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MITOCHONDRIAL-DNA AND NUCLEAR-GENE DIFFERENTIATION IN NORTH AMERICAN PRAIRIE GROUSE (GENUS *TYMPANUCHUS*)

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ABSTRACT.—The fragmentary effects of Pleistocene glacial activity have been implicated in speciation among avifauna endemic to the Central Plains of North America. The prairie-grouse complex (genus *Tympanuchus*), distributed throughout the central United States and Canada, contains three sister taxa believed to have originated from the expansion of late Pleistocene refugial populations. We assayed mitochondrial-DNA (mtDNA) restriction-site and allozyme variation in prairie grouse obtained from localities throughout their current range in North America to examine the nature and timing of events promoting differentiation in *Tympanuchus*. The genetic data were not consistent with Pleistocene isolation of sufficient duration to allow a taxonomically or geographically structured pattern of genetic variation to emerge. No clear genetic differences among species were observed. Allozymes could not distinguish populations belonging to different species and frequencies were generally similar across taxa. The mtDNA differentiation was characterized by a predominant haplotype shared by all taxa; the remainder (15) were generally infrequent and closely related to the prevalent (and presumably ancestral) haplotype. The presence of unique mtDNA haplotypes within species and absence of certain allozyme alleles from particular taxa implied a degree of isolation and restrictions to gene flow. However, the mtDNA haplotypes did not sort phylogenetically, which suggests recent common ancestry of the lineages and may explain the lack of congruence between genetic variation and species designations. Despite the absence of quantitative genetic differentiation, considerable morphological and behavioral differences are apparent among the putative species. Adult male plumage, vocalization structures, and courtship behaviors form the basis for taxonomic divisions among prairie grouse, but, considering their close association with reproduction, such characters may be subject to sexual selection and may evolve rapidly relative to mtDNA or allozymes. *Received 27 January 1993, accepted 19 August 1993.*

RANGE FRAGMENTATION and isolation associated with Pleistocene glaciation have been widely implicated in the differentiation of various closely related, but largely allopatric, avian species (Rand 1948, Mengel 1964, 1970, Selander 1965). The Central Plains of North America, in particular, have been viewed as an isolating agent promoting speciation among avian taxa that now occupy eastern deciduous and western montane forest (Mengel 1964, 1970, Bermingham et al. 1992). However, the role of glacial activity in the speciation of avifauna endemic to the Central Plains is not well understood. Many subspecies and species in the Great Plains are assumed to have arisen through geographic isolation in refugia during Pleistocene glacial maxima (Selander 1965, Hubbard 1973). Conversely, the low species diversity of grassland birds and paucity of closely related congeners led Mengel (1970) to hypothesize that there has been little past fractionation of the Central Plains into isolated environments. Therefore, it is unclear as to whether differentiation and speciation among birds of the Central Plains may be attributed to allopatric (due to isolation) or sympatric (without geographic subdivision) mechanisms.

The prairie grouse (genus *Tympanuchus*) comprise an interesting "species complex" for examining the competing hypotheses regarding the fate of the Central Plains during the Pleis-

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tocene and possible modes of differentiation/speciation among birds of the North American grasslands. The prairie-grouse complex contains three primarily grassland adapted sister taxa distributed throughout the central United States and Canada. Two extant forms of *T. cupido* (Greater Prairie-Chicken) occur primarily in remnant tall-grass prairie in Kansas, Nebraska, Oklahoma, and South Dakota (*T. c. pinnatus*) and in small endangered populations along the Texas Gulf Coast (*T. c. attwateri*; Westemeier 1980). *Tympanuchus pallidicinctus* (Lesser Prairie-Chicken) occupies the shrub grasslands of southwestern Kansas southward through Oklahoma to eastern New Mexico (Waddell and Hanzlick 1978, Cannon and Knopf 1980, Taylor and Guthery 1980), and *T. phasianellus* (Sharp-tailed Grouse) is resident to the north-central United States and central Canada to Alaska (Miller and Graul 1980).

Tympanuchus cupido and *T. pallidicinctus* are recognized as distinct species (AOU 1983) due to differences in behavior, habitat affiliation, and social aggregation (Grange 1940, Jones 1964, Sharpe 1968). However, the differences are not as great as those seen among other well-defined grouse species, causing some researchers (Short 1967, Johnsgard 1983) to regard these taxa as allopatric subspecies. Conversely, *T. phasianellus* is morphologically distinct from the "prairie chickens." Elongated central and reduced peripheral rectrices as well as rudimentary pinnae and cervical apteria distinguish *phasianellus* from *cupido* and *pallidicinctus* (Ridgway and Friedmann 1946). In fact, *phasianellus* (formerly *Pedioecetes phasianellus*) was once placed in a distinct monotypic genus, but *Pedioecetes* has been synonymized with *Tympanuchus* to reflect physiological (Hudson et al. 1959, 1966) and demographic similarities.

Wisconsin glaciation has been cited as the primary factor promoting subdivision and divergence among prairie-grouse populations. Hubbard (1973) hypothesized that prairie grouse were continuously distributed throughout much of the Great Plains during the Sangamon interglacial, but that considerable range fragmentation and isolation of populations occurred during the Wisconsin glacial period. Differentiation within isolated refugia and postglacial reinvasion of the plains may thus have produced the distribution of extant taxa. Therefore, prairie grouse are useful for characterizing genetic variation among morphologically divergent avian sister taxa believed to have originated from the expansion of late Pleistocene refugial populations. In this study, we assessed mtDNA and allozyme variation in prairie grouse and tested previous hypotheses regarding differentiation and speciation among the taxa. Specifically, we addressed the following questions: What is the nature of genetic differentiation in the prairie-grouse complex? Are the patterns of genetic variation and estimated times since divergence among mtDNA lineages consistent with late Pleistocene (recent) vicariance? Has sporadic interspecific hybridization induced by human activity influenced the pattern of differentiation among taxa? How do levels of genetic differentiation compare with morphological and behavioral divergence among the putative species?

METHODS

Specimens.—Tissues (brain, heart, liver) were obtained from 86 prairie grouse and frozen on dry ice. Most birds were harvested by hunters, but samples from the endangered *T. c. attwateri* were taken from incidental mortalities at the Attwater Prairie-Chicken National Wildlife Refuge. Collection sites, listed below by state and county (USA) or province and municipality (Canada), were located throughout the current range of each taxon: *T. c. pinnatus*, ILLINOIS, Jasper (2), Marion (2), KANSAS, Butler (3), Chase (5), Lyon (2), Shawnee (4), NEBRASKA, Rock (3), Thomas (3), OKLAHOMA, Osage (3), SOUTH DAKOTA, Lyman (6); *T. c. attwateri*, TEXAS, Colorado (1), Refugio (1); *T. pallidicinctus*, KANSAS, Clark (9), Morton (7); *T. phasianellus*, COLORADO, Routt (2), MANITOBA, CANADA, Coldwell (4), Chetfield (2), MINNESOTA, Aitkin (4), Lake of the Woods (3), NEBRASKA, Thomas (5), Rock (1), NORTH DAKOTA, Billings (1), Burleigh (1), Grant (2), McKenzie (1), Morton (1), SOUTH DAKOTA, Campbell (1), Dewey (2), Lyman (2), QUEBEC, CANADA, Ungava Comté (3).

Mitochondrial-DNA analysis.—In the laboratory, mtDNA was isolated from frozen tissue and purified on cesium chloride density gradients (Carr and Griffith 1987). Mitochondrial DNA from 51 prairie grouse (*T. c. pinnatus*, 21; *T. c. attwateri*, 2; *T. pallidicinctus*, 6; *T. phasianellus*, 22) representative of localities from the Texas Gulf Coast to Hudson Bay was digested with 12 six-base-recognizing (*Bam*H I, *Cla* I, *Dra* I, *Eco* RI, *Eco* RV, *Hind* III, *Nde* I, *Pst* I, *Ssp* I, *Sst* I, *Stu* I, *Xba* I) and 4 four-base-recognizing (*Hha* I, *Msp* I, *Rsa* I, *Taq* I) restriction enzymes. Fragments were end-labeled with ^{32}P-deoxynucleotides, separated by molecular weight on 1.2% agarose or 4% acrylamide gels, and visualized by autoradiography. Fragment sizes were estimated from comigrating molecular size standards

composed of lambda DNA and PM2 DNA digested with *Hind* III.

Unique fragment patterns produced by each restriction enzyme were designated alphabetically in chronological order of discovery. Restriction sites were easily inferred from the fragment data and used to assign each individual a composite haplotype that reflected a unique pattern of site variation across all restriction enzymes. The composite haplotypes were arbitrarily assigned numerical designations. To determine the most-parsimonious relationships among the composite haplotypes, a presence/absence matrix of the inferred restriction sites was analyzed using the HEURISTIC-SEARCH option in PAUP 3.0n (Swofford 1990). A strict-consensus tree was constructed containing information common to all equally-parsimonious solutions. Haplotypes unique to a single species were projected onto a map of North America and a minimum-mutation network summarizing the consensus tree was constructed by linking the haplotypes in an unrooted phylogenetic network so as to minimize the number of mutational steps.

The proportion of shared restriction sites was used to estimate the extent of nucleotide sequence divergence among the haplotypes (Upholt 1977). Within each species, two indices of mtDNA variation were calculated: (1) nucleotide diversity (π; Nei and Tajima 1981), which is a measure of mtDNA polymorphism across all individuals; and (2) nucleon diversity ($\hat{h}$; Nei and Tajima 1981) reflecting the diversity of haplotypes within each taxon. Mitochondrial-DNA differentiation in the prairie-grouse complex was estimated by determining the proportion of mtDNA variation attributable to differences among (versus within) populations (G_{ST}; Takahata and Palumbi 1985). Due to small sample sizes, we combined some geographically proximate populations in the G_{ST} analysis. The G_{ST} statistic was interpreted by a comparison with values derived from 100 permutation tests in which individuals were drawn randomly from the entire data set (from any species) and assigned to hypothetical populations with the same dimensions as the field-sampled populations.

Allozyme analysis.—Horizontal starch-gel electrophoresis (Selander et al. 1971, Harris and Hopkinson 1976) conducted at the Savannah River Ecology Laboratory was used to resolve 30 presumptive genetic loci in 60 prairie grouse from localities throughout the United States and Canada. Appropriate tissues from the endangered *T. c. attwateri* were unavailable for the allozyme analysis. The five buffer systems and tissue types (H = heart, L = liver) employed to visualize specific proteins (designated according to McAlpine et al. [1989] and followed by International Union of Biochemistry [1984] EC numbers) are indicated. **(1)** Amine-citrate (Clayton and Tretiak 1972): fumarate hydratase (L) (FH; 4.2.1.2); glucose dehydrogenase (L) (GDH; 1.1.1.47); glucose phosphate isomerase (L) (GPI; 5.3.1.9); glyceraldehyde-3-phosphate dehydrogenase (L) (GAPD; 1.2.1.12); αglycerol-3-phosphate dehydrogenase (L) (GPD1; 1.1.1.8); sorbitol dehydrogenase (L) (SORD; 1.1.1.14); and xanthine dehydrogenase (L) (XDH; 1.1.1.204). **(2)** Continuous tris citrate II (Selander et al. 1971): aconitase 1, soluble, aconitase 2, mitochondrial (L) (ACO1, ACO2; 4.2.1.3); glutamate dehydrogenase (L) (GLUD; 1.4.1.2); isocitrate dehydrogenase 1, soluble (L) (IDH1; 1.1.1.42); lactate dehydrogenase A (L) (LDHA; 1.1.1.27); malate dehydrogenase, NAD (soluble), malate dehydrogenase, NAD (mitochondrial) (H) (MDH1, MDH2; 1.1.1.37); phosphogluconate dehydrogenase (L) (PGD; 1.1.1.44); and phosphoglucomutase 1,2 (L) (PGM1, PGM2; 5.4.2.2). **(3)** Lithium hydroxide (Selander et al. 1971): esterase (L) (ES; 3.1.1.1). **(4)** Poulik (Poulik 1957): albumin (L) (ALB); mannose phosphate isomerase (L) (MPI; 5.3.1.8); nucleoside phosphorylase (L) (NP; 2.4.2.1); dipeptidase A, C (L) (PEPA, PEPC; 3.4.13.11); peptidase E (L) (PEPE; 3.4.11.1); and superoxide dismutase 1, soluble (L) (SOD1; 1.15.1.1). **(5)** Tris-maleate (Manlove et al. 1975): adenosine deaminase (L) (ADA; 3.5.4.4); creatine kinase, brain form, creatine kinase, muscle form (L) (CKBB, CKMM; 2.7.3.2); glutamic-oxaloacetic transaminase 1, soluble, and glutamic-oxaloacetic transaminase 2, mitochondrial (L) (GOT1, GOT2; 2.6.1.1).

The common allele at each locus was designated 100 or −100 to denote respective anodal or cathodal migration. Other alleles were labeled numerically by expressing the electrophoretic mobility of their protein products relative to the common allele. Indices of diversity and polymorphism including mean heterozygosity over all loci ($\bar{H}$) and the percentage of polymorphic loci (P) were calculated from the allozyme data. Genetic distances among populations and among species were calculated according to Rogers (1972), and patterns of similarity based on the genetic distances were delineated by UPGMA cluster analysis (Sneath and Sokal 1973).

Results

Mitochondrial-DNA differentiation.—From fragment profiles with comigrating molecular size standards, we estimated the length of the mtDNA molecule in prairie grouse to be 16,600 base pairs (bp), which is typical for avian species (Shields and Helm-Bychowski 1988). All individuals possessed a single mtDNA haplotype and no length variation was observed. Sixteen composite haplotypes (Table 1) each consisting minimally of 122 inferred sites were identified. Of the 12 six-base-recognizing endonucleases, 7 produced only one fragment profile in all individuals. The remaining 5 six-base-cutting enzymes, as well as all four-base-recognizing

TABLE 1. Mitochondrial DNA haplotypes in prairie grouse generated by 12 six-base-recognizing and 4 four-base-recognizing restriction enzymes. Fragment patterns for each restriction endonuclease designated alphabetically and combined as composite haplotypes across all restriction enzymes. Consecutive alphabetical designations do not imply genetic relatedness.

n	Composite haplotype[a]
	T. cupido
8	1 AAAAAAAAAAAA AAAA
6	2 ....B
1	3 .....B..... ...B
1	4 ..... .B..
1	5 ..... B...
2	6 .....C..... ...B
1	7 .....B..... ....
1	8 .....B..... ..CB
1	9 .B..... ..D.
1	10 .B..... ....
	T. pallidicinctus
2	1 ....
3	10 .B..... ....
1	11 .B..... ..B.
	T. phasianellus
17	1 ....
1	12 .....B... ....
1	13 ..B..... ....
1	14 .....C... ....
1	15 ..... C...
1	16 ....B..... ....

[a] Composite haplotypes numbered consecutively. Letters within haplotypes from left to right represent fragment profiles for following restriction endonucleases: (six-base-recognizing enzymes) *Bam*H I, *Cla* I, *Dra* I, *Eco* RI, *Eco* RV, *Hin*d III, *Nde* I, *Pst* I, *Ssp* I, *Sst* I, *Stu* I, *Xba* I; (four-base-recognizing enzymes) *Hha* I, *Msp* I, *Rsa* I, *Taq* I.

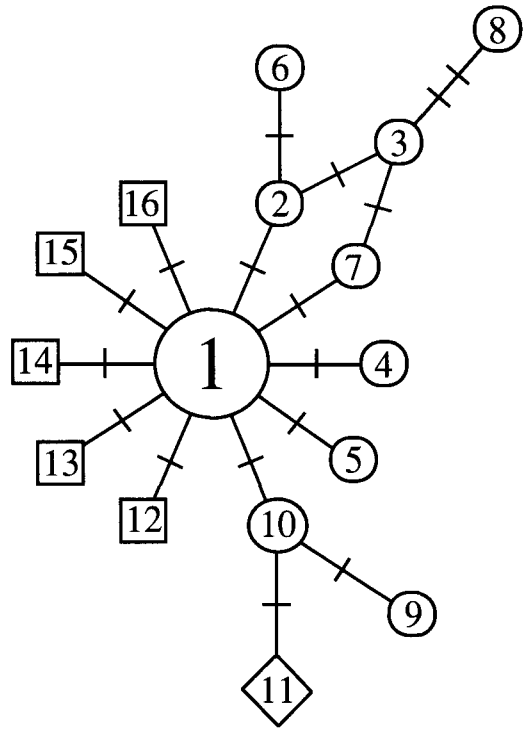

Fig. 1. Parsimony network interrelating all mtDNA haplotypes observed in prairie grouse. Haplotypes identified by number as in Table 1. Haplotypes shared among taxa (1 and 10) indicated by circles; those unique to a given taxon are depicted symbolically: oval, *T. cupido*; diamond, *T. pallidicinctus*; square, *T. phasianellus*. Cross bars on lines interconnecting haplotypes show inferred number of restriction-site differences (gains or losses) between them.

endonucleases, generated more than one pattern.

The HEURISTIC-SEARCH option in PAUP for the most-parsimonious relationship(s) among the haplotypes determined six equally parsimonious trees of 16 steps (consistency index = 0.938). In the minimum-mutation network (Fig. 1), the majority of haplotypes, whether species specific or shared among taxa, differed from a prevalent haplotype by a single mutational event (a restriction-site gain or loss). The central haplotype in Figure 1 (number 1) predominated in abundance and geographic distribution. This particular haplotype was common to all three prairie-grouse species, identified at most localities, and present in 53% of the 51 individuals examined. A second haplotype (number 10) was shared by *T. cupido* and *T. pallidicinctus* in Kansas, but the others were unique to a given taxon (Fig. 2).

Prairie-grouse mtDNA haplotypes were not partitioned along species boundaries. Estimates of nucleotide-sequence divergence within species exceeded interspecific values in many instances. The percent sequence divergence among all haplotypes averaged 0.22 and ranged from 0.07 to 0.54. The minimum sequence divergence between two haplotypes unique to different taxa (0.14%) was very low even by avian standards. The extent of differentiation was greater among haplotypes unique to *T. cupido* (P averaged 0.25%) than among haplotypes restricted to *T. phasianellus* (P = 0.15%). *Tympanuchus cupido* also possessed greater nucleon diversity and nucleotide diversity than *T. phasianellus* (Table 2).

The level of mtDNA differentiation in prairie grouse indicated a high degree of similarity between geographically-distant conspecific pop-

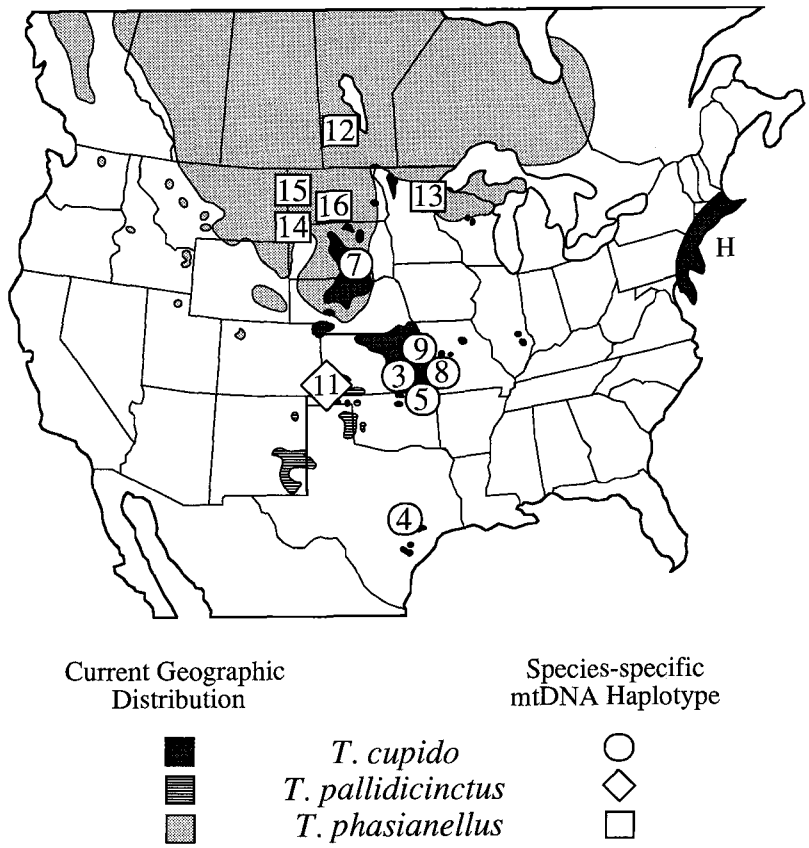

Fig. 2. Geographic orientation of species-specific mtDNA haplotypes in prairie grouse. Haplotypes identified by number as in Table 1. Haplotypes 2 and 6 (not shown) were observed in more than one *T. cupido* population. Original range of extinct Heath Hen (*T. c. cupido*) indicated along northeastern Atlantic coast (H).

ulations, as well as between groups of populations belonging to different species. The G_{ST} statistic (0.282) representing the proportion of mtDNA variation attributable to genetic differences among localities did not exceed 95% of the G_{ST} values derived from the permutation tests. Therefore, we did not observe geographic subdivision for mtDNA among prairie-grouse populations and detected no quantitative differentiation among the putative species.

Allozyme variation.—Six of the 30 allozyme loci exhibited variation in prairie grouse. Levels of heterozygosity and polymorphism within species (Table 2) were within the range of values reported for other galliform birds (Corbin 1987, Gutiérrez et al. 1983, Zink et al. 1987, Ellsworth et al. 1989). No fixed allelic differences among the taxa were observed. Overall, the distribution and frequency of alleles across all prairie grouse were similar (Table 3, Fig. 3). However, several low-frequency alleles at specific loci were absent from particular species. Although *T. cupido* and *T. pallidicinctus* were more similar to each other ($D = 0.011$) than either was to *T. phasi-*

TABLE 2. Indices of mtDNA and nuclear-gene diversity in prairie grouse.

Taxon	mtDNA		Allozymes	
	$\hat{h} \pm$ SE	$\pi \pm$ SE[a]	$\bar{H} \pm$ SE	P
T. cupido	0.826 ± 0.059	0.0013 ± 0.0005	0.039 ± 0.017	20.0
T. pallidicinctus	0.733 ± 0.155	0.0007 ± 0.0006	0.054 ± 0.026	20.0
T. phasianellus	0.411 ± 0.131	0.0004 ± 0.0003	0.046 ± 0.020	20.0

[a] π values (and SEs) calculated using the SEND program written by L. Jin (Nei and Jin 1989).

TABLE 3. Allele frequencies at polymorphic allozyme loci in prairie grouse.

Allele	Taxon (*n*)		
	T. cupido (25)	*T. pallidicinctus* (13)	*T. phasianellus* (22)
		***ACO* 1**	
121	0.040	0.038	0.227
100	0.960	0.962	0.773
		ADA	
125	—	—	0.045
100	0.920	0.923	0.955
74	0.080	0.077	—
		***IDH* 1**	
185	0.318	0.308	0.150
100	0.682	0.692	0.850
		MPI	
106	0.040	—	0.068
100	0.940	0.731	0.864
93	0.020	0.269	0.068
		PEPC	
104	0.040	0.077	0.091
100	0.800	0.846	0.841
98	0.040	—	0.045
93	0.120	0.077	0.023
		PGD	
117	0.020	0.038	—
100	0.940	0.962	0.977
80	0.040	—	0.023

anellus ($D = 0.020$ and 0.023, respectively), conspecific populations did not cluster together in UPGMA analysis (Fig. 4).

DISCUSSION

The most striking aspects of genetic differentiation in the prairie-grouse complex were the absence of phylogenetic resolution among the mtDNA haplotypes, and lack of association between allozyme genotype and the putative species. We observed no clear distinctions between taxa for either mtDNA or allozymes. For example, some mtDNA haplotypes observed only in *T. cupido* were more closely related to lineages unique to *T. phasianellus* than they were to other *T. cupido* haplotypes. Additionally, allozymes could not distinguish populations belonging to different species. The pattern of mtDNA differentiation in prairie grouse was similar to that observed in other birds such as the Red-winged Blackbird (*Agelaius phoeniceus*; Ball et al. 1988) and Common Grackle (*Quiscalus quiscula*; Zink et al. 1991). The limited degree of mtDNA differentiation in these species was attributed to recent and extensive range expansion, life histories conducive to dispersal, and the absence of long-term zoogeographic barriers to movement that mask the relationship between phylogeny and geography (category IV; Avise et al. 1987, Avise 1989). However, Red-winged Blackbirds and Common Grackles exhibit greater dispersal tendencies than prairie grouse. Banding-recovery data indicate that 85–90% of young Red-winged Blackbirds and Common Grackles may disperse as much as 60 km, and the remainder may potentially move much farther (over 700 km; Moore and Dolbeer 1989). In contrast, dispersal distances in prairie grouse are typically less than 7 km (Hamerstrom and Hamerstrom 1951, Copelin 1963, Robel et al. 1970, 1972). Additionally, the range of prairie grouse has diminished significantly in recent times because of human activity (Johnsgard and Wood 1968).

For prairie grouse, there are two primary hypotheses that may account for the high degree of genetic similarity among taxa despite marked morphological and behavioral differences. Hypothesis one posits that differentiation among prairie grouse occurred in geographic isolation during Pleistocene glacial advances, but genetic evidence of such an event has been lost upon secondary contact due to hybridization (past or on-going) that has facilitated gene flow and introgression among taxa. The second hypothesis predicts that subdivision among prairie grouse occurred during the Wisconsin (Hubbard 1973), which was sufficiently recent such that all populations retain (share) ancestral genetic polymorphisms that arose prior to divergence. Morphological and behavioral differentiation among species, therefore, may have proceeded rapidly relative to the rate of genetic change.

We acknowledge that sporadic hybridization among prairie grouse does occur, but we do not believe it is a significant factor in determining the high degree of genetic similarity among taxa or their phylogenetic status. *Tympanuchus cupido* and *T. phasianellus* were largely allopatric throughout most of their original (pre-settlement) ranges (Coues 1874) with only narrow zones of sympatry (Leopold 1931). The minor geographic overlap coupled with ecological separation in areas of sympatry suggest very limited pre-settlement hybridization between *cupido* and *phasianellus*. With the settlement of

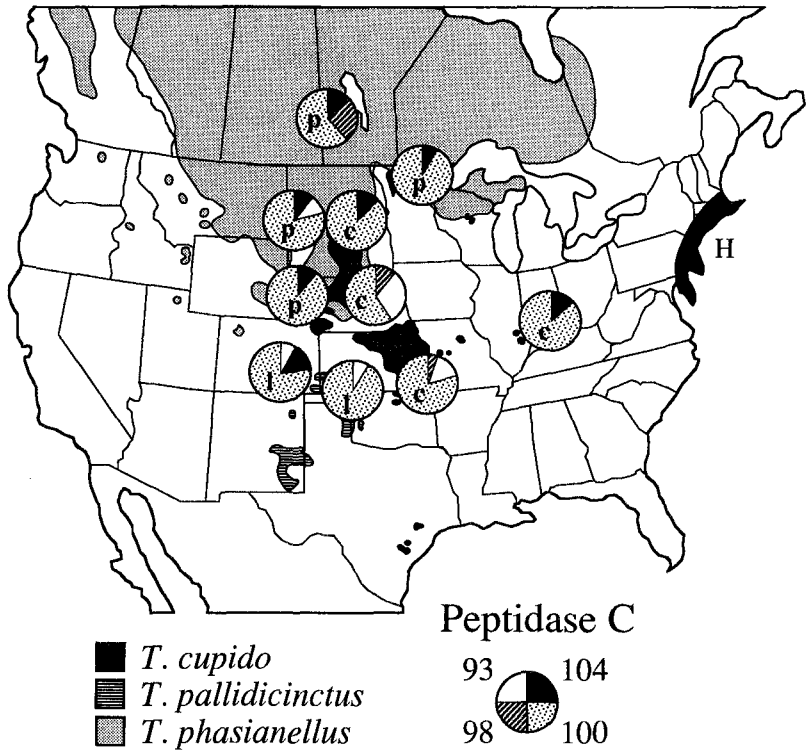

Fig. 3. Geographic distribution of allele frequencies at peptidase c locus in prairie grouse. Allele frequencies expressed as proportional area of circle occupied. Species-specific populations denoted by letters within each pie diagram: (c) *T. cupido*; (l) *T. pallidicinctus*; (p) *T. phasianellus*.

the prairie, agricultural practices and development have increased the incidence of interspecific hybridization (Bent 1932, Evans 1966, Johnsgard and Wood 1968), which may have facilitated recent gene flow and introgression among taxa. However, we sampled localities far from potential zones of hybridization such as Colorado and Quebec, Canada for *T. phasianellus*, and the Attwater Prairie-Chicken subspecies of *T. cupido* in Texas. Due to our findings that (1) a mtDNA haplotype common to all three species occurred at most localities (including geographically distant sites), (2) many unique haplotypes were present within species, and (3) certain low-frequency alleles were absent from particular taxa, we believe hybridization and current gene flow have had little effect on the overall pattern of genetic differentiation in prairie grouse.

The genetic data do not provide evidence of an early Pleistocene separation of sufficient duration to allow a taxonomically or geographically ordered pattern of genetic variation to emerge. The concept of historical refugia predicts that haplotype diversity within a historically isolated assemblage will be drastically reduced (Ferris et al. 1983) due to substantial fluctuations in population size and the significance of effective population sizes in models of stochastic mtDNA lineage survivorship (Av-

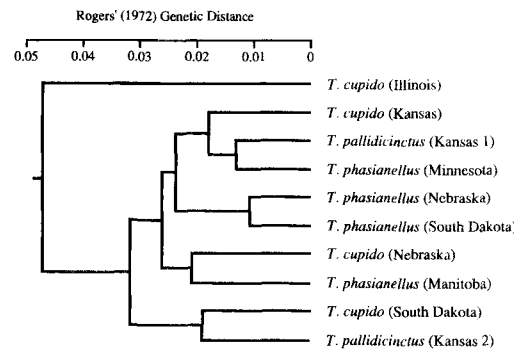

Fig. 4. UPGMA dendrogram depicting allozyme similarity among populations of prairie grouse. *Tympanuchus pallidicinctus* collected from: (1) Clark Co., Kansas; and (2) Morton Co., Kansas. Cophenetic correlation was 0.856.

ise et al. 1984). If prairie grouse were subdivided during the early Pleistocene, we may expect stochastic lineage loss to have occurred within isolated refugia and an accumulation of genetic differences between separated populations (taxa). However, mtDNA variation in prairie grouse (Fig. 1) was characterized by a predominant haplotype shared by all taxa and a number of others that differed from the common haplotype by single mutational events. The pattern of differentiation suggests that the less-frequent lineages were derived from the prevalent and presumably ancestral lineage. The putative ancestral haplotype was likely present in prairie grouse prior to subdivision because it is broadly distributed geographically and is shared by all taxa. Most of the infrequent haplotypes were species-specific, indicating that they may have arisen after "speciation."

The presence of species-specific mtDNA haplotypes and absence of several allozyme alleles from certain taxa imply a degree of isolation and restriction to gene flow among the species. However, we detected no clear phylogenetic resolution among the mtDNA haplotypes and no congruence between allozyme variation and species designations. These observations suggest subdivision among taxa may have occurred relatively recently. Recently speciated taxa may exhibit a pattern of mtDNA differentiation where sequence divergence within species exceeds interspecific values because mtDNA reflects change that has accumulated since the time of last common female ancestor irrespective of the time since speciation (Avise et al. 1983). It is highly probable that species defined by morphological characteristics will appear polyphyletic in terms of mtDNA lineages for many generations following a speciation event (Avise et al. 1984, Neigel and Avise 1986). Therefore, prairie-grouse mtDNA lineages may not have had sufficient time to achieve a condition of reciprocal monophyly (i.e. to sort phylogenetically) such that each taxon constitutes a distinct lineage.

Correspondence between estimated times of divergence among the prairie-grouse mtDNA lineages and the timing of Wisconsin glaciation would be consistent with Hubbard's (1973) vicariance-speciation hypothesis. However, predicted dates of geological events in earth's history are merely approximations, and calibrations of a molecular clock for mtDNA in birds (Shields and Wilson 1987) permit only crude estimates of divergence times among lineages. The differences among mtDNA haplotypes in prairie grouse may reflect evolutionary change occurring both prior and subsequent to "speciation," making it difficult to estimate accurately the time since divergence among *Tympanuchus* species. Nevertheless, the greatest nucleotide-sequence divergence values suggest that the maximum time elapsed since divergence among lineages has been approximately 270,000 years. More recent "speciation" is highly likely because the greatest sequence divergence values reflect intraspecific differences. Thus, the mtDNA data are roughly consistent with a late Pleistocene separation among the taxa.

Although prairie grouse are genetically similar and appear to have been subdivided recently, considerable morphological and behavioral differentiation is apparent among the species, particularly between the "prairie chickens" and *T. phasianellus*. Prairie grouse are sexually dimorphic and males exhibit complex stereotyped behaviors associated with reproduction (Johnsgard 1983). Adult male plumage, vocalization structures, and courtship behaviors comprise the primary differences among the taxa. Thus, several explanations may account for the morphological and behavioral divergence among prairie grouse despite the absence of diagnostic genetic differences. First, morphological and behavioral differentiation may be attributable to sexual selection. Male prairie grouse aggregate and aggressively defend small display territories (leks; Hamerstrom and Hamerstrom 1973, Ballard and Robel 1974) and overtly compete with one another for mates by ritualized display behaviors that include tail fanning, erection of specialized neck feathers (pinnae), and vocalizations produced by inflatable esophageal air sacs (Sharpe 1968, Hjorth 1970). Dominant males occupy the central territories on the "booming/dancing grounds" and participate in the great majority of matings (Ballard and Robel 1974). The display structures of males form the basis for taxonomic distinctions among prairie grouse, but considering their prominent role in reproduction, they may be subject to sexual selection and thus may evolve rapidly. Second, the morphological attributes that differ among taxa may, to an extent, be ecophenotypic. James (1983) has demonstrated a nongenetic component to geographic character variation in Red-winged Blackbirds. As prairie-grouse taxa have distinctly different

habitat requirements (Johnsgard 1983), morphological differentiation may, in part, reflect adaptation to regional environmental conditions. A third possibility is that genes responsible for morphology and behavior evolve much more rapidly than mtDNA (or allozymes). Racial differentiation in color and size of House Sparrow (*Passer domesticus*) populations has been shown to occur extremely rapidly (Johnston and Selander 1964). Similarly, geographic differentiation in prairie grouse may have occurred over a time span that was too short for mtDNA restriction site or isozyme differences to accrue.

In summary, the genetic data were not consistent with a Pleistocene vicariance of sufficient duration to allow substantial genetic differentiation between isolated populations (species) to accumulate. However, the presence of unique mtDNA haplotypes within species and absence of specific alleles from particular taxa imply some degree of separation. Mitochondrial-DNA and allozyme variation in prairie grouse suggest that gene flow is not responsible for the high degree of similarity among taxa or the absence of geographically and taxonomically structured patterns of genetic differentiation. Rather, recent population fragmentation and isolation may account for the lack of clear genetic distinctions among species. Morphological and behavioral differentiation among prairie grouse has probably been driven by sexual selection and appears to have progressed rapidly relative to either mtDNA or allozymes.

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