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SYSTEMATICS OF GROUSE AND PTARMIGAN DETERMINED BY NUCLEOTIDE SEQUENCES OF THE MITOCHONDRIAL CYTOCHROME-*B* GENE

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ABSTRACT.—The delineation of species groups and determination of relationships among taxa within the Tetraoninae (grouse and ptarmigan) have relied heavily on anatomical and behavioral specializations associated with reproduction. As a result, the various classifications of tetraonines differ considerably with respect to the phylogenetic relationships depicted for the primary species groups. We used nucleotide sequence data derived from the mitochondrial cytochrome-*b* gene to examine relationships among all New World taxa and the major Old World "superspecies" groups of grouse and ptarmigan. The cytochrome-*b* sequence data: (1) indicated an early divergence of the *Bonasa* species; (2) grouped *Dendragapus obscurus* with *Tympanuchus* and supported an association of these taxa with *Lagopus* and *Tetrao*; and (3) suggested that tetraonines underwent a period of rapid diversification in North America. The molecular phylogeny provides support for adaptive interpretations of morphological and behavioral variation among grouse and ptarmigan and indicates that the complex reproductive systems of tetraonines reflect homoplasy probably as a result of convergent evolution. We recommend that grouse and ptarmigan taxonomy incorporate genetic considerations and that classifications reflect common ancestry. *Received 9 October 1995, accepted 5 March 1996.*

GROUSE AND PTARMIGAN constitute a generalized subfamily (Tetraoninae) of gallinaceous birds that collectively have a Holarctic distribution in the Northern Hemisphere (Johnsgard 1983). Eight species are endemic to North America, seven forms occur exclusively in the Old World, and two ptarmigan species, the Willow Ptarmigan (*Lagopus lagopus*) and Rock Ptarmigan (*L. mutus*), occupy tundra and alpine habitats in both North America and Eurasia (Table 1). Currently, 17 tetraonine species are recognized, but several closely related "superspecies" groups may reflect geographic races derived from Pleistocene separation (Janossy 1976, Ellsworth et al. 1994) rather than specifically distinct taxa (Short 1967, Johnsgard 1983).

Morphological features including marginal comb-like membranes (pectinations) on the toes and feathered nostrils and tarsi distinguish the Tetraoninae from other galliform groups (Peters 1934, Ridgway and Friedmann 1946, Wetmore 1960), but systematic relationships among grouse and ptarmigan have been controversial. Various taxonomic treatments of tetraonines differ considerably with respect to the interrelationships among primary species groups. Traditional classifications of grouse and ptarmigan used behavioral patterns and morphological features associated with reproduction to define generic and species limits and to infer systematic relationships among taxa (Short 1967, Fjeldså 1977, Johnsgard 1983, Potapov 1985). However, these anatomical and behavioral traits are adaptive and subject to convergent evolution. Therefore, classifications derived from such characters may contain groupings that reflect evolution toward a similar function rather than recent common ancestry.

Ellsworth et al. (1995) examined evolutionary relationships among North American tetraonids using restriction fragment analysis of mitochondrial DNA (mtDNA). Their phylogenetic hypothesis was derived from molecular characters, thereby representing an independent phylogeny from those produced using plumage patterns and courtship behaviors. The restriction fragment data supported the association among *Lagopus*, *Tetrao*, and *Dendragapus* [*obscurus*] suggested by Short (1967) on the basis of hybridization frequencies and similarities in plumage patterns. Relationships among the *La-*

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gopus species previously proposed by Short (1967) and others (Höhn 1980, Johnsgard 1983) also were confirmed. However, several aspects of the molecular phylogeny were not congruent with any previous classifications of the Tetraoninae. In particular, *Dendragapus canadensis* and *D. obscurus* were genetically divergent and did not comprise a monophyletic group. Additionally, the mtDNA fragment data did not adequately resolve relationships among the primary tetraonid lineages.

In this study, we produce a more detailed molecular data set and expand our study to include representatives of several Palaearctic taxa. These new data are used to test previous phylogenetic hypotheses and further resolve evolutionary relationships among grouse and ptarmigan. Our analysis includes 13 species encompassing all New World taxa as well as representatives of the major Eurasian species groups. We chose comparative nucleic acid sequencing of the mitochondrial cytochrome-*b* gene to exploit the phylogenetic utility of mtDNA and because nucleotide sequence data from the cytochrome-*b* gene have proven useful for determining relationships within several avian families (Richman and Price 1992, Helm-Bychowski and Cracraft 1993, Krajewski and Fetzner 1994, Lanyon and Hall 1994).

METHODS

Specimens.—Brain and liver tissues were collected from representatives of all New World grouse and ptarmigan species, several Old World grouse taxa, and two related galliform species that served as outgroups (Table 1). Mitochondrial DNA from 36 individuals was isolated from frozen tissue and purified on cesium chloride density gradients (Carr and Griffith 1987). Several individuals from most species were sequenced in order to minimize the possible effects of intraspecific variation on our phylogenetic hypotheses (Smouse et al. 1991). Existing sequences from the White Leghorn Chicken (*Gallus gallus domesticus*; Desjardins and Morais 1990) also were included as an outgroup.

Asymmetric PCR amplification and nucleotide sequencing.—For each individual, a 723-base-pair (bp) region of the mitochondrial cytochrome-*b* gene was amplified by subjecting a 50 ng aliquot of purified mtDNA to asymmetric polymerase chain reaction (PCR) amplification (Saiki et al. 1988) following the protocol of Allard et al. (1991). Available cytochrome-*b* sequences from divergent avian and mammalian taxa were aligned to identify conserved regions for the modification/construction of the oligonucleotide primers L14841 5′-CCATCCAACATCTCTGCTTGATGAAA-3′ (Kocher et al. 1989) and H15518 5′-GGTTACTAGTGGGTTTGCTGG-3′. The primers are named such that the letters L and H refer to the light and heavy strands, respectively, of the mtDNA molecule, and the numbers relate the position of the 3′ base of each primer to the human mtDNA sequence (Anderson et al. 1981). Amplification of double-stranded DNA (dsDNA) was preformed in 100-μL volumes containing 2.5 units of *Taq* DNA polymerase, an unbalanced primer ratio (50 pmol:5 pmol), 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin, and 200 μM each dNTP. The thermocycle profile for generating dsDNA consisted of 25 cycles with 1 min denaturation at 95°C, 1 min annealing at 50°C, and 1 min 15 s polymerization at 72°C. Following amplification, the entire reaction volumes were centrifuged in Centricon® 30 microconcentrators (Amicon) and washed twice with 2 ml ddH_2O to remove excess PCR reactants and concentrate the amplification products. A 2 μL aliquot of dsDNA was then used in a 100 μL reaction to generate single-stranded DNA (ssDNA) suitable for direct sequencing (Gyllensten and Erlich 1988, as modified by Allard et al. 1991). The ssDNA amplifications contained 50 pmol of the excess primer (no limiting primer) and involved 20 cycles with an annealing temperature of 55°C. Nucleotide sequences were obtained from the ssDNA template with the extension-dideoxy-chain termination method (Sanger et al. 1977) by incorporating the limiting primer in the sequencing reactions. All fragments were sequenced on both strands to increase accuracy. Complete sequences were aligned using the LINEUP option of the GCG software package (Devereux et al. 1984).

Phylogenetic analysis.—To estimate evolutionary relationships among taxa, we analyzed the nucleotide sequence data using maximum parsimony and distance methods. In the parsimony approach, we selected the HEURISTIC search option in PAUP (Swofford 1990) with a RANDOM taxon addition sequence (10 replications) and the TREE-BISECTION-RECONNECTION swapping algorithm to search for trees with the fewest number of evolutionary events (i.e. nucleotide substitutions). All uninformative nucleotide positions were excluded from the data set prior to analysis. Substitutions at the third positions of codons were much more common than substitutions at first or second positions, and few first or second positions were phylogenetically informative. We believe that differentially weighting a few sites can exaggerate the random component of nucleotide substitution and obscure the phylogenetic signal present in the data. We therefore applied equal weight to all sites. Two independent analyses were conducted because of a substantial transition bias (transition substitutions A ↔ G and C ↔ T greatly outnumber transversions A or G ↔ C or T) in the cytochrome-*b* gene of tetraonids and other avian species (Edwards et al. 1991): (1) tran-

TABLE 1. Classification and distribution of grouse, ptarmigan, and related galliform taxa examined in this study.[a]

Taxonomy	Distribution	n[b]
Family Phasianidae		
Subfamily Tetraoninae		
Genus *Bonasa*		
Subgenus *Bonasa*		
B. umbellus (Ruffed Grouse)	Nearctic	4
Subgenus *Tetrastes*		
B. bonasia (Hazel Grouse)	Palaearctic	1
B. sewerzowi (Black-breasted Hazel Grouse)	Palaearctic	na
Genus *Centrocercus*		
C. urophasianus (Sage Grouse)	Nearctic	1
Genus *Dendragapus*		
Subgenus *Canachites*		
D. canadensis (Spruce Grouse)	Nearctic	2
D. falcipennis (Sharp-winged Grouse)	Palaearctic	na
Subgenus *Dendragapus*		
D. obscurus (Blue Grouse)	Nearctic	5
Genus *Lagopus*		
L. lagopus (Willow Ptarmigan)	Holarctic	2
L. leucurus (White-tailed Ptarmigan)	Nearctic	2
L. mutus (Rock Ptarmigan)	Holarctic	2
Genus *Tetrao*		
Subgenus *Lyrurus*		
T. tetrix (Black Grouse)	Palaearctic	1
T. mlokosiewiczi (Caucasian Black Grouse)	Palaearctic	na
Subgenus *Tetrao*		
T. urogallus (Capercaillie)	Palaearctic	1
T. parvirostris (Black-billed Capercaillie)	Palaearctic	na
Genus *Tympanuchus*		
T. cupido (Greater-Prairie Chicken)	Nearctic	4
T. pallidicinctus (Lesser-Prairie Chicken)	Nearctic	3
T. phasianellus (Sharp-tailed Grouse)	Nearctic	3
Subfamily Meleagridinae		
Genus *Meleagris*		
M. gallopavo (Wild Turkey)	Nearctic	2
Subfamily Odontophorinae		
Genus *Colinus*		
C. virginianus (Northern Bobwhite)	Nearctic	3

[a] Adapted from Johnsgard (1983). Brackets denote "superspecies" groups that may reflect geographic races rather than distinct species.
[b] n is number of individuals sequenced for 609-bp portion of mitochondrial cytochrome-*b* gene; na, not available.

sitions and transversions were weighted equally, and (2) transversion substitutions were preferentially weighted (2:1). We constructed majority-rule consensus trees from all equally parsimonious solutions produced by the various approaches and evaluated support for particular clades with bootstrap estimates (Felsenstein 1985, 1988). Bremer support indices (Bremer 1988, Källersjö et al. 1992) also were calculated as a measure of stability for monophyletic groups. We then constructed cladograms that were constrained by stipulating that currently recognized genera (*Bonasa, Lagopus,* and *Dendragapus,* respectively) remain monophyletic to assess the degree to which the molecular data supported conventional taxonomic groupings of grouse and ptarmigan.

We used the DNADIST program in the Phylogeny Inference Package (PHYLIP; Felsenstein 1989) to calculate sequence divergence values between species based on Kimura's (1980) two-parameter model. Kimura's method yields the number of nucleotide substitutions per site (K_{ij}) between two sequences i and j by taking into account unequal transitional and transversional rates and assuming no rate heterogeneity among nucleotide positions along the sequence. We assumed a transition:transversion ratio of 20:1 to accommodate the previously mentioned transition bias. Evolutionary relationships among taxa were estimated from the genetic distance matrix using the FITCH algorithm in PHYLIP with the GLOBAL search and RANDOM sequence input options in effect. The FITCH program estimates a phylogeny using Fitch-Margoliash (1967) and least squares criteria (Cavalli-Sforza and Edwards 1967). The robustness of the consensus tree topology was evaluated by running the SEQBOOT, DNADIST, FITCH, and CONSENSE programs sequentially.

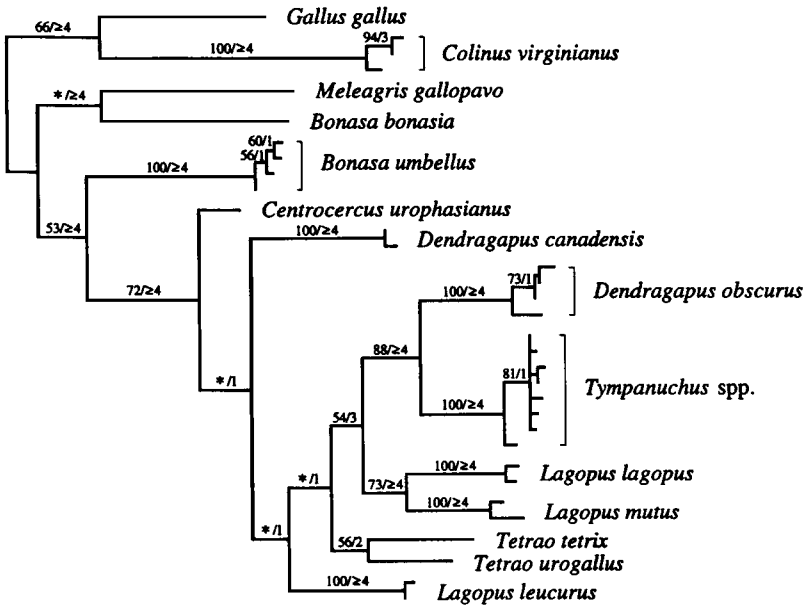

FIG. 1. Majority-rule consensus tree (consistency index = 0.935) based on 609 nucleotides of the mitochondrial cytochrome-*b* gene depicting phylogenetic relationships among grouse and ptarmigan. Nucleotide positions were treated as unordered with equal weight and randomly sampled with replacement in 500 bootstrap replications using the HEURISTIC search option, RANDOM taxon addition (10 replications), and TREE-BISECTION-RECONNECTION swapping algorithm in PAUP 3.1 (Swofford 1990). Percentage of iterations supporting putative clades is indicated along respective branches followed by Bremer support indices. Branches appearing in <50% of trees are denoted by asterisks. Branch lengths proportional to number of substitutions supporting each group.

The nucleotide sequence alignment reported in this paper may be obtained from the European Bioinformatics Institute by sending an e-mail message to ⟨netserv@ebi.ac.uk⟩ and including the line HELP ALIGN: or GET ALIGN:DS24871.DAT.

RESULTS

Parsimony analysis.—Complete nucleotide sequences for a 609-bp portion of the mitochondrial cytochrome-*b* gene were obtained for all taxa. Among the 36 individuals analyzed, 32 distinct sequences were observed. The sequences contained 173 (28.4%) variable sites, of which 95 were phylogenetically informative. The vast majority of informative sites (83) were located at the third codon position. Some informative sites were detected at the first codon position (12), but the second position was devoid of phylogenetically informative variation.

Five equally parsimonious trees of length 487 were obtained in the cladistic analysis. The resultant trees were topologically similar, with differences among them solely attributable to the alternative grouping of individuals within *Tympanuchus*. We used successive approximations (Carpenter 1988) in an attempt to resolve these equally parsimonious solutions, but several iterations failed to delineate a single most parsimonious tree and could not partition *Tympanuchus* individuals along "species" boundaries. Differential weighting of transversions and transitions (2:1) in an independent search produced five trees (620 steps) identical in structure to those observed in the above analysis.

The monophyly of several tetraonid genera was not supported in the consensus of the five equally parsimonious trees derived from the cytochrome-*b* sequence data (Fig. 1). Although the *Tetrao* species grouped with one another, *Dendragapus obscurus* was strongly associated with *Tympanuchus* and showed no affinity toward *D. canadensis*. Likewise, *Lagopus lagopus* and *L. mutus* were affiliated with the *Dendragapus obscurus*—*Tympanuchus* clade; however, *L. leucurus* did not cluster with the other ptarmigan and fell into the unresolved portion of the tree. *Bonasa bonasia* and *B. umbellus* were outside the

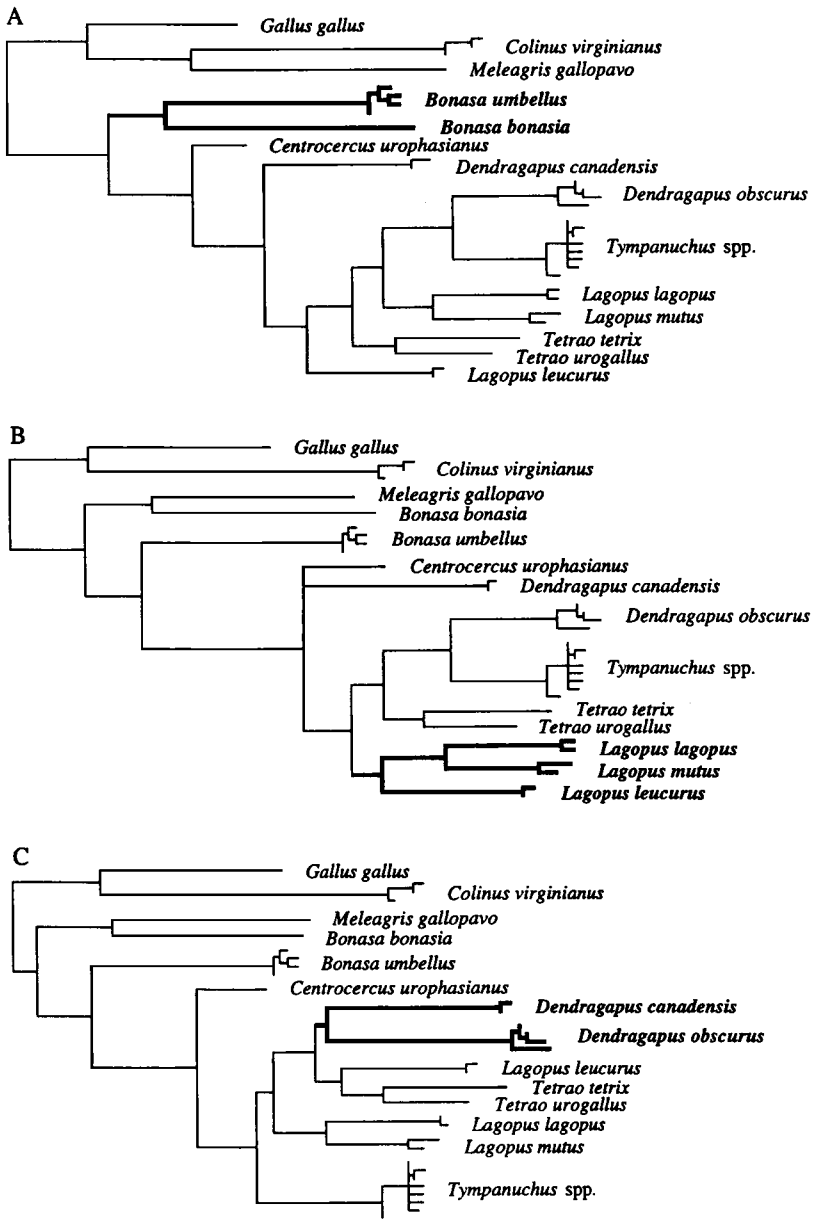

FIG. 2. Alternative phylogenies for tetraonines obtained in analyses constrained by the *a priori* stipulation that the monophyly of currently recognized genera (in bold) be retained. (A) Consensus of five cladograms (493 steps) in which *Bonasa bonasia* and *B. umbellus* were held together as a monophyletic group. (B) Consensus of 10 equally parsimonious trees (length 493) produced when *Lagopus* species were retained as a clade. (C) Phylogenetic consensus of five trees 501 steps in length obtained by *a priori* grouping of the *Dendragapus* species. Parsimony analyses conducted on cytochrome-*b* DNA sequences using PAUP.

main tetraonid clade and did not comprise a monophyletic group. We determined whether the relative rates of nucleotide substitution differed between the two species of *Bonasa* using the binomial test of Mindell and Honeycutt (1990) to investigate the possibility that the grouping of *B. bonasia* with *Meleagris* was attributable to differential rates of molecular evolution. Two different outgroups, chicken and quail, were used to test for deviations from rate

TABLE 2. Percent nucleotide sequence divergence (Kimura 1980; lower matrix) and transition : transversion ratios[a] (upper matrix) in a 609-bp portion of the mitochondrial cytochrome-*b* gene among grouse, ptarmigan, and related galliform taxa.

Taxon	Taxon								
	1	2	3	4	5	6	7	8	9
1. *Gallus gallus*	—	1.02	1.00	1.00	1.45	2.03	2.00	2.07	2.10
2. *Colinus virginianus* (A)	19.19	—	3/0	7.00	0.75	1.05	1.07	1.09	1.07
3. *C. virginianus* (B)	18.94	0.49	—	6.00	0.79	1.09	1.11	1.14	1.11
4. *C. virginianus* (C)	18.39	1.33	1.16	—	0.79	1.07	1.11	1.14	1.12
5. *Meleagris gallopavo*	19.39	20.08	20.62	20.04	—	2.74	2.63	2.78	2.83
6. *Bonasa umbellus* (A)	17.79	18.90	19.40	18.60	17.09	—	4.00	3/0	2/0
7. *B. umbellus* (B)	18.31	19.71	20.22	19.66	17.36	0.83	—	3.00	4.00
8. *B. umbellus* (C)	18.03	19.40	19.92	19.36	17.32	0.49	0.66	—	3/0
9. *B. umbellus* (D)	18.27	19.15	19.66	19.11	17.56	0.33	0.83	0.49	—
10. *Bonasa bonasia*	17.86	20.70	20.70	20.39	17.70	15.61	16.32	16.29	16.06
11. *Centrocercus urophasianus*	17.94	18.68	18.68	18.89	17.62	12.27	13.15	12.91	12.27
12. *Dendragapus canadensis* (A)	19.00	20.96	20.96	20.91	17.55	12.81	13.69	13.45	12.81
13. *D. canadensis* (B)	19.24	20.96	20.96	20.91	18.03	12.81	13.69	13.45	12.81
14. *Dendragapus obscurus* (A)	18.67	23.38	23.65	23.32	16.53	12.55	13.42	13.18	12.97
15. *D. obscurus* (B)	18.67	22.02	22.29	21.97	17.47	12.13	13.00	12.76	12.55
16. *D. obscurus* (C)	18.43	23.65	23.93	23.59	16.76	12.76	13.64	13.40	13.18
17. *D. obscurus* (D)	18.47	24.40	24.68	24.33	16.50	12.06	12.92	12.68	12.48
18. *Lagopus lagopus* (A)	17.79	19.06	19.06	18.77	17.71	13.02	13.91	13.66	13.45
19. *L. lagopus* (B)	17.55	19.06	19.06	18.77	17.71	13.02	13.91	13.66	13.45
20. *Lagopus leucurus* (A)	17.31	18.39	18.39	18.10	15.38	12.81	13.69	13.45	13.23
21. *L. leucurus* (B)	16.84	18.14	18.14	17.85	14.92	12.81	13.69	13.45	13.23
22. *Lagopus mutus* (A)	17.79	20.01	20.01	19.71	17.79	12.43	13.31	13.07	12.86
23. *L. mutus* (B)	17.35	20.85	20.85	20.53	18.31	12.25	13.12	12.88	12.67
24. *Tympanuchus* spp.[b]	17.71	20.60	20.60	20.29	17.00	11.71	12.57	12.34	12.13
25. *Tympanuchus cupido* (A)	17.95	20.34	20.34	20.03	17.47	12.13	13.00	12.76	12.55
26. *T. cupido* (B)	17.00	20.38	20.38	20.08	17.03	12.15	13.02	12.78	12.57
27. *T. cupido* (C)	17.95	20.60	20.60	20.29	17.23	11.92	12.78	12.55	12.34
28. *Tympanuchus pallidicinctus* (A)	17.03	20.43	20.43	20.12	16.83	11.76	12.62	12.38	12.17
29. *T. pallidicinctus* (B)	17.71	20.60	20.60	20.29	16.07	12.13	13.00	12.76	12.55
30. *Tympanuchus phasianellus* (A)	17.23	21.12	21.12	20.81	17.00	12.13	13.00	12.76	12.55
31. *T. phasianellus* (B)	17.95	20.34	20.34	20.03	17.23	11.92	12.78	12.55	12.34
32. *Tetrao tetrix*	16.80	19.52	19.52	18.72	17.51	11.53	12.39	12.15	11.94
33. *Tetrao urogallus*	17.15	21.97	21.97	21.64	18.35	12.86	13.75	13.07	13.29

[a] Number of transitions/transversions for comparisons with zero transitions or transversions.
[b] Sequence observed in *Tympanuchus cupido* and *T. phasianellus*.

homogeneity, but no significant differences between either species relative to the *Gallus* and *Colinus* outgroups were observed ($P = 0.24$ and 0.38, respectively).

Subjecting the cladistic analyses to the *a priori* constraint that the monophyly of currently recognized genera be retained demonstrated a lack of support for traditional classifications of tetraonids. Stipulating the monophyly of *Bonasa* produced five trees of 493 steps (six steps longer than the most parsimonious solutions; Fig. 2A). Similarly, when the *Lagopus* species were retained as a clade, 10 equally parsimonious trees of length 493 were observed (Fig. 2B). The *a priori* grouping of *Dendragapus canadensis* and *D. obscurus* produced five cladograms (length 501) that were 14 steps longer than the shortest trees (Fig. 2C).

Transition and transversion substitutions.—Transition-to-transversion ratios indicate that the outgroup taxa have reached saturation of transition substitutions (transition:transversion ratio ≤ 2.83) with respect to each other and in comparison with grouse and ptarmigan. Among tetraonines, a transition bias similar to other avian species (Edwards et al. 1991, Krajewski and Fetzner 1994) was observed. The ratio of transitions to transversions ranged from 2.29 between *Bonasa bonasia* and *Lagopus mutus* to 19.50 between *Dendragapus obscurus* and *Tympanuchus cupido* (Table 2).

Distance analysis.—The overall extent of nucleotide sequence divergence (Table 2) within species generally was $\leq 1\%$. The maximum intraspecific divergence occurred in *Dendragapus obscurus* (2.02%) between a lineage from Van-

TABLE 2. Extended.

Taxon											
10	11	12	13	14	15	16	17	18	19	20	21
1.84	1.84	2.21	2.24	2.41	2.41	2.37	2.25	2.03	2.00	1.97	1.90
1.20	1.07	1.23	1.23	1.43	1.32	1.45	1.34	1.17	1.17	1.00	0.98
1.20	1.07	1.23	1.23	1.45	1.34	1.48	1.36	1.17	1.17	1.00	0.98
1.23	1.14	1.28	1.28	1.49	1.37	1.51	1.39	1.20	1.20	1.02	1.00
1.64	2.00	2.00	2.07	2.07	2.22	2.11	2.19	2.26	2.26	1.89	1.81
3.00	3.06	3.86	3.86	4.58	4.42	4.67	6.22	3.93	3.93	3.86	3.86
2.95	3.06	3.80	3.80	4.46	4.31	4.54	5.90	3.87	3.87	3.80	3.80
3.15	3.25	4.07	4.07	4.83	4.67	4.92	6.56	4.14	4.14	4.07	4.07
3.10	3.06	3.86	3.86	4.75	4.58	4.83	6.44	4.07	4.07	4.00	4.00
—	2.70	3.56	3.67	4.21	4.36	4.14	3.29	3.75	3.75	3.81	3.81
14.32	—	5.25	5.50	8.00	8.33	8.17	5.22	4.50	4.50	5.38	5.13
15.99	9.05	—	2/0	14.00	15.50	14.25	8.00	5.63	5.63	7.17	7.50
16.45	9.44	0.33	—	14.00	15.50	14.25	8.00	5.88	5.88	7.50	7.83
13.88	9.81	10.93	10.93	—	8/0	1/0	0.33	6.83	6.83	12.50	12.00
14.31	10.20	12.15	12.15	1.33	—	9/0	3.00	7.83	7.83	13.00	12.50
13.66	10.00	11.13	11.13	0.16	1.50	—	0/3	7.00	7.00	12.75	12.25
13.96	10.26	11.60	11.60	0.66	2.02	0.50	—	4.56	4.56	7.14	6.86
14.59	10.08	9.61	10.00	8.40	9.57	8.59	9.03	—	2/0	6.67	7.00
14.59	10.08	9.61	10.00	8.40	9.57	8.59	9.03	0.33	—	7.00	7.33
14.81	9.25	8.79	9.18	9.73	10.13	9.93	10.39	8.21	8.59	—	2/0
14.81	8.85	9.18	9.57	9.34	9.73	9.54	9.99	8.59	8.98	0.33	—
13.39	8.89	10.61	11.01	8.59	9.38	8.79	9.23	6.50	6.50	8.40	8.02
13.20	9.30	10.83	11.24	8.22	9.00	8.42	8.85	7.08	7.08	9.00	8.80
13.23	9.61	10.13	10.53	6.46	6.83	6.27	6.87	8.98	8.59	10.33	9.93
13.23	10.00	10.13	10.53	6.83	7.20	6.64	7.24	8.59	8.21	10.33	10.33
13.05	10.02	10.15	10.55	6.47	6.84	6.28	6.88	8.80	8.42	10.35	9.95
13.45	9.81	9.93	10.33	6.64	7.02	6.46	7.05	8.79	8.40	10.13	10.13
12.64	9.44	10.57	10.97	5.93	6.30	5.74	6.33	9.02	8.63	10.17	9.77
13.23	8.82	10.13	10.53	6.09	6.46	5.91	6.49	8.98	8.59	9.54	9.15
13.23	10.00	10.53	10.93	6.46	6.83	6.27	6.87	9.38	8.98	10.73	10.33
13.23	9.81	10.33	10.73	6.64	6.64	6.46	7.05	9.18	8.79	10.13	9.73
14.40	9.43	9.55	9.95	9.32	10.11	9.52	9.97	8.58	8.58	8.97	8.58
14.27	8.49	9.61	10.00	8.21	8.59	8.40	8.64	9.44	9.44	8.24	7.85

couver Island and other conspecific populations in Colorado. We also obtained a divergence value of 1.67% between two *Lagopus mutus* individuals collected from localities in North America and Scandinavia. Genetic divergence values among congeneric species were widely disparate. For example, distances among "species" of *Tympanuchus* averaged only 0.5% and were equivalent to or less than intraspecific values observed in other species. In fact, an identical sequence was shared by *T. cupido* and *T. phasianellus*. In contrast, the average differentiation between *Bonasa umbellus* and *B. bonasia* exceeded 16%, the maximum divergence observed between any species (including all intergeneric comparisons). Average differentiation among other congeners ranged from 6.79% between *Lagopus lagopus* and *L. mutus* to 11.45% between *Dendragapus canadensis* and *D. obscurus*. Nucleotide sequence divergence between the various tetraonid species and the outgroup taxa was in the range of 14.92% to 24.68%.

Evolutionary relationships among grouse and ptarmigan determined by genetic distances (Fig. 3) differed in some respects from those derived from parsimony analysis. In the Fitch-Margoliash tree: (1) *Bonasa umbellus* was the basal tetraonid lineage; (2) *B. bonasia* did not associate with *Meleagris gallopavo;* and (3) a very weak affiliation was evident among *Dendragapus canadensis, Centrocercus urophasianus,* and *Lagopus leucurus*. Phylogenetic discordance between the parsimony and distance methods occurred in regions of the trees that were not robust to bootstrap resampling. Relationships defined by both methodologies included: (1) a well supported

TABLE 2. Extended.

Taxon											
22	23	24	25	26	27	28	29	30	31	32	33
2.03	1.87	2.26	2.30	2.30	2.30	2.27	2.26	2.19	2.30	2.00	1.74
1.04	1.06	1.31	1.29	1.29	1.31	1.29	1.31	1.36	1.29	1.27	1.10
1.04	1.06	1.31	1.29	1.29	1.31	1.29	1.31	1.36	1.29	1.27	1.10
1.07	1.09	1.34	1.32	1.32	1.34	1.32	1.34	1.39	1.32	1.25	1.13
2.03	2.00	2.15	2.22	2.15	2.19	2.11	2.00	2.15	2.19	2.11	1.90
3.13	2.82	4.25	4.42	4.42	4.33	4.25	4.42	4.42	4.33	3.77	3.25
3.12	2.83	4.15	4.31	4.31	4.23	4.15	4.31	4.31	4.23	3.71	3.24
3.31	3.00	4.50	4.67	4.67	4.58	4.50	4.67	4.67	4.58	4.00	3.31
3.25	2.94	4.42	4.58	4.58	4.50	4.42	4.58	4.58	4.50	3.92	3.38
2.50	2.29	4.00	4.00	3.93	4.07	3.79	4.00	4.00	4.00	3.41	2.70
3.90	3.64	7.83	8.17	8.17	8.00	7.67	7.17	8.17	8.00	6.43	3.70
6.25	5.56	13.00	13.00	13.00	12.75	13.50	13.00	13.50	13.25	9.60	5.63
6.50	5.78	13.50	13.50	13.50	13.25	14.00	13.50	14.00	13.75	10.00	5.88
7.00	5.57	17.50	18.50	17.50	18.00	16.00	16.50	17.50	18.00	16.33	6.67
7.67	6.14	18.50	19.50	18.50	19.00	17.00	17.50	18.50	18.00	17.67	7.00
7.17	5.71	17.00	18.00	17.00	17.50	15.50	16.00	17.00	17.50	16.67	6.83
4.67	3.90	6.80	7.20	6.80	7.00	6.20	6.40	6.80	7.00	8.17	4.33
5.17	4.71	7.33	7.00	7.17	7.17	7.33	7.33	7.67	7.50	8.60	4.20
5.17	4.71	7.00	6.67	6.83	6.83	7.00	7.00	7.33	7.17	8.60	4.20
6.83	6.14	13.25	13.25	13.25	13.00	13.00	12.25	13.75	13.00	9.00	4.75
6.50	6.00	12.75	13.25	12.75	13.00	12.50	11.75	13.25	12.50	8.60	4.50
—	9.00	6.83	6.83	6.67	7.00	6.50	6.50	7.17	7.00	7.80	3.60
1.67	—	6.00	6.00	5.57	6.14	5.43	5.71	6.00	6.14	6.67	3.36
8.40	8.80	—	2/0	2/0	1/0	2/0	4/0	2/0	1/0	15.33	7.50
8.40	8.80	0.33	—	3/0	1/0	3/0	6/0	4/0	3/0	15.33	7.83
8.22	8.24	0.33	0.50	—	3/0	2/0	6/0	2/0	3/0	14.33	7.67
8.59	9.00	0.16	0.16	0.50	—	3/0	5/0	3/0	2/0	15.67	7.67
8.05	8.06	0.33	0.50	0.33	0.50	—	3/0	2/0	3/0	14.67	7.33
8.02	8.42	0.66	0.99	1.00	0.83	0.50	—	6/0	5/0	16.00	7.17
8.79	8.80	0.33	0.66	0.33	0.49	0.33	0.99	—	3/0	15.33	7.83
8.59	9.00	0.16	0.49	0.50	0.33	0.50	0.83	0.49	—	15.67	7.33
7.81	8.21	8.74	8.74	8.18	8.94	8.39	9.13	8.74	8.94	—	4.43
8.27	8.67	9.18	9.57	9.39	9.38	9.02	8.79	9.57	8.98	6.70	—

clade containing *Dendragapus obscurus* and *Tympanuchus*; (2) an association of *D. obscurus*—*Tympanuchus* with a group that includes *Lagopus lagopus* and *L. mutus* and a clade containing the *Tetrao* species; and (3) placement of the *Bonasa* species outside the primary tetraonid group.

DISCUSSION

Patterns of phylogenetic divergence.—The earliest fossil remains of tetraonid-like birds have been dated to the early Miocene (Brodkorb 1964), and all pre-Pliocene fossils have been recovered exclusively from North American localities. The fossil record and present diversity of endemic species have been interpreted as suggesting a North American origin for the Tetraoninae (Johnsgard 1983). The genetic data support the early divergence of the *Bonasa* species, but they do not provide insight into the location of evolutionary origin for grouse and ptarmigan. *Bonasa umbellus*, which is endemic to North America, is the most basal tetraonid lineage in the distance (FM) tree, but *B. bonasia* (endemic to Eurasia) falls outside all other tetraonids in the parsimony tree.

A middle Pleistocene fossil species from Europe (*Bonasa praebonasi*) is believed to be ancestral to the three extant *Bonasa* species (Janossy 1976) and may indicate that *Bonasa* evolved in Europe or western Asia. According to this scenario, *Bonasa umbellus* colonized North America relatively recently, and its promiscuous mating system (with nonvocal acoustical signals and sedentary drumming display) evolved after separation from *B. bonasia* (Johnsgard 1983). The cytochrome-*b* data, however, suggest an early separation between these two species and do not support the monophyly of *Bonasa*. *Bonasa bonasia* and *B. umbellus* were traditionally placed

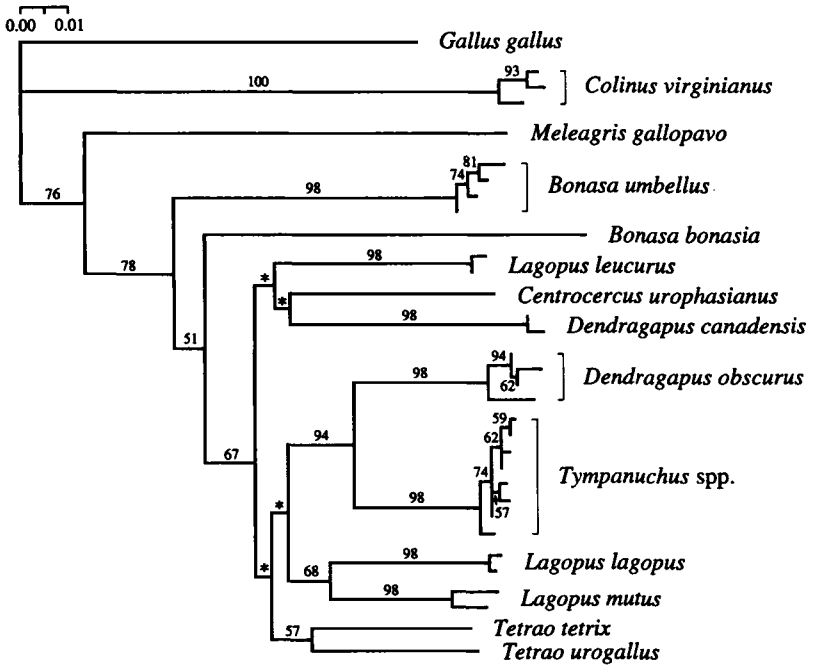

FIG. 3. Fitch-Margoliash tree (average percent standard deviation = 5.18) derived from genetic distances (Kimura 1980) among grouse and ptarmigans shown in Table 2. Branch length units represent substitutions per site. Branches within species less than 0.001 in length are not shown. Numbers along branches reflect percentage of 100 bootstrap replicate trees containing putative groupings. Asterisks denote branches supported in <50% of bootstrap iterations.

in separate genera until Short (1967), using morphological, behavioral, and ecological similarities, merged the monotypic genus *Bonasa* (containing only *umbellus*) with the Eurasian genus *Tetrastes* containing the two closely related forms of Hazel Grouse (*B. bonasia* and *B. sewerzowi*). In this study, *Bonasa bonasia* and *B. umbellus* were genetically well differentiated and comprised basal lineages in both the parsimony and distance trees. Assuming the most parsimonious solution (Fig. 1) represents a better phylogenetic hypothesis than the tree in which *Bonasa* was constrained to be monophyletic (Fig. 2A), the two *Bonasa* forms appear to have originated independently at an early stage of tetraonid evolution.

Attempts to reconstruct the phylogenetic history of groups that have diverged over short time periods (star phylogenies) often yield conflicting results because there has been insufficient time for genetic change to accumulate. As a result, few genetic characters support clades defined by short branches, and the cladistic structure of the phylogeny will be topologically unstable to bootstrap manipulations (i.e. random resampling of characters). Evolutionary relationships among grouse and ptarmigan derived from cytochrome-*b* sequences and mtDNA restriction fragments (Ellsworth et al. 1995) both exhibit branching patterns that are characterized by short internodal distances among several of the primary lineages and branches that are not well supported by bootstrapping. It is important to note that the area in which resolution is problematic for the genetic data is precisely where traditional classifications (Short 1967, Fjeldså 1977, Johnsgard 1983, Potapov 1985) commonly disagree. The inability of genetic, morphological, and behavioral characters to resolve relationships in a defined region of the phylogeny suggests that a number of tetraonid groups may have diversified within a relatively short period of time.

Our phylogenetic hypothesis cannot pinpoint the center of evolutionary origin for grouse and ptarmigan, but by inferring the minimum number of transcontinental colonization events required to explain the distribution of fossil and extant species, we hypothesize that the rapid diversification of tetraonine taxa occurred in North America. If the episode of prolific speciation occurred on the North Amer-

ican continent, two independent migration events would have been sufficient to transmit lineages originating during the purported radiation to Eurasia: (1) colonization of the Old World by an ancestral form of *Tetrao* sometime after the North American radiation but before the separation of *Tetrao tetrix* and *T. urogallus;* and (2) recent immigration of *Dendragapus falcipennis* to Eurasia. The converse hypothesis, that the cladogenic events occurred in the Palaearctic, requires transcontinental movement of at least four independent lineages that are endemic to North America: (1) the ancestor of *Dendragapus obscurus* and *Tympanuchus,* (2) *Lagopus leucurus,* (3) *Centrocercus urophasianus,* and (4) *Dendragapus canadensis.*

Adaptation in tetraonines.—When studying the evolution of ecological segregation among sympatric warbler species (*Phylloscopus*), Richman and Price (1992) found strong support for adaptation as the cause of morphological and behavioral variation when the effects of common ancestry were removed from the analysis. Similarly, our results provide support for adaptive interpretations of morphological and behavioral variation among tetraonines and indicate that traditional classifications contain groupings that largely reflect adaptation to similar environments rather than monophyletic assemblages united by common ancestry. For example, the molecular phylogeny suggests that the diverse reproductive systems characteristic of grouse and ptarmigan often evolved independently. We have identified several instances where distantly related taxa are sympatrically distributed or occupy similar habitats (ecological equivalents) and exhibit similar reproductive strategies. These findings indicate that tetraonid mating systems are attributes (sensu Mickevich and Weller 1990) rather than homologous characters and predict that if these complex reproductive systems are reduced to basic components and examined within the framework of phylogenetic history, differences due to convergent evolution will be evident.

Alternative phylogenetic hypotheses.—Classifications of tetraonids based on behavioral, demographic, and morphological similarities (attributes) may be erected with little consideration of evolutionary relatedness and may produce groupings with no phylogenetic context. The parsimony approach assumes that trees containing the fewest evolutionary events (the most parsimonious solutions) represent the best phylogenetic hypotheses and that trees of increasing length are increasingly less reliable. In our analyses, cladograms constrained by stipulating the monophyly of currently recognized genera were invariably longer than the most parsimonious solutions (Fig. 1). In particular, the trees retaining *Dendragapus canadensis* and *D. obscurus* as sister taxa (Fig. 2C) were considerably longer (14 steps) than the shortest trees, indicating that these species do not comprise a monophyletic group and questioning their congeneric status. Our phylogenetic hypothesis did not support the monophyly of *Lagopus* or *Bonasa,* but trees forced to maintain the monophyly of these genera were only five steps longer than the most parsimonious solutions. Conventional relationships among the ptarmigan species were resolved by mtDNA restriction fragment data (Ellsworth et al. 1995); therefore, lack of support for the monophyly of *Lagopus* by the cytochrome-*b* sequences may be attributable to the previously mentioned radiation such that few genetic characters actually support this group. Similarly, the *Bonasa* species may have diverged soon after the separation of tetraonids from other galliform groups.

In summary, our studies of tetraonine systematics using molecular genetic data were consistent with previous classifications in some respects but identified relationships that differ from traditional phylogenies. Additional studies are needed to clarify specific aspects of the evolutionary history of grouse and ptarmigan. We recommend that classifications of tetraonines incorporate genetic considerations to reflect common ancestry and that future studies interpret the evolution of adaptive strategies and reproductive systems within the framework of phylogenetic history.

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