we captured everything correctly.