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CHAPTER 10

RAPID EVOLUTION IN LEKKING GROUSE: IMPLICATIONS FOR TAXONOMIC DEFINITIONS

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Abstract.—Species and subspecies delineations were traditionally defined by morphological and behavioral traits, as well as by plumage characteristics. Molecular genetic data have more recently been used to assess these classifications and, in many cases, to redefine them. The recent practice of utilizing molecular genetic data to examine taxonomic questions has led some to suggest that molecular genetic methods are more appropriate than traditional methods for addressing taxonomic uncertainty and management units. We compared the North American Tetraoninae—which have been defined using plumage, morphology, and behavior—and considered the effects of redefinition using only neutral molecular genetic data (mitochondrial control region and cytochrome oxidase subunit 1). Using the criterion of reciprocal monophyly, we failed to recognize the five species whose mating system is highly polygynous, with males displaying on leks. In lek-breeding species, sexual selection can act to influence morphological and behavioral traits at a rate much faster than can be tracked genetically. Thus, we suggest that at least for lek-breeding species, it is important to recognize the possibility that morphological and behavioral changes may occur at an accelerated rate compared with the processes that led to reciprocal monophyly of putatively neutral genetic markers. Therefore, it is particularly important to consider the possible disconnect between such lines of evidence when making taxonomic revisions and definitions of management units.

Key words: grouse, sexual selection, speciation, species concepts.

Evolución Rápida en los Tetraoninae con Asambleas de Cortejo: Implicaciones para las Definiciones Taxonómicas

Resumen.—Las delimitaciones de especies y subespecies han sido tradicionalmente definidas con base en caracteres morfológicos y de comportamiento, como también por características del plumaje. Recientemente también se han usado datos moleculares genéticos para evaluar estas clasificaciones, y en muchos casos, para redefinirlas. La práctica reciente de usar datos moleculares genéticos para responder preguntas taxonómicas ha llevado a algunos a sugerir que estos métodos son más apropiados que los métodos tradicionales para abordar la incertidumbre taxonómica y definir unidades de manejo. Comparamos las especies norteamericanas de Tetraoninae—las cuales han sido definidas utilizando caracteres del plumaje, morfológicos y de comportamiento–y consideramos los efectos de redefinir estas especies usando sólo datos moleculares genéticos neutrales (región control mitocondrial y subunidad 1 de la citocromo oxidasa). Usando el criterio de monofilia recíproca, no fuimos capaces de reconocer las cinco especies que tienen un sistema de apareamiento altamente poligínico, con machos que se exhiben en asambleas de cortejo. En las especies que se reproducen en asambleas de cortejo, la acción de la selección sexual puede

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influenciar a los caracteres morfológicos y de comportamiento a una tasa mucho más rápida de la que se puede detectar genéticamente. Por esto, sugerimos que, al menos en especies con asambleas de cortejo, es importante reconocer la posibilidad de que los cambios morfológicos y de comportamiento pueden ocurrir a una tasa acelerada en comparación con los procesos que llevan a la monofilia recíproca de los marcadores genéticos presumiblemente neutrales. Por lo tanto, es particularmente importante considerar la posible desconexión entre esas líneas de evidencia al hacer revisiones taxonómicas y definir unidades de manejo.

THE DEBATE AS to how to classify organisms into species has been ongoing for over 150 years (Darwin 1859, Mayr 1942b, Wiley 1978, Cracraft 1983, de Queiroz 1998, Wheeler and Meier 2000). New species concepts are added almost continually (Hey 2001) to address perceived failures of the ones in use, and the debate continues as biologists attempt to place discrete boundaries on a continuous process (Winker et al. 2007). Consensus on how to define units below the species level is even more difficult to achieve, because subspecific boundaries are necessarily even less discrete and more changeable through time. As a result, the utility of the subspecies as a taxonomic rank has been debated widely (e.g., Wilson and Brown 1953, Gill 1982, Mayr 1982a, Storer 1982, Cracraft 1983, Haig et al. 2006, Phillimore and Owens 2006). If correctly delineated, however, intraspecific taxonomic units can be important for conservation efforts because they represent evolutionary capability within a species and likely represent incipient species in some cases (Moritz 1999, Haig et al. 2006). Additionally, such units provide an avenue for protection, at least within North America, where legislation recognizes a range of designations below the species level (Haig et al. 2006; Haig and D'Elia, this volume).

Although subspecies (and species) have traditionally been defined using traits related to plumage, morphology, and behavior, advances in molecular biology have led to the availability of relatively simple genetic markers that measure patterns of genetic variation contained in discrete loci that are presumed to be selectively neutral. In many cases, molecular data sets are not congruent with subspecies defined by traditional methods (Zink 1989, O'Brien and Mayr 1991, Ball and Avise 1992, Burbrink et al. 2000, Zink 2004). Further, Zink (2004) argued that subspecies defined with traditional methods may actually misinform conservation efforts, through misrepresentation of underlying patterns of intraspecific variation. This lack of concordance among approaches has led some to suggest that molecular methods should be used as the primary approach to defining such units for conservation (Moritz 1994, Zink 2004). More specifically, it has been suggested that the criterion of reciprocal monophyly among mitochondrial sequences (i.e., all members of a group share a more recent common ancestor with one another than with other such monophyletic groups on a phylogenetic tree) should be used to define such units (Moritz 1994, Zink 2004). Others have advocated more inclusive approaches that combine data from plumage, morphology, and behavior with neutral molecular markers (Dizon et al. 1992, Vogler and DeSalle 1994, Haig et al. 2006).

Here, we highlight a situation that illustrates the continuing importance of considering both molecular genetic and more traditional types of data when making inferences about species and subspecies delineations. Specifically, in taxa with highly skewed mating systems that are subject to sexual selection, patterns of variation in neutral molecular genetic markers may not appropriately reflect patterns of genetic variation that underlie traditional characteristics such as plumage, morphology, or behavior that may be subject to strong selection. Within these taxa, using data from neutral molecular markers alone or elevating their significance in relation to other forms of evidence may also misinform conservation efforts.

Many instances of accelerated evolutionary change resulting from natural or sexual selection have been examined (Meyer 1993, Nagel and Schluter 1998, Uy and Borgia 2000, Panhuis et al. 2001, Genner and Turner 2005, Spaulding 2007). Organisms that are subject to strong sexual selection because of highly skewed reproductive success among males can undergo rapid changes in morphology and behavior that can be the driving force in speciation (Ellsworth et al. 1994, Uy and Borgia 2000, Panhuis et al. 2001, Spaulding 2007). Among three lekking species of prairie grouse, Ellsworth et al. (1994) noted a disconnect between strong morphological and behavioral differences and relatively low levels of mitochondrial and nuclear differentiation. They suggested that changes in morphology and behavior in these species occurred more rapidly than usual, compared with rates of change in mitochondrial and nuclear markers (Ellsworth et al. 1994). Thus, taxa with

skewed mating systems and strong sexual selection may, as a general rule, accumulate differences in morphology and behavior at a greater rate, in relation to the amount of differentiation of neutral molecular markers, than is typical in species with more balanced mating systems. Consequently, if predetermined amounts or patterns of differentiation in neutral genetic markers are used as a criterion in species or subpecies definitions (such as a requirement for reciprocal monophyly), the magnitude of morphological and behavioral differences separating recently diverged species will differ depending on the natural history—particularly the mating systems—of the organisms under consideration. Therefore, examining only neutral genetic data or elevating the importance of such data over morphological and behavioral characteristics may mislead the conservation of real evolutionary units in these organisms.

The molecular phylogeny of grouse (Tetraoninae) and other galliforms has been studied previously using various mitochondrial and nuclear markers (Gutiérrez et al. 2000, Lucchini et al. 2001, Dimcheff et al. 2002, Drovetski 2002). These studies examined the historical relationship among all Tetraoninae and, in some cases, their placement within Galliformes. In the present study, we used North American Tetraoninae to reexamine phylogenetic relationships with a focus on the role of mating systems. Building on the work of Ellsworth et al. (1994), we investigated the relationship between mating systems and speciation by examining the group of grouse (family Phasianidae, subfamily Tetraoninae) found in North America, which includes a range of morphologically distinct species, widely accepted by taxonomists, with mating systems that vary from monogamous to highly skewed (Wittenberger 1978). Our objective was to overlay taxonomic delineations determined using traditional morphological, behavioral, and geographic methods with molecular genetic data. We examined the level of concordance between data types and determined whether discontinuities were consistent with different mating systems. We hypothesized that in species subjected to strong sexual selection either now or in the recent past, there would be less concordance between traditional and molecular methods than in species without such strong sexual selection.

Methods

Most previous molecular studies of grouse characterized each species using a single exemplar for phylogenetic reconstruction (Gutiérrez et al. 2000, Lucchini et al. 2001, Dimcheff et al. 2002). Drovetksi (2002), however, used multiple individuals from each species to reconstruct phylogenies using different genes. In the present study, we chose mitochondrial genes for which multiple exemplars from each taxon could be included. We obtained all published complete mitochondrial control-region sequence for North American grouse species that were available through GenBank, including species with three types of mating systems: monogamous, promiscuous with males dispersed, and highly promiscuous with lekking males (Wittenberger 1978). These three groups of species included Willow, Rock, and White-tailed ptarmigan, all considered monogamous; Ruffed Grouse, "Blue Grouse" (see below), and Spruce Grouse, all considered promiscuous, with males dispersed; and Greater Sage-Grouse, Gunnison Sage-Grouse, Sharp-tailed Grouse, Greater Prairie-Chicken, and Lesser Prairie-Chicken, all considered highly promiscuous, with lekking males (Wittenberger 1978; scientific names of species are given in Table 1). For most of these species, there were only a few complete control-region sequences, and these were used in our analysis. There were 59 sequences for Blue Grouse, so we chose 13 of those sequences loosely representing different geographic locations and spanning the two subspecies that are now recognized as full species, Dusky Grouse (*Dendragapus obscurus*) and Sooty Grouse (*D. fuliginosus*) (Barrowclough et al. 2004). We refer to both these species as "Blue Grouse" (*D. obscurus*) because this is how they were defined originally using morphological characters. Within each Blue Grouse location, we randomly chose one sequence. Additionally, we sequenced the entire control region in five Gunnison Sage-Grouse and an additional seven Greater Sage-Grouse known to represent both clades described by Kahn et al. (1999), because the complete control-region sequences for Greater Sage-Grouse available in GenBank represented only one of two deeply divergent clades.

To amplify the complete mitochondrial control region in Greater and Gunnison sage-grouse, a 25-μL polymerase chain reaction (PCR) was performed with primers 16775L (Quinn 1992) and H595 (Oyler-McCance et al. 2007) using the following thermal profile: preheat at 94°C for 2 min followed by 35 cycles of denature at 94°C for 40 s, anneal at 55°C for 1 min, and extend at 72°C for 4 min. The reactions concluded with a 10-min postheat at 72°C. The PCR products were prepared for

TABLE 1. Species included in the study, their mating system as defined by Wittenberger (1978), and the GenBank accession numbers of the sequences used in the study.

Latin name	Common name	Mating system	Control-region accession numbers	COI accession numbers
Lagopus muta	Rock Ptarmigan	Monogamous	AF184299, AF532445, AF532447, AF532449, AF532446, AF184294	DQ433739, DQ433738, DQ433737, DQ433736, DQ433734
L. lagopus	Willow Ptarmigan	Monogamous	AF532444, AF532440, AJ297169, AF532443, AF532442, AF532441	DQ433713, DQ433712, DQ433711, DQ433710
L. leucura	White-tailed Ptarmigan	Monogamous	AF532437, AF532439, AJ297167, AJ297168, AF532438	DQ433714, DQ433717, DQ433718, DQ433715, DQ433716
Bonasa umbellus	Ruffed Grouse	Promiscuous, males dispersed	AF532415, AF532416, AF532417, AJ297157	DQ432768, DQ434343, AY666563, AY666214
Dendragapus obscurus	Dusky Grouse ("Blue Grouse" herein; see text)	Promiscuous, males dispersed	AY570309, AY570302, AY570308, AY570318, AY570310, AY570331, AY570356, AY570347, AY570346, AY570352, AY570354, AY570343, AY570332	DQ433565, DQ433564, DQ433563, DQ433562, DQ433561, DQ432884
Falcipennis canadensis	Spruce Grouse	Promiscuous, males dispersed	AF532454, AF532453	DQ432923, DQ433635, DQ433636, DQ433637
Centrocercus urophasianus	Greater Sage-Grouse	Promiscuous, lek breeding	AY569303, AF532424, AJ297158, AJ297159, AF532423, GQ902779, GQ902780, GQ902781, GQ902782, GQ902783, GQ902784, GQ902785	DQ433466, DQ433465, DQ433464, DQ433463, DQ432834, GQ902786, GQ902787, GQ902788, GQ902789
C. minimus	Gunnison Sage-Grouse	Promiscuous, lek breeding	AF532425, GQ902774, GQ902775, GQ902776, GQ902777, GQ90278	DQ432833, DQ432832
Tympanuchus phasianellus	Sharp-tailed Grouse	Promiscuous, lek breeding	AJ297176, AF532436, AF532435, AJ297177, AY569304	DQ434206, DQ434205, DQ434204
T. cupido	Greater Prairie-Chicken	Promiscuous, lek breeding	AY569305, AJ297171, AJ297172, AF532432, AF532431, AF532435	AY666333
T. pallidicinctus	Lesser Prairie-Chicken	Promiscuous, lek breeding	AF532434, AJ297174, AJ297175, AF532433	DQ434203, DQ434202, DQ434201, DQ434200, DQ434199

sequencing by adding 5 U exonuclease I (10 U μL^{-1}, USB, Cleveland, Ohio) and 0.5 U shrimp alkaline phosphatase (1 U μL^{-1}, USB) and incubating at 37°C for 30–45 min. The enzymes were denatured by a 15-min 80°C incubation. Sequencing was performed using 2 µL prepared template and a Quick Start Kit (Beckman Coulter, Fullerton, California) following the manufacturer's protocol except using half reaction volumes (10 µL). Each product was sequenced using five primers to increase accuracy: 521H (Quinn and Wilson 1993), 16775L, H595, grouse internal CR A (AGTGTCAAGATGATTCCCCATAC), and grouse internal CR B (CTCTGGTTCCTCGGTCAG). Sequences were visualized on a CEQ8000 XL DNA Analysis System (Beckman Coulter).

For the aforementioned grouse taxa, we also obtained all published sequences of a portion of the mitochondrial cytochrome-*c* oxidase I gene (COI), also known as the barcoding gene (Hebert et al. 2003). There were fewer published sequences in this region, and all the sequences were

published in two studies (Hebert et al. 2003, Kerr et al. 2007). To ensure that the COI sequences for Greater Sage-Grouse represented individuals from both of the deeply divergent control-region clades (Kahn et al. 1999), we sequenced an additional four Greater Sage-Grouse known to be representative of both clades.

To sequence the Greater Sage-Grouse COI gene, 25-μL PCRs were performed using primers Bird F1 and Bird R1 (Kerr et al. 2007) with the following touch-down thermal profile: denature at 94°C for 30 s, anneal at 60°C for 1 min, and extend for 2 min at 72°C; subtract 1°C from the annealing temperature per cycle for 12 cycles; continue for 23 cycles with a 30-s denature at 94°C anneal for 1 min at 45°C and extend at 72°C for 2 min. The reactions concluded with a 20-min post-heat at 72°C. The products were prepared for sequencing and sequenced as above using both Bird F1 and Bird R1 primers.

Sequences from both mitochondrial regions were aligned in SEQUENCHER, version 4.5 (Gene Codes, Ann Arbor, Michigan). Wild Turkey (*Meleagris gallopavo*) control region and COI sequences (AF532414, DQ433016) were used as outgroups. Phylogenetic analyses were performed on both data sets using Bayesian inference within MRBAYES, version 3.12 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). Analysis of aligned sequences from each mitochondrial region was done by running four chains in each of the two independent analyses that MRBAYES executes as a default. The chain heating temperature was set to 0.2. Tree and parameter values were recorded every 100 generations. At the end of the analysis, the first 25% of stored trees were eliminated and the remaining trees were compiled into a consensus tree by the program. For both data sets, 1 million generations were completed, at which time the final convergence diagnostic (average standard deviation of split frequencies) was 0.0073 for the COI data set and 0.0119 for the control-region data set.

To determine whether different analytical methods gave congruent results, phylogenetic analyses of these data were also done with maximum-parsimony analysis using the heuristic search algorithm of PAUP*, version 4.0b10 (Swofford 2003). Maximum trees saved (MaxTrees) was set at 10,000 and random branch swapping was done using tree bisection–reconnection (TBR). Gaps were scored as a fifth base. A consensus of 1 million bootstrap replicates was used for the final tree.

Results

DNA sequence alignments were straightforward across COI, with 73% of sites (414 of 566) completely conserved. For the control-region sequences, 67% of sites (788 of 1,177) were completely conserved. This is consistent with the relative ease of alignments using coding regions (i.e., COI) as compared with those using non-coding regions (i.e., control region).

Phylogenetic analyses (Bayesian and maximum parsimony) of the control-region sequence were concordant with taxonomic delineations defined using traditional methods in all non-lekking grouse. All formed well-supported reciprocally monophyletic groups, each with a posterior probability of 100% (Bayesian) and a bootstrap value of 100 (maximum parsimony). Blue Grouse formed two reciprocally monophyletic clades corresponding to the split described by Barrowclough et al. (2004) that ultimately led to the recent elevation of these two groups to full species status. The five taxa that exhibit lekking behavior did not form reciprocally monophyletic groups with either Bayesian analysis (Fig. 1) or maximum-parsimony analysis (not shown). Kahn et al. (1999) reported that sequence data from the control region yield two deeply divergent clades within Greater Sage-Grouse. Gunnison Sage-Grouse fell into one of those two distinct Greater Sage-Grouse clades; thus, some Greater Sage-Grouse are more closely related in mitochondrial DNA (mtDNA) to Gunnison Sage-Grouse than they are to members of their own species. The remaining three lekking grouse (Sharp-tailed Grouse, Lesser Prairie-Chicken, and Greater Prairie-Chicken) were even less well resolved and did not form reciprocally monophyletic groups (Fig. 1). Maximum-parsimony analysis of the same data yielded a bootstrap consensus tree with the same key features described above. However, among deeper topological features, there was no support (bootstrap < 50%) for placing the *D. obscurus* and *Tympanuchus* complex as sister clades, nor was there support for the deeper clade that includes those two plus the *Centrocercus* group.

Analysis of the COI region revealed that all non-lekking grouse formed well-supported reciprocally monophyletic clades that match prior species designations with posterior probabilities of 100 (Fig. 2, Bayesian) and bootstrap values ≥97. Among the lekking grouse, none formed reciprocally monophyletic groups despite there

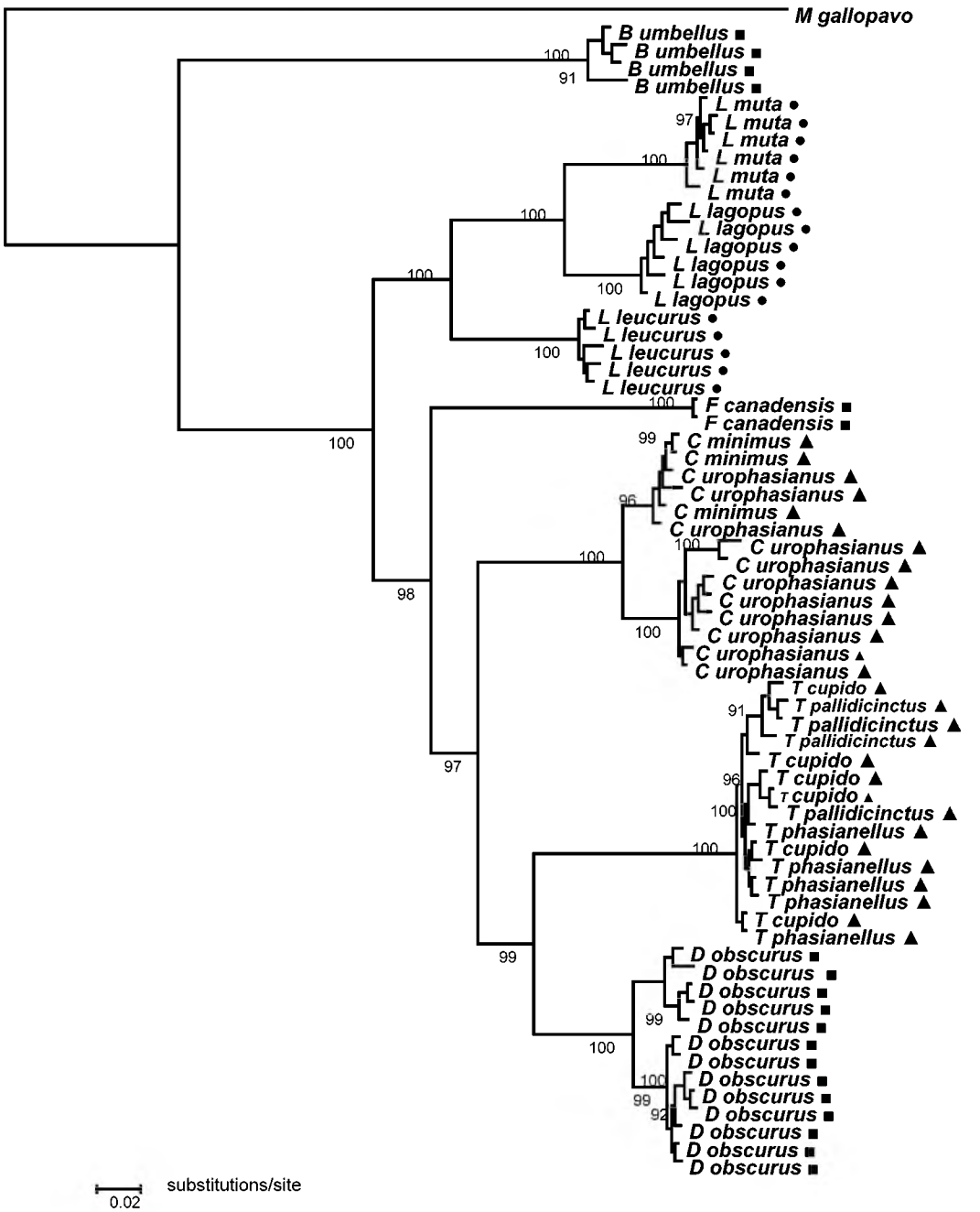

FIG. 1. Phylogenetic tree based on Bayesian analysis of the control region. Circles represent species with monogamous mating systems, squares represent promiscuous species with dispersed males, and triangles represent promiscuous species with a lek mating system. The numbers are posterior probabilities as calculated by Bayesian analysis.

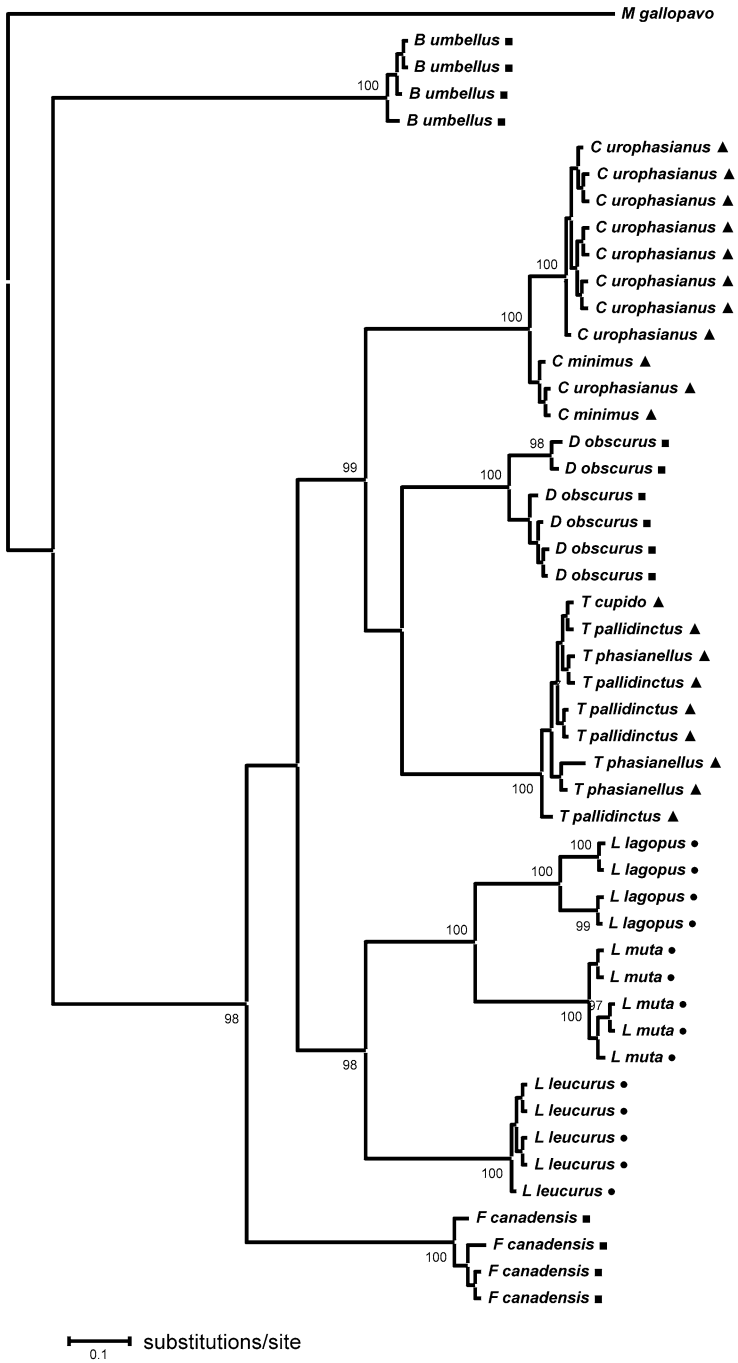

FIG. 2. Phylogenetic tree based on Bayesian analysis of the mitochondrial cytochrome-*c* oxidase I gene (COI). Circles represent species with monogamous mating systems, squares represent promiscuous species with dispersed males, and triangles represent promiscuous species with a lek mating system. The numbers are posterior probabilities as calculated by Bayesian analysis.

being more than one defined species in each case. Gunnison Sage-Grouse haplotypes were allied with one of the two deep clades of Greater Sage-Grouse (Fig. 2). Similar to the results for the control-region, Lesser Prairie-Chicken, Greater Prairie-Chicken, and Sharp-tailed Grouse were all intermixed within a single clade. Maximum-parsimony analysis of the same data revealed a similar topology but did not place *L. leucurus* as a sister group to the *L. muta* + *L. lagopus* clade.

Discussion

Our findings support the hypothesis that in lekking species subjected to strong sexual selection either now or in the recent past, there was less concordance between traditional and molecular methods than in species without such strong sexual selection. Like Drovetski (2002), we found that all non-lekking taxa formed monophyletic groups. And like Ellsworth et al. (1994) and Drovetski (2002), we found that none of the three taxa within the *Tympanuchus* group were reciprocally monophyletic. Ellsworth et al. (1994) and Drovetski (2002) suggested that speciation within this group is very recent. Johnson (2008) proposed that this group experienced rapid diversification in the late Pleistocene (10,000–18,000 years ago), which resulted in species with little or no interchange since divergence. Our data expand upon those from Drovetski (2002) by including additional samples from Greater Sage-Grouse and multiple samples from Gunnison Sage-Grouse. Drovetski (2002) showed Gunnison Sage-Grouse and Greater Sage-Grouse as sister groups exhibiting reciprocal monophyly. By including additional samples in the present study, we detected both of the deep clades present in the Greater Sage-Grouse (Kahn et al. 1999), rather than the single clade represented in previous phylogenetic studies (Drovetski 2002). Gunnison Sage-Grouse fell within one of those clades (Figs. 1 and 2), as previously recognized. Thus, Greater Sage-Grouse and Gunnison Sage-Grouse lack reciprocal monophyly, which is consistent with the pattern that we see in the other lekking species in North America. We suggest that speciation within this *Centrocercus* group is probably recent, like that within the *Tympanuchus* group.

Within North American grouse, non-lekking species are reciprocally monophyletic for neutral molecular markers. By contrast, among lekking species, taxonomic boundaries based on observations of plumage, morphology, and behavior are not reflected by similar diagnostically consistent characters at the molecular level. In the lek mating system, in which a few males do most of the mating, sexual selection can act to influence morphological and behavioral traits (Spaulding 2007) at a rate much faster than can be tracked using neutral genetic markers (Ellsworth et al. 1994). In some cases, reciprocal monophyly may appear long after complete and irreversible isolating mechanisms are in place. Further, the time that it takes to reach reciprocal monophyly in mitochondria depends on multiple factors, such as the effective population size of females (Avise and Wollenberg 1997).

Although most of the analysis presented here has focused on the species level, there are obvious implications for subspecies delineations as well. Species are more reasonably expected to be reciprocally monophyletic than subspecies. Here, we have shown that even at the species level, lekking grouse are not reciprocally monophyletic, and, thus, we should not expect such a relationship at the subspecies level. Lesser and Greater Prairie-Chickens exhibit distinct differences in behavior, plumage, morphology, habitat affiliation, and social aggregation that led to their recognition as distinct species (Grange 1940, Jones 1964, Sharpe 1968, Johnsgard 2002), although some have considered them subspecies in the past (Short 1967, Johnsgard 1983). There are currently no defined subspecies of Lesser Prairie-Chicken, and Greater Prairie-Chickens are divided into two subspecies (one being the endangered Attwater's Prairie-Chicken, *T. c. attwateri*). Compared to Lesser and Greater prairie-chickens, Sharp-tailed Grouse are even more distinct, particularly in morphology (Johnsgard 2002), and were at one time considered a distinct monotypic genus (Ellsworth et al. 1994). Seven subspecies of Sharp-tailed Grouse have been identified, primarily on the basis of subtle morphological differences and geographic distribution (Connelly et al. 1998). Like Ellsworth et al. (1994), Drovetski (2002), and Johnson (2008), we suspect that speciation in *Tympanuchus* is recent. Additionally, Greater Sage-Grouse and Gunnison Sage-Grouse exhibit distinct morphological, plumage, and behavioral characteristics (Hupp and Braun 1991; Young 1994; Young et al. 1994, 2000) and appear to be reproductively isolated (Young 1994, Young et al. 1994, Kahn et al. 1999, Oyler-McCance et al. 1999), which suggests that

speciation within this group is recent as well. There are no recognized subspecies of Gunnison Sage-Grouse, whereas Greater Sage-Grouse were previously split into two subspecies (Eastern and Western), although the validity of this division has been questioned (Benedict et al. 2003).

Our data are consistent with the hypothesis that the strong force of sexual selection driving rapid changes in morphology, plumage, and behavior has led to rapid reproductive isolation and speciation within these lekking taxa. As such, there may not have been sufficient time to reach reciprocal monophyly even at the species level in these groups. Thus, if one were to examine only data from neutral genetic markers among these taxa, important evolutionary processes would be overlooked. Identification of subspecies within these lekking taxa is likely to require an especially astute analysis of plumage, morphology, and behavior and may be misled in cases where genetic data alone are considered. Further, the conservation of these subspecies is vital because they may ultimately represent incipient species in a time-frame shorter than that experienced by non-lekking taxa. Because such processes are imperative for conservation efforts, we think that a pluralistic approach involving morphological, behavioral, and genetic data should be used, particularly when assessing taxa with highly skewed mating systems, such as lekking grouse.

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