CHAPTER FIVE

Molecular Insights into the Biology of Greater Sage-Grouse

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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.

Key Words: Centrocercus urophasianus, genetics, lek evolution, mating system, population genetics, taxonomic boundaries.

Nuevos Conocimientos Sobre la Biología Molecular del Greater Sage-Grouse

Resumen. Estudios recientes sobre la genética del Greater Sage-Grouse (*Centrocercus urophasianus*) han revelado algunos hallazgos importantes. Primero, la paternidad múltiple en las crías es mas prevalente que lo que se creía anteriormente, y los leks (asambleas de cortejo) no están compuestos por grupos de parentesco. Segundo, el Greater Sage-Grouse es genéticamente distinto del Gunnison Sage-Grouse (*C. minimus*). Tercero, la población de Lyon–Mono en el Mono Basin, en los límites entre Nevada y California, tiene unas características genéticas únicas. Cuarto, la delineación previa entre el Western Greater Sage-Grouse (*C. u. phaios*) y el Eastern Greater Sage-Grouse (*C. u. urophasianus*) no tiene soporte genético. Quinto, dos poblaciones aisladas en Washington muestran indicios de que la diversidad genética ha sido perdida debido a la disminución y al aislamiento de las poblaciones.

Palabras Clave: Centrocercus urophasianus, evolución de los leks, fronteras taxonómicas, genética, genética de poblaciones, sistema reproductivo.

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Molecular genetic approaches to the study of wildlife began in the late 1970s and early 1980s, but their initial use was very limited, in part because of the amount of expertise in molecular genetic techniques that was then both necessary and rare. In the 1980s, the development of the polymerase chain reaction (PCR) and concurrent improvements in DNA sequencing technology led to a major increase in efficiency and decrease in cost. As a result, it is now commonplace to gather DNA sequence information from targeted regions of the mitochondrial or nuclear genomes. Such techniques are now being widely applied to investigate relationships among species, populations, family groups, and individuals, even in species for which little was previously known at the genetic level (Haig 1998, DeYoung and Honeycutt 2005, Oyler-McCance and Leberg 2005).

In the nuclear genome, tandem repetitive DNA sequences, such as microsatellites, tend to undergo rapid change in the number of tandem repeats (Li et al. 2002). Within populations, such regions thereby generate numerous alleles that can be used in various ways to estimate different aspects of the individual population, such as past effective population size; to estimate levels of historical gene flow between populations; and even to determine parentage of offspring. While such repetitive regions are typically but not always absent in most vertebrate mitochondrial genomes, the rate of base substitution in parts of the mitochondrial genome typically exceed those of comparable regions in the nuclear genome (Pesole et al. 1999). One of the most widely targeted hypervariable mitochondrial regions is found at both ends of the major noncoding control region (Baker and Marshall 1997). It is these and other rapidly evolving segments of DNA that have provided a great deal of new insight into relationships among vertebrate populations and closely related species in recent decades (Avise 1994).

Sage-grouse (*Centrocercus* spp.) are ground-dwelling galliforms found exclusively in North America. Historically, sage-grouse occupied virtually any habitat dominated by sagebrush (*Artemisia spp.*), including much of the western United States (Johnsgard 1983). Until recently, all sage-grouse were considered to be one species. In 2000, sage-grouse were split into two species (Fig. 5.1): Greater Sage-Grouse (*C. urophasianus*) and Gunnison Sage-Grouse (*C. minimus*), following Young et al. (2000). The range of Greater Sage-Grouse historically spanned 15 western states and three Canadian provinces (Schroeder et al. 2004), yet they currently occupy only 56% of the range

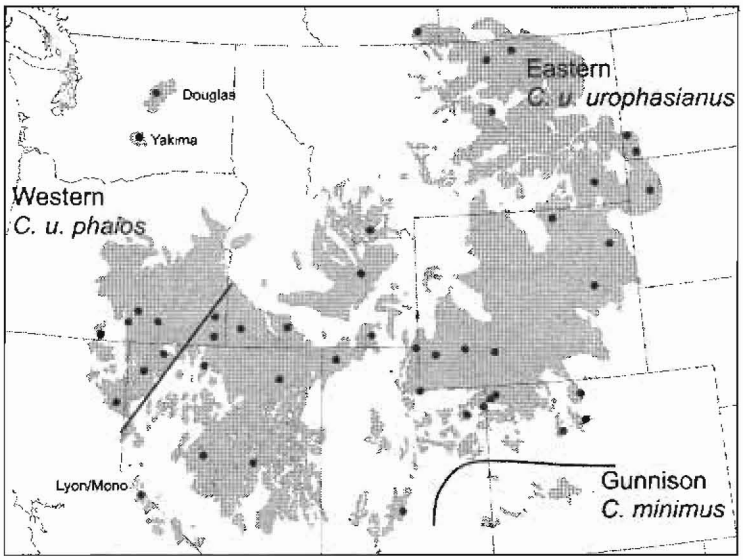

Figure 5.1. Distribution of both Greater Sage-Grouse and Gunnison Sage-Grouse with historical subspecies noted. Dots represent sampling sites for the population genetic studies. The two populations in Washington and the population along the border of Nevada and California are noted, as they are discussed in detail.

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occupied before settlement by Europeans (Fig. 5.2) and were extirpated in at least four states and one province (Connelly and Braun 1997, Schroeder et al. 2004). Regional population declines have been dramatic, ranging from 17% to 47% (Connelly and Braun 1997). These declines are likely linked to the loss, fragmentation, and degradation of sagebrush habitat (Braun 1998), resulting in the isolation of small populations that formerly existed in more contiguous habitat (Fig. 5.2).

As sagebrush obligates, Greater Sage-Grouse rely on sagebrush for virtually all aspects of their life cycle (Patterson 1952, Braun et al. 1977, Connelly et al. 2000c). Seasonal movements of Greater Sage-Grouse have been reported to be quite variable, depending on the population being studied. Some populations are considered to be resident, with little movement throughout the year, while others have been documented to move up to 161 km (Patterson 1952). Still other populations have been described as migratory, with consistent seasonal movements up to 82 km (Connelly et al. 1988). With that stated, the amount of movement among populations and connectedness of populations remain largely unknown.

Greater Sage-Grouse have a polygynous mating system that has been the focus of much research (Wiley 1974, Wittenberger 1978, Gibson and Bradbury 1986, Bergerud 1988a, Gibson et al. 1991). In the spring, males gather together on leks, where they engage in an elaborate strutting display for females. The consistency of the behavioral components of these strut displays, even when compared between widely separated populations, led Wiley (1973a) to use them as an example of a fixed action pattern. Males establish territories on leks and defend them throughout the breeding season (Gibson and Bradbury 1986). Females arrive on leks, usually in groups, and are attracted to dominant males (Wiley 1978). While observational studies have provided some guidance on what proportion of males actually breed (Patterson 1952, Wiley 1973b), until recently this information had not been complemented with studies at the genetic level.

Because of declining habitat and population numbers, Greater Sage-Grouse have become the focus of conservation concern. As a result, nearly all aspects of their ecology are being examined in an attempt to better understand and manage this species. Several molecular genetic approaches have

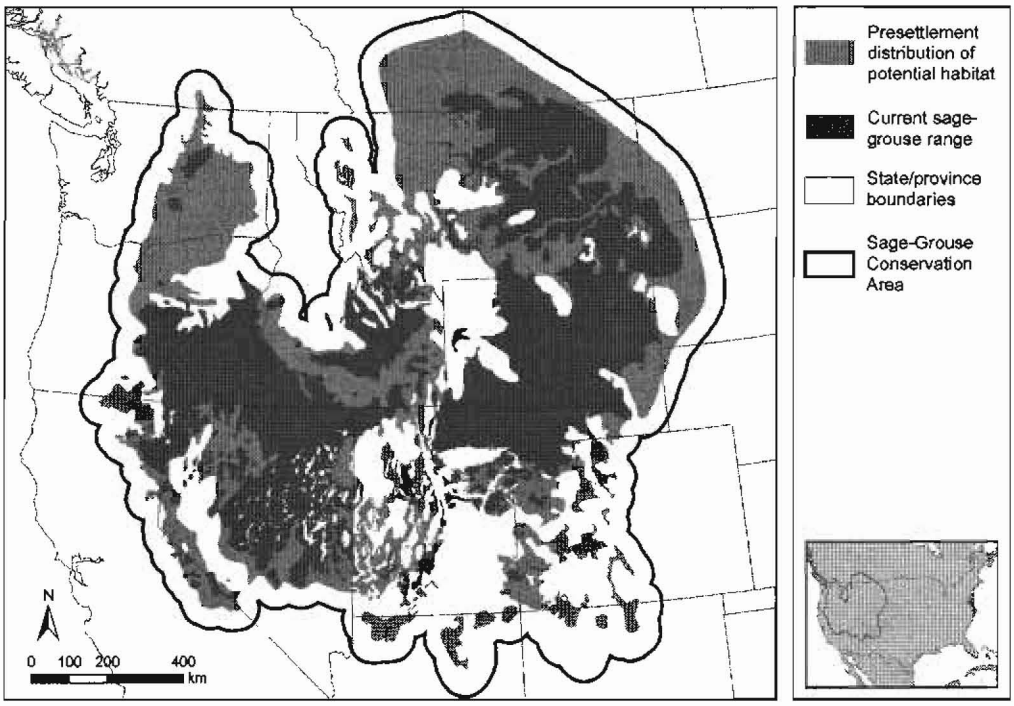

Figure 5.2. Historical and current distribution of the Greater Sage-Grouse (from Connelly et al., 2004, adapted from Schroeder et al. 2004).

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been employed to aid in this effort. Because molecular methods can be used to more definitively assess parentage, such techniques have been used to augment observational studies of the mating system of Greater Sage-Grouse and to investigate rates of multiple paternity. Such information is needed for management to better estimate effective population sizes. Additionally, molecular methods can be used to reassess taxonomic boundaries defined using morphological and behavioral characters. Finally, such methods can also be used to examine population structure and gene flow, i.e., connectivity of populations, as well as to document levels of genetic diversity. All those metrics are paramount for conservation efforts. This chapter reviews the current published literature using molecular genetic techniques to obtain information useful for the conservation and management of Greater Sage-Grouse and put it into the context of avian ecology using selected molecular studies.

Molecular genetic studies of sage-grouse have focused on several questions. One study investigated the mating system of Greater Sage-Grouse by comparing behavioral data and genetic data (Semple et al. 2001). Another study examined the evolution of lek formation in Greater Sage-Grouse by determining whether leks consist of kin groups (Gibson et al. 2005). Four other studies have looked at taxonomic boundaries and population genetics (Kahn et al. 1999, Oyler-McCance et al. 1999, Benedict et al. 2003, Oyler-McCance et al. 2005b). We provide a review of these studies and discuss how they can be used in the management and conservation of Greater Sage-Grouse and how they fit into a larger ecological perspective.

INVESTIGATING THE LEK MATING SYSTEM

Behavioral observations of the lek mating system of Greater Sage-Grouse have led to the suggestion that mating distributions are strongly skewed and that females typically mate with only one dominant male (Wiley 1973b, Gibson and Bradbury 1986, Gibson et al. 1991). Evidence suggests that a component to the mating system exists that was unobserved by previous studies, whereby females may be mating off the lek with purportedly nondominant males. This can be inferred in part by the study of Semple et al. (2001), who assessed the parentage of 10 broods of Greater Sage-Grouse in Long Valley, California, near Mono Lake (Fig. 5.1). In this study, behavioral observations on attendance, territoriality, and mating behavior of males on one lek were made. Each male was uniquely identified using colored leg bands. Both males and females associated with this lek were trapped, and blood samples were collected. Females were radio-collared and followed throughout the breeding process. After 7 to 9 days of incubation, nests were located, all eggs were collected, and embryos were sacrificed for genetic analysis.

Six variable microsatellite loci were used to identify the parentage of all embryos. The genotypes of the embryos were compared to the associated mother to identify which alleles were maternal and which were paternal. The number of paternal alleles was then used to determine whether the brood had been sired by more than one male. An attempt was made to exclude potential males as fathers based on their microsatellite genotypes.

Semple et al. (2001) found that all broods had genotypes consistent with their putative mother and that eight of 10 broods showed results consistent with only one father. Two broods did show results suggesting that at least two males had fathered the brood. Of the 10 females with broods, only four mated on the lek from which behavioral observations were made and thus were seen copulating. Three of those four females mated with males that had been genotyped, and in each case, the putative father could not be excluded as the father based on genotype data. The fourth female mated with a male who was not banded or genotyped. Semple et al. (2001) conclude that in all cases for which they had data, the genetic data were consistent with behavioral data. Although previous studies suggested very low instances of multiple paternity, this molecular genetic study showed that it may be more prevalent than was once thought.

Such comparisons between observation-based studies and molecular ones have been conducted in other species (Kellogg et al. 1995, Coltman et al. 1999, Heckel et al. 1999). The addition of molecular data in this case can add another layer of information, as mating behavior does not always reflect parentage (Coltman et al. 1999), and can provide better estimates of effective population size, which is important for the management of species (Lande and Barrowclaugh 1987).

Among avian lekking species, many have been shown to exhibit the behavior of polyandry (Trail 1985, Petrie et al. 1992, Fiske and Kålås 1995), although fewer studies have actually been able to document parentage (Lanctot et al. 1997, Lank et al.

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2002). Only one other lekking grouse has been examined in this way. The mating behavior and genetic paternity of Black Grouse (*Tetrao tetrix*) have been documented in several studies (Alatalo et al. 1996, Kokko et al. 1999, Lebigre et al. 2007). Unlike the results found by Semple et al. (2001) in Greater Sage-Grouse, multiple mating and multiple paternity in Black Grouse were found to be rare (Alatalo et al. 1996, Lebigre et al. 2007). The studies of Black Grouse were larger, examining 135 broods as opposed to 10 in Greater Sage-Grouse, and included data from multiple leks (Lebigre et al. 2007). Given this difference in observed trends between related species, larger-scale studies of Greater Sage-Grouse are suggested in order to better estimate rates of multiple paternity. Although the study by Semple et al. (2001) provides an indication that some females are mating with more than just the dominant males on leks, it would be prudent to extend such studies to larger sample sizes and additional leks in order to better understand the impact that such behaviors have on the effective population sizes of Greater Sage-Grouse in general.

In another study that included molecular genetic data, Gibson et al. (2005) tested the hypothesis that lekking behavior evolved in Greater Sage-Grouse because kin selection favored subordinate males joining the leks of their close relatives in order to increase their inclusive fitness. This might occur if females were more attracted to leks with larger numbers of attending males. The authors collected blood samples and made behavioral observations at three major leks in one population in Long Valley, California, near Mono Lake (Fig. 5.1). Their data included two time periods (1984–1988 and 1997–2001, except 1999). Males were categorized as territorial or nonterritorial based on daily observations as to whether males aggressively excluded other males from territories on leks. A measure of spatial association of males off leks was also estimated using the degree to which males with overlapping ranges moved collectively from day to day as an index of association. These behavioral observations were coupled with inferences that could be made from their molecular data set. The mean relatedness within leks was estimated using 11 highly variable microsatellite loci, and it was then compared to mean relatedness estimates from within known family groups. They determined that the mean relatedness values on leks were statistically indistinguishable from zero, suggesting that males on leks are not closely related. Additionally, those mean relatedness values were significantly lower than relatedness values calculated among known family members (Gibson et al. 2005). They found no evidence for local clustering of related individuals while on leks, yet they did find that related individuals tended to cluster together when off the lek. Their data reveal that Greater Sage-Grouse leks consist of groups of largely unrelated males with little if any spatial association among kin either within or between leks.

Several other avian lekking species have been examined in a similar way, testing whether or not males on leks are more related than random. Similar to the findings of Gibson et al. (2005), males on leks were not found to be related in both the Long-tailed Manakin (*Chiroxiphia linearis*; McDonald and Potts 1994) and the Great Bustard (*Otis tarda*; Martin et al. 2001). Studies of the Peacock (*Pavo cristatus*) and White-bearded Manakin (*Manacus manacus*), however, have documented that males on leks are related (Petrie et al. 1999, Shorey et al. 2000, Höglund and Shorey 2003). Among lekking grouse, both Black Grouse and Lesser Prairie-Chicken (*Tympanuchus pallidicinctus*), have been examined in this manner, and both have shown males on leks to be related (Höglund et al. 1999, Bouzat and Johnson 2003), contrary to what has been found for Greater Sage-Grouse (Gibson et al. 2005). Again, it would be informative for future studies to assess additional populations in this regard to determine whether the population studied in Long Valley, California, is typical of the species. Ultimately such knowledge impacts management in the sense that genetic diversity contained within single leks may (or may not be) reduced relative to the species as a whole.

REASSESSING TAXONOMIC BOUNDARIES

Investigating a Morphologically and Behaviorally Different Group

Because morphological (Hupp and Braun 1991) and behavioral (Young et al. 1994) evidence suggested the sage-grouse in southwestern Colorado and southeastern Utah were distinct from sage-grouse elsewhere, two molecular studies were initiated examining molecular genetic variation across the boundary of these two groups of sage-grouse in Colorado (Kahn et al. 1999,

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Oyler-McCance et al. 1999). The goal of the two genetic studies was to determine whether there were genetic differences between sage-grouse from southwestern Colorado and those from northwestern Colorado.

Kahn et al. (1999) sequenced 141 base pairs from a rapidly evolving part of the control region of mitochondrial DNA (mtDNA) from approximately 20 individuals each from seven populations. Six of those populations were within the range of the larger-bodied Greater Sage-Grouse, sampled in northern Colorado and Utah. The remaining population was within the range of the smaller-bodied grouse, sampled in the Gunnison Basin of Colorado. That population, along with others nearby, was later assigned species status as the Gunnison Sage-Grouse (Young et al. 2000).

This study revealed that the Greater Sage-Grouse populations were more diverse than the Gunnison population, with many mtDNA haplotypes present per population (average of 7.3), while the Gunnison Sage-Grouse population had only two haplotypes present. The Greater Sage-Grouse populations had four ubiquitous haplotypes, and the Gunnison Sage-Grouse population had only one of those four haplotypes and also included a haplotype that was unique. Using maximum parsimony analysis, Kahn et al. (1999) revealed that all haplotypes fell into one of two deep clades. All populations of Greater Sage-Grouse had individuals from both clades. From these observations, they hypothesized that the two deep clades might reflect what were historically two distinct populations or metapopulations that began diverging at least 850,000 years ago during the Pleistocene. More recently, the two groups intermingled, leading to the modern pattern.

Oyler-McCance et al. (1999) expanded on the mitochondrial study of Kahn et al. (1999) by including data from four nuclear microsatellites and by adding both mitochondrial and nuclear data from three additional populations of Gunnison Sage-Grouse. They, too, showed that the Greater Sage-Grouse had more genetic diversity in mitochondrial haplotypes, and their microsatellite data showed concordant patterns in the nuclear genome, with higher levels of allelic diversity among the Greater Sage-Grouse (average of 5.7 alleles per locus) than among the Gunnison Sage-Grouse (average of 2.6 alleles per locus). Population genetic analysis revealed a lack of evidence of gene flow among the two groups of sage-grouse, which was consistent with the idea that the Gunnison Sage-Grouse should be recognized as a new species (Young et al. 2000). Furthermore, Oyler-McCance et al. (1999) showed that levels of gene flow were much higher among populations of Greater Sage-Grouse ($F_{ST} = 0.03$) than among populations of Gunnison Sage-Grouse ($F_{ST} = 0.21$).

These two genetic studies provided evidence of a lack of gene flow between the two morphologically and behaviorally distinct groups of sage-grouse in Colorado. This was consistent with the previous morphological (Hupp and Braun 1991) and behavioral (Young et al. 1994) evidence suggesting that the small-bodied sage-grouse in southwestern Colorado were, in fact, a distinct species. This newly recognized species was subsequently named and described (Young et al. 2000).

Examining a Subspecific Boundary

The establishment of the Gunnison Sage-Grouse as a separate species was a recent event, and because its range is comparatively small, the Greater Sage-Grouse range was affected only slightly when this distinction was made. Within the remaining larger range of the Greater Sage-Grouse, the species has been divided into eastern (*C. u. urophasianus*) and western (*C. u. phaios*) subspecies (Aldrich 1946). This delineation was based on plumage and coloration differences in just eight individual Greater Sage-Grouse collected from Washington, Oregon, and California (Aldrich 1946). The western subspecies presumably occurred in southern British Columbia, central Washington, east-central Oregon, and northeastern California (Aldrich 1946). Populations in other areas of the range were considered to be the eastern subspecies (Fig. 5.1). The validity of the subspecies distinction was later questioned, as many biologists were unable to recognize this boundary in the field (Johnsgard 1983). Benedict et al. (2003) published a molecular investigation that covered the western part of the range of the Greater Sage-Grouse, spanning the boundary between the two subspecies, as described by Aldrich (1946, 1963).

In that study, tissue samples from 16 populations crossing the boundary separating the eastern and western subspecies, as described by Aldrich (1946, 1963), were collected. These were used to sequence part of the mitochondrial DNA

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control region (Kahn et al. 1999). Among the 332 individuals sampled across the range, Benedict et al. (2003) identified a total of 38 haplotypes, all falling into either one of the two monophyletic clades described by Kahn et al. (1999). Five of these were common, widespread haplotypes, and upon examination of their distribution, no obvious genetic subdivision between the eastern and western subspecies existed. On this basis, the authors concluded that no genetic evidence supported the original subspecies distinction, although they did point out that further behavioral and morphological measurement across the boundary should be considered.

In the course of addressing the subspecies question, the authors found that several individual sampled populations were notable for other reasons. In their broad survey, they noted that novel haplotypes—those found in only one population—were common, but also that they usually occurred at low frequency, typically <10% of the individuals sampled within each population (Benedict et al. 2003). Along the border between Nevada and California, however, they found an unusual population (Lyon–Mono, Fig. 5.1) in which 87.5% of the haplotypes found were unique, constituting 97.7% of the birds sampled. This led the authors to suggest that the Lyon–Mono population has been isolated from neighboring populations for a considerable amount of time. Because this population has closely related but novel haplotypes belonging to each clade, they believe that it is likely that this isolation occurred after the intermixing of populations representing the two major haplotype clades, as originally hypothesized by Kahn et al. (1999). Presumably, over tens of thousands of years of isolation, factors such as mutation and genetic drift resulted in the significant divergence of the Lyon–Mono population from other Greater Sage-Grouse populations. Benedict et al. (2003) indicate that the preservation of genetic diversity represented by the unique allelic composition of the Lyon–Mono population is of particular importance for conservation. Because the likelihood that the distinctiveness of neutral genetic markers extends to genes under adaptive selection, the authors believe that this population should be managed independently to avoid the translocation of other Greater Sage-Grouse into this area. They also maintain that it will be critical that additional morphological and behavioral studies of the Lyon–Mono population be undertaken to address taxonomic questions.

In addition, Benedict et al. (2003) found that the two populations in Washington (Fig. 5.1) contained the lowest level of haplotype diversity observed, perhaps resulting from a recent genetic bottleneck given that these populations now occupy just 8–10% of their original range (Friedman and Carlton 1999) and have shown significant declines in population size (Schroeder et al. 2000). Benedict et al. (2003) suggest that the probable loss of genetic variation caused by this bottleneck and its potentially long-term adverse impact (Bouzat et al. 1998, Le Page et al. 2000) should be addressed as management strategies are developed for these populations. They advocate that active management, such as translocation of birds, may be justified to ensure their continued persistence.

Examining Taxonomy and Population Genetics Across the Range

In order to investigate taxonomic questions and examine levels of gene flow and connectedness among populations, Oyler-McCance et al. (2005b) greatly extended the sampling range and density of previous studies and provided a comprehensive examination of the distribution of genetic variation across the entire range of Greater Sage-Grouse using both mtDNA sequence data and data from nuclear microsatellites. They collected 1,080 samples from 46 populations from all U.S. states with populations of Greater Sage-Grouse (11) and one Canadian province (Alberta), spanning the entire range of the species.

Overall, Oyler-McCance et al. (2005b) found that the distribution of genetic variation showed a gradual shift across the range in both mitochondrial and nuclear data sets. They indicate that this pattern suggests localized gene flow with isolation by distance—that is, movement among neighboring populations yet not likely across the range. Results from their nested clade analysis of the mitochondrial data confirm this finding, with seven clades characterized by restricted gene flow with isolation by distance. Analysis of their microsatellite data showed similar results, with a positive correlation between genetic distance and geographic distance. Furthermore, a genetic clustering analysis (STRUCTURE; Pritchard et al. 2000) revealed that unique genetic clusters were made up of populations geographically adjacent to one another, and while most genetic

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clusters consisted of many populations, the smaller, more fragmented populations on the periphery of the range in Colorado, Utah, Lyon–Mono in Nevada and California, and Washington made up their own clusters, suggesting lower amounts of gene flow in these areas (peripheral isolates). These data are consistent with previous research on dispersal (Dunn and Braun 1985) suggesting that gene flow is likely limited to the movement of individuals between neighboring populations and not likely the result of long-distance movements of individuals across large portions of the range. Oyler-McCance et al. (2005b) believe that this information is important because conservation efforts often consider translocations and augmentation of existing populations using animals from outside populations. Their data suggest linkages among neighboring populations and differences among distant populations, raising the possibility that local adaptations may exist and that translocations should involve neighboring populations rather than geographically distant populations.

Isolation by distance has been reported in many other grouse species, including Red Grouse (*Lagopus lagopus scoticus*; Piertney et al. 1998), Black Grouse (Caizergues et al. 2003b), Capercaillie (*Tetrao urogallus*; Segelbacher et al. 2003), Greater Prairie-Chickens (*Tympanuchus cupido*; Johnson et al. 2003), White-tailed Ptarmigan (*Lagopus leucura*; Fedy et al. 2008), and Rock Ptarmigan (*Lagopus mutus*; Caizergues et al. 2003a). Thus, it is not surprising that, in general, grouse exhibit this pattern, being ground-dwelling birds with short-distance seasonal movements compared to other avian migratory species.

Because of the comprehensive sampling regime and addition of nuclear markers, Oyler-McCance et al. (2005b) were also able to address some of the issues raised by Benedict et al. (2003). Similar to the findings of Benedict et al. (2003), Oyler-McCance et al. (2005b) discovered that in both mtDNA and microsatellite data sets, the least amount of genetic diversity was in the two Washington populations, likely due to habitat loss and subsequent population decline. The authors also reinforced the observation that the Lyon–Mono population was genetically unique compared to all other populations and that the difference was striking. While an additional 24 populations were added in their study, the fact remained that 93% of individuals in the Lyon–Mono population contained novel haplotypes not found elsewhere across the range. The genetic diversity present in Lyon–Mono was comparable to, if not higher than, most other populations, suggesting that the differences were not due to a genetic bottleneck or founder event. Their nuclear data corroborated these facts, because Lyon–Mono was significantly different from almost all other populations; and in their STRUCTURE analysis, the Lyon–Mono population was the only population forming its own cluster.

Taxonomic classifications have traditionally been defined using morphological and behavioral characteristics. With the advent of PCR and with advances in molecular techniques, genetic information is now being gathered and used to address such issues. Taxonomic delineations derived only from morphological characteristics can be erroneous (Avise 1989), as they can either fail to recognize distant forms (Avise and Nelson 1989) or recognize forms that exhibit little evolutionary differentiation (Laerm et al. 1982). While classifications based on morphology and behavior have been acceptable, use of molecular techniques can often resolve discrepancies and help to augment or refine taxonomic definitions. This is particularly important as it applies to listing under the Endangered Species Act (ESA), for defining management units, and for recovery of the species/subspecies, because funding priorities generally are based on taxonomic status (O'Brien and Mayr 1991).

The taxonomic status of many different species has recently been reevaluated using genetic data. In addition to the Gunnison Sage-Grouse, several new species have been recognized, including the Kemp's ridley sea turtle (*Lepidochelys kempii*), the north Pacific right whale (*Eubalaena japonica*), and Perrin's beaked whale (*Mesoplodon pewini*), all the result of molecular genetic analyses (Bowen et al. 1991, Rosenbaum et al. 2000, Dalebout et al. 2002). More common, however, is the use of molecular genetic data to investigate and often redefine subspecies. As difficult as it is for scientists to concur on a definition of species (Avise 1994), it is even more difficult to come to an agreement on the appropriate way to identify subspecies (Zink 2004, Cronin 2006, Haig et al. 2006). This is perhaps why the assessment of subspecies, fueled by the potential economic impacts of their protection, can become so controversial and divisive (Ramey et al. 2005, King et al. 2006, Vignieri et al. 2006). Examples of such studies

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include examination of the Preble's meadow jumping mouse (*Zapus hudsonius preblei*) and the Northern Spotted Owl (*Strix occidentalis*), both of which have been listed under the ESA and have been studied extensively by more than one genetics lab (Barrowclaugh and Gutiérrez 1990; Haig et al. 2001, 2004; Barrowclaugh et al. 2005; Ramey et al. 2005; King et al. 2006; Vignieri et al. 2006).

Several authors have investigated phylogenetic relationships among grouse, including the Greater Sage-Grouse (Ellsworth et al. 1996, Gutiérrez et al. 2000, Drovetski 2002). The focus of these studies was to elucidate the evolutionary relationships among grouse. At a finer scale, genetic data (Barrowclaugh et al. 2004) have recently been used to raise two subspecies of Blue Grouse (*Dendragapus obscurus*) to species level, now recognizing the Dusky Grouse (*D. obscurus*) and the Sooty Grouse (*D. fuliginosus*) as separate species. Additionally, the boundaries among several subspecies of Sharp-tailed Grouse (*Tympanuchus phasianellus*) have recently been questioned because of analysis of genetic data (Spaulding et al. 2006). It is clear that this type of information will continue to aid in the refinement of taxonomic classifications in the future.

Given the anomalous nature of the Lyon–Mono population relative to the numerous other populations of the species that were sampled, it becomes interesting to compare it to the newly recognized and closely related Gunnison Sage-Grouse. Oyler-McCance et al. (2005b) noted that the Lyon–Mono population is at least as divergent from other populations of Greater Sage-Grouse as Gunnison Sage-Grouse are from Greater Sage-Grouse, by virtue of the large number of new haplotypes unique to that population (novel haplotypes). Gunnison Sage-Grouse were recognized as a new species based on morphological, behavioral, and genetic data (Young et al. 2000), and those authors felt it important to consider these features in concert, as it can be difficult to define species based on molecular data alone, at least if one is applying the biological species concept. Such considerations led Taylor and Young (2006) to initiate a behavioral study of the Lyon–Mono population whereby they compared the strutting behavior of males from that population with several other populations of Greater Sage-Grouse. Their comparisons of behavior revealed few or no differences (Taylor and Young 2006). This suggests that while Lyon–Mono may have been isolated for an amount of time similar to the isolation of Gunnison Sage-Grouse, they have not experienced a significant divergence in behavioral characteristics as has been documented in Gunnison Sage-Grouse (Young et al. 2000), which ultimately led to their reproductive isolation (Oyler-McCance et al. 2005a). Precisely because of findings such as this one, some argue that morphological and behavioral data as well as genetic data are essential in the description and revision of taxonomic boundaries (Haig et al. 2006).

The study of Oyler-McCance et al. (2005b) reinforced the findings of Benedict et al. (2003) that the Lyon–Mono population is sufficiently genetically different to warrant special attention. They appear similar to other populations in behavior (Taylor and Young 2006), but Oyler-McCance et al. (2005b) believe that more comprehensive morphological comparisons should be performed before a change in taxonomic status should be considered. Regardless of the label placed on this population, Oyler-McCance et al. (2005b) believe that if the population could be managed separately and protected due to its genetic distinctiveness, it may become important to the survival of the species over large timescales.

The molecular examination of the mating system of Greater Sage-Grouse by Semple et al. (2001) and Gibson et al. (2005) focused on leks in Long Valley, California, near Mono Lake (Fig. 5.1), which is the same general area (Lyon–Mono) described to be genetically unique by Benedict et al. (2003) and Oyler-McCance et al. (2005b). Therefore, it would be very interesting to conduct studies similar to that of Semple et al. (2001) and Gibson et al. (2005) in areas outside of the Mono Basin to determine whether the patterns documented in the Mono Basin (Lyon–Mono) hold true outside of the genetically unique population. It is possible that while the isolation of the Mono Basin birds did not lead to the gross morphological and behavioral changes that have been documented in Gunnison Sage-Grouse, there may be more subtle differences in territoriality of males or in overall lek behavior between the Mono Basin group and Greater Sage-Grouse elsewhere.

CONSERVATION IMPLICATIONS

Molecular genetic techniques have only recently been used to help achieve a better understanding of avian biology and the interaction between

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species and their changing environments. Here we have attempted to discuss the contributions to Greater Sage-Grouse biology that molecular genetic techniques have afforded. Advances toward a better understanding of the mating system have already been made, and increased availability of microsatellite markers and technological improvements will undoubtedly lead to more broad and comprehensive studies in the future. Similarly, more fine-scale population-level analyses of Greater Sage-Grouse will ensue as localized questions arise. Finally, these studies have provided an interesting case study for examining the implications of sexual selection generated by the different evolutionary pathways of the Gunnison Sage-Grouse and the Lyon–Mono population of Greater Sage-Grouse.

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