

From: [Smith, David](#)
To: [Chris Funk](#); [Robert Gibson](#); [Mevin Hooten](#); [Maldonado, Jesus](#); [Sara Oyler-McCance](#); [Jeffrey Olsen](#); [Christin L. Pruett](#); grouse@homenetnw.net; [Schwartz, Michael K.-FS](#)
Cc: [Craig Hansen](#); [Steven Morey](#); [Jonathan Cummings](#); [Sarah Converse](#)
Subject: review of notes and report from genetics workshop
Date: Wednesday, December 17, 2014 9:14:53 AM
Attachments: [2014-10-21_Workshop_Notes_Set_2_AB.docx](#)
[2014-10-21-Workshop_Notes_Set_1_HF.docx](#)
[2014-12-16_DRAFT_FULL_REPORT_GRSG_Genetics_workshop_for_review.docx](#)

Greetings,

This email is to request your review of the attached summary report and notes from the greater sage-grouse genetics workshop.

The U.S. Fish and Wildlife Service (Service) greatly appreciated your participation in the workshop - your expertise contributed significantly to the workshop. At this time, the Service requests that you review for accuracy the attached workshop products.

Please return corrections by email to Dave Smith, drsmith@usgs.gov by 20 January 2015.
Please also contact Dave or Craig Hansen, craig_hansen@fws.gov, with any questions.

Best regards and happy holidays,
Dave

David R. Smith
USGS - Leetown Science Center
11649 Leetown Road
Kearneysville, WV 25430
drsmith@usgs.gov
304-724-4467
<https://profile.usgs.gov/drsmith>

Expert Elicitation Workshop on Genetics of the Greater Sage-Grouse

Wednesday, Oct. 22 and Thursday, Oct. 23

Fort Collins, Colorado

Notes – Angela Burgess

Day 1: Wednesday, Oct. 22

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 is 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-

Chris Funk – Colorado State University

Michael Schroeder – Washington Dept. of Fish and Wildlife

Christin Pruett – Florida Institute of Technology

Robert Gibson – University of Nebraska - Lincoln

Mike Schwartz – University of Montana and USFS

Sara Oyler-McCance – USGS Fort Collins Science Center

Mevin Hooten – USGS Colorado Cooperative Fish and Wildlife Research Unit

FWS- Information packets – various maps provided, also hanging on the wall. Agenda also included. Sara will provide short discussion on genetics, followed by a break and then into Exercise 1.

USGS- genetics presentation primarily to assist everyone to be on the same page and help provide additional information for FWS and USGS facilitators. Any questions? No questions.

FWS- meeting notes being taken, will be collated and combined and passed along to experts for review prior to finalization.

Sara Oyler-McCance: Introduction to Genetics - The Basics

Primarily for those non-genetic experts in the room.

DNA – tool to answer genetics questions. Also limitations to genetics

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

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

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

Sources of DNA: biological tissue or fluid: blood, saliva, urine, semen, feces...

Polymerase Chain Reaction (PCR): 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

The Cell: DNA in Cell Nucleus and Mitochondria – two sources of DNA have different properties

Mitochondrial DNA: Maternally Inherited; Small, circular genome (~16,000 bp in birds), evolves more quickly than the nuclear genome. Chicken genome – interested in phylogenetic study – might target cytochrome B that evolves more slowly

MTDNA Sequencing: Haplotype A; Haplotype B (Assigned to an individual based on what their sequence looks like). Different based on substitutions.

Look at distribution of haplotypes for one population compared to haplotypes from another population. For example, comparing groups you may see many similarities, where you may assume there is a lot of gene flow between populations.

Closer MTDNA Sequence shows they shared a genetic ancestor more recently than those with more substitutions within the sequence.

Haplotype distribution can tell us a lot about gene flow.

Nuclear DNA: Large, typically evolves slowly, some regions evolve fast, inherited from both parents

Microsatellites typically used for Nuclear DNA studies: Regions in nuclear genome that are characterized by short, random repeats. Mutations may occur in locations where you're adding a repeat.

Single Nucleotide Polymorphisms (SNPs): A region in the genome where individuals vary by a single nucleotide; most common type of genetic variant; SNPs can occur in coding regions

Most genetic studies you're looking at thousands of SNPs

Microsatellite Genotyping: Most studies looking at more than 1 loci – now most studies use ~15 microsatellite loci.

Questions interested in: Assessment of Parentage: Which chick does not belong to the hen? Who are parents, how many of males are doing breeding?

How connected populations are to one another? Inferences between gene flow among populations.

Calculate genetic differences between populations: build a matrix that shows how similar and different populations may be from one another

Structure Analysis – individual based methods – take all individuals, use clustering algorithm, each individual assigned a probability of belonging to genetic clusters. Population information AND info about individuals

Genetic Diversity- can be measured many ways- look at allelic richness: how many alleles there are in a population, corrects for number of samples in each population.

Landscape genetics: powerful field which combines genetics, landscape information, spatial statistics

“Population” genetics, where: Populations defined ahead of time; genetic distance calculated between all pairs of “populations”; coarse scale

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; identify connectivity between barriers to gene flow

Random gene flow; Isolation by distance; Isolation by barrier; Isolation by distance with barrier; Isolation by resistance.

Questions:

- 1) USGS: Reciprocal Monophyly: One set of cluster looked different from rest, is that what mono comes from?
 - Those that share same node on tree would be reciprocally monophyletic.
 - If they are specifically monophyletic, they may be considered by some to be separate species
 - Some would argue that's not a good way to define a species because there may not have been enough time.
- 2) Expert : SNPs - looking at microsatellite data, there are typically thousands, correct
 - Yes, typically thousands 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 SNPs, that does not mean that microsatellite data is inferior.

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

Looking at greater sage-grouse or possibly Gunnison sage-grouse

Background: Historical distribution/Current distribution of greater sage-grouse. Some populations small and isolated

Regional pop. Declines, fragmentation and degradation of habitat

Movement: seasonal movements are variable; some pops are described as mig.; movements and connectivity among pops. 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

Historically, 1 species of sage grouse; 2 described subspecies – eastern and western.

Gunnison sage grouse recognized (*C. minimus*) – all other sage grouse renamed “Greater sage-grouse”

Published Studies: Investigating the lek mating system

Parentage:

- 1) Microsatellite analysis of female mating behavior in lek-breeding sage grouse: Semple, Wayne, and Gibson

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 previously thought

- 2) Bird et al. 2012 – Behavioral ecology

Most of clutches had single mother and father but there was evidence of multiple paternity (2.2%) and intraspecific nest parasitism (7.9%); The proportion of all sampled males who fathered offspring was 45.9%.

- 3) Gibson et al. 2005 – molecular ecology

Test whether lekking behavior evolved in GRS because kin selection...

Microsatellite markers; CA

Mean relatedness values on leks were not different from 0; Males on leks 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

- 4) Bush et al. 2010 – The Auk

Microsatellites; Alberta

Levels of genetic diversity were high and did not change over 9 years of study; Did not observe isolation by distance among leks; Leks were not differentiated from each other suggesting substantial gene flow across study area; No evidence of male philopatry

Taxonomy

Large-bodied vs. small-bodied sage grouse

- 1) Kahn et al. – The Auk 1999

Determine if genetic difference existed between large and small bodied sage grouse

mtDNA – CO

Haplotypes fell into 2 divergent clades; est. that the 2 clades began diverging at least 850,000 years ago; Haplotype frequencies in the small bodied pop were different and less diverse than the large bodied pops; absence of gene flow between 2 groups

- 2) Oyler-McCance et al 1999 – Mol. Ecology

Determine whether there was gene flow between large and small bodied birds

mtDNA and microsatellites

Sig. differences between large and small birds using both marker types

- 3) Young et al 2000 – Wilson Bull.

- 4) Benedict et al. 2003 – Cons. Genetics:

No genetic evidence was found to support the delineation of these subspecies; pop straddling the border between CA and NV (Bi-state) had an unusually high proportion of unique haplotypes, consistent with genetic isolation; had been isolated for thousands of years

Pop. Genetics:

- 1) Oyler-McCance et al – Journal of Wildlife Management

Assess the extent of pop structure and gene flow; High degree of pop structure and low amounts of gene flow among pops; Pop structure for GUSG was higher than had been reported

- 2) Oyler-McCance et al 2005 – Mol. Ecology

Examined the dist. Of genetic variation across range of GRSG; Pop. Follow an isolation-by distance model of restricted gene flow; Ident. 2 pops in WA with low levels of genetic variation; Another pop in Strawberry Valley had low genetic; Bi-state genetically distinct

- 3) Bush et al. 2011 – Conserv. Genetics

Birds from N. MT, Alberta, and Sask. were identified as a single pop that exhibited isolation by distance with Milk R demarcating 2 subpopulations

Both subpops had high genetic diversity; River valleys and a large ag region serving as barrier

4) Breidinger et al. 2013 W. North American Naturalist:

mtDNA control region data

No unusual haplotype compositions in the 3 remote pops; haplotype comp of the Anthro Mountain and Strawberry Valley reference pops differed from other NE UT

5) Zink 2014 – Open Ornithology

mtDNA Control region, microsatellites; Rangewide

With the exception of the WA pop, no consistent relationships were found between estimates of genetic variation and demographic trends across the range at either of the 2 spatial scales

GRSG does not exhibit genetics of declining pops

6) Oyler-McCance et al Cons. Genetics 2014?

mtDNA and microsatellites – supported the idea that Bi-state represents a genetically unique pop

7) Schulwitz et al The Condor 2014

Microsatellites

Found 4 genetic clusters: 1) Pinedale and Casper; 2) Powder R. Basin and SE MT 3) Jackson Hole 4) Gros Ventre

All samples outside JH and GV showed isolation by distance

8) Thompson et al –Chapter 5 of dissertation: Submitted to Condor – In Review

Patterns of dispersal, gene flow, and genetic structure – NW CO

All leks had high genetic diversity

Moderate-high gene flow between neighboring PMZ

PMZs showed isolation-by-dist. With a gene flow following a directional or 2-dimensional Stepping stone pattern that was local and between neighboring leks

9) Dawn Davis – Chapter 5 of dissertation –Univ. of Idaho- Journal of Wildlife Management – in review

Microsatellites

Sign. Isolation by distance among males, suggesting that males are more philopatric sex and females have a greater predisposition to disperse

Stronger spatial structuring for males vs. females

10) Chapter 2 - Tebbenkamp, Univ. of Idaho. – submitted to Conservation Genetics

Pairwise F_{ST} est. and Bayesian clustering methods provided evidence for 5 genetic pops within the bi-state.

Misc. studies:

Sex Determination

- 1) Bush et al. 2005- Conservation Genetics
- 2) Baumgardt et al.: Primer Notes; Genomic Applications

Papers in Review:

- 1) Oyler-McCance et al – submitted to the Auk – Re-examining genetic variation
- 2) Oyler-McCance et al – submitted to Heredity
- 3) Hanks et al. – submitted to Biometrics – Latent spatial models and sampling design for landscape genetics; Microsatellites- rangewide
- 4) Fedy et al. submitted to Ecological Applications
- 5) Row et al. Submitted to Ecology and Evolution – Microsatellites in WY

Research Efforts in Progress:

- 1) Assess impacts of translocations in WA – Schroder
- 2) Assessing impacts of translocations into Strawberry Valley, UT –
- 3) Landscape Genetics: Mike Schwartz began to do it in MT; Oyler-McCance in WY; now working together
- 4) Intensive landscape genetic efforts in WY and MT and for Gunnison Sage-grouse in CO
- 5) Rangewide Connectivity Assessment – comprehensive, rangewide assesement

Delineate the genetic pop structure of GRSB across range; quantify genetic connectivity

Collect genetic data across the range; collect feathers at leks in all states and provinces; process feathers in lab to obtain genetic info; use genetic data in combination with spatial analysis of landscape components; huge collaborative effort

- 6) Development of a high resolution genome-wide SNP array

Allow to look at adaptation as well – Todd Cross – look for genes that he knows are functional genes- characterized from the chicken because they are not that far apart. Should be able to find adaptive SNPs and Neutral SNPs. See how well they compare to the current microsatellite markers

- 7) Identify Conservation Units inferred from genetic variation across the landscapes: Do CUs designated using microsatellites differ from CUs delineated from SNPs

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.
- 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?
 - 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.
 - Expert: More of a hierarchical structure: individual, lek, etc.
 - 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. Useful to sample birds because they are all 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?
 - 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 – if fidelity to an area would outweigh moving to a new location
 - Females will first visit area where they will eventually nest, and then go to lek, and go back to nest. Significant fraction of females who will go to peripheral areas 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 N are larger than birds farther south. May have implications to translocation. Body size alone is not enough to distinguish them as different species.

USGS: 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 E and W.
- Expert: Rangelwide study of genetics could be re-examined with E/W 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 E/W 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 lit. until 1930s. Lewis and Clark did not see GRS 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 GRS 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 geneticists). 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. 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 will look at all available information. We want to know uncertainties and rationale for thoughts (i.e., I'm uncertain about XYZ, but I think it will be clarified by X upcoming research...)

USGS: First question: Evaluating gene flow between the E and W portions of the GRS 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.

E/W 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 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 E/W 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 E/W paper, but there was a lot more information from that study that could be looked to attempt to look at that.
- 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
- 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 ind. 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 E/W, 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 analysis of dispersal vs. genetic analysis. Genetic analysis is much more reliable because of bias related to behavioral research.
- FWS: first question – line of E/W boundary – potentially based on lek location?
- Expert: Hypothesis – evidence does not support gene flow between E/W?

Exercise – use sticky notes to write your estimate.

Results of Exercise:

USGS: Some folks saw symmetric distribution, some had a tail to them but were largely at one end...

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 E/W patches?
- Expert: Snake River, Idaho- higher human population, ag., become 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: Are we having enough gene flow to have X% gene flow? Mills et al. 2000? Mammal communities in pacific NW – Lowe and Allendorf - 2012 – give numbers of migrants per

generation. Low 0-1, med 1-10, high >10. Meaningful level to get at – gene flow between 2 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 E/W 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 E/W - 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
- USGS: Need is for large scale, recent time.
- Expert: In the grand scheme of things, there haven't been a lot of things changing in the dividing area (Yellowstone, Tetons, etc.)
- Expert: Definition of recent and historic: Recent – now; historic – before Lewis and Clark?

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 GRSB gene flow, if any.

Break out into 3 groups (30 minutes), use distribution map to identify no more than 5 barriers to GRSB 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 detectable 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 had 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: with new genetic information potentially available 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 GRS 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 historically
- 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. This is just one piece of information for our listing decision. Policy decision at a later time – we do not want you to be involved in that decision. When we start receiving more information, we feel we will be more qualified to process additional genetic information as it comes in as related to decision.

- Expert: Decisions should be data based as opposed to opinion based, which is where some of the uncomfortableness from this morning.
- FWS: The conversation from these exercises is key and provides a good foundation of information and background- helpful to those of us in 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 their mal-adapted because of genetic drift. Looking at what they've done with Attwater's prairie chicken – instead of translocating LEPC from Kansas, they've chosen not to.
- 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, 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 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.
- 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 to infer differences genetically or potentially phenotypes
- USGS: Has genetics been able to identify GRSB 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?
- 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 GRSB, 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

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, E/W Montana, MO river

- 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 – 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 shows 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 in N. Sea – 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 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's not 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 pop – 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 E/W 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 ESUs/Management Units, would it make sense that there's any disconnection between the E/W 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 GRS? 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 disproportionately 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 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 Mountain 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 example in IL 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: $\text{Survival (pop or ind)} = \text{threat} + \text{inbreeding} + \text{threat} * \text{inbreeding}$; 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 a candidate
- 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). Fragmenters 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 GRS habitat is on Federal lands, which has dual use management. Question: We are constantly 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 much more relaxed. Hope is that the current range map does not turn into PAC map in the future. Connectivity map shows links.
- FWS: On W side of PACs- other threats such as fire and invasive may relate to genetic implications than what may otherwise appear when looking at PACs.
- 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 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? Is that objective of the question “how much is too much”?
- 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 ask, “So what if you lose the Columbian Basin, what does that mean for genetic diversity for GRS? How much emphasis should be placed on protecting that?”
- 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: WNV, are there any individuals that have been seen to be more fit genetically to survive?
- FWS: Individuals that do get WNV die- transmission issues and collection of mortalities – potential die offs that are easy to miss. Biggest die-offs are in areas with coal-bed methane and surface water.

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.

Expert Elicitation Workshop on the Genetics of the Greater Sage-Grouse
Wednesday October 22, 8:00 AM – 5:00 PM and
Thursday October 23, 8:00 AM – 12:00 PM
Fort Collins, Colorado

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

8:00 – Welcome, logistics, and introductions – purpose

CH: Gathering best available for sage-grouse listing decision

Decision is FWS-only; this meeting is purely for information-gathering only, no decision-making involved
FWS has a deadline of September 30 2015 for listing decision.

We lack technical expertise to evaluate available genetic information - gathered you all together to help us out with that and to elicit additional information from you.

[GROUND RULES DOCUMENT]

Introductions:

Craig – FWS writing team, species report

Dave – increasingly involved in expert elicitation to fill information gaps and decision-analysis

Jonathan – post-doc in Leetown Science Center with Dave, manning computer

Steve – helping Dave and Jonathan keep the ball moving and on track

Jesse – R1 Candidate Conservation coordinator

Kate – R6 project manager for status review

Mary – R8 senior biologist on sage-grouse

Theo – R6 public affairs office, here in case media shows up

HF – R1 RO writing team, species report

Pat – National Sage-grouse cons coordinator - Cheyenne

Angela – R6 RO writing team, species report

Lief – R6 sagegrouse ecologist Cheyenne

Experts:

- Jesus Maldonado – Smithsonian. Focused on endangered mammals
- Jeff Olson – Cons genetics lab in Anchorage AK; mainly salmonid fishes
- Chris Funk – Conservation genomics of vertebrates
- Mike Schroeder – sage-grouse non genetics since 1981
- Christian – conservation genetics birds
- Robert Gibson – behavioral ecologist
- MikeSchwartz – USFS monitoring methods, research solutions, only wildlife genetics in USFS
- Sara Oyler- McCance – research geneticist – genetics/genomic sage-grouse and other species
- Mevin Hooton – co-op unit here – statistician – had a Gunnison's student, work with Mike and Sara and others on optimal feather collection

CH: wall maps, sticky notes for scoring, info packets

DS: asked Sara for background on genetic methods for non-geneticists in the room, as well as sage-grouse. Will end by 5 and start promptly at 8 tomorrow. Agenda – specific information we will cover starting after break after Sara's talk.

CH: security in bldg. Letter from Congress; we are recording notes from meeting – will circulate draft notes and exec summary for review. All will go into our admin record.

Sara:

Dave asked her for a couple of things: basic genetics (background for benefit of non-geneticists), then talk specifically about sage-grouse genetics – what’s out, what’s in review, etc., for everyone’s benefit.

Background

Use DNA – genetics is a tool used to answer many questions. Like any tool, has pros and cons – not be-all, end-all. Limitations.

Basics: DNA is coiled and folded into chromosomes which are located in the nucleus of a cell.

Gene = sequence of DNA that codes for some gene product (a protein or RNA)

Not all DNA codes for products – some coding, not coding.

Locus = where a gene is located.

Genotypes = pairs of genes or non-coding sequences at a locus. Homozygosity vs heterozygosity (latter important for genetic diversity)

Sources of DNA = any biological tissue or fluid

PCR = Targeting a certain location in the genome, go in and target a certain area of genome and make many copies of it

Molecular markers – regions in the genome targeted

Coding (non-neutral markers) – proteins, RNA

Non-coding (neutral markers) – introns, microsatellites

DNA in nucleus and in mitochondria

mtDNA

Has been used a lot, especially in past. Maternally inherited (one copy). Small, circular genome (16k basepairs in birds, e.g.) – lots of genes in mt

Some evolve quickly = control region (population differences)

Some evolves more slowly = (e.g.) Cytochrome B (phylogenetics/taxonomy)

Amplify and sequence: read every base-pair in the product that is amplified

Assign a haplotype depending on what the product looks like

If there’s a base substitution, then different haplotype

Compare distribution of haplotypes between populations, can say something about gene flow between them.

How related are haplotypes to each other? Different by one base-pair substitution, more substitutions?

The more substitutions, the greater the difference, the more divergent, and the common ancestor is more removed in history.

Relationships among haplotypes and distribution on landscape – phylogenetic tree.

A chunk all the same = reciprocally monophyletic

Often more messy, however. May shed light on what has occurred in evolutionary time

Nuclear DNA

Most of it evolves very slowly, some evolves very quickly and targeted for landscape studies

Inherited from both parents

Types of markers

1. Microsatellites: regions in nuclear genome comprised of short repeated sequences

Instead of base substitutions, looking for mutations that, for example, add or delete repeats. Fragments are different sizes and can be measured on a gel. Size of fragment defines genotype

2. Single nucleotide polymorphisms (SNPs)

Regions where genome vary by a single nucleotide – can occur in coding and non-coding regions. Can look at genetic variation. More common.

Looking at large numbers of SNPs. They occur a LOT

Microsatellite genotyping – looking these days at as many as 15 loci. In contrast, with SNPs, genotyping based on clouds of data points

Questions investigated with genetic methods:

- Assessing parentage – how many of the males are breeding (e.g., which hen doesn't belong to the hen)
- How connected are populations – compare allele frequencies among populations to assess gene flow
- Calculate genetic distance between populations – build matrix of relatedness
 - o Can do pop studies based on individuals (vs pops) – take all of individuals use clustering algorithm, what is most appropriate or likely clustering? Each individual given a probability of belonging to each cluster identified
- Genetic diversity – many ways to measure. With microsats, allelic richness. Corrects for differences in sample sizes.
- Landscape genetics – newish field; amalgamation of pop genetics, landscape ecology, and spatial statistics.

Most recent developments: Moving from population approach (going in ahead of time, identifying pops, and sampling accordingly, then calculating genetic distances) to a more landscape-focused approach, with many more individuals sampled homogeneously across landscape (without any assumptions about populations). Instead of genetic distance between populations, genetic distance between all pairs of individuals. Assess connectivity and ID barriers to gene flow. Gene flow in more fragmented landscapes.

Frees up hypothesis-generating across the landscape. Isolation by distance? Isolation by barrier?

Isolation by distance with a barrier? Isolation by resistance (accounts for cost for individual to move across landscape – integrates habitat quality, etc.)?

QUESTIONS

Q: reciprocal monophyly?

A: Sometimes guides definition of species – given enough time, all species become reciprocally monophyletic. But not all species are. Depends on definition of species being used.

Q: SNPs vs. microsatellites? – spreadsheet slide – SNPs table would be vast, right?

A: Yes. Microsats – could have many states at one locus (reef fish, for example). Microsat and SNP loci are different.

[exchange about differences between microsats and SNPs]

Q: are both microsats and SNPs used to characterize allele frequency?

A: can use SNPs similarly to look at pop structure, maybe not comparable with microsats for assessing genetic diversity. Allelic richness measure from SNPs? Heterozygosity, but also different from microsats. Can also look at SNPs that are under selection (as well as non-coding SNPs).

A: Diversity metrics: expect more heterozygosity in micros than SNPs – more states at each locus. But more SNPs should be more powerful than microsats because larger data sets, and can look at local adaptation. Results should be correlated with each other if looking at mutation, genetic drift and other random or non-coded processes. SNPs not as useful with non-invasive sampling – higher drop-out rates(e.g., from dropped feathers), e.g., working with ancient DNA. Microsats very standardized now, SNPs obtained many different ways.

Q: 2005 markers vs. new study now?

A: using more markers now.

Overview of GRSG genetics

Sara reviewed all the literature she could find. Looked at studies to do with species-level analysis (a little about Gunnison's)

Background about GRSG

- Mike Schroeder's rangemap. Lots of range-contraction, has left many pops small and isolated
- Have been significant regional pop declines – 17-47% Loss, frag, degradation of habitat
- Movements are highly variable. Seasonal movements, some migratory over 80km Connectivity largely unknown
- Mating system – polygynous, males defend leks and display to attract females. Usually only dominant males breed – big impact on effective pop size and overall genetic diversity of species.
- Historically one species of SG. Used to be a W and E subspecies. In 2000, GUSG recognized. All the rest then renames GRSG.

Published Studies – lek system, taxonomic, population genetics, misc. studies

Parentage and mating systems

Semple et al. – year? - Investigate mating systems by comparing behavioral data and genetic data – Microsats, pop in CA

20% of broods had multiple fathers – maybe more prevalent than thought

In all cases with data, genetic and obs data matches up.

Bird et al. 2012 – Determine paternity and polygamy

Alberta, microsats

Most clutches had a single mother and father, but evidence of multiple paternity (8%)

Intraspecific nest parasitism -

7-year study, proportion of sampled males that fathered broods – 47%

Gibson et al. – 2005 – test whether lekking behavior evolved in sage-grouse because of kin selection

Microsats, California

Mean relatedness values on leks were not different than zero – males on leks not closely related

Lower than known family members

No evidence of clustering. Related individuals tend to cluster off leks

Leks largely unrelated males

Bush et al. 2010 – assess genetic diversity in Alberta and sex-specific relatedness within and among leks

Microsats, Alberta

Genetic diversity on leks high and didn't change over 9-yr study

Substantial gene flow across study area

Relatedness on leks low – no evidence of male philopatry

Taxonomy (e.g., GRS/GUSG)

Kahn et al. – 1999 – Determine whether genetic differences exist between large and small bodied forms of sage grouse and estimate a crude timeframe in morph and behavior differences between forms

Microsats, Colorado

Two divergent clades, represented ancestrally divergent pops that subsequently intermixed

Estimated beginning divergence about 850K ybp, absence of gene flow today between two groups

Oyler-McCance et al. 1999 – expanded on previous, included more pops. Determine whether there was gene flow

Significant differences between large and small using both mtDNA and microsats

Lack of reciprocal monophyly

Further support recognition of distinct species based on biological species concept

Young et al. 2000 – Gunnison's sage grouse

Benedict et al. 2003 – evaluate subspecies evaluation in GRS

Western part of range – mtDNA (control region)

No real genetic evidence to support subspecies distinction

Lyon-Mono pop different – unusually high proportion of unique haplotypes – consistent with long isolation.

Low genetic diversity in WA state (two pops)

Gene flow, connectivity, genetic diversity.

Oyler-McCance et al, 2005 – pop subdivision in GUSG

mtDNA control and microsats

High degree of pop structure and low gene flow.

Structure higher than had been reported for GRSg

Low diversity in Dove Creek

Oyler-McCance et al. 2005 – GRSg dist of genetic variation across the range

Mt DNA control and microsats – whole range

Pops tended to fall into isolation-by-distance model

Reidentified low diversity in WA – decline

Strawberry Valley Utah – low diversity

Lyon-Mono distinct

Bird et al 2011 – Alberta, MT, WY

Range edge dynamics, microsats

Birds from this region – single population, isolation by distance (milk river separating two sub pops)

Both subpops had high genetic diversity

Barriers to gene flow

Maintaining genetic connectivity in fragmented habitat

McBride et al

Assess mtDNA diversity in three remote pops in Utah

No unusual haplotype combos in these 3 pops

Antho mtn and Strawberry ref pops differed from rest of Utah

Desolation Canyon barrier to gene flow?

Zink – 2014 – compare published genetic and demographic data – reduction in genetic diversity?

mtDNA and micro sat (previously published)

With exception of WA pops, no consistent relationships between genetic var and demographic data

Measure inbreeding not related to pop trends – doesn't show expected genetic signatures of declining pops

Oyler-McCance et al. 2014 – better understanding of boundaries of Bi-State pop and examining genetic structure within it

mtDNA and microsats – both support idea that bi-state is genetically unique

significant genetic structure – N-S, evidence for 3 subpopulations

Schulwitz (sp.?) et al – just now – Jackson Hole and Gros Ventre pops in MT

Microstats

Pinedale and Casper, PRB, and SE MT, and Jackson hole and Gros Ventre

Low genetic diversity and small pops.

Historical gene flow

Thompson et al. (diss) Pop genetics - NW CO

Microsats

All leks – high genetic diversity, moderate to high flow between management zones

Isolation by distance

Male-biased dispersal – male moving further

Higher genetic structure in females

Dispersal rare of more than 40-60 km, both sexes

Davis et al (diss) – in revision for JWM

Patterns of dispersal in NE CA

Microsats

Significant isolation by distance among males; more phylopatric than females

Stronger structure for males than females

Tebbenkamp et al (thesis) – in review

Genetic structure within bistate

Microsats

Pairwise evidence for 5 genetic populations

No documented dispersal events, but genetic assignment suggests recent dispersal.

Misc:

Bird et al.

Baumgardt et al.

Primer notes (several papers)

Genomic applications (several papers)

Papers in Review – Taxonomy, pop/landscape genetics

Oyler-McCance – in review

SNP approaches, GUSG, GRSG, bi-state

Where in genome biggest diffs between GRS & GUSG

Hanks et al. in review – spatially explicit models and sampling design – how to use to optimize landscape genetic analyses - microsats

Fedy et al. – submitted – genetic and demographic data to assess population risk

Row et al. in review – landscape genetics in WY, importance of seasonal habitat distribution – connectivity

microsats

Ongoing Research

impacts of translocations
pop/landscape genetics
rangewide connectivity
devel. of genomic markers

Assessing impacts of translocations in WA – Schroeder et al.

Increasing genetic diversity?

Assessing impacts of translocations into Strawberry Utah

Landscape genetics in progress:

Many attempts to start doing this for sage-grouse

Schwartz and Cross in MT, O-McC and Fedy in WY Joined forced

Murphy at U WY – landscape studies in WY

Grad student on GUSG in CO

Oyler-McCance et al. Rangewide connectivity assessment: delineate genetic population structure across range, relative importance of landscape features to influence gene flow

Collect feathers across range during lek counts in all states/provinces

Processing thousands of feather samples

Will use in combination with spatial analysis of landscape components

Good data from MT and WY, more coming on line soon

GIGANTIC collaborative effort

Microsats now

Difficulty – comparing between labs, etc.

Adding SNPs – trying to do both this and microsats to allow assessment of local adaptation

Mainly Todd Cross

Look for genes he knows are functional, identify candidate SNPs under selection as well as neutral SNPs.

Also want to see how they compare with microsat data

Identify conservation units: neutral processes as well as genes subject to selections

Would CUs designated based on microsats be the same at those designated based on SNPs and local adaptation?

QUESTIONS:

Comment: know that during period mating is occurring, some males not on leks – what are they doing?

In black grouse, about 20% of mating happens off leks. Very small-scale study, tried to catch all females at one lek. K Bird's study has much larger samples of birds, but all feathers of males collected on leks, so these were the most likely to breed – biased sample. Conclusions resting on that assumption. Do we have genetic data that can translate into effective population size.

Comment: Males on leks can mate a lot of times in one morning – females will copulate with more than one male.

Comment: no time to read all the papers – not a GRS person. Is the lek is the unit of sample? Putative population?

A: 2005 paper – hunter collected wings, so not lek sampled. Hunt units. New range-wide study is basically tied to leks, but leks not interchangeable with pops. Hope to let biology determine pops.

Comment: The birds use different parts of habitat at different time of year. When snow melts, males go to leks. Females go looking for someplace to nest. Individual females may nest as far as 20km from lek where they mated. Once all that's over and habitat dries up, birds move to mesic areas and upslope. Distribution in landscape then different again. So, hard to say that lek = population. Lek is a useful place to sample birds at a certain time of year, because that's where the birds are concentrated.

Comment: at least this is a consistent sampling method. Samples from hunter kills problematic!

Comment: only data on non-migrant population – a little telemetry data, old data. No such thing as a “non-migratory population.” Only exists w.r.t. individuals, individual phenotype, or extreme isolation. Depends on distribution of seasonal habitats.

Comment: if you radio-track enough birds, widely differing.

Q: What's known about frequency and decision-process in distance of nesting from lek?

A: had females fail then move 20 km and nest again. Depends. Success plays a role, fidelity plays a role, habitat change – if an area burns, don't know what response is, etc.

A: Studied in some detail in CA: large flocks in winter. As snow melts, females look for places to nest, males go to leks. Before females mate, find nesting area. More likely that females will go to leks that are ??? away, but still significant proportion of females that will disperse farther. Didn't study ecological factors that determined these patterns, just looked at factors.

Comment: high variation in body size. N-S gradient with N being larger. (Display behavior pretty comparable across range. GUSG display very different. Easy to see that they're different.) What are consequences for translocation?

DS: Early on, mentioned data alluding to E-W split?

SO-McC: Paper evaluating subspecies delineation. Was it supported by genetic data? No. Original delineation was based on coloration in museum specimens.

DS: any other large-scale E-W structure?

SO_McC: Range-wide paper didn't look at that, but data could be reexamined. Current range-wide work will answer this question.

PD: in 2010 looked at this question, couldn't find behavioral, movement, or genetic support for this delineation, although this was informal. AOU didn't have time to revisit nomenclature problem.

Comment: **There could be a different line out there. Green River? Core of Idaho? Red desert? Could find variation across some big landscape gaps. Ruffed grouse don't cross it.**

Comment: Have to be careful with STRUCTURE analyses – have to be careful that samples aren't missing across a gradient. Have to make sure differences are biological and not just statistical.

Comment: in 1940s and 50s, dealing with crop damage issues and trapping thousands of sage-grouse and hauling them everywhere. Not always good records of where they were released...Oregon?

Wyoming? Parts of Montana? Thousands of birds. WA – NM in 1958, but didn't arrive in good shape.

Comment: What's going on in landscape of sage-grouse? Fragmented, but what's happening over thousands of years?

Comment: Based on pollen, packrat middens, etc., expanding. Have expanded northward in Holocene. Maybe into Canada? Lewis and Clark didn't see them in eastern Montana, not until Maraias River. Maybe the grouse and prairie chicken were expanding north before latest contraction (and population declines). We don't know what was going on 100 years ago – absence of data.

| PART 2: BARRIERS TO GENE FLOW AND GENETIC DIVERGENCE IN SAGE-GROUSE | | |
|---|---|--|
| 10:15 am | Barriers to Gene Flow (Exercise 1): EVALUATE GENE FLOW BETWEEN THE EASTERN AND WESTERN PORTIONS OF THE GREATER SAGE-GROUSE'S RANGE. | Dave Smith Steve Morey Jonathan Cummings |

DS: Will focus discussion on specific questions –

SM: Spirit of expert elicitation: some of the questions that FWS is facing can't be answered by consulting a few published papers. We could ask somebody, but if we can ask the group in this forum we think we will have a much richer response. Diversity in this kind of group is a great thing and we rely on that for the richest discussion. We invited you because you have a great track record, but all have different academic, geographic frames of reference. That diversity means that when we through a question at you, all will have different levels of familiarity and frames of reference. By working together we can do what you're already doing. Discussion is to elevate and level the playing field. Consider next day and a half expansion of Q&A period we've just had. Not just those who have questions right in their wheelhouse, it's the people who are the most courageous to ask the naïve questions.

The agenda builds, each discussion rests on what we've discussed previously. Even if questions don't seem like they're in your area of expertise, they really are, because you're all great naturalists, and everyone's participation will be of greatest interest for the group and greatest value to FWS.

There's a tension between great participation and adhering to schedule. Remember to keep your remarks on topic and in the spirit of elevating and leveling the field.

There's no expectation of there being any right or wrong answer – just seeking variety of perspectives. We're interested in full characterization of your beliefs about whatever we're talking about.

DS: Format and roadmap: The format of this is that the questions are structured around FWS information needs. Four or five topics. We will introduce topics, discussion to clarify question/topic, clarify terms, etc. Will have to do with genetic patterns. Discussion about evidence. Then formal question to (a) take narrative discussion and make it more specific, and (b) capture uncertainty associated with judgment. Also want to capture differences in uncertainty between experts. Agenda builds on itself: Barriers to gene flow in sage-grouse. Will start with a question about a barrier and what we anticipate the gene flow to be across this barrier. Get our legs, define terms, get used to format. Then, further/more expansive discussion about barriers (in breakout groups), then come together to combine info. Then look at all barriers and rank them. Then genetic diversity questions and implications for barriers. May or may not be done by end of day – may have a homework assignment tonight and pick up tomorrow. Then issues of genetic divergence and persistence. Where in range might low diversity affect persistence. Then time to do a check-in, and revisit upcoming research.

Q: Sara's talk: ongoing research, high quality, high density. We'll be giving our opinions about these questions, so how you will use information that comes along after this workshop?

DS: Depends on timing. There's a timeline for the decision – 30 Sep 2015. Information has to be available prior to that. FWS is very interested in knowing what new information will be coming along. If you think some future research may answer the question better than we can do today, that could be represented in your characterization of your uncertainty and rationale. You've all been invited because of what you know now and your background and expertise, so we are interested in your input now.

EXERCISE 1: EVALUATING GENE FLOW BETWEEN E & W PORTIONS OF GRSG RANGE

Low and high diversity and low gene flow across barriers came up in Sara's talk, but we want to be more specific and have a common understanding of what, for example, "low" and "high" mean.

Distribution map (red range map): look at E vs W. General pattern apparent. Someone mentioned difference in haplotype frequency in MT, for example, distinguishing NE vs SW. Someone else mentioned Green River as a potential barrier. Too, distribution of threats may vary across range and inform this question too.

Round 1: Look at whole range and consider evidence of potential barrier to E-W flow. Spread 100 points among following responses:

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

Then discuss.

Round 2: Then individually assign 100 points to responses and express rationale.

Comment: it's one thing to say there's variable gene flow, it's something else to say there's a barrier.

Are we hooked on this barrier concept, or is there a continuum?

Comment: profoundly uncomfortable with this process, and it's antithetical to what everyone around the table does as science. This isn't how you do this. Would be okay with coming to a consensus about what low gene flow means. Why would we go with an opinion-guided process? Not trying to throw a wrench in this process?

DS: Good point. If there is a published paper that addresses these particular questions, we can identify that and use it. Often times, the question in a decision-makers mind doesn't come packaged that way. We're not seeking opinion in the isolation of evidence. You know the evidence and the inference that can be drawn from this evidence. There's literature that says that this IS the way to do this.

Comment: when there's gaps, I agree with you. But within six months we're going to have a paper that addresses this whole question. We can come up scientifically with this answer within a few months.

DS: but Right Now there is uncertainty, and the workshop outcomes will reflect that. E.g., right now, if you're completely uncertain, then each category gets 20 points. Period. Not the first time this question has come up, although your specific wording was unique.

SM: One way of looking at it is that these regulatory processes don't stop, they march forward with preset deadlines.

Comment: Agree, but this seems harmless. So we put a bunch of junk down and talk about it. Can't see the point of delaying. Round 1 is simply to get the ball rolling.

Comment: So we're going to do this, so how do we define E and W portions of range? Also need some idea of what the genetic groupings are and see what's known about gene flow among them.

DS: Take first thing: What makes sense re E vs W?

Comment: Seems like has to be based on Sara's 2005 paper – best information available now. Looking at it now, not really distinct genetic groups – isolation by distance.

Comment – yes, that's what they found. Point of paper was not to look for barriers. Could you rerun the data to answer that question? STRUCTURE had just come out, and may be more sophisticated analyses you could do now to look at barriers. Looks for unique clusters and assigns individuals to clusters. Did the easiest thing you could do: This population has the greatest proportion of "blue" (but there's more info in each blue dot you could use to get at barrier question).

Comment: For clarification – should we ask more clarification about this question?

DS: YES – will keep talking until we get some clarity

Comment: Tough to define gene flow. What temporal scale? Genome evolves at various rates in various regions (of genome), and with respect to landscape. Short term? Long term?

DS: We want your opinion – what is best temporal scale to consider

Comment: pretty easy if you say gene flow tomorrow or in a million years, but difficult as you slide along that scale.

Comment: Historical or current barriers?

DS: Would contemporary gene flow be the place to start (rather than historical)?

Comment: Let's stick with Sara's study. Differences in haplotype frequencies – evolutionary history of sage-grouse. Gene flow now? A complete guess.

Comment: what's the shortest timescale?

Comment: Allele-based (past) vs. assignment. Averaged over time effects? That's what 2005 paper looks like. Can say there have been simulations and empirical analyses when known barrier created and can see if you can ascribe differences to those barriers. Just because you see a genetic difference, can't say how long it's been there. Theory tell us that patterns at microsat scale (effective pop size - N_e) – if $N_e + 20$, it takes about 80 generations for patterns to form. Couple hundred years for GRS? Haven't seen N_e for GRS.

Comment: N_e size will be affected by distribution in space. Could have really small N_e and see rapid change on the landscape at a smaller scale than what you want to consider as distinct segments.

Comment: if you want to look at contemporary gene flow have to look at telemetry or assignment tests.

DS: So, genetic evidence is really for evaluating historical gene flow – but doesn't say when in the past. Could be wide temporal scale.

Comment: There's multiple signals. mtDNA different timeframe than microsat.

Comment: also gender-specific – mtDNA = female dispersal only.

Comment: mtDNA doesn't evolve as fast as hypervariable microsat regions.

Comment: male vs. female – several papers on reading lists addressing this issue. No consistent pattern in sage grouse. Some male-biased dispersal, some female-biased dispersal. So maybe we can't worry about mtDNA vs. microsat rates – bigger issues.

DS: check in with FWS –

JD: how about other sources of info, e.g., lek connectivity. Might be helpful to look at other data sources

Comment: long tension between behaviorally vs genetic evidence of dispersal. Telemetry biased toward very short-term processes. Genetic evidence far, far better. Not saying we shouldn't look at other stuff, but shouldn't give it greater weight than genetic data.

JD: just pointing out that there's other information that people can draw on to discuss where an E-W split might be. That's the first question: where are we talking about? If you look at lek distribution map, there's a clear line that can be drawn.

Comment: can't we rule out A and E? There is gene flow. Given data in hand, clearly there is not no gene flow, and it's not panmyctic.

Comment: how about frame it as a hypothesis...

DS: ready to do Round 1? On sticky notes: name, and distribution of points A through E. Craig will collect.

ROUND 1 TABLE OF SCORES:

DS: talk about differences, rationales. Issue of different timescales and views of gene flow, etc.

Comment: seems like in order to answer this question we have to consider the spatial scale. Opposite ends of range, there may be none, center of range there may be a lot.

Comment: Talking about birds associated with a particular ecosystem and variety of habitats within it to make it through the year. The way that maps on this area is highly complex. Has become more complex because of what humans have done on the landscape. Will vary tremendously based on underlying heterogeneity. Can't sort it out on the back of an envelope. There are some specific studies of areas where geography is well known and there's enough context to discuss this, but there are other areas where there's little information.

DS: can we talk about spatially averaged patterns across the range? Between these two big patches? Intuition about this?

Comment: Lots of time in SE ID looking at STGR. Seen a lot of GRSG and know where they are. Look at Snake River – historically that was GRSG habitat. Now there's people living in it, converted for cropland, etc. Now no leks near the river. However, that doesn't necessarily mean they don't cross it. Not habitat they'd nest in, but a bird traveling in November may have no problem crossing it. Even so, that's a recent thing in the past century. Other areas are a little more natural – SW corner of Montana. Birds on W side of Tetons connect to rest of ID. Do the birds on the east side, at Jackson Hole, get to the birds on the west side? They don't have to go very far, but Tetons in the way. Really best habitat for getting from E to W is SE, fragmented habitat. Likely there are birds making that transit, but seldom shows up in connectivity work. In most of range there's no restrictions. Lean toward low gene flow – a fraction of “unrestricted” movement. Small populations: movement is a function of area of occupancy/pop size. In small populations, movement is restricted.

DS: How are folks

Comment: one was geneticists think about gene flow, # of migrants or proportion of migrants from pop A to pop B per generation. Rules of thumb. Standard for low = 1 migrant per generation. Pretty substantial differences in allele frequencies over time. F_{st} values.

Comment: could look at this a variety of ways. What kind of connectivity do you want to preserve? Is there enough gene-flow to prevent demographic problems. Have to define these questions and thresholds to answer these questions. 0-1, 1-10, >10 migrants per generation – rule of thumb, but depends on functional definitions – what’s “enough”? (Mills et al. 200X? Book chapter. Mammal communities in Pacific Northwest.; Lowe and Allendorf 2012(?).)

Comment: isn’t a function of the pop size you’re going to or coming from? E.g., if Wyoming doesn’t get any migrants from the west, not going to change.

Comment: if you have a huge population, it doesn’t matter.

Comment: pretty clear from score distributions that no one is sure what the definitions of low, medium, and high are.

Comment: It would take a long time for lack of gene flow to result in reciprocal monophyly.

Comment: Presume what you guys are ultimately interested in is when whether gene flow poses a threat to demography and threatens population collapse. Right?

[[[This didn’t get corrected – this is NOT the only aim of this]]]

Comment: We don’t even know what the E-W split it, would like to have the person in the room who knows more about GRSG than anyone go draw a line on the map. To clarify, we’re trying to understand if there’s connection between one half of the range and the other half of the range, not whether there’s movement between CA and ND, right?

Comment: “Is there enough flow to prevent breeding collapse” is one question, is another the question of distinct population segments?

Comment: not making policy decision here, gathering info to make policy decisions.

Comment: are you asking if there are distinct biological, ESUs?

Comment: Differences in haplotype frequencies – deep ancestral patterns. Do we actually know very much about historically whether range has contracted into distinct units?

Comment: on broad scale, pretty good evidence for example that sage-grouse occurred all over NM, but which species? Dunno. No one has looked at those bones. Formerly found all the way to Mexico border. Big change we know about. And know they were once more widely distributed in ID, owing to formerly greater extent of sagebrush habitat.

Comment: Sara had nice graphics about landscape genetics. All kinds of hypotheses about isolation by X. We all agreed we’re talking about a barrier orientied N-S. If we’re just talking about this barrier, would score differently. There’s isn’t one sort of continuous barrier from top to bottom. So how much of that N-S oriented barrier is operating as a barrier?

DS: Round 2: want to open it up again to see if any more clarifying questions, comments.

JD: Curious how many people are looking at this map, and Sara’s cluster map. Lot of colors different between E and W. Big Horn, for example, has a color more prevalent in the West. How are you looking at these clusters in your evaluation?

[Sara is looking at it and wishing she’d done it differently!]

Comment: would need to look at the data different ways to answer the question at hand, because the dots are not absolute. There is a F_{st} matrix, but it’s not in the paper.

Comment: I don’t want to pick on any one person. Still don’t understand the question or how to revisit my scores. This is all dependent on scale, we’re using different definitions. There’s someone who

thought there's a 50% chance of complete mixing, panmyxia. This person is looking at it at a different scale than I am. What did you mean?

Comment: I was thinking across the range, not a particular spot. Thinking that there are areas in the range where mixing IS completely unrestricted. Not using strict definition of "panmyxia."

Comment: so we need to get on the same page and ensure we're all using the same definitions.

Comment: need to tell us or have us define what the scales are. Need to come up with our own question. On average, how much gene flow do we think is going on in specific places.

Comment: at huge scale, could probably come to some agreement.

Comment: could come up with a ranking for the whole two halves, and one for a couple of populations that are 50 miles apart.

Comment: but the question is about a barrier, not a scale. Can we talk about a barrier or barrier-with-distance.

Comment: So maybe first question is, "is there or isn't there a barrier?"

Comment: Or, "is flow homogeneous or heterogeneous across range?"

DS: the question IS large scale, and refers to N-S barrier. Distance could be influencing this. Is barrier defining an eastern portion and a western portion of range.

Comment: suggest ditching the distance component.

DS: An operational definition could be derived from the previous discussion, focusing on migrants-per-generation, and we're looking at the best genetic evidence over a long period of time, historically (right?).

Comment: seems to me question you're asking is about contemporary gene flow. So not sure mtDNA is relevant. Should be focusing on microsat data. Not enough in 2005 map to tell you how many migrants between two points. There is a mixture of colors in each dot – current dots simply represent majority.

Comment: isolation by distance map in paper. R_{st} values high for a bird. Some populations so have a lot of divergence between them, typically the ones that are farthest apart.

Comment: have to be careful about how you divide your populations when you have isolation by distance.

Comment: this doesn't show potential barriers – that would be indicated by spread.

SM: Need to restate the question for Round 2.

First a diagram. Time and space matrix – recent/historical, small/large scale. Which box are we in?

DS: understand we are in recent, large-scale box. Gene flow across barriers that extend N to S and split range of GRSG into Eastern and Western components.

Comment: depends why we're bringing this up. If you're doing this to ID DPSs, then go historic. If you're talking influence of oil and gas, etc., leave it where it is (recent, large-scale).

Comment: E.g., Snake River – a division, now, but not historically.

Comment: The big issue isn't the Snake River or Teton/Yellowstone complex. Haven't changed. SE ID has a lot of landuse but probably not changing sage-grouse distribution that much. Historically (say, 200 years ago, but acknowledging anthro landscape change long before that) or recently, probably hasn't changed much.

Comment: is historic timeframe relevant?

Comment: YES ice-age refugia and influence on genetics, especially with respect to DPS.

DS: FWS: what are the info needs?

JD: Recent, large-scale.

DS: Round 2: The question is this - Recent, large-scale gene flow across barriers that extend N to S and split range of GRSG into Eastern and Western components. Distribute 100 points.

[lunch]

ROUND 2 TABLE

DS: Will use results to structure subsequent questions

Exercise 2: Identify potential barriers. Use distribution map to discuss what might be no more than five barriers to gene flow. Location, source, why barriers, how long they've been there, and to what degree they're barriers (scale of gene flow discussed earlier, in terms of migrants/generation). Indicate spatial and temporal scale considered (use four-cell matrix). Each group will have someone familiar with sage-grouse data.

Q: actually put them on the map?

DS: Yes.

Q: So, these really aren't barriers, right?

DS: Not impermeable.

Q: One of us is involved with a paper on gene-flow in Wyoming. That gets after this exact question about barriers, right?

A: some of them, yes.

Q: Can we see some of those data here and now?

DS: Each group is going to report out, so that would be a time to reference this.

A: The paper is in review. Can't share results now.

DS: There will be an opportunity tomorrow to discuss current analyses that would inform the status review.

MAP WITH LINES DEPICTING EACH GROUP'S LINES (BARRIERS)

Brief presentations from each group.

Group #1: Chose factors they thought would be the biggest. Settled on the three natural and anthropogenic landscape features that influence movement.

Mountains (Wind Rivers, Bighorns, Teton/Yellowstone, S. CO) natural plus anthro. Heterogeneity. Don't think elevation per se is an issue, but habitat features may be.

Rivers (Missouri, including reservoirs and Columbia – 4 mile-wide, owing to depth and breadth of valley...don't fly across very often. Pops separated by Columbia).

Desert (Southern Nevada – habitat is up higher; reverse of what you find further north. Sky-island situation).

Cropland – forces birds into small patches of habitat. Lots of cropland in Montana, Utah, SE Idaho. Not always the case that sage-grouse only persist in contiguous habitat. Most leks in N WA are in wheat fields. Nice and open and good if surrounded by shrub-steppe.

Transmission lines – connectivity study in WA, used multiple types of models, including a genetic model. Genetic and lek persistence models = underestimated impact of transmission lines of persistence (may

not apply as much to rest of range. WA lines are gigantic. No longer have birds occupying areas beyond transmission lines. Also lines are oriented in a way that maximizes collision. "Normal" (220 kv) lines also has negative effect.

Highways (e.g., I-80) – 24-hour roads. All day, every day (except when closed by snow). Lek distribution and persistence and connectivity were effected. Recent feature, so probably would be hard to see genetic marker of this. Not in same class as many other highways that don't get as much traffic.

Q: Sage-grouse don't like trees, right? What about forests?

A: In Utah, regularly seen in/around aspen trees. So not definitively a barrier. Will see them making long flights (over inhospitable habitat)

A: key issue is raptors – trees provide perches.

Q: Missouri River line: first look at genetic data – Montana-centric. Continental Divide = first break. There is a split, a perceived barrier.

JD: if you were only looking at genetic data, how different would the line be?

A: Obviously bi-state and WA are out there. Rest of range? It's all a matter of degree. Less movement associated with features, not no movement. So seeing that reflected in the genetic data would be subtle, gradual, take a long time to show up. Distance is the obvious scale. I-80 adds distance. On the landscape scale, there is a website of reports <http://waconnected.org/>

DS: Comparing powerlines and highways. Powerlines more substantial?

A: in WA, yes. Model validation: models where importance of powerlines was increased generated better fit with lek data. Do have an interstate dividing pops in WA, but better to look at I-80 in southern Wyoming. Goes right through habitat. Samples from 20 years ago and now would be informative with respect to impact of I-80.

Group #2 – Potential barriers fairly similar to #1.

Low hot desert

Hi elevation coniferous forests

Large habitat gaps –anthropogenic

Roads?

Ag fields?

Approach: took what they knew about genetic data, not having same on-the-ground habitat knowledge as #1.

Knew Bi-state was different. Inhospitable habitat to east and north. Historical separation.

Knew WA was different. Believed no natural gene-flow from WA. More recent separation.

Knew Jackson Hole pop relatively unique – barrier between e and w Montana (forested area)

Snake River? But based on genetic data, don't see big differences on either side of it.

Qs about roads, but not enough info to say.

Q: Weezer (sp.?) area, NW of Boise, unique? E of OR/ID border. Hell's Canyon.

A: first look: not separating out.

QUESTIONS:

Comment: Why WA separation more contemporary?

A: mtDNA analysis of Bi-state – isolated for a long time. Because there were sagebrush areas between OR and WA more recently, thought separation more recent.

Comment: What did Nevada look like during last ice-age. (Lake Lahontan) Easy to envision separation from that time.

LW: Jackson pop is hooked – is that a continuous line along MT –ID border?

A: only because they knew something about that population

LW: If you had enough samples on either side of I-80, how about SNPs – any possibility of seeing internal differentiation, or too short a timescale?

A: need to look at it. So far, no, but would be interesting to look specifically at I-80. Haven't done that.

LW: Leks out to 4 miles either side of I-80 now inactive. What's a reasonable timescale on which to measure the loss?

Comment: have played around with microsat data re highways: highways are not randomly located and tend to follow major river features. Could be one or the other – can't separate.

Comment: NOT TRUE FOR I-80 – so ideal situation to look at.

Group #3

Looked at genetic evidence to see where strong evidence for breaks.

WA, Bi-state, Jackson, between ID and NW WY and SW MT, Strawberry Valley

QUESTION

Comment: what's a mechanism and what's data-based evidence for a break? High mountains, extensive forested areas, agricultural areas, large deserts and water bodies = mechanisms. There are groups of people studying effects of hypothesized mechanisms on distribution (with many species). Models developed for sampling, not to relate mechanisms to patterns. Most felt uncomfortable drawing lines on the map because it conflates mechanism and data.

Comment: others agree.

Comment: thought about energy development, too, but tends to be discontinuous. Can go around it. May slow you down but not stop you. Maybe.

Comment: some groups mentioned Jackson Hole as an isolated population. Is there a difference in the way these long-term isolated pops act genetically compared with less isolated populations? Is there a way to look at this with samples coming from across the range?

Comment: people have asked this a lot about island species. Can we detect how long a population has been isolated. SNPs look for long stretches of genome that are homozygous. Developing diagnostics for bottlenecks. Second part is what does that mean for you? If you make it onto an island and can purge deleterious mutations, no problem. Should be straightforward to do with enough GRS samples. Good paper recently published for fish in S Sierras.

Comment: agree – can test for long vs recent isolation. Probably historically large/contiguous pops that are fragmented are more vulnerable than small pops that have been isolated for a long time.

Comment: Translocations are popular, are you better off getting birds from a long-isolated source?

Comment: No - better off taking birds from a more diverse population. More adaptive potential in new situation.

Comment: Strawberry Valley population (e of Provo) – after finding low genetic diversity, local people claimed there was a recent, predation-caused bottleneck. Now birds moved around so much, hard to tell what's going on.

Q: are there any places within range where energy development is so extensive that it could create barriers?

A: Lots of wind turbines, but still a blob on the landscape. What happens if you do that over miles and miles and create a major landscape feature? That would result in landscape conversion. Once energy development is so broad, it will behave like a barrier. Could be important in Wyoming, if not in WA.

Comment: interesting question – energy development. Certainly isolating mechanism in areas with low lek density. What happens when you have high-density leks and you start removing them? Do you remove the stepping stones? Hoping questions 3 and 4 will get at this. Energy devel in E Montana/Wyoming could remove critical steppingstones within the range. How will that affect connectivity. More than absolute barriers.

Comment: Whole stepping stone issue. Birds go out an explore – looking for habitat. One thing that determines this behavior is whether or not they find birds. In translocations, you're forcing that issue. The single most important factor is whether or not they find other birds. Social component to this. Declining density = social role becomes more important.

Comment: People doing translocations have unique perspective on this.

Comment: scalewise, have to consider dampening effect in Wyoming of energy development. Should put another line.

PD: Where in Wyoming would you put those lines?

Comment: NE WY, Pinedale anticline area? I-25? Tough – fine-scale, diffuse. Combination of all the energy development infrastructure, no one single thing.

JD: Two classes of info on map: potential and actual barriers.

Comment: Continental Divide, Missouri River Valley – hypothesized.

LW: K Bush has done some of that. Differentiation N. of Milk River.

DS: Back to stepping stones – more discussion on this?

Comment: Goes to getting beyond absolute barriers. Giant E-W, more minor barriers. Gotta get beyond barriers, What about distance as a mechanism over space? Effects that degrade habitat and make genetic distances look longer than they would otherwise. Overall porosity of landscape. Think of core areas being protected – but don't want to save a bunch of isolated little zoos. Need stepping stones between them. At what levels do leks become so sparse that they cease to function as stepping stones? Do these themes have meaning from a genetic standpoint?

SM: DS & JD: It is worth filling in gaps in that table?

DS: Review tonight and decide.

SM: The next hopefully not secret agenda item to raise next, but one other thing warrants discussing: Lots of discomfort about scoring exercise and about putting these lines on map. We did rein in some of our semantic uncertainty, started to talk a more common language, were able to take more in terms of time and spatial scales. Results of this kind of workshop become part of a real-world decision...these are tangible things, and there's discomfort and people may not be comfortable with the use to which these may be put. Don't know what the resolution is, but want to continue to have great talks about all of these topics. Deadlines for these decisions don't change. So these explorations of some of these

topics will happen with or without workshop. There will be follow-up on this – hope this generates some ongoing dialog on these topics that continues after workshop ends. What we talked about is more important than the tangible products. Good to continue to put a gun to your heads and put you outside of your comfort zones to do these exercises. Don't know how to increase comfort with exercises or results on either side. Want to have a short discussion about comfort levels with what we're doing.

[15 min break]

SM: Explore this topic some more. You want to be useful but not used. Don't have exact answer, but shining a little light on why these questions are useful to us. They are kind of specific and realize they kind of threw you for a loop. Took some doing even to articulate them. Not in a decision-making role in this. Let's take 15 minutes here – Craig organized this workshop, give him a moment to say a few words, then go around the room so people can say what they're thinking about.

CH: Address uncertainty about your uncertainty. Used to dealing with species that are more restricted in abundance and distribution. This is not the final decision point, just one piece of the analysis. These are questions that are playing in our minds. This is basic biology. The policy decisions are coming along farther down the line. Tried to structure the questions to get to the basic biological issues of persistence on the landscape, connectivity, etc. But we may have to rethink how we ask these questions. This has so far helped a lot – we are already much better informed than we were. We're not here to do policy. We thought of these as seminars for us. Hope that helps ease some of the discomfort that cropped up earlier.

SM: go around the room – please bring up what's weighing on you.

Comment: when you make decisions about stuff, it should be data-based. Not based on unsupported decisions. You were asking me about things I don't know a lot about. Somewhat reassuring that everyone came up with much the same conclusions on the map. Hope you won't use results from earlier this morning in policy decisions.

Comment (MG): have participated in three or four of these. This conversation has happened in some form at all of them. Always a level of discomfort, because Have never yet seen that information made based on a map or a score, it's the discussion and increased intelligence about the data that results that is helping us.

KN: The conversation and hearing some of the perspectives are very helpful – need to be able to express where there are gaps and differences of opinion, and this helps a lot.

Comment: What's neat about this is that we got to get a feel for the real scientific expertise in the room. The bothersome part is that we can do this – with enough resources and time – in a year, or so. Feel like you're in your defense or prelim and being asked questions you don't have the answers for.

Comment: really appreciate your perspective that this is a tool to discuss and argue and take home more information. Would be good to articulate that from the outset. Have seen maps like this end up in all kinds of places, and that scares the daylights out of me. Appreciate that this map is just a conduit of information to you, then I'm comfortable. But we can answer every single one of these questions, but better if we'd been sent off to answer them and then come back and have a data-driven discussion.

That's how you can use data for management, not like this. From our perspective as scientists, we're using crayons here and this is goofy.

Comment: Would not want to be in your shoes, for sure. We all appreciate the work you're doing and how difficult it is. I am paranoid and don't want to see what we're doing get into the wrong hands. But do appreciate what you're trying to do. This is useful, though. E.g., the I-80 question – we have the data but didn't look at it that way, so dialog is useful in that way – learning what you guys need to know.

Comment: Looked at the hypothesis testing part of it and getting ideas on paper...I-80, powerlines, etc. Interesting to look at where people put lines and think about why. Besides bi-state and WA, we don't know.

Comment JD: We have two maps here: genetic evidence, and hypotheses.

Comment: that approach would have generated a different discussion in breakout groups.

JC: tried to capture hypotheses on map with dashed lines.

Comment: Evidence for some of those lines, some are ideas. Maybe more ideas for data analysis, so getting a lot out of this. Hope we can accurately capture that. Hope we all have a chance to review (maps) before they go anywhere.

SM: Thank you, and thanks for being constructive. NOW, DS will share with you another practical, real-world question that FWS has.

DS: What analyses could be done to assess the loss of genetic diversity for the GRS if any portion of the range were lost?

- What metrics 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?

Comment: How do you lose blocks? Around the edges? More likely all over. What is the pattern of loss? Might be two questions. Losing a portion of the range might not matter, isolating a portion of the range might.

Comment: difference in mechanisms – if losing a portion of the range may increase genetic drift risk. If one pop is divided into subpops and you lose a subpop, not just decreasing size, but losing unique genetic diversity.

Comment: lots of methods to test for bottlenecks with decrease in pop size. First have to delineate units on landscape, then apply tests to each. Tricky with continuously distributed species like GRS. Without discrete pops hard to define.

Comment: Have moved heaven and earth to keep local genes on the landscape. Do whatever you can to keep them, even if they're maladaptive, still trying to maintain local genes. Look at pygmy rabbit, Atwater's prairie chicken.

Comment: there's about 10 different topics there –your conflating all kinds of genetic issues. Argue that you don't know anything about genes under selection. Often want to use the closest geographic population, most conservative. Talk about neutral genes: maintain variation is first priority. Way to do is maintain gene flow and connectivity. Talking about spatial scales – long-term deep phylogenetic splits – don't want to change those. Recent fragmentation is the problem and want to repair those and not lose genetic variation and downward spiral to extinction. Need to silo these concepts. Are there

metrics to characterize genetic variation? Yes. Have to be careful. Pops going downhill demographically, but maybe plenty of gene flow. Much harder to say what isolation is when you're not in an island situation. When we see a loss of genetic variation wrt surrounding populations that's a red flag. Sure we'll be looking at this using multiple methods/metrics.

Comment: You are wanting to know what the genetic trajectory is of a declining population? Then you first have to get at demographics and pop dynamics. That's a prospective way of doing this. Rather than using existing genetic variation for a retrospective look.

Comment: Concept to think about is red flags. Spatial and temporal context to think about red flags for specific populations. Scan landscape, find whatever you delineate as populations, find one that's lower than surrounding, that's a red flag. Temporal component, even better: Sara has a time-series of data now. If you see a dip over time, that's a red flag.

Comment: Strawberry Valley population (predator-caused bottleneck), and WA (no-brainer).

Comment: need monitoring to do this?

Comment: Yes. Best way – monitoring on a regular schedule. Also can use programs to estimate historic population size.

Comment: No one has brought up phenotype issues. Weight differences across range. Plasticity? Follows a nice gradient. Not the only thing. Some examples: WA – number of tail feathers is fixed at 20. No less. Across range, you see 20 and 18. GUSG – 18. Why is a trait like that mixed across range, but fixed in WA? Clutch size – 9 in NC Washington, but elsewhere in range 7.5. Highest rate of nesting and re-nesting. Doesn't crop up in genetics. Why haven't we paid attention to this? There may be many things we don't know because we don't pay attention. Should we ever consider translocating birds from someplace else? May change these unique characters. Decided not to bring in birds from the east. We don't know if it's a big deal, but want to stay focused on regions were formerly connected to.

Comment: Certainly won't find these differences using neutral markers.

Comment: know we're not supposed to talk about DPS or ESU, but in any such units want to take into account phenotypic variation and neutral marker variation. In terms of monitoring vulnerability – maybe have lost variation in those traits through drift. Yes, keep those source pops until you know more.

Comment: info we do have for a lot of the 2005 data points. Would wing measurements be of interest?

Comment: OR birds smaller.

DS: more thoughts on phenotype connection?

Comment: could be useful, but in order to assess and get a handle on these – localized studies of fitness and see how those phenotypes affect fitness. Should consider this.

Comment: Yes agree – evaluate same trait in different groups in standard environment and see if they hold or are plastic.

Comment: Weight plasticity, now they've been doing translocations for a while. East edge primarily release site have been reproducing successfully, check weights and see if they weigh what WA birds weigh.

Comment: don't know if could use phenotypic traits to determine loss of genetic diversity.

Comment: IF you do want to get to DPS language: discrete (movement), significant (adaptation). Any phonological or other uniqueness. Can still get at significance question through depth of genetic signal, add temporal significance (length of branch) – those get at adaptation (– adaptive vs. neutral markers).

DS: Initial comment; Step 1: delineate populations. Are we there?

Comment: certainly loosely defining populations in 2005. From wings in wing barrels. Wouldn't want to do that based on 2005. Data being collected now will answer that question in the future.

Comment: back to specific question: what would you need to assess loss of diversity. If we had a good model of how individuals move around and how genes transfer, could model removal of certain areas, increase resistance, etc.

Comment: Are modeling these things – what if you increase development in certain areas, etc. Gene-flow based.

Comment: how to separate out recent, anthropogenic signal? [Hex-sim modeling]

Comment: Always an issue. If we start to get effect of population size, have an improved frame of reference for interpreting how recent signal is.

Comment: what sort of data would be needed to properly inform such a model? Need temporally collected data – time series. Not snapshot. But not sure.

Comment: Seems clear that part of what you're asking has to do with designing studies going forward. The way question is posed, you want to know what will happen in the future. One approach might be build very complex model of how birds interact with landscape and infer gene-flow. But problem is you're building assumptions on assumptions. Have to build in real uncertainty, then you will wind up with useless projections. Looks at the data we have now and decide what should continue to monitor.

DS: What if some portion of range is lost – what is effect on genetic diversity?

Comment: there isn't a history range-wide now with which to examine this question. But there may be coming up.

Comment: trying to develop a set of markers with which to evaluate change through time by picking up feathers at leks on a regular schedule.

Comment: had opportunity to jump right into SNPs. Strength of microsats is that there's already a time series. But want to start building baseline with SNPs. Should be able to translate them in the future to whatever technology comes next.

Comment: also possibility of going back at coarse scale to 2005 and take similar subsample of data, in general this is what we see during the intervening time period.

Comment: change over time to evaluate change in N_e .

DS: last questions/comment before closing?

DS: red flags – Strawberry? Any surprises, other red flags?

Comment: would be surprised if there aren't more surprises.

SM: Red flags – are the people in this room even able to have that discussion? Or we have to be really teed up?

Comment: can go through the literature and there probably are a few more things...Anthro Mountain? Others? Probably can pick out a few more. Yes could have now, but wouldn't take very long.

Comment: Seems like Steve Knick is an important person to talk to about this.

DS: Will start at 8:00. Half-day. Want to make the most of that time. Are there areas in the range that have low diversity that might have an effect on persistence? Are there things that will or could be done to address genetics that are relevant to the status review? What could be done to help address these

questions about loss of diversity and mechanisms of persistence that are important to consider in the status review?

Thursday morning – revised agenda

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 has been hypothesized?

MAP: lines based on genetic evidence for low-no gene flow. Evidence-based lines on map [[[GET LIST OF THESE]]]

Comment: not sure I agree that Strawberry Valley has low gene-flow. Low diversity, yes, because they had a predator problem.

Comment: some are likely to have more than low gene flow.

LW: When you're talking about gene flow - functional definition - is it one or two dispersing, or enough migrants or interchange to affect one of these groups (am I even asking the question the right way)?

How big are the holes in the colander? Could we capture that by making the lines thicker or thinner?

Comment: Certainly there's probably a couple of ESUs sitting there, beyond that there are certainly places with restricted gene flow - exchanging migrants, but not on separate evolutionary trajectories.

LW: an example - Bi-State vs. Missouri River

Comment: Three levels: Low enough level that they're on separate trajectories (ESUs)

Comment: Adaptive markers to identify ESUs (C. Funk's work) Different types of intraspecific units - ESUs are big units - almost subspecies, isolated a long time, maybe adaptive differences. With SNP data you get some that are potentially adaptive. To delineate ESUs, use all your loci - neutral (historical isolation), adaptive loci. Management units - demographically isolated units. Mainly determined by births/deaths (id by neutral loci only - because looking at genetic drift internally). FIGURE OUT IF YOU HAVE ESUs and if you have management units within them. These breaks and others that might exist might demarcate ESUs, but not clear to me that existing, published data has been collected in a way that would identify these. But will have it pretty soon.

Comment: early spring, soonest.

DS: Degree of divergence, right?

Comment: Yes. ESUs are units that have little gene flow between them, but may have phenotypic or adaptive differences. Those would be more differentiated than management units (demographically isolated by no other differences).

Comment: Management unit might be discrete but not significant.

Comment: ESUs and management units in salmon - watershed based mgmt. units. So fisheries are managed to achieve certain number of spawning adults, escapement goals, etc. Those aren't ESUs, which would be on a broader scale. Temporally or spatially confined (e.g., spawning season). ESU spatial scale broader than management scale.

Comment: So with salmonids, ESU might be a total drainage in the Columbia [[haha, au contraire!]]

DS: taps others in the room who haven't said much.

Comment: ESUs traditionally based on mtDNA and recip monophyly. Morph, behave, and habitat-based criteria added.

Comment: Bi-state is not recip mono with rest of GRSg, because you've always had the deep clades.

Comment: that definition often too stringent for birds, esp at subspecific level. Significant differentiation and other criteria (plus now adding adaptive markers).

Comment: any decisions made based on adaptive markers?

Comment: Not yet, but yes based on phenotypic differences. Now instead of using a single adaptive marker can look across the genome to look for adaptive markers. New technology.

Comment: lot of debate about choosing something that is actually under selection and determining whether something presumed adaptive is adaptive.

Comment: examples from North Sea stocks – haven't status been revised now based on adaptive genes.

Comment: some marine species – herring or cod? – also recently atlantic salmon.

Comment: will always be

Comment: trying to develop SNP arrays to screen for variation in a diversity of species. Collaborating on projects that use neutral markers but also look for adaptive markers, e.g., in island fox.

Comment: obviously, the boundaries have been drawn now – there are likely to be more when the current work is done. When you look at the current evidence, can say that there are these but likely are more.

Comment: if we had to say now, we definitely have some strong breaks and could have strong agreement on these. Probably secondary ones, and tertiary ones, maybe others deserve more scrutiny (e.g., Missouri R). Are there other ESUs on this map?

Comment: would be interesting to look at Jackson Hole – compare with Bi-state. Washington: had common haplotypes, but low diversity (suggesting isolation). Translocations – not even sure for range-wide how you deal with that situation. Probably not going to have birds moving from OR to WA on their own now. See WA as a completely different category than Bi-State. [[[??? What does this mean?]]]

Comment: know where samples are. Samples came from NC WA. Main translocation area is to the east. Have put birds in Yakima, but don't know a lot of what's going on in there – Tribe doesn't share a lot. Pop furthest east, also. Those are the two translocation pops. Those are going to be OR or NV birds. Have been bringing birds into Training Area and collecting feathers. Haven't analyzed to see provenance of birds. Birds were radio-marked but not a lot of success. Not optimistic about having moved genes in to that populations.

Comment: what about break generated in SW MT, Bitterroots. Is that a significant break?

Comment: Based on range-wide data. What we've looked at so far, that break shows up pretty strongly. Not ESU-level, but worth looking at. Keep thinking it's a sampling effect, but it keeps not going away.

Comment : The goal of recognizing ESUs is to maintain those divisions and adaptive potential. Very different parts of range – Alberta vs G Basin. Would have a hypothesis that those would be distinct ESUs. WA is tricky because relatively recently – last 100-200 year –was contiguous with rest of range. Would you consider an ESU something that has been fragmented by humans. ESUs should be large, naturally divergent units. BUT WA is relatively distant from rest of range – has seen unique or less variable phenotypes, etc.

Comment: if you have a cline in some trait or marker, across a broad geographic area, how does that play into the decision-making process. Yes, Alberta and G Basin very difference, but striking fact about

sage-steppe is very different rainfall patterns from east to west. More summer rain in east. Would be very surprised if there were adaptive differences in grouse across that range. But if there's no break, but there is a cline, how do you deal with that? Numerous examples of this.

Comment: Even if no breaks, must recognize the variation across that cline to maintain adaptive variation within the species.

DS: "Representation" – is a concept in use in the decision-making.

Comment: There's a big change between genetic change from drift (recent, short-term pop dynamics, WA) **Not significant from adaptive side.** [[Why not significant?]]

Comment: There are 110 museum samples. Could get something going there in short time. Lots of them are at Burke.

Comment: Look at even 10. Send 'em, we'll do it. Look at fisher work on west coast - similar. **If all of a sudden we looked at historic samples and saw that there was no gene flow historically, that would be pretty important. WA: Probably just drift, not adaptive, but there might be something else going on.**

We don't have the genes to test, so using time as a proxy. Ring species concept. Bitterroot break = can see how that's two ends of the spectrum but don't know what is actually going on – haven't tested that.

Comment: would like to look at mtDNA from Jackson hole – hard to say without additional data.

Comment: Speculative. Going to guess there are more management units in there. Bet if you look at Jackson Hole it will pop up. mtDNA differences are pretty great. (Pie-charts map)

Comment: Bi-State pop has completely different haplotypes than anywhere else. Orange is "everything else" lumped together, so a little misleading. If you had all individual haplotypes on map, could tell a different story. Common haplotypes shift as you go across the range. Consonant with isolation by distance, but there is more detail you could look at in there.

MAP: Hypothesized lines

DS: Could be limited gene flow based on landscape features. Are there any of these places where existing data could be tapped to test the line?

Comment: **the I-80 question is definitely testable.** First paper that's in review – they looked at whole state, what are biggest drivers of gene flow. Roads as a category didn't pop out, but when they talked this morning, could take the data, esp. from SW WY, and test that hypothesis with data that we had. Could be published and useful by September.

Comment: Look at 50 genotypes from N and 50 from S of I-80 and look at F_{st} . Because you wouldn't know how much E-W is masking a signal. Null would be 100 random (fake) highways and test N & S.

Comment: if it doesn't show up with I-80, probably nowhere else. Goes through the best habitat in the range. It'll show up there if anywhere. If not there, what does that say about highways elsewhere? Wouldn't the null model be isolation by distance? If you have a rectangle with I-80 bisecting it, isn't that the null model?

Comment: would be concerned about distribution of samples. E-W effects possible.

Comment: think sample distribution probably is OK.

Comment: map of sample design? Could project it.

DS: Which other hypothetical lines could be tested with existing data?

Comment: Yes, a lot of them. Anything that was in range-wide analysis.

Comment: map of the leks we know about. Black ones have been sampled. I-80: have sampled both N and S.

Comment: want to stretch it out as long as possible along E-W access. If connectivity on both N and S, important part of the equation.

Comment: some big gaps in sampling.

Comment: N-S valleys and ridges and other areas that don't support sage-grouse.

PD: between "wamsutter" and rock springs – big gap. Getting into Red Desert.

Comment: Yes can totally examine hypothetical lines using existing data.

[[variable opinions about how to do that.]]

JD: Threats on east side of range are different than west. Oil/gas vs. fire/invasives. We will be asked about this – differences between these areas. Management units – does an E-W split make sense there? Discontinuity, if not genetic differences?

Comment: Where do you think the line should be?

Comment: Before I'd be comfortable saying anything about that, would want to take another look at the data in that paper. Wouldn't be hard to do. Don't know how much of it is a gradient. Will have a better answer when range-wide stuff is done, but might be able to tell you something in a couple of weeks.

Comment: The pie charts suggest something is going on. Nothing is known now, but it will come out of the range-wide data.

DS: When ?

Comment: Have the data now. Could run STRUCTURE on the existing new range-wide data and do it now. Could run a PCA. Take a plenary look at this – ID and CO samples. Can figure this out.

Comments: They can talk about doing it. Nervous about doing things before it's all done. Don't give any information out before you're done and it's right and complete. Totally at your disposal to help out.

Comment: when is deadline and we don't want any more information? Haven't released any information.

JD: we have to make a recommendation around April. It'll be harder to incorporate new info after that.

KN: The door doesn't close – not hard and fast, want to be mindful of new, dramatic info. The sooner we have it, the better.

Comment: can look at old (2005) data and try to take a new look at it. New data: will be harder to figure out how to address this with respect to policy about releasing data prior to completing analysis, peer-review etc.

Identify areas within the greater sage-grouse range where low genetic diversity or inbreeding depression is likely to affect greater sage-grouse persistence.

Columbia Basin, Strawberry Valley. (not Bi-State – different, not less)

Comment: should tap people at table to figure out best/fastest method to screen for red flags.

Comment: need population data. Do you have N_e values?

Comment: Merging demographic and genetic data has to be data from the same sites.

Comment: How to downscale lek data to population size estimate?

Comment: find little isolated areas and check those out, also choose places based on demographic data. Use multiple screening criteria.

Comment: Advocate for using many metrics. Bottleneck tests, heterozygosity tests, multiple indices to see if there's a problem.

Comment: Gauge whether an ESU has a problem or a population within a broader ESU.

[[**ESU definition**: these guys say by definition: evolutionary only. Not anthropogenic.]]

What is the significance of these, or any/all combinations...?

Comment: large literature about importance of peripheral populations. Margins of species' range. Maybe be adaptive to environmental conditions – climate change, etc. I would say those peripheral populations are really important for the species as a whole for that reason. Lots of literature on this. Could be disproportionately important.

[[[Contrast with Mike's comment about non-significant of WA because represents drift]]]

Comment: K. Bush diss: General perception of Alberta as sink populations sustained by immigration from MT. Would then Alberta pops be locally adapted? Are they important peripheral populations? Could judge based on demography of peripheral populations. High genetic diversity and gene flow, but demographically a sink – maybe just a weird situation.

Comment: that's why red-flag approach is good. Need to be informed by ecology on the ground; genetic screening will only tell you so much. Not going to provide a silver bullet. Will have to bring multiple different approaches to informing this. Local knowledge (e.g., Strawberry – predation problem), demographic info.

How much can be lost before we go from “so what” to “oh darn it”?

Comment: this is exactly the network analysis – E.g., Knick and Hanser. – and stepping stones. Again, planning to look at this with range-wide.

Comment: run different projections to look at different fragmentation scenarios.

[[10 m break]]

Channeling a decision-maker's perspective, are there areas where genetics exacerbate the effect of threats on species persistence?

SM: Decision-makers want the info in the form they want it. Interplay between decision-maker objectives and scientists. Want to lay out a real-world manager objective and get your conceptual reaction to it. I'm a manager, have sage-grouse listing decision coming at me, all kinds of modeling underway, etc. Want to make sure I have the best stuff in front of me when I make this decision. Opportunity to have geneticists in the room. Are there any areas where the genetics itself is kind of a threat on some level? Are there areas where, if I don't lay on the genetics on top of that I might overestimate how good things are?

There are two reactions to this: the conceptual and the downscaled.

Conceptual reaction? How to refine this question in order to answer it?

Comment: a statistical comment – sounds like what you're asking is if there's an interaction between genetics and other threats. If they birds are living in a perfect world, then the genetic process does what it's going to do. Now you've thrown in other processes, how do they influence that process?

Comments: The way we're using genetics – all we're doing is looking at pieces of the genome to characterize what's happening on the landscape. No different than measuring the dbh of trees. Almost nonsensical question. Missing what this question is asking. All we're doing is measuring.

Comment: if genetic processes, we'd all say it's about small populations.

Comment: do other threats exacerbate genetic problems

Comment: interaction between vital rates and inbreeding depression – extinction vortex. There's two reasons that genetics are important in mgmt. and conservation: a tool to measure things - demography, adaptation and assess fitness. Are there populations where genetic var is so low or there are so few individuals where you have inbreeding depression?

Comment: can we assume managers understand extinction vortices?

CH: No

Comment: WA would be a candidate population for assessing relationship between fitness and genetic variation. Maybe bi-state? But has been isolated for a long time, high genetic variation.

Comment: goes back to genetic variation that we asked earlier. Can estimate N_e in small isolated areas we IDd earlier to find red flags. N_e measures: 50-500 rule = when genetics might become a problem (red flag – entering extinction vortex?) IS there inbreeding depression?

Comment: GUSG – little fragmented populations – some are experiencing inbreeding depression. Yes there are estimates of N_e but don't necessarily need to time slices to estimate these.

Comment: there are a lot of one-sample estimates. Can get artificial estimates of N_e .

Comment: ask decision makers: tell me about other layers of info – one should be population size.

Based on that, look further – at genetic data, at vital-rate data (is inbreeding depression happening).

Comment: When I first read the question was also struggling. Example pointed out in Strawberry Valley. Genetic information insufficient to see the whole picture. If the managers are thinking of instances where genetic information is exacerbating other perturbations, have to make sure you have multiple lines of evidence.

Comment: once we learned what was happening on the ground in Strawberry, there was a good (recent) explanation, and a management alternative.

[[[Still don't understand this – how would recent predator pressure become expressed in reduced genetic variability?]]]

Comment: there's amazing demographic data. Could look at juvenile survival. It's a common place to see inbreeding effects. Marked reductions in juvenile survival relative to surrounding populations?

[[[Could be disease, predation, bad winter, etc.]]]

Comment: can't think of an example with GRSG, but LEPC – decline over years, reduced genetic variation. Detected in hatchability of eggs. Normal: 9.5/10 eggs hatch. Pop dropped to <50, and hatchability dropped to 6/10. Infertile or chicks not strong enough to get out of egg. Brough birds in from Kansas, Nebraska, Minnesota, and hatchability improved. Data do exist for GRSG, but most people collecting it would notice that kind of change.

Comment: Have found in Gunnison's.

Comment: Take out juvenile survival and put in hatch success. Less other potentially confounding ecological factors. Still, the one-line answer to the question is “small population”.

Comment: don't forget that effective population size is a lot smaller than the observed pop size (N_c).

Don't know what ratio is for GRSG, but maybe 100 individuals may result in N_e of <50 .

Comment: demographic model. Can't recall results.

Comment: GRSG weird breeding system.

Comment: female biased sex ratio. It's been done, but still have to make assumptions about how many males are breeding – hard data to get.

Comment: if you can estimate N_e based on molecular approaches and census the population in the field, can figure out the ratio.

Comment: recent paper **“Simple life history traits ... across taxa” Waples 2013 or 2014**. Calculates $N:N_e$ ratio for multiple species.

Comment: even if this paper has AN estimate of N_e for GRSG, know that the ratio could vary across the range.

Comment: two ways to do this –demographic study, genetic methods. Latter incorporates bottlenecks and systematically produces smaller N_e , most influenced by years when you have areally small population. If you do genetically asks question about history of population. If demographically, the answer is what is happening Right Now, but hard to do right, lots of guesswork.

Comment: in recovery plans, target is about 2000 birds/population. So even with a ratio of 25%, still have N_e of 500. So know what kind of population we want to have to consider downlisting (in state).

Comment: Lekking species liable to have lower N_e .

Comment: With some N_e measurements, in some cases like WA comfortable saying it's a discrete population, but tougher to say in the thick of the range.

Comment: not sure that I see exactly how to answer this question. Is N_e the answer? The answer is really about interaction with other threats. But we don't know how these threats interact with small population size. Counts, capture-recapture rates, etc. But how do you tie genetic information into that. Common: Mark-recap survival analysis, could include individual levels of genetic variation as a covariate. Do individuals with lower genetic variation have lower survival? Ask those kinds of questions. Could be additive OR interaction. Additive interaction of individual inbreeding coefficient with survival. Genetic threats to pop persistence, added impact of inbreeding coefficient on fitness measures. This will be exacerbated during harsh climate years, for example. Could include other external threats in such analyses. Delineate population across range and compare heterozygosity and pop or demographic data over time.

Survival (pop or ind) = threat + inbreeding + threat*inbreeding.

Correlation between demography and inbreeding.

Comment: in an average year, these things seldom manifest.

Comment: practical tools for managers, don't recommend long-term studies. Don't disagree, but 15-year studies of song-sparrows are the exceptions. Need quick and dirty.

Comment: there's some value in this though because there will be work ongoing after September 2015, where these kinds of efforts could be undertaken and these metrics used.

SM: For now, though, managers what to know where on the map we have a problem NOW.

Spent some good time talking about Columbia Basin, good candidate. Are there other places on the map?

Comment: Jackson Hole has been declining for years.

Comment: not willing to circle spots on the map. There will be, soon.

Comment: Weiser ID, Klamath area (isolated pocket in the middle of nowhere, maybe no way to save it), Utah and Colorado fragmented areas, some more isolated pockets S-C Utah isolated pocket, areas in NE interior of Utah, Peyonce (sp?) area – Christian Hagen, Brett Walker worked there, Colorado fragmented areas. Colorado pops in very bad shape, some may be gone. Couple populations in NV that may be gone. (Some – Pine Nut included in Bi-State). Dinosaur area of Utah.

PD: Belt Mountain, MT?

Comment: Wisdom, Red Rocks, Belt – little SW MT pieces.

SM: Want to catch up on anything that didn't get said yesterday? Not leave anything hanging...

Comment: Non-genetic issue. Pointed out yesterday that translocation birds only stay if they find other birds. Realized that there's a substantial literature on territory settlement - colonial and non-colonial species. Conspecific cueing. Males looking for territory will look for other males. Widespread phenomenon, esp in breeding passerines. At least in N Hemisphere. Clustering is more than habitat gradients. Not sure if relevant, but wanted to mention it.

Comment: Talking about WA museum specimens: 25 specimens at Burke or Puget or WSU museum. Burke already excited about helping.

Comment: at least do the mtDNA sequencing, at least have a historical haplotype pie-chart.

Comment: 1000+ specimens in database. Also will check for OR specimens, would be good to include nearest pop.

Comment: NE OR? Would be important to include these.

Comment: in 40s and 50s moving birds from Wyoming to N OR, but didn't work.

Comment: remember objective: get an indication of drift in this population. If underlying mechanism is drift, then expectation is that haplotypes would be a subsample of OR pie charts, but different than WA pie charts today.

Chance for other questions from around the room:

JD: Another question that will come up (in addition to E-W), what about central ID: is that an appropriate management unit. I-84, ag devel., expanding human population. Will new data tell us anything about this?

Comment: Good question.

Comment: Does genetic differentiation matter?

JD: it will matter – can be subtle, won't have to be significant.

Comment: New data definitely will shed light on this. Not available now. Working on it. We might have answers by April.

PD: Comment earlier about changes occurring at different speeds on landscape. Possible intensity of threats elsewhere ability to generate same situation we see in WA? Can threats increase rate of genetic change/isolation.

Comment: If threats reduce pop to a certain point, yes.

Comment: Seems like greatest risk for GRSB is in core of range, not in periphery. Don't forget about the middle part!

Comment: have seen the PAC map – priority areas in Wyoming are scattered all around. IT was negotiated with politicians, energy industry, etc. to identify these areas. Don't think genetics played a role in that decision. If you look at Wyoming, if it ends up that way, will be little pockets of occupancy surrounded by energy development.

Comment: in that case, definitely WILL have a problem with genetics (and a lot of other things).

PD: Previous comment channeled her idea – PAC map problems.

Comment: the idea is not to ignore GRSB outside of PACs.

PD: wanted to ask genetic questions about PAC map. Tasked in 2011 to identify where were the most important areas to manage GRSB. Went to state partners to develop that map of PACs – state people said if we have these areas in our states can manage GRSB for long-term persistence. Reason why we looked at these, more than half of GRSB on Federal lands. Multiple uses, but had to look at what we can do without. Some are randomly lost to wildlife, esp in west. We're constantly asked, despite the fact that only 64% of range is represented, if we can allow development within PACs to meet multiple-use goals on federal lands. Primary areas focusing on conservation. But if can't get it all, our direction in COT is that we really have to keep these areas on the landscape. People immediately went after there being more than enough here. Can we let development happen if it "doesn't effect" the birds? Get this question daily. These are key to conservation of the species – always our answer. But those other agencies are mandated to manage for multiple resource uses.

From a genetic perspective, are there genetic considerations that would help us direct our Fed land mgmt. partners?

Comment: don't you have cumulative impacts considerations? This has to supersede any genetic considerations we could provide. What are the regulations for cumulative impacts? Geneticists can't say "don't remove this population" Tiny bandaid, when real issue is cumulative effects analysis.

PD: No, how do we include genetic consideration in that cumulative effects analysis? Are there key areas where genetics might be a disproportionate part of that analysis?

Comment: Use PAC map as your network

Comment: Yes, we are looking at key connective leks. How do areas interact with each other? What are key interstitial stepping stones? Similar to Jesse's ID question. Are looking for those leks that are between core breeding areas that are disproportionately affecting gene flow. The "hubs."

Comment: One specific scenario would be interesting: in NE WY, there are just a few little PACs, all contiguous range. Surrounded by coal-bed methane. Similar situation in MT. Contrast range with this and compare how much non-PAC area (coal-bed methane).

Comment: for some states PACs was a mathematical exercise to identify the smallest area containing the greatest number of birds. E.g., OR.

PD: WY looked at NE WY, already leased to coal-bed methane and already has declining birds. So eliminated those areas. Lot of private land. Why invest in restoration instead of focusing on conservation.

Comment: now more birds in WA than the Dakotas and NE CA. Not because we're doing well in WA. AL and SA going down the tubes.

Comment: PD question really important.

PD: making these mgmt. decisions without considering underlying genetic components.

Comment: would love to have a map that shows what happens if you remove this lek or that one. An interactive tool.

Comment: Did that in WA – a centrality map for STGR and GRSG. Which leks are most important – shown on a scale. Centrality and connections to maintain network. Again look at waconnect website.

Comment: think about corridors between PACs so you don't end up isolating pops that aren't isolated now.

PD: was a recommendation in the COT. Outside of these areas, of course, management restrictions are much related.

LW: Different and scarier discussion about PAC map becoming GRSG range map. Overlay connectivity map on PAC map.

JD: More on PAC map: West may look rosier with respect to land allocation, but we have landscape scale disturbances that we can't control – fire.

Comment: SE OR doesn't look any better than east.

Comment: risk of massive fragmentation. Massive climatic changes. Certainly on Western side, naturally fragmented, e.g., Bi-State.

Comment: what are the invasives concerns?

JD: cheatgrass. Burns more frequently.

Comment: landscape stuff focused on Wyoming and Montana. IF had data layers, would look at cheatgrass, fire, juniper encroachment.

PD: Utah – fox infestation in Strawberry Valley associated with humans benefitted synanthropic species. Small GRSG population, no predator savvy. This pattern of fragmentation and predation cropping up elsewhere. Predator control may help as a temporary fix but doesn't get at underlying problem. But can predator control help beyond that?

LW: example from GUSG.

Comment: have they gotten rid of preds in Strawberry Valley?

PD: maintaining expensive pred control program.

PD: We had talked a little about maintaining peripheral populations. Look at natural fragmentation in NV and UT. Does genetic info suggest that there areas there that we may or may not lose significant genetic information if they blinked out? Could ask same question about other components – ecological variation, etc.

Comment: could do an exercise where you look at proportion of whole genetic variation that would be lost if you lost this or that population. Proportion of haplotypes, proportion of alleles. Lose some, recalculate total variation, etc.

Graph: Y = diversity, X = area removed. The key is identifying when you have a problem, but it depends on which areas you take out. Can you identify an inflection point where the loss is "too much." Would curve differ if you took out random populations/leks, vs. peripheral populations, etc. How would that

affect the inflection point. Worst case scenario starting in middle and going out. Could compare current distribution and PAC or connectivity map. Assess what you already have lost, even.

Inflection point is when rate of change changes. When you get to a point where all your isolated populations are recip mono, you have a real problem.

Comment: that would take a long time.

Comment: dangerous thing about looking for inflection point is the perception that you can remove and remove to that point. Significant isolation could happen well before you reach inflection point.

Comment: another type of inflection – remove lek by lek and examine reduction in connectivity.

Connectivity might go well before loss of diversity.

JD: How we'll be asked the question: so what if you lose the Columbia Basin? What does that mean for the species as a whole? How about western portion of range?

Comment: non-genetic answers to that question – ecological consequences.

JD: Hypothetical questions is importance of preserving that unit.

Comment: looking at neutral markers, don't get at adaptive importance.

Comment: SNPs get at both – just look at those.

Comment: WILL NOT have that information by April. May have microsat data by April. **Probably could find in WA something that's totally different once you have adaptive markers.** But WA may always be on its own trajectory from here forward, with no input from elsewhere.

JD: Don't know what I'd do to generate the graph. Sense of simple measures of diversity in different parts of range. Is this fairly simple to do.

Comment: can look at existing data, no unique haplotypes in WA, and only neutral microsats. **If lost WA, won't lose any unique haplotypes, might not lose any of the neutral variation across the range, but really don't know about adaptive variation – existing data don't answer that question.**

Comment: problem with graph – has all sorts of genetic considerations lumped together on Y axis.

Comment: Amount of total variation in species could be relatively insensitive to area. You could lose 50% of your area and maintain 90% of your allelic diversity or other measure of genetic variation.

Misleading picture of health and viability.

Comment: climate change and Nward (and upward) shift of sagebrush. White Mountains in CA, little bit of sagebrush up high. A few decades of warming, that will disappear off the top. Scenario likely to play out on southern end of range. Some PACs won't matter anymore.

JD: Yes we are working with modelers to address this.

Comment: if you want to say something intelligent and specific about climate change, you'll have to have specialists involved.

Comment: important to maintain connectivity to provide best chances for adaptation to climate change – adaptive potential and ability to track shifting habitat. Assisted migration – moving warm-adapted individuals north, etc.

Comment: controversial idea.

Comment: WNV?

Comment: highly susceptible.

PD: birds that get it die. Some birds may have been exposed that seem to have survived. Difficult to monitor. Have to get carcasses before they decay. Some mortalities every year. [[[2010 and 2011?]]]
Think we just missed a big die-off in Dakotas and SE MT. Conditions were right for WNV.

Comment: arid-land birds

PD: worst in SE WY. Links with coal-bed methane ponds (Powder River Basin). Esp in late summer when hens bring broods to mesic habitats. Intensity/distribution of disease is anthropogenic.

SM: wrapping up.

CH: Will circulate notes and craft executive summary for review.

Summary Report: Workshop for Experts on the Genetics of the Greater Sage-Grouse

*Held at the USGS Fort Collins Science Center in Fort Collins, Colorado, on
October 22 and 23, 2014*

Prepared by the Workshop Planning Team

Executive Summary

Genetic differences exist between individuals and populations across the range of the greater sage-grouse, or sage-grouse (*Centrocercus urophasianus*). However, the degree to which these genetic differences represent distinct populations, isolated populations, or other genetically different units of sage-grouse is largely unclear. Additionally, the techniques and metrics used to evaluate and describe genetic isolation, divergence, and diversity have changed or improved since previous genetic studies were published in 2005 and summarized in 2011 (Oyler-McCance *et al.* 2005; Oyler-McCance and Quinn 2011). New research on the genetics of sage-grouse has also been completed, is in progress, or is potentially needed. Therefore, as we gather information from a variety of sources for the status review of the species, the U.S. Fish and Wildlife Service (Service) with assistance from the U.S. Geological Survey (USGS) organized a workshop for scientific experts to meet and discuss questions and uncertainty about patterns of, and potential impediments to, gene flow across the range of the greater sage-grouse.

The purpose of the workshop was to collect information from scientific experts on the sources, magnitudes, and implications of potential impediments, or barriers, to gene flow across the range of the greater sage-grouse. This information could also help identify populations or areas with low genetic diversity or unique genetic characteristics. The workshop also provided an opportunity for scientific experts to discuss ongoing and upcoming studies and research ideas related to greater sage-grouse genetics.

The workshop provided the following information:

- The available genetic data does not explain 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 movement, 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 have low levels of gene flow. The Columbia Basin and Jackson Hole populations are also small, isolated, and less genetically diverse than the bi-state population.
- 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, but not necessarily low gene flow.
- Small, isolated populations present the greatest genetic risk. Threats that fragment habitats and isolate populations further reduce genetic diversity and increase genetic risk.
- Populations at the periphery of the range may have low genetic diversity, but may also be uniquely adapted to specific environmental conditions. These unique adaptations add genetic diversity of the species and may be beneficial in the future.
- Upcoming research, particularly the range-wide landscape connectivity study, will provide clarity regarding barriers and gene flow across the overall range of the greater sage-grouse.

The following report summarizes the workshop organization and proceedings, with supporting materials attached in appendices.

Organizing the Workshop

The workshop brought together a diverse group of scientific experts to share, discuss, and debate their scientific knowledge on genetic techniques, conservation genetics, and the greater sage-grouse. Before the workshop, 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 workshop planning team used best practices for eliciting information from experts to identify and invite scientific experts to participate in the workshop (Burgman 2005). The planning team first 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 based on an expert'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). The USGS facilitators emailed the invitations and served as the primary points of contact for the experts. If an expert declined an invitation, the facilitators invited a replacement from the planning team's list.

Invited experts represented a diversity of expert judgment on greater sage-grouse genetics within the scientific community and would effectively contribute to group discussions. The facilitators limited the meeting size to nine experts in order to maximize open, scientific 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 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 provided their curricula vitae and completed screening forms to identify any conflicts 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.

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 described below, the workshop proceedings generally adhered to the agenda.

Summary of Workshop Exercises and Discussions

The workshop planning team used meeting notes taken by three note takers to draft the following summary of the workshop exercises and discussions. The experts were given an opportunity to review the meeting notes and summary report.

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:

- Geneticists assign individuals a haplotype based on their sequenced mitochondrial, maternally-inherited DNA (mtDNA). Individuals with different haplotypes have different mtDNA sequences.
- Comparing the distribution of haplotypes between two populations tells genetics something about the flow of genes between the populations. The more substitutions, the greater the genetic difference, the more divergent, and the longer the separation from a common ancestor.
- Groups are reciprocally monophyletic if they share the same genetic code. Groups that are not reciprocally monophyletic are genetically different and could be classified as different species.
- New laboratory techniques that use single nucleotide markers, or SNPs, as genetic markers rather than mtDNA microsatellites to evaluate genetic code, are more powerful and provide greater insight into adaptive traits.

Dr. Oyler-McCance's second presentation provided a summary of the available literature, papers in review, and upcoming research on sage-grouse genetics.

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 across the eastern and western portions of the greater sage-grouse's overall range (Figure 1). One or many north-to-south oriented barriers could limit gene flow between large, eastern and western portions of the range, potentially resulting in genetic differences from east-to-west.

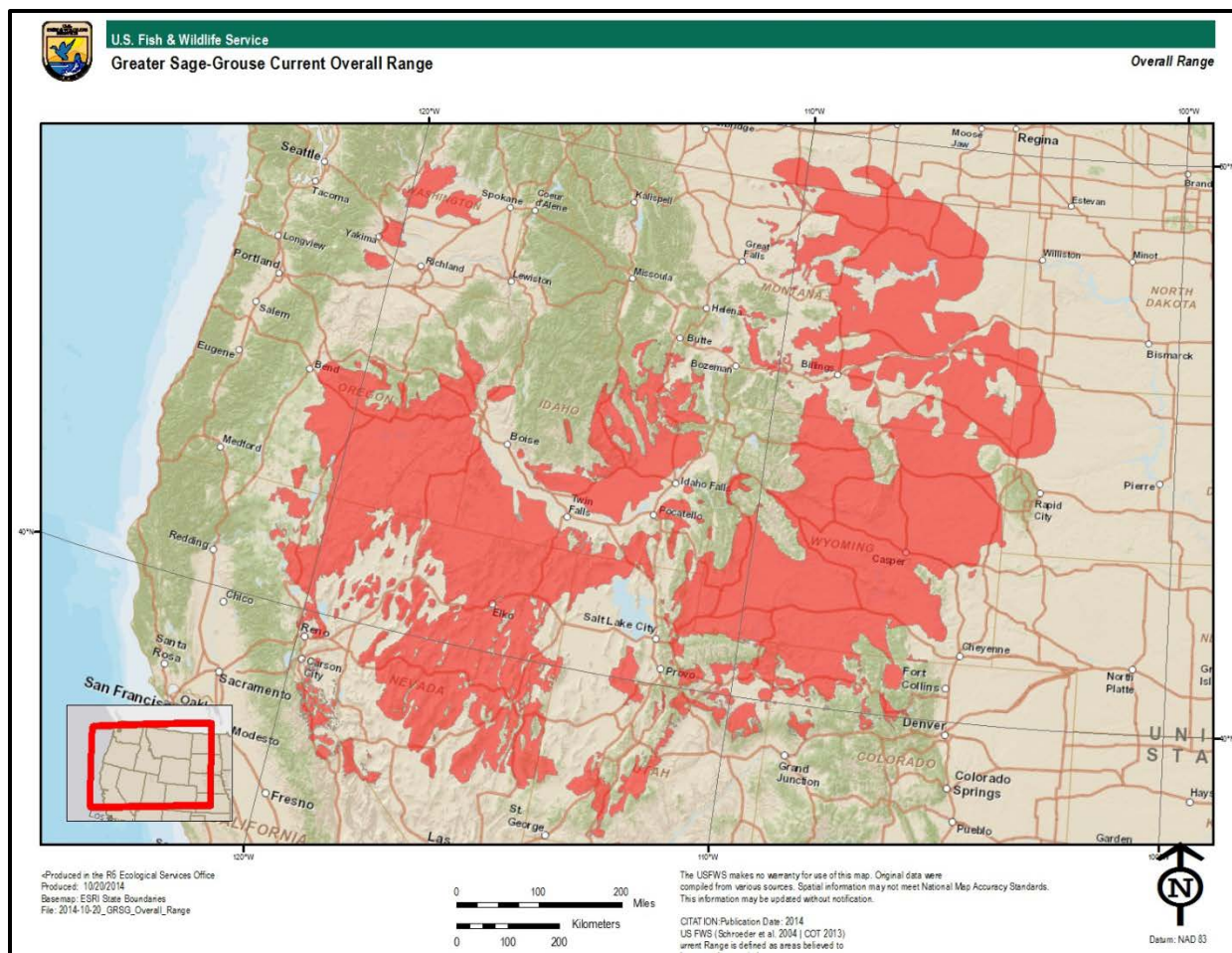


Figure 1. Overall range of the greater sage-grouse. The Experts used this blank map in Exercises 1 and 2 to discuss potential barriers .

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 migrates 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 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 described a barrier 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 difference 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); and
- Upcoming research from a range-wide genetics assessment expected to publish in the fall of 2015 will answer 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 the workshop questions to allow the experts to categorize information they provided as either a hypothesis or evidence supported by the available scientific literature.

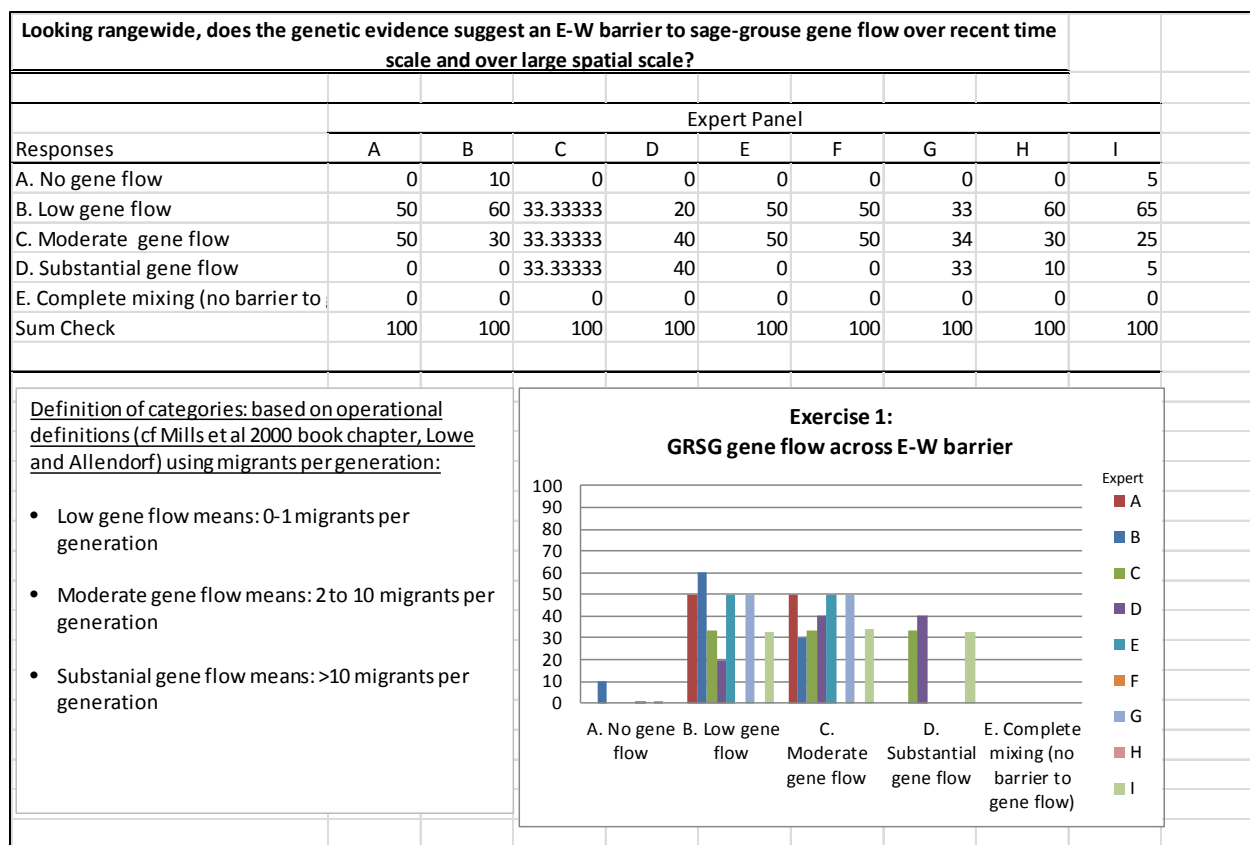


Figure 2. Exercise 1 asked each expert to rank a barrier to gene flow between eastern and western portions of the range.

The experts discussed 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 is best described as a cline, or gradient, with more genetic divergence between the easternmost and westernmost extremes of the range. Additionally, smaller, more isolated populations likely experience more genetic divergence, or drift. However, reanalysis of the existing data would specifically and numerically address this question. Reanalyzing data collected in 2005 and the upcoming range-wide connectivity study will specifically address this question. to investigate an east-to-west split and the range-wide connectivity study expected in the fall of 2015 will also address this question.

To summarize Exercise 1, the available genetic data does not specifically address landscape-level gene flow between large, eastern and western portions of the range. There is likely a gradation of genetic diverge from east to west, with the greatest genetic difference between the eastern and western extremes of the range and small, isolated populations. Existing data will be reanalyzed and new data will soon be available to specifically investigate genetic divergence from east-to-west across the range of the greater sage-grouse.

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 3 experts. The facilitators asked each group to draw lines that represent potential barriers to gene flow (Figure 1).

Each group characterized a line as either:

- A real barrier supported by existing, data-driven, genetic evidence (Figure 3); or
- A hypothetical barrier based on qualitative information 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 restricted gene flow that have not been analyzed or considered. Further, ongoing research in Wyoming and the range-wide connectivity study will specifically and numerically address the question.

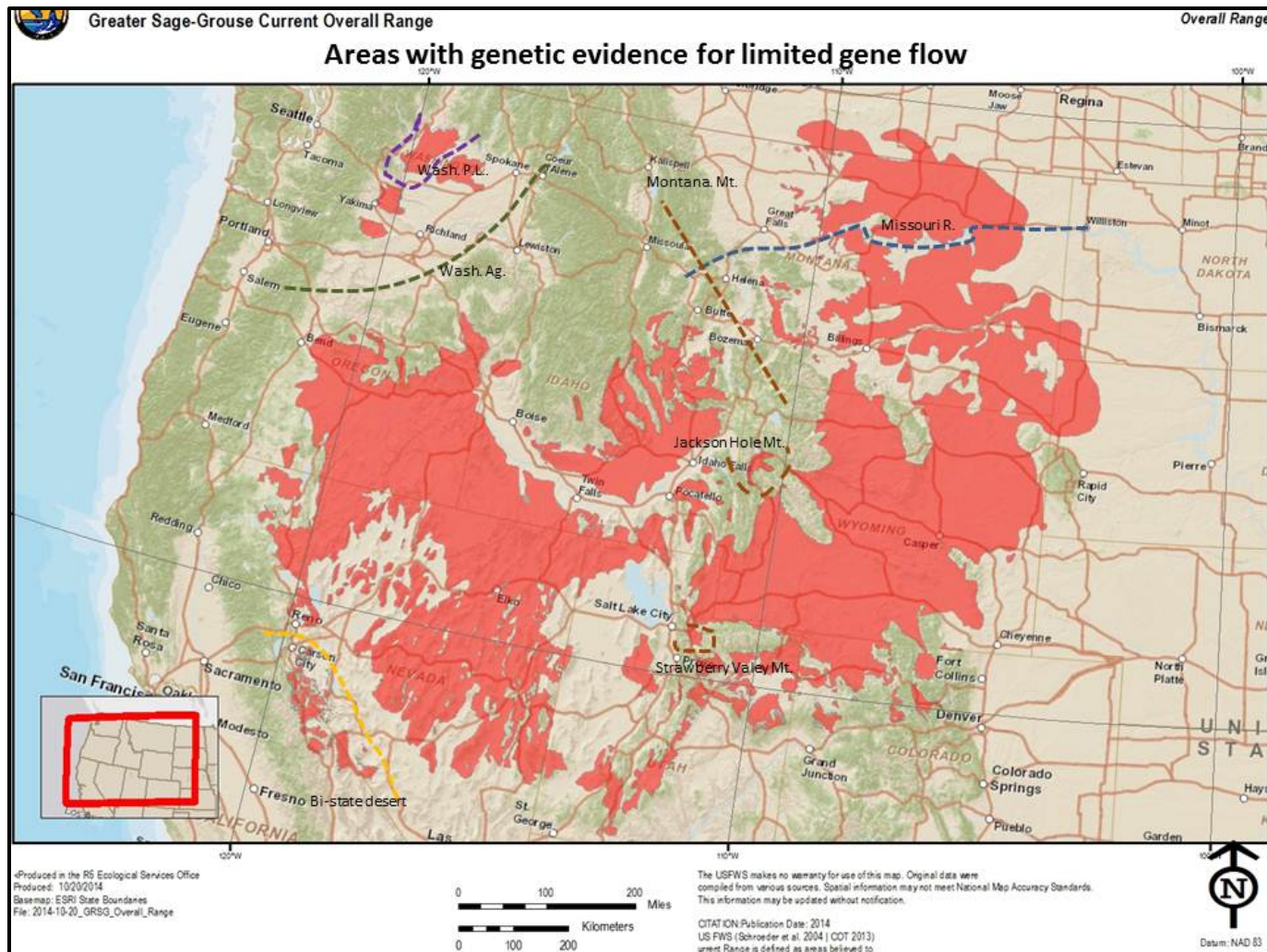


Figure 3. Barriers to gene flow supported by existing genetic evidence discussed by the expert groups in Exercises 2 and 3. Blue line is river, Brown is mountains/forest, Green is cropland, Orange/Yellow is desert, Purple is high capacity power-lines. Some locations were given labels to benefit in workshop communication, but they do not necessarily correspond directly to an otherwise mapped feature.

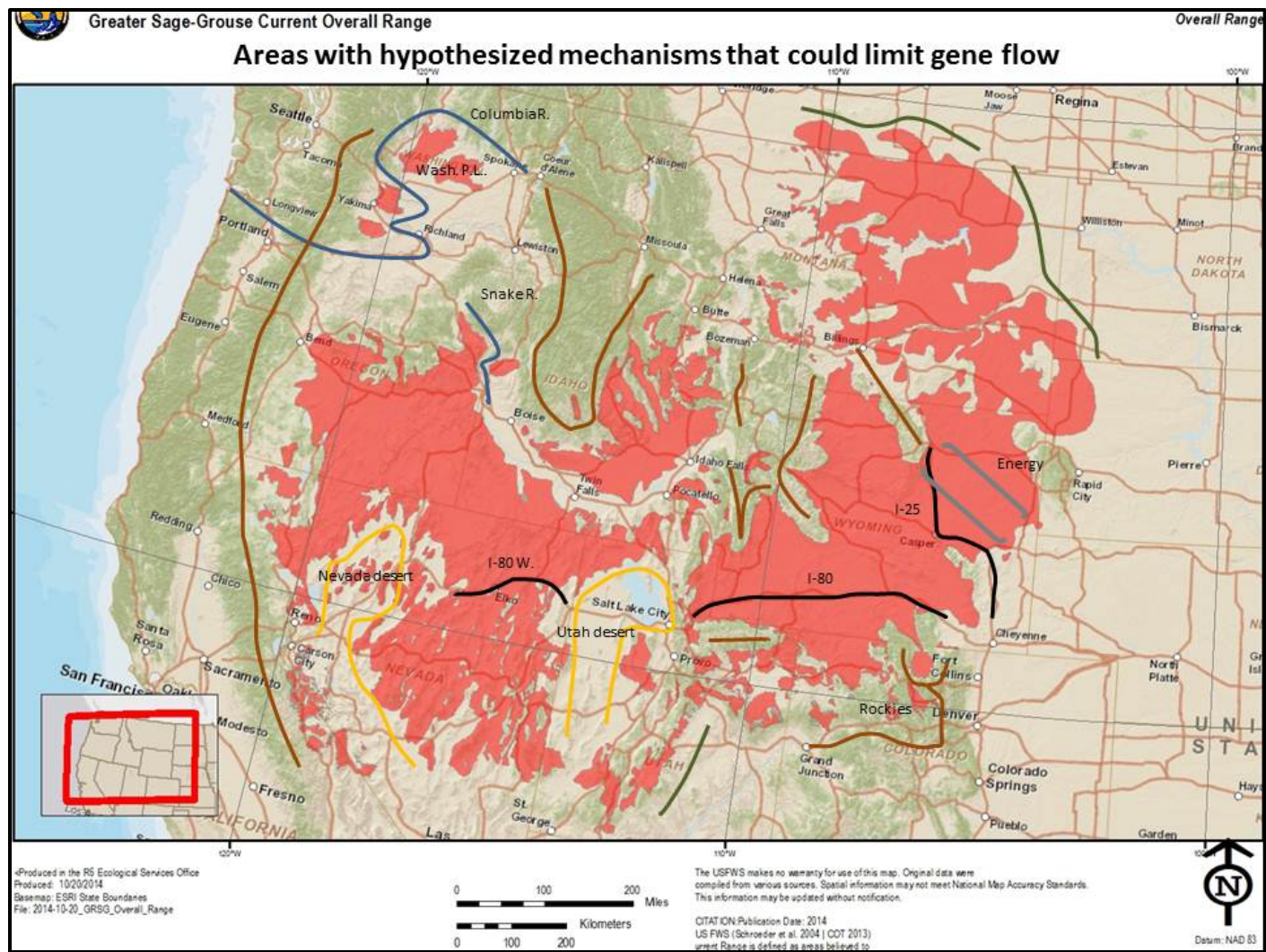


Figure 4. Hypothetical barriers to gene flow discussed by the experts in Exercises 2 and 3. Blue is river, Brown is mountains/forest, Green is cropland, Orange/Yellow is desert Black is interstate highway, Grey area is energy development. Some locations were given labels to benefit in workshop communication, but they do not necessarily correspond directly to an otherwise mapped feature.

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 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, and the available genetic data does not indicate that there are genetic differences on either side. Conversely, sage-grouse are not likely able to fly across the 4-mile wide Columbia River, with its deep, broad valley. Further, elevation itself may not be a barrier, but the habitat features associated with elevation extremes likely act as a barrier to gene flow.

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

- Large reservoirs, such as those along the Missouri River.
- Agricultural fields.
- Electrical power lines.
- Highways and roads, such as Interstate 80 (I-80) in southern Wyoming.
- Energy development and its associated infrastructure.

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 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*) have been found from *data-driven genetic evidence* and the places where limited gene flow have been *hypothesized*?

Exercise Discussion:

The experts again stressed that the identified barriers and areas with limited gene flow that they are definitive or comprehensive. Due to the lack of available data, there are likely other barriers and places on

the landscape that have restricted gene flow that have not been analyzed or considered. In the future, more data could identify additional barriers to gene flow and the upcoming range-wide connectivity study will answer this question with greater certainty.

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

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

| AREA OR POPULATION WITH LIMITED GENE FLOW BASED ON THE AVAILABLE GENETIC EVIDENCE | STATE(S) | HYPOTHESIS OR BASED ON EXISTING GENETIC EVIDENCE? | SUMMARY OF DISCUSSION BY THE EXPERTS |
|---|-----------------------|---|--|
| Bi-State | California and Nevada | Based on existing genetic evidence | <ul style="list-style-type: none"> • The bi-state population has completely different haplotypes than the rest of the range. • Isolation is by distance. • The genetic evidence indicates that the bi-state population has been isolated for approximately 1,000 to 10,000 years. • During the last ice age (the Pleistocene), Lake Lahontan may have acted as a barrier. • Due to the established clades, the bi-state population is not reciprocally monophyletic with the rest of the range; it is different genetically. • Although genetically different, the bi-state population is genetically diverse, which is indicative of a long history of isolation. |
| Columbia Basin | Washington | Based on existing genetic evidence | <ul style="list-style-type: none"> • The Columbia Basin population is small and isolated. • Isolation is by distance. • 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. • The Columbia Basin population has likely experienced genetic drift, but it is not likely adaptively different from the rest of the range. • Analysis of historic gene flow would help age and explain the genetic difference. • The genetic isolation of the Columbia Basin likely occurred more recently than the bi-state population, because prior to conversion to croplands, sagebrush habitats connected Oregon and Washington more recently than in Nevada. • Two populations in eastern Washington have translocated sage-grouse from Oregon and Nevada, which could complicate an analysis of contemporary gene flow. |

| AREA OR POPULATION WITH LIMITED GENE FLOW BASED ON THE AVAILABLE GENETIC EVIDENCE | STATE(S) | HYPOTHESIS OR BASED ON EXISTING GENETIC EVIDENCE? | SUMMARY OF DISCUSSION BY THE EXPERTS |
|---|----------|---|---|
| Jackson Hole | Wyoming | Based on existing genetic evidence | <ul style="list-style-type: none"> 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. Due to its isolation and small size, sage-grouse in Jackson Hole may have unique haplotypes that differ from those found in the bi-state population. |
| Missouri River Valley | Montana | Based on existing genetic evidence | <ul style="list-style-type: none"> Preliminary data expected to publish by the fall of 2015 suggest that the Missouri River Valley inhibits sage-grouse movement, which has resulted in genetic difference on either side of the river. Isolation is by a geographic barrier. The Missouri River is not necessarily a complete barrier, but there is limited gene flow across the river. |

To summarize Exercises 3 and 4, the experts identified evidence-based and hypothetical barriers to gene flow across the range of the greater-sage grouse. Hypothetical barriers not yet considered that could reduce gene flow include natural and anthropogenic features, such as forested mountains, electrical distribution 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 is 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.

Part 3 and Part 4: Genetic divergence and persistence

Exercise 4 - Areas with low-genetic diversity across the range and areas where genetic process 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:

- Small, isolated populations experience less genetic diversity and are more susceptible to genetic risks, such as inbreeding depression and genetic drift.
- Fragmentation and isolation decreases population connectivity, reduces gene flow, and reduces genetic diversity, and consequently may also decrease the size of the overall range.

Table 2 summarizes the discussion regarding small, isolated populations of greater sage-grouse with low genetic diversity. The experts again stressed that results from the upcoming range-wide connectivity study will help evaluate the genetic diversity in these areas, and could reveal more areas of concern with low genetic diversity. Additionally, the range-wide connectivity study will 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 processes that reduce genetic diversity, such as inbreeding depression and 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 the future as the species adapts to changing environmental conditions. Therefore, from a geneticist's perspective, threats that fragment habitats and isolate small populations would be the most concerning for the maintenance of genetic diversity.

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

| AREA(S) OR POPULATION(S) THAT MAY BE LESS GENETICALLY DIVERSE AS A RESULT OF SMALL POPULATION SIZE & ISOLATION | STATE(S) | HYPOTHESIS OR BASED ON EXISTING GENETIC EVIDENCE? | SUMMARY OF DISCUSSION BY THE EXPERTS |
|--|--|---|--|
| Columbia Basin | Washington | Based on existing genetic evidence | <ul style="list-style-type: none"> Populations in the Columbia Basin are small and isolated, and less genetically diverse. Sage-grouse are not likely able to move naturally from Oregon to Washington. As a result, Columbia Basin populations have haplotypes common to the rest of the range, but they are less genetically diverse, suggesting isolation by distance. Additional research is required to evaluate the time of isolation. The existing data does not provide insight into adaptive variation of the Columbia Basin. |
| Jackson Hole | Wyoming | Based on existing genetic evidence | <ul style="list-style-type: none"> The Jackson Hole population is small and isolated, with less genetic diversity. |
| Strawberry Valley | Utah | Based on existing genetic evidence | <ul style="list-style-type: none"> The population in the Strawberry Valley has reduced genetic diversity, largely the result of over-predation, but the population does not necessarily have low gene flow. Translocations complicate genetic analyses. |
| Klamath area | Oregon | Hypothesis | <ul style="list-style-type: none"> Isolated pocket of occupied habitat. |
| Weiser | Idaho | Hypothesis | <ul style="list-style-type: none"> Isolated pocket of occupied habitat. |
| Belt Mountains | Montana | Hypothesis | <ul style="list-style-type: none"> Isolated pocket of occupied habitat. |
| Populations at the periphery of the range | S. Utah N. Colorado N. Montana S. Idaho | Hypothesis | <ul style="list-style-type: none"> 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, so less genetically diverse. 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. |

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 on the current understanding of sage-grouse genetics.

Exercise Discussion:

As summarized in Table 3, several upcoming or recently completed genetic studies will provide additional scientific clarity regarding gene flow, barriers, and genetic diversity across the range of the greater sage-grouse. The experts were generally reluctant to express their judgments on how new studies and research could change the current understanding of sage-grouse genetics. However, the upcoming range-wide landscape connectivity study could influence the workshop discussions by modifying or identifying new barriers.

Table 3. Recently published studies, ongoing research, and research ideas regarding the genetics of the greater sage-grouse discussed by the experts.

| PROJECT NAME | CITATION OR RESEARCHERS | LOCATION | PROGRESS | SUMMARY OF DISCUSSION BY THE EXPERTS |
|---|------------------------------|-----------------------|----------------------------------|---|
| Low neutral genetic diversity in isolated Greater Sage-Grouse (<i>Centrocercus urophasianus</i>) populations in northwest Wyoming | Schulwitz <i>et al.</i> 2014 | Jackson Hole, Wyoming | Recently completed and published | <ul style="list-style-type: none">• 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.• The experts discussed how it would be interesting to compare the haplotypes from Jackson Hole with the haplotypes in the Bi-State population.• Looking at the mtDNA would also be important to confirm the Jackson Hole population's difference. |
| Genetic differentiation using SNPs | Oyler-McCance | Range-wide | Currently in review | <ul style="list-style-type: none">• Differentiation of the greater sage-grouse, the bi-state population, and the Gunnison sage-grouse using SNPs. |
| Landscape genetics in Wyoming | Knick <i>et al.</i> | Wyoming | Currently in review | <ul style="list-style-type: none">• Landscape genetics of sage-grouse in Wyoming, focusing on the importance of seasonal habitats to drive landscape connectivity. |

| PROJECT NAME | CITATION OR RESEARCHERS | LOCATION | PROGRESS | SUMMARY OF DISCUSSION BY THE EXPERTS |
|---|-----------------------------|------------|------------------------|---|
| Range-wide landscape connectivity | Knick <i>et al.</i> | Range-wide | Ongoing | <ul style="list-style-type: none"> Range-wide landscape connectivity analysis using SNPs. This study will scientifically address many of the workshop's questions. Previous analyses looked at only a handful of locations across the overall range and at 14 microsatellite loci. This new research with SNPs looks at hundreds of locations across the range, with over 8,000 samples, and at potentially more than 100,000 loci. Completion timeline is unclear: Ideally available by spring 2015, but not likely available before fall 2015. |
| Impact of translocations in Washington | Schroeder | Washington | Ongoing | <ul style="list-style-type: none"> Study investigating the impact of translocations on sage-grouse genetics in Washington. Availability timeframe unknown. |
| Impact of translocations in Strawberry Valley | Unknown | Utah | Ongoing | <ul style="list-style-type: none"> Study investigating the impact of translocations on sage-grouse genetics in the Strawberry Valley. Availability timeframe unknown. |
| Connectivity across the Missouri River | Schwartz <i>et al.</i> | Montana | Ongoing | <ul style="list-style-type: none"> 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 | <ul style="list-style-type: none"> 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 | <ul style="list-style-type: none"> Investigate I-80 as a potential barrier to gene flow using available data. Available in a few months. |
| Historical connectivity of the Columbia Basin | Schroeder and Oyler-McCance | Washington | Unfunded research idea | <ul style="list-style-type: none"> 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. |

| PROJECT NAME | CITATION OR RESEARCHERS | LOCATION | PROGRESS | SUMMARY OF DISCUSSION BY THE EXPERTS |
|---|-------------------------|----------|------------------------|---|
| Time of isolation for the Jackson Hole population | Unknown | Wyoming | Unfunded research idea | <ul style="list-style-type: none"> Evaluate the time of isolation for the Jackson Hole population. |

Literature Cited

Burgman, M. 2005. Risks and decisions for conservation and environmental management. Cambridge University Press, Cambridge, UK.

Drescher, M., A. H. Perera, C. J. Johnson, L. J. Buse, C. A. Drew, and M. A. Burgman. 2013. Toward rigorous use of expert knowledge in ecological research. *Ecosphere* 4:Article 83. <http://dx.doi.org/10.1890/ES12-00415.1>

Lowe, W. H., and F. W. Allendorf. 2010. What can genetics tell us about population connectivity? *Molecular Ecology* 19:3038-3051.

Mills, L. S., and F. W. Allendorf. 1996. The one-migrant-per-generation rule in conservation and management. *Conservation Biology* 10:1509-1518

Mills, L. S., M. K. Schwartz, D. A. Tallmon, and K. P. Lair. 2003. Measuring and interpreting changes in connectivity for mammals in coniferous forests. Pages 587-613 in C. J. Zabel and R. G. Anthony, editors. *Mammal Community Dynamics: Management and Conservation in the Coniferous Forests of Western North America*. Cambridge University Press, New York, USA.

Oyler-McCance, S.J., S.E. Taylor, and T.W. Quinn. 2005. A multilocus population genetic survey of the greater sage-grouse across their range. *Molecular ecology* 14(5):1293-1310.

Oyler-McCance, S.J., and T.W. Quinn. 2011. Molecular Insights into the Biology of Greater Sage-Grouse. Pages 85-94 *In* S.T. Knick, and J.W. Connelly, eds. *Greater Sage-Grouse: ecology and conservation of a landscape species and its habitats*. Studies in Avian Biology, 38th.

Schulwitz, S., Bedrosian, B., & Johnson, J. A. 2014. Low neutral genetic diversity in isolated Greater Sage-Grouse (*Centrocercus urophasianus*) populations in northwest Wyoming. *The Condor* 116(4): 560-573.

U.S. Environmental Protection Agency. 2011. Expert elicitation task force white paper. Science and Technology Policy Council, USEPA, Washington DC. (<http://www.epa.gov/stpc/pdfs/ee-white-paper-final.pdf>)

Appendix 1: Workshop Planning Team and Agenda

The workshop planning team (Table 1) developed the workshop's purpose, invitation list, and agenda. Thirteen of the 19 planning team members observed the meeting. *Outside parties were not invited to observe the meeting.* Meeting observers did not participate as experts and outside observers were not invited to participate. Three members of the planning team took notes during the workshop. 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.

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

| PLANNING TEAM MEMBER NAME | ORGANIZATION | PRIMARY ROLE |
|---------------------------|--|--|
| Craig Hansen | U.S. Fish and Wildlife Service Region 6 | Workshop coordinator, observer, and note taker |
| David Smith | U.S. Geological Survey | Facilitator |
| Steve Morey | U.S. Fish and Wildlife Service Region 1 | Facilitator |
| Sarah Converse | U.S. Geological Survey | Facilitator |
| Steve Abele | U.S. Fish and Wildlife Service Region 8 | Planning |
| Angela Burgess | U.S. Fish and Wildlife Service Region 6 | Observer and note taker |
| Dawn Davis | U.S. Fish and Wildlife Service Region 1 | Planning |
| Pat Deibert | U.S. Fish and Wildlife Service Region 6 | Observer |
| Jesse D'Elia | U.S. Fish and Wildlife Service Region 1 | Observer |
| Jeff Everett | U.S. Fish and Wildlife Service Region 8 | Planning |
| Holly Freifeld | U.S. Fish and Wildlife Service Region 8 | Observer and note taker |
| Carey Galst | U.S. Fish and Wildlife Service Headquarters | Planning |
| Kate Norman | U.S. Fish and Wildlife Service Region 6 | Observer |
| Genevieve Skora | U.S. Fish and Wildlife Service Region 8 | Planning |
| Kris Tita | U.S. DOI Office of the Solicitor | Planning |
| Lief Wiechman | U.S. Fish and Wildlife Service Region 6 | Observer |
| Jonathan Cummings | U.S. Geological Survey | Facilitator |
| Mary Grim | U.S. Fish and Wildlife Service Region 8 | Observer |
| Theo Stein | U.S. Fish and Wildlife Service Region 6 | Observer |

Workshop Agenda:

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

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

Thursday October 23, 8:00 AM – 12:00 PM

Fort Collins, Colorado

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

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 captured 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 9 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

| EXPERT NAME | ORGANIZATION | CATEGORY 1 = GREATER SAGE-GROUSE EXPERTS 2 =RELATED SPECIES EXPERTS 3 = NON-GRSG EXPERTS IN CONSERVATION GENETICS | RSVP (Yes or NO) |
|--------------------|---|--|--|
| Mevin Hooten | Colorado Cooperative Fish and Wildlife Research Unit | 1 | Yes |
| Michael Schwartz | USFS | 1 | Yes |
| Sara Oyler-McCance | USGS | 1 | Yes |
| Mike Schroeder | Washington State Department of Fish and Wildlife | 1 | Yes |
| Christin Pruett | Florida Institute of Technology | 2 | Yes |
| Jesus Maldonado | Smithsonian Center for Conservation and Evolutionary Genetics | 3 | Yes |
| Chris Funk | Colorado State University | 3 | Yes |
| Robert Gibson | University of Nebraska – Lincoln | 2 | Yes |
| Jeffrey Olsen | USFWS Conservation Genetics Laboratory Alaska | 3 | Yes |
| Robert Zink | University of Minnesota | 3 | Initially agreed to participate, but later withdrew due to prior commitments |
| Steve Knick | USGS | 1 | No |
| Brad Fedy | Univ. of Waterloo | 1 | No |
| Lisette Waits | Univ. of Idaho | 3 | No |
| J.L. Bouzat | University of Illinois at Urbana-Champaign | 2 | No |
| Andrew Martin | University of Colorado – Boulder | 3 | No |
| Rob Fleischer | Smithsonian Center for Conservation and Evolutionary Genetics | 3 | No |
| Melanie Murphy | University of Wyoming | 2 or 3 | |
| Ephraim Hanks | Penn State | 3 | |

| EXPERT NAME | ORGANIZATION | CATEGORY 1 = GREATER SAGE-GROUSE EXPERTS 2 =RELATED SPECIES EXPERTS 3 = NON-GRSG EXPERTS IN CONSERVATION GENETICS | RSVP (Yes or NO) |
|---------------------|---------------------------|---|----------------------------|
| Todd Cross | University of Montana | 1 | |
| Sergei Drovetski | University of Alaska | 3 | |
| Tom Quinn | University of Denver | 1 | |
| Krissy Bird (Bush) | University of Alberta | 1 | |
| Christian Hagen | Oregon State University | 2 | |
| Sue Haig | Oregon State University | 3 | |
| Ron Van Den Bussche | Oklahoma State University | 3 | |

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>. You will need to see the receptionist on arrival to sign in. Please bring a photo ID.
- To get to Fort Collins from DIA:
 - SuperShuttle: 970-482-0505; <http://www.supershuttle.com/Locations/DENAirportShuttleFortCollins.aspx>
 - Green Ride CO: 970-226-5533; <http://www.greenrideco.com/>
- 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

Ground Rules for the
Genetics Expert Elicitation Workshop Conducted as
Part of the Greater Sage-Grouse Status Review

U.S. Fish and Wildlife Service
October 9, 2014

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.¹ 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 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.

¹ 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](#).

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

Baumgardt, J.A., C.S. Goldberg, K.P. Reese, J.W. Connelly, D.D. Musil, E.O. Garton, and L.P. Watts. 2013. A method for estimating population sex ratio for sage-grouse using noninvasive genetic samples. *Molecular Ecology Resources* 13(3):393-402.

<http://onlinelibrary.wiley.com/doi/10.1111/1755-0998.12069/pdf>

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.

Benedict, N.G., S. Oyler-McCance, S.E. Taylor, C.E. Braun, and T.W. Quinn. 2003. Evaluation of the eastern (*Centrocercus urophasianus urophasianus*) and western (*Centrocercus urophasianus phaios*) subspecies of sage-grouse using mitochondrial control-region sequence data. *Conservation Genetics* 4(3):301-310.

http://download.springer.com/static/pdf/769/art%253A10.1023%252FA%253A1024089618546.pdf?auth66=1412710440_a44d491b1e23c11af156ee0d066f3a9b&ext=.pdf

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.

Bird, K.L., C.L. Aldridge, J.E. Carpenter, C.A. Paszkowski, M.S. Boyce, and D.W. Coltman. 2013. The secret sex lives of sage-grouse: multiple paternity and intraspecific nest parasitism revealed through genetic analysis. *Behavioral Ecology* 24(1):29-38.

<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.

Breidinger, L.S., K.E. Mock, and T.A. Messmer. 2013. Greater Sage-Grouse and natural gas development in Utah: using population genetic data for conservation efforts. *Western North American Naturalist* 73(2):177-183.

<http://utahcbcp.org/files/uploads/publications/BreidingerEtAl2013LeahSmith.pdf>

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.

Bush, K. L., C. K. Dyte, B. J. Moynahan, C. L. Aldridge, H. S. Sauls, A. M. Battazzo, B. L. Walker, K. E. Doherty, J. Tack, J. Carlson, D. Eslinger, J. Nicholson, M. S. Boyce, D. E. Naugle, C. A. Paszkowski, and D. W. Coltman. 2011. Population structure and genetic diversity of Greater Sage-grouse (*Centrocercus urophasianus*) in fragmented landscapes at the northern edge of their range. *Conservation Genetics* 12: 527-542.

<http://link.springer.com/article/10.1007/s10592-010-0159-8#>

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.

Bush, K. L., C. L. Aldridge, J. E. Carpenter, C. A. Paszkowski, M. S. Boyce, and D.W. Coltman. 2010. Birds of a feather do not always lek together: Genetic diversity and kinship structure of Greater Sage-grouse (*Centrocercus urophasianus*) in Alberta. *Auk* 127: 343-353.

<http://aoucospubs.org/doi/pdf/10.1525/auk.2009.09035>

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.

Bush, K.L., M.D. Vinsky, C.L. Aldridge, and C.A. Paszkowski. 2005. A comparison of sample types varying in invasiveness for use in DNA sex determination in an endangered population of greater Sage-Grouse (*Centrocercus uropihasianus*). *Conservation Genetics* 6(5):867-870.

http://www.aviangenetics.com/Sage-Grouse/downloads/bush_et_al_2005_dna_extract.pdf

[No abstract]

Davis, D. M. 2012. Population structure of Greater Sage-grouse in northeastern California: Implications for conservation in a declining peripheral population. Ph.D. dissertation, University of Idaho, Moscow.

[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 (A_{IC}) 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.

Gibson, R. M., D. Pires, K. S. Delaney, and R. K. Wayne. 2005. Microsatellite DNA analysis shows that greater sage grouse leks are not kin groups. *Molecular Ecology* 14: 4453–4459.

<http://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2005.02746.x/pdf>

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.

Oyler-McCance, S.J., M.L. Casazza, J.A. Fike, and P.S. Coates. 2014. Hierarchical spatial genetic structure in a distinct population segment of greater sage-grouse. *Conservation Genetics* .

<http://link.springer.com/article/10.1007/s10592-014-0618-8/fulltext.html>

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.

Oyler-McCance, S.J., and T.W. Quinn. 2011. Molecular Insights into the Biology of Greater Sage-Grouse. Pages 85-94 *In* S.T. Knick, and J.W. Connelly, eds. Greater Sage-Grouse: ecology and conservation of a landscape species and its habitats. *Studies in Avian Biology*, 38th.

<https://www.fort.usgs.gov/sites/default/files/products/publications/22121/22121.pdf>

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.

Oyler-McCance, S.J., S.E. Taylor, and T.W. Quinn. 2005. A multilocus population genetic survey of the greater sage-grouse across their range. *Molecular ecology* 14(5):1293-1310.

<http://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2005.02491.x/pdf>

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.

Oyler-McCance, S., J. St. John, S.E. Taylor, A.D. Apa, and T.W. Quinn. 2005. Population Genetics of Gunnison Sage-Grouse: Implications for Management. *The Journal of Wildlife Management* 69(2):630-637.

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.

Quinn, T.W., N.W. Kahn, J.R. Young, N.G. Benedict, S. Wood, D. Mata, and C.E. Braun. 1997. Probing the evolutionary history of sage grouse *Centrocercus urophasianus* populations using mitochondrial DNA sequence. *Wildlife Biology* 3(3-4):291.

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.

<http://wdfw.wa.gov/publications/01305/wdfw01305.pdf>

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.

Taylor, R.L., D.E. Naugle, and L.S. Mills. 2012. Viability analyses for conservation of sage-grouse populations: Buffalo Field Office, Wyoming. Bureau of Land Management, Buffalo Field Office, Buffalo, Wyoming.

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 sagegrouse. 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 mi²(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.

Taylor, S.E., S. Oyler-McCance, and T.W. Quinn. 2003. Isolation and characterization of microsatellite loci in Greater Sage-Grouse (*Centrocercus urophasianus*). Molecular Ecology Notes 3(2):262-264.

<http://onlinelibrary.wiley.com/doi/10.1046/j.1471-8286.2003.00424.x/pdf>

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.

Tebbenkamp, J.M. 2014. Greater Sage-Grouse in the Bi-State Distinct Population Segment: An evaluation of genetic structure, connectivity, and vital rate in Mono County, California. Master of Science. University of Idaho.

[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 F_{ST} 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-telemetry, 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.

Thompson, T. R. 2012. Dispersal ecology of Greater Sage-grouse in northwestern Colorado: Evidence from demographic and genetic methods. Ph.D. dissertation, University of Idaho, Moscow.

http://cpw.state.co.us/Documents/Research/Birds/Thompson_dissertation_2012FINAL.pdf

ABSTRACT

The greater sage-grouse (*Centrocercus urophasianus*; here after 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.

Zink, R.M. 2014. Comparison of Patterns of Genetic Variation and Demographic History in the Greater Sage-Grouse (*Centrocercus urophasianus*): Relevance for Conservation. The Open Ornithology Journal 7:11.

<http://www.miningamerica.org/wp-content/uploads/Zink-Report.pdf>

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 $N_e < 50$ in 30 years and $P(N_e < 500)$ 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

**Stiver, J. R., A. D. Apa, T. E. Remington, and R. M. Gibson.** 2008. Polygyny and female breeding failure reduce effective population size in the lekking Gunnison sage-grouse. *Biological Conservation* 141:472-481.

[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%2B2008%2BPolygyny%2Band%2Bfemale%2Bbreeding%2Bfailure%2Breduce%2Beffective%2Bpopulation%2Bsize%2Bin%2Bthe%2Blekking%2BGunnison%2Bsage-grouse%26btnG%3D%26as\\_sdt%3D1%252C5%26as\\_sdt%3D#search=%22Stiver%202008%20Polygyny%20female%20breeding%20failure%20reduce%20effective%20population%20size%20lekking%20Gunnison%20sage-grouse%22](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%2B2008%2BPolygyny%2Band%2Bfemale%2Bbreeding%2Bfailure%2Breduce%2Beffective%2Bpopulation%2Bsize%2Bin%2Bthe%2Blekking%2BGunnison%2Bsage-grouse%26btnG%3D%26as_sdt%3D1%252C5%26as_sdt%3D#search=%22Stiver%202008%20Polygyny%20female%20breeding%20failure%20reduce%20effective%20population%20size%20lekking%20Gunnison%20sage-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 ( $N_e$ ) 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  $N_e$  for the study population. We also simulated the effects of population size, skewed vs. random mating, and female breeding failure on  $N_e$ . 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  $N_e$  (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,  $N_e$  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.

**Young, J. R.** 1994. The influence of sexual selection on phenotypic and genetic divergence among sage-grouse populations. Dissertation. Purdue University, West Lafayette, Indiana, USA.

<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.<sup>^</sup> 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.<sup>^</sup> 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.



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

Attach presentations.

## **Appendix 7: Workshop Notes**

Attach three sets of notes.