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# PHYLOGENETIC RELATIONSHIPS AMONG NORTH AMERICAN GROUSE INFERRED FROM RESTRICTION ENDONUCLEASE ANALYSIS OF MITOCHONDRIAL DNA[1]

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*Abstract.* Systematic relationships among North American grouse and ptarmigans (Tetraoninae) are not well defined because traditional classifications were based on morphological and behavioral characters with limited taxonomic utility. Restriction enzyme analysis of mitochondrial DNA (mtDNA) was used to generate a phylogeny for North American tetraonines that was then utilized to test previous phylogenetic hypotheses for the group and to examine the origin and evolution of complex reproductive behaviors and morphological features characteristic of grouse and ptarmigan species. Nucleotide sequence divergence among congeneric species derived from mtDNA restriction fragment patterns varied extensively, ranging from 0.28% in prairie grouse (*Tympanuchus*) to 4.06% among ptarmigans (*Lagopus*) and 10.15% between Blue Grouse (*Dendragapus obscurus*) and Spruce Grouse (*D. canadensis*). Using the Northern Bobwhite (*Colinus virginianus*) as an outgroup, the molecular phylogeny partitioned species into three primary groups: (1) *Tympanuchus*; (2) *Lagopus, Dendragapus obscurus,* and *Tetrao urogallus* (the European Capercaillie); and (3) the Ruffed Grouse (*Bonasa umbellus*), *Dendragapus canadensis,* and Sage Grouse (*Centrocercus urophasianus*). Prairie grouse were genetically distinct from other grouse species, but a polyphyletic distribution of haplotypes and limited mtDNA differentiation within *Tympanuchus* suggest that divergence among the prairie grouse occurred very recently. Within *Lagopus,* the Willow (*L. lagopus*) and Rock (*L. mutus*) Ptarmigans were more closely related to each other than either was to the White-tailed Ptarmigan (*L. leucurus*). *Dendragapus canadensis* grouped with *Bonasa umbellus*; whereas *D. obscurus* was allied with *Lagopus* and *Tetrao.* Thus, the genus *Dendragapus* as currently constructed is polyphyletic (i.e., *D. canadensis* and *D. obscurus* have had separate evolutionary histories) and the morphological similarities between the two species may be attributable to convergent adaptation to coniferous forest. We inferred from the molecular phylogeny that the complex reproductive systems in tetraonines have arisen independently and that corresponding morphological and behavioral specializations may reflect parallel evolutionary trends.

*Key words: Tetraoninae; grouse; ptarmigans; mtDNA; systematics; evolution.*

## INTRODUCTION

Grouse and ptarmigans comprise a group of galliform birds that collectively have a circumpolar distribution in the Northern Hemisphere. There are currently ten recognized species partitioned into five genera in North America. Traditionally, grouse and ptarmigans have been viewed as a distinct family (Tetraonidae) on the basis of morphological features such as feathered tarsi and nostrils and pectinate toes (Peters 1934, Ridgway and Friedmann 1946, Wetmore 1960). However, the Tetraonidae has been relegated to subfamilial status (Tetraoninae; Brodkorb 1964, Holman 1964) within the Phasianidae along with pheasants, peafowl, junglefowl, partridges, francolins, and Old World quail (Sibley and Ahlquist 1990).

Previous classifications of grouse and ptarmigan species have been derived from subjective evaluations of morphological characters (such as natal plumage, egg color, number of rectrices) and behavioral patterns associated with courtship display (Short 1967, Fjeldså 1977, Johnsgard 1983, Potapov 1985). Morphological and behavioral similarities among taxa may result from homology (a common ancestry) or analogy (derived from independent evolution toward a common function); however, only homologous characters provide reliable information for reconstructing evolutionary relationships. The anatomical and behavioral features utilized in the classification of grouse and ptarmigans have limited taxonomic utility because they: (1) are adaptive and subject to convergent evolution; (2) tend to evolve rapidly in avian species (Johnston and

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Selander 1964); and (3) may have an environmental (non-genetic) component influencing their expression. As a result, the classifications based on such characteristics differ greatly in topology and tend to contain monotypic genera.

Grouse and ptarmigans exhibit complex social behaviors and anatomical specializations that are associated with unique life history and reproductive strategies, including monogamy and several distinct types of polygyny (Oring 1982; Johnsgard 1983, 1994). Many species are sexually dimorphic and dichromatic, with a variety of novel behaviors (ritualized postures, vocalizations, aerial displays) and morphological characteristics (inflatable esophageal air sacs, feather specializations) present in males. Reproductive systems in grouse and ptarmigans have been intensively studied; however, there is currently no solid framework (a phylogeny derived from independent characters) on which to interpret and analyze the evolution of these complex behavioral patterns and morphological features.

Genetic approaches have facilitated our ability to make inferences about evolutionary relationships among birds. Analyses of mitochondrial DNA (mtDNA) have proven to be particularly useful for determining systematic relationships among species (Kessler and Avise 1984, Ovenden et al. 1987, Gill and Slikas 1992) and for differentiating morphologically similar taxa (Mack et al. 1986, Avise and Nelson 1989). In this paper, restriction endonuclease analysis of mtDNA was used to examine the evolutionary affinities of all North American grouse and ptarmigan species. Our study: (1) tests previous phylogenetic hypotheses derived from morphology, plumage, and behavior with a molecular data set; (2) clarifies the origin and evolution of complex reproductive systems and associated morphological/behavioral traits in tetraonines; and (3) contributes to our understanding of the evolution of grouse and ptarmigan species.

## METHODS

Brain and/or liver samples were collected from specimens distributed throughout the geographic range of all North American grouse and ptarmigans as follows: Ruffed Grouse (*Bonasa umbellus*) $n = 6$; Sage Grouse (*Centrocercus urophasianus*) $n = 6$; Spruce Grouse (*Dendragapus canadensis*) $n = 6$; Blue Grouse (*D. obscurus*) $n = 4$; Willow Ptarmigan (*Lagopus lagopus*) $n = 7$; White-tailed Ptarmigan (*L. leucurus*) $n = 4$; Rock Ptarmigan (*L. mutus*) $n = 3$; Greater Prairie-Chicken (*Tympanuchus cupido*) $n = 6$; Lesser Prairie-Chicken (*T. pallidicinctus*) $n = 5$; and Sharp-tailed Grouse (*T. phasianellus*) $n = 7$. A European grouse, the Capercaillie (*Tetrao urogallus*, $n = 1$), was included in the analysis and the Northern Bobwhite (*Colinus virginianus*, $n = 1$) was used as an outgroup. Detailed information regarding collecting localities is presented in Appendix 1.

Mitochondrial DNA from 54 individuals was isolated from frozen tissue and purified on cesium chloride density gradients (Carr and Griffith 1987). We digested aliquots of purified mtDNA with 14 restriction enzymes: *Apa* I, *Bam*H I, *Bcl* I, *Cla* I, *Hin*d III, *Hpa* I, *Kpn* I, *Nco* I, *Nde* I, *Pst* I, *Pvu* II, *Sal* I, *Sst* I, and *Xba* I. The resulting fragments were end-labeled with $\alpha^{32}$P-nucleotide triphosphates using DNA polymerase (Klenow fragment), separated on 1.2% vertical agarose gels, and visualized by autoradiography. Fragment sizes were estimated by direct comparisons with co-migrating standards of known size (a mixture of lambda DNA and PM2 DNA digested with *Hin*d III).

For each restriction enzyme, we assumed that fragments exhibiting identical mobilities were homologous (i.e., attributable to the same restriction sites across taxa). To minimize the possibility of scoring nonhomologous fragments of similar size as identical due to indistinguishable differences in migration, bands of questionable size identity were compared side-by-side on 1% vertical agarose gels. Fragments that appeared to be similar in size on 1.2% gels, but which could be differentiated on the 1% gels, were labeled alphabetically (e.g., 800a and 800b) and treated as separate fragments (Appendix 2). However, certain enzymes produced only one fragment in several different species. We determined whether these fragments were homologous (attributable to the same site) or nonhomologous (attributable to different sites) by digesting the sample with a second restriction enzyme that cut the DNA only at sites known to be identical in all taxa. If the fragments were homologous, identical profiles were observed in the double digests. Fragments deemed nonhomologous by the double digestions were also treated as different fragments.

Each individual was scored for the presence or absence of each restriction fragment and assigned a composite haplotype based on fragment profiles across all restriction enzymes. The propor-

TABLE 1. Average estimates of percent nucleotide sequence divergence between mtDNA haplotypes within (in parentheses along diagonal) and among species of North American grouse and ptarmigans.[1] The Northern Bobwhite (*Colinus virginianus*) was used as an outgroup.

| Species | Species | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| 1. *Bonasa umbellus* | (0.58) | | | | | | | | | |
| 2. *Centrocercus urophasianus* | 7.39 | (0) | | | | | | | | |
| 3. *Dendragapus canadensis* | 7.43 | 7.43 | (0.30) | | | | | | | |
| 4. *Dendragapus obscurus* | 9.61 | 4.44 | 10.15 | (1.45) | | | | | | |
| 5. *Lagopus lagopus* | 9.24 | 5.95 | 9.55 | 5.04 | (0.66) | | | | | |
| 6. *Lagopus leucurus* | 8.86 | 4.93 | 8.68 | 3.99 | 3.75 | (0.21) | | | | |
| 7. *Lagopus mutus* | 7.79 | 7.10 | 11.06 | 5.63 | 3.31 | 5.13 | (0.55) | | | |
| 8. *Tympanuchus* spp. | 9.29 | 7.14 | 8.48 | 5.52 | 6.89 | 5.79 | 6.82 | (0.28) | | |
| 9. *Tetrao urogalhus* | 10.36 | 8.39 | 11.84 | 7.03 | 8.87 | 8.51 | 8.32 | 9.05 | (–) | |
| 10. *Colinus virginianus* | 16.24 | 14.61 | 23.91 | 17.04 | 19.38 | 16.04 | 19.22 | 13.45 | 19.28[2] | (–) |

[1] A European grouse, the Capercaillie (*Tetrao urogallus*), was included in the analysis.
[2] The *T. urogallus-C. virginianus* comparison included two fragments produced by the restriction enzyme *Sst* II.

tion of shared fragments (F; Nei and Li 1979) was used to estimate the extent of nucleotide sequence divergence (*P*) among the composite haplotypes. We then determined the phylogenetic relationships among species from the matrix of *P*-values using the FITCH (with GLOBAL optimization and RANDOM addition of taxa) and NEIGHBOR options in PHYLIP (Felsenstein 1989). The FITCH algorithm uses Fitch-Margoliash (Fitch and Margoliash 1967) and least-squares methodology (Cavalli-Sforza and Edwards 1967) to construct a phylogenetic network. NEIGHBOR implements the distance matrix (Neighbor-Joining) method of Saitou and Nei (1987) to infer a tree that may have the smallest sum of branch lengths. Neither approach assumes that rates of genetic change are uniform among lineages.

## RESULTS

Fragment profiles and co-migrating size standards were used to estimate the length of the mtDNA molecule in Tetraonines to be approximately 16.6 kb, which is typical for many avian species (Shields and Helm-Bychowski 1988). All individuals possessed a single mtDNA haplotype and no length variation due to insertions or deletions was detected.

The restriction enzyme analysis produced 248 unique fragments comprising 24 composite mtDNA haplotypes (Appendix 2). Estimates of nucleotide sequence divergence among haplotypes within species were generally less than 1.00 (Table 1). However, a divergent lineage of *Dendragapus obscurus* from Vancouver Island differed from other conspecific populations in Colorado and Montana by 1.45%. No mtDNA variation was detected in the *Centrocercus urophasianus* samples obtained from California, Colorado, and Idaho.

Estimates of mtDNA differentiation among congeneric species varied extensively. Within the *Tympanuchus* complex we identified 4 mtDNA haplotypes, but they were not strictly partitioned along species boundaries. Two of the haplotypes were unique to *T. cupido* (No. 19 and No. 20), one haplotype was shared by *T. cupido* and *T. pallidicinctus* (No. 21), and one haplotype was common to all three species (No. 22) (Appendix 2). Sequence divergence among the prairie grouse haplotypes (maximum differentiation = 0.40%) was equivalent to or less than the divergence estimates observed *within* other species of North

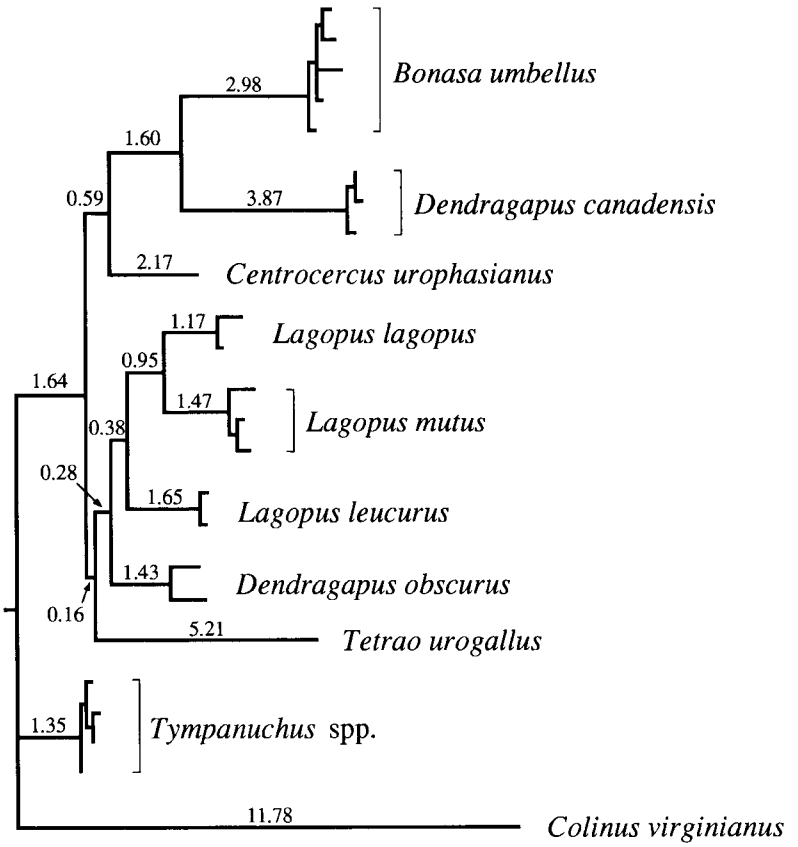


FIGURE 1. Fitch-Margoliash tree (average %SD = 9.99) derived from a matrix of mtDNA sequence divergence values depicting evolutionary relationships among all species of North American grouse and ptarmigans. Branch lengths within species were generally <1 and are not shown. The tree is "rooted" by the outgroup taxon, *Colinus virginianus*.

American grouse. Differentiation among the ptarmigan species (*Lagopus*) ranged from 3.31% to 5.13%. In contrast to both *Tympanuchus* and *Lagopus*, the two species of *Dendragapus* (*canadensis* and *obscurus*) differed by 10.15%, one of the highest *P*-values observed between any pair of taxa (including the intergeneric comparisons).

Mitochondrial DNA sequence divergence between *Colinus* and the various grouse species ranged from 13.45% vs. *Tympanuchus* to 23.91% vs. *Dendragapus canadensis*. We believe *C. virginianus* was an appropriate outgroup because: (1) all of the outgroup–ingroup comparisons exceeded the estimates of differentiation among the grouse species indicating that *Colinus* lies outside the Tetraoninae; (2) a New World quail was not too distant to provide useful outgroup information because the minimum level of mtDNA divergence between *Colinus* and grouse (13.45%) only slightly exceeded the maximum estimate of differentiation among the grouse taxa (11.84%); and (3) odontophorines are related to tetraonids by a variety of morphological and behavioral characteristics (Johnsgard 1973).

Trees derived by the Fitch-Margoliash (Fig. 1) and Neighbor-Joining (Fig. 2) algorithms were identical in overall topology. Both methodologies partitioned species into three primary groups: (1) *Tympanuchus*; (2) *Lagopus, Dendragapus obscurus, Tetrao urogallus*; and (3) *Bonasa umbellus, Dendragapus canadensis, Centrocercus urophasianus*. Members of the genus *Tympanuchus* fall outside the group containing all other North American grouse and ptarmigans. *Bonasa umbellus* and *Dendragapus canadensis* group together, and *Centrocercus* appears to be affiliated with *Bonasa* and *D. canadensis* but the association is relatively weak. The fragment analysis

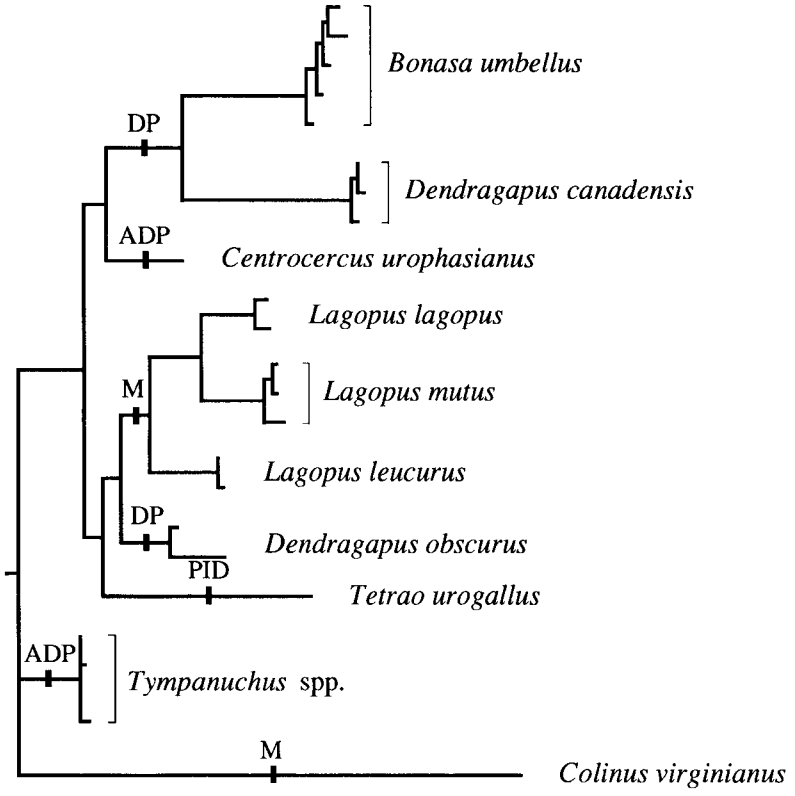


FIGURE 2. Network of relationships for North American grouse and ptarmigans based on the Neighbor-Joining method (Saitou and Nei 1987). The branching pattern of the Neighbor-Joining tree is identical to that of the Fitch-Margoliash network (Fig. 1), but branch lengths differ slightly. Mating systems of the taxa under consideration are plotted on the tree (M = monogamy, DP = dispersed polygyny, PID = polygyny with intermediate dispersion, ADP = arena display polygyny).

partitioned the ptarmigans into a single clade and resolved relationships among species that are consistent with previous classifications (Short 1967, Fjeldså 1977, Johnsgard 1983). *Tetrao* and *D. obscurus* are included in the group with *Lagopus*; however, the branches defining these relationships are relatively short. Nevertheless, our phylogeny indicates that *Dendragapus canadensis* and *D. obscurus*, which are currently considered congeneric, have had separate evolutionary histories.

## DISCUSSION

### GENUS *TYMPANUCHUS* (PRAIRIE GROUSE)

Prairie grouse are adapted to grassland and grassland-woodland ecotone throughout the central United States and Canada. Prairie grouse are considered to be distinct from other genera because their plumage differs extensively from other species (being almost entirely barred) and males possess numerous morphological structures associated with complex courtship (lekking) behavior (Johnsgard 1983, 1994). *Tympanuchus cupido* and *T. pallidicinctus* are recognized as distinct species (American Ornithologists' Union 1983); however, the differences (in behavior, habitat, and social aggregation) that distinguish these taxa (Grange 1940, Jones 1964, Sharpe 1968) are relatively minor compared to those observed between other well-defined species. Thus, some researchers (Short 1967, Johnsgard 1983) consider *T. cupido* and *T. pallidicinctus* allopatric subspecies. *Tympanuchus phasianellus* (formerly *Pedioecetes phasianellus*) was once placed in a separate genus due primarily to differences in morphology (Ridgway and Friedmann 1946), but *Pedioecetes* has been synonymized with *Tympanuchus* (Short 1967).

The mtDNA fragment data suggest that *Tympanuchus* is distinct at the generic level. However, the extent of interspecific differentiation was far less than that observed between other congeneric grouse species (such as *Lagopus*) and approximated the lowest mtDNA distances seen among closely related avian species (Avise and Zink 1988). A second striking aspect of mtDNA differentiation within *Tympanuchus* was the observation that several haplotypes were shared among species. Ellsworth et al. (1994) examined mtDNA variation in *Tympanuchus* and concluded that the low level of interspecific divergence and polyphyletic distribution of haplotypes is most likely attributable to recent speciation, possibly resulting from subdivision caused by Pleistocene glacial activity.

### GENUS *LAGOPUS*, THE PTARMIGANS

Ptarmigans are small to medium sized grouse that inhabit tundra and alpine habitats. Features distinctive of *Lagopus* such as feathered toes with reduced lateral pectinations and multiple complex molts generally reflect adaptation to cold northern environments. *Lagopus lagopus* and *L. mutus* are sympatrically distributed in the tundra and northern boreal forests of the Holarctic; whereas, *leucurus* is restricted to high altitude regions in western North America (Johnsgard 1983).

There is controversy surrounding the phylogenetic relationships of ptarmigans; two opposing classifications have been proposed. Plumage, egg color, and the progression of molts may suggest that *L. leucurus* diverged prior to the separation of *L. lagopus* and *L. mutus* (Short 1967, Höhn 1980, Johnsgard 1983). However, behavioral patterns have been interpreted as evidence that *L. lagopus* was the earliest divergent lineage (Johansen 1956, Braun 1969). The mtDNA data support the classification of Short (1967) that places *L. lagopus* and *L. mutus* as being more closely related to each other than either is to *L. leucurus*.

*Tetrao urogallus*, the largest member of the grouse family, occupies coniferous forests in Europe and Asia (Johnsgard 1983). Based primarily on similarities in female and natal plumage and the frequency of hybridization, Short (1967) considered *Lagopus* and *Tetrao* to comprise a major monophyletic lineage of grouse that exhibits more distant affinities with *Dendragapus* (*D. canadensis* in particular). An association among *Lagopus*, *Tetrao*, and *Dendragapus obscurus* is evident in the mtDNA phylogeny, but relationships among these taxa are not confidently resolved.

### *DENDRAGAPUS*, A POLYPHYLETIC GENUS

The range of *D. obscurus* is closely associated with the distribution of true fir and Douglas fir in western North America (Beer 1943); whereas, *D. canadensis* occurs transcontinentally in boreal coniferous forests (Aldrich 1963). From 1899 until 1967, *D. canadensis* (formerly *Canachites canadensis*) and *D. obscurus* were placed in different genera. Short (1967) merged *Canachites* with *Dendragapus* on the basis of similarities in overall body proportions, bill, wing, and tail morphology, egg color, and juvenile plumage. In this study, *D. canadensis* and *D. obscurus* were among the most genetically divergent tetraonine taxa in North America. Relationships inferred from molecular characters indicate that these two species do not constitute a monophyletic lineage. *Dendragapus canadensis* groups with *Bonasa umbellus*, but *D. obscurus* is affiliated with *Lagopus* and *Tetrao*. Thus, the morphological similarities between the species may be attributable to convergent evolution associated with adaptation to coniferous forest habitats.

### EVOLUTION OF TETRAONID MATING SYSTEMS

Grouse and ptarmigans exhibit a variety of complex reproductive systems, ranging from nearly monogamous to polygynous (male-dominance). Male ptarmigans establish large territories and usually form a pair bond with only one female (Johnsgard 1983), but in the polygynous species males mate with several females by overtly competing with one another by ritualized display. The polygynous mating systems are divisible into three types based on the degree of male clustering during reproductive competition (Oring 1982): (1) dispersed polygyny where males occupy large and widely spaced territories is typical of the forest dwelling grouse (*Dendragapus canadensis*, *D. obscurus*, and *Bonasa umbellus*) (Lumsden 1968, Hjorth 1970); (2) intermediate dispersion, exemplified by *Tetrao urogallus*, in which males display from sites that are markedly clumped; and (3) the complex arena display of *Centrocercus* and *Tympanuchus* where males aggregate and aggressively defend small display territories

known as leks (Hamerstrom and Hamerstrom 1973, Ballard and Robel 1974).

Other galliform groups such as quails and partridges retain a strongly monogamous system; thus monogamy in the ptarmigans is believed to represent an ancestral condition. The polygynous systems in the other grouse species were believed to be independently derived (Short 1967, Johnsgard 1983); however, this hypothesis could not be critically tested because systematic relationships among tetraonines were based primarily on similarities for morphological and behavioral characters that are closely associated with reproduction.

Relationships among taxa suggested by the mtDNA analysis allow us to make preliminary inferences regarding the evolution of the various reproductive systems in grouse and ptarmigans using a phylogeny derived from independent characters. The molecular data are consistent with the independent origins of both the dispersed polygynous and communal display behaviors (Fig. 2). For example, *Bonasa umbellus* and *Dendragapus canadensis* share the dispersed polygynous system with *D. obscurus*. All three species are adapted to northern coniferous or deciduous forest. *Dendragapus* males make aerial display flights in which "clapping" or "drumming" of the wings may occur (Blackford 1963, MacDonald 1968). *Bonasa* males also establish territories and advertise for mates with a drumming display, but extensive wing beating has been substituted for flight (Hjorth 1970). In our phylogeny, *B. umbellus* and *D. canadensis* group together; whereas, *D. obscurus* is genetically distinct. Thus, the development of dispersed polygyny and similarities in male courtship that *B. umbellus* and *D. canadensis* share with *D. obscurus* might reflect selective pressures unique to northern forest environments.

Similarly, *Centrocercus* and *Tympanuchus* are lek-forming taxa that occupy open shrubland/grassland habitats in central and western North America. Males advertise to attract females with a complex sequence of stereotyped behaviors that includes tail fanning, erection of specialized neck feathers, and vocalizations produced by brightly colored inflatable air sacs (Sharpe 1968, Hjorth 1970). *Centrocercus* and *Tympanuchus* are not closely related genetically and are partitioned into different groups by the molecular phylogeny. These observations suggest that: (1) an "aggregative polygynous mating system" has evolved independently in these taxa; (2) the display behaviors and morphological specializations in males that are associated with reproduction are attributable to parallel evolutionary trends; and (3) such displays are not homologous characters and similarities between reproductive systems do not imply phylogenetic relatedness.

## MITOCHONDRIAL DNA RESTRICTION FRAGMENTS FOR PHYLOGENETIC ANALYSIS

Although restriction sites are often more informative than fragments, concurrent analyses of fragments and sites have produced congruent results in several systematic studies involving birds (Zink 1991, Zink and Dittmann 1991, Gill and Slikas 1992). Additionally, the average *P*-values for all pairwise comparisons among the grouse and ptarmigan species (Table 1) were less than 12%, which is below the 15% maximum divergence suggested by Upholt (1977) and Dowling et al. (1990) for fragment comparisons. The proportion of fragments shared by at least two ingroup taxa (41%) was also far greater than the minimum level (25%) proposed by Kessler and Avise (1985).

Our phylogeny for the Tetraoninae based on mtDNA restriction fragments is a preliminary estimation of the evolutionary affinities among grouse and ptarmigans. Resolution was sufficient to confidently discern relationships (or lack thereof) among congeneric species, to evaluate the phylogenetic significance of current taxonomic groupings, and to make inferences regarding the evolution of mating systems and selected morphological traits. However, several problematic areas such as the interrelationships among several primary lineages and the placement of other tetraonid species remain.

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APPENDIX 1. Locality data for North American grouse and ptarmigan specimens used in this study.

Ruffed Grouse (*Bonasa umbellus*) (ALASKA; North Star Borough, $n = 1$; MONTANA; Fergus Co., $n = 1$; ONTARIO, CANADA; Ignace Municipality, $n = 1$; PENNSYLVANIA; Erie Co., $n = 1$; QUEBEC, CANADA; Duplessis Comté, $n = 1$; WISCONSIN; Langlade Co., $n = 1$).

Sage Grouse (*Centrocercus urophasianus*) (CALIFORNIA; Lassen Co., $n = 2$; COLORADO; Gunnison Co., $n = 1$; Jackson Co., $n = 1$; Saquache Co., $n = 1$; IDAHO; Clark Co., $n = 1$).

Spruce Grouse (*Dendragapus canadensis*) (ALASKA; vicinity of Anchorage, $n = 1$; MANITOBA, CANADA; Grand Rapids Municipality, $n = 2$; NOVA SCOTIA, CANADA; Victoria Municipality, $n = 1$; ONTARIO, CANADA; Ignace Municipality, $n = 1$; QUEBEC, CANADA; Saguenay Comté, $n = 1$).

Blue Grouse (*Dendragapus obscurus*) (COLORADO; Gunnison Co., $n = 1$; Routt Co., $n = 1$; MONTANA; Fergus Co., $n = 1$; BRITISH COLUMBIA, CANADA; Vancouver Island, $n = 1$).

Willow Ptarmigan (*Lagopus lagopus*) (MANITOBA, CANADA; Leaf Rapids Municipality, $n = 2$; NEWFOUNDLAND, CANADA; $n = 2$; QUEBEC, CANADA; Ungava Comté, $n = 2$; YUKON TERRITORY, CANADA; 299 km S Eagle Plains, $n = 1$).

White-tailed Ptarmigan (*Lagopus leucurus*) (ALASKA; vicinity of Paxson, $n = 2$; COLORADO; Larimer Co., $n = 2$).

Rock Ptarmigan (*Lagopus mutus*) (ALASKA; vicinity of Fairbanks, $n = 1$; NEWFOUNDLAND, CANADA; $n = 1$; QUEBEC, CANADA; Ungava Comté, $n = 1$).

Greater Prairie-Chicken (*Tympanuchus cupido*) (ILLINOIS: Marion Co., $n = 1$; KANSAS; Butler Co., $n = 1$; Shawnee Co., $n = 1$; NEBRASKA; Thomas Co., $n = 1$; OKLAHOMA; Osage Co., $n = 1$; SOUTH DAKOTA; Lyman Co., $n = 1$).

Lesser Prairie-Chicken (*Tympanuchus pallidicinctus*) (KANSAS; Clark Co., $n = 3$; Morton Co., $n = 2$).

Sharp-tailed Grouse (*Tympanuchus phasianellus*) (COLORADO; Routt Co., $n = 1$; MANITOBA, CANADA; Coldwell Municipality, $n = 1$; MINNESOTA; Aitkin Co., $n = 1$; NEBRASKA; Thomas Co., $n = 1$; NORTH DAKOTA; Billings Co., $n = 1$; QUEBEC, CANADA; Ungava Comté, $n = 1$; SOUTH DAKOTA; Lyman Co., $n = 1$).

Capercaillie (*Tetrao urogallus*) (NORWAY; captive flock, $n = 1$).

Northern Bobwhite (*Colinus virginianus*) (ILLINOIS; Williamson Co., $n = 1$).

APPENDIX 2. Presence/absence matrix for 248 restriction fragments among all species of North American grouse and ptarmigans, a European grouse (*Tetrao urogallus*), and the outgroup taxon *Colinus virginianus*. Estimated fragment sizes (×100) are listed vertically below each restriction enzyme and letters (a–c) differentiate fragments of similar size.[a]

| Species | Haplotype | *n* | *Apa* I | *Bam*H I | *Bcl* I | *Cla* I | *Hind* III | *Hpa* I |
|---|---|---|---|---|---|---|---|---|
| | | | 11<br>5197644<br>76502119554<br>ab ab | 11<br>55987554443211<br>2039795831258442 | 1<br>6887766555443322222111<br>685877052073727654431085432 21<br>ab ab | 111111<br>666211543321<br>66099247708776<br>ab | 1111111<br>5311100974322111111<br>74842981266317553209866643 2<br>ab abc | 1111<br>53319843332221<br>052628641186468432<br>ab |
| *Bonasa umbellus* | 1 | 2 | 10000001000 | 0000011100000010 | 00000101000001000000010000100 | 00010000100000 | 00000001000100110100010010 0 | 00010001000010000 |
| | 2 | 1 | 10000001000 | 0000011100000010 | 00000101000000000100011000100 | 00010000100000 | 00000001000100110100010010 0 | 00010001000010000 |
| | 3 | 1 | 10000001000 | 0001000100010010 | 00000101000001000000010000100 | 00010000100000 | 00000001000100110100010010 0 | 00010001000010000 |
| | 4 | 1 | 10000001000 | 0000011100000010 | 00000101000001000000010000100 | 00010000100000 | 00000001000100110010010010 1 | 00010001000010000 |
| | 5 | 1 | 10000001000 | 0000011100000010 | 00000101000001000000010000100 | 00010000100000 | 00000001000100110100010010 0 | 00010001000010000 |
| *Centrocercus urophasianus* | 6 | 6 | 10000001000 | 1000000000000100 | 10000000000000000000000000000 | 00001001000000 | 00000010000110000010000010 0 | 00010000000111001 |
| *Dendragapus canadensis* | 7 | 4 | 00010111100 | 0100000000000101 | 00001000100010000000000000000 | 00100000000001 | 00000000110100100000010010 0 | 00010000000101100 |
| | 8 | 1 | 00010111100 | 0100000000000101 | 00001000100010000000000000000 | 00100000000001 | 00000000110100100000010010 0 | 00010000000101100 |
| | 9 | 1 | 00010111100 | 0100000000000101 | 00001000100010000000000000000 | 00100000000001 | 00000000110100100000010010 0 | 00010000000101100 |
| *Dendragapus obscurus* | 10 | 3 | 10000001000 | 0010010000000100 | 01010000000000000000000000000 | 00001001000000 | 00001000000100001010100000 0 | 00010000100001001 0 |
| | 11 | 1 | 10000001000 | 0010010000000100 | 00010000000101000001000000000 | 00001001000000 | 00000000101100001010100010 0 | 00100000100000001 0 |
| *Lagopus lagopus* | 12 | 3 | 10000001000 | 0010010000000100 | 00100000010000000010000100100 | 00000101000010 | 01000000000100000000000100 10 | 01000000100000000 |
| | 13 | 4 | 10000001000 | 0010010000000100 | 00100000010000000010000001110 | 00000101000010 | 00000100000101000000101001 0 | 01000000100000000 |
| *Lagopus leucurus* | 14 | 3 | 10000001000 | 0010010000000100 | 00001000010010000000000000100 | 00001001000000 | 00100000000110000000000100 0 | 01000000100000000 |
| | 15 | 1 | 10000001000 | 0010010000000100 | 00001000010010000000000000100 | 00001001000000 | 00100000000110000000000100 0 | 01000000100000000 |
| *Lagopus mutus* | 16 | 1 | 10000001000 | 0010010000000100 | 00100001000000000010000000100 | 00000101000010 | 00010000000100000000111101 0 | 00001010001000000 |
| | 17 | 1 | 10000001000 | 0010010000000100 | 00100000010000000010000100100 | 00000101000010 | 00010000000100000000111101 0 | 00000110001000010 0 |
| | 18 | 1 | 10000001000 | 0010010000000100 | 00100000010000000010000100100 | 00000101000010 | 00010000000100000000111101 0 | 00001010001000000 |
| *Tympanuchus cupido* | 19 | 2 | 01000101000 | 0010010000000100 | 01010000000000000000000000000 | 00000011101000 | 00000000110110000000000100 | 00000100110001000 0 |
| | 20 | 2 | 01000101000 | 0010010000000100 | 01010000000000000000000000000 | 00000011101000 | 00000000110110000000000100 | 00000100110001000 0 |
| | 21 | 1 | 01000101000 | 0010010000000100 | 01010000000000000000000000000 | 00000010111100 | 00000000110110000000000100 | 00000100110001000 0 |
| | 22 | 1 | 01000101000 | 0010010000000100 | 01010000000000000000000000000 | 00000011101000 | 00000000110110000000000100 | 00000100110001000 0 |
| *Tympanuchus pallidicinctus* | 21 | 3 | 01000101000 | 0010010000000100 | 01010000000000000000000000000 | 00000010111100 | 00000000110110000000000100 | 00000100110001000 0 |
| | 22 | 2 | 01000101000 | 0010010000000100 | 01010000000000000000000000000 | 00000011101000 | 00000000110110000000000100 | 00000100110001000 0 |
| *Tympanuchus phasianellus* | 22 | 7 | 01000101000 | 0010010000000100 | 01010000000000000000000000000 | 00000011101000 | 00000000110110000000000100 | 00000100110001000 0 |
| *Tetrao urogallus* | 23 | 1 | 00101001000 | 0000010010101100 | 00000010000100011000100000001 | 10000000000000 | 00000010000100001010100010 0 | 01000000100000000 |
| *Colinus virginianus* | 24 | 1 | 10000000011 | 0000100101000000 | 01000000001000100000000010000 | 01000000000000 | 10000000000000000000010000000 | 10000000000010000 |

[a] Haplotypes numbered consecutively; *n* = number of individuals in which haplotype was observed.

APPENDIX 2. Extended.

| Species | *Kpn* I | *Nco* I | *Nde* I | *Pst* I | *Pvu* II | *Sal* I | *Sst* I | *Xba* I |
|---|---|---|---|---|---|---|---|---|
| | 11111<br>6531099655433321<br>6500096965463061 | 1111<br>6664877665332222<br>6613373515438632665<br>ab ab | 1<br>3886655433221111<br>4427741391827753<br>ab ab | 11111<br>6665487666532111<br>6665097653156521<br>abc | 11111<br>654315111<br>6495157422 | 1111<br>6210644<br>6071596 | 1111111<br>66322008654444432221<br>66863982875653205873 1<br>ab | 1111111<br>64431009776665554444322211<br>67569835215309847631476192 98 |
| *Bonasa umbellus* | 0100000000000001 | 0010000000000000001 | 0001000010101010 | 1000000000000000 | 0000110000 | 0001100 | 0000100000000010000000 | 0000000000001100100000000000 |
| | 0100000000000001 | 0010000000000000001 | 0001000010101010 | 1000000000000000 | 0000110000 | 0001100 | 0000100000000010000000 | 0000000000100001100000000000 |
| | 0100000000000001 | 0010000000000000001 | 0001000010101010 | 1000000000000000 | 0000110000 | 0001100 | 0000100000000010000000 | 0000100000000000100000000000 |
| | 0100000000000001 | 0010000000000000001 | 0001000010101010 | 1000000000000000 | 0000110000 | 0001100 | 0000100000000010000000 | 0000000000100000110000000001 |
| | 0100000000000001 | 0010000000000000001 | 0001000000111110 | 1000000000000000 | 0000110000 | 0001100 | 0000100000000010000000 | 0000000000100001100000000000 |
| *Centrocercus urophasianus* | 0000000110000101 | 0000001010000100100 | 0001100000001010 | 0000001000100111 | 0001001011 | 0001100 | 0000000010100100000000 | 0000000000010000001010100000 |
| *Dendragapus canadensis* | 1000000000000000 | 0000010010000001010 | 0001010000001011 | 0100000000000000 | 1000000000 | 0001100 | 0001000000000000100000 | 1000000000000000000000000000 |
| | 1000000000000000 | 0000011000000000010 | 0001010000001011 | 0100000000000000 | 1000000000 | 0001100 | 0001000000000000100000 | 1000000000000000000000000000 |
| | 1000000000000000 | 0000010010000001010 | 0001010000001011 | 0100000000000000 | 1000000000 | 0001100 | 0100000000000000000000 | 1000000000000000000000000000 |
| *Dendragapus obscurus* | 0000000011100001 | 0000000110100000100 | 0001001001001000 | 0000010000100101 | 0001001011 | 1000000 | 0000000000001001100101 | 0000001000010000000000000000 |
| | 0100000000000001 | 0000000110100000100 | 0001001001001000 | 0000000001101101 | 0001001011 | 1000000 | 0000000010000110010 0 | 0000001000010000000000000000 |
| *Lagopus lagopus* | 0000001000001011 | 1000000000000000000 | 0001010000001011 | 0000010000100101 | 0001001011 | 1000000 | 0000001000000000010010 | 0000000101000000000000000000 |
| | 0000001000001011 | 1000000000000000000 | 0001010000001011 | 0000010000100101 | 0001001011 | 0001100 | 000000100000000010010 | 0000000101000000000000000000 |
| *Lagopus leucurus* | 0100000000000001 | 0000010010001000000 | 1000000000001010 | 0000010000100101 | 0001001011 | 1000000 | 0000000100001000010 01 | 0010000000000000000000010000 |
| | 0100000000000001 | 0000010010001000000 | 1000000000001010 | 0000010000100101 | 0001001011 | 1000000 | 0000000100001000010 01 | 0001000000000000000000010010 |
| *Lagopus mutus* | 0000100001000001 | 0001000000000010000 | 0001000010101010 | 0000100000000101 | 0001001011 | 1000000 | 1000000000000000000000 | 0000010000000010000000000000 |
| | 0000100001000001 | 0001000000000010000 | 0001000010101010 | 0001000000000001 | 0001001100 | 1000000 | 1000000000000000000000 | 0000010000000010000000000000 |
| | 0000100001000001 | 0001000000000010000 | 0001000010101010 | 0000100000000101 | 0001001011 | 1000000 | 1000000000000000000000 | 0000010000000010000000000000 |
| *Tympanuchus cupido* | 0000010010000001 | 0100000000000000000 | 0001010000101000 | 0000010100000001 | 0001001100 | 0010010 | 0000010000010000000001 | 0000010000000000010000000100 |
| | 0000010010000001 | 0100000000000000000 | 0001000110001000 | 0000010100000001 | 0001001100 | 0010010 | 0000010000010000000001 | 0000010000000000010000000100 |
| | 0000010010000001 | 0100000000000000000 | 0011000000001000 | 0000010100000001 | 0001001100 | 0010010 | 0000010000010000000001 | 0000010000000000010000000100 |
| | 0000010010000001 | 0100000000000000000 | 0011000000001000 | 0000010100000001 | 0001001100 | 0010010 | 0000010000010000000001 | 0000010000000000010000000100 |
| *Tympanuchus pallidicinctus* | 0000010010000001 | 0100000000000000000 | 0011000000001000 | 0000010100000001 | 0001001100 | 0010010 | 0000010000010000000001 | 0000010000000000010000000100 |
| | 0000010010000001 | 0100000000000000000 | 0011000000001000 | 0000010100000001 | 0001001100 | 0010010 | 0000010000010000000001 | 0000010000000000010000000100 |
| *Tympanuchus phasianellus* | 0000010010000001 | 0100000000000000000 | 0011000000001000 | 0000010100000001 | 0001001100 | 0010010 | 0000010000010000000001 | 0000010000000000010000000100 |
| *Tetrao urogallus* | 0001000010000000 | 0000001001010000001 | 0001000110001000 | 0010000000000000 | 0100000010 | 1000000 | 1000000000000000000000 | 0100000000000000000000001000 |
| *Colinus virginianus* | 0010000000010000 | 0000000000000000000 | 0110000000000000 | 0000000110010000 | 0010001000 | 0100001 | 0010000000000000001000 | 0000000010000000000101100000 |