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Recent Range Expansion and Divergence among North American Prairie Grouse

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Abstract

Prairie grouse (genus: *Tympanuchus*) once existed throughout much of North America but have recently experienced significant population declines, isolation, and extinction. In previous molecular studies, contrasting patterns or an unresolved polytomy among *Tympanuchus* taxa (*Tympanuchus phasianellus*, *Tympanuchus pallidicinctus*, and *Tympanuchus cupido*) have resulted from traditional phylogenetic methods. As an alternative approach, the timing of expansion and the demographic processes that may have lead to this association among haplotypes, namely incomplete lineage sorting or migration, were explicitly investigated by comparing pairwise mitochondrial DNA control region nucleotide differences and through the use of a isolation with migration coalescent model. The timing of geographic expansion and population divergence time estimates generated under these models support previous inferences that *Tympanuchus* experienced a rapid expansion and diversification in the late Pleistocene 10,000–80,000 years before present. Further, morphological and behavioral differences originally used to describe *Tympanuchus* species were substantiated with little or no migration identified since population divergence. However, estimates of population divergence and migration between a number of morphologically similar subspecific taxa, including the greater prairie chicken (*Tympanuchus Cupido pinnatus*), the endangered Attwater's prairie chicken (*Tympanuchus Cupido attwateri*), and the extinct heath hen (*Tympanuchus Cupido cupido*), suggest these taxa are as differentiated with each other as they are from other *Tympanuchus* species. This information will prove useful in conservation efforts by providing estimates of demographic history that have helped shape the evolutionary relationships among *Tympanuchus* grouse.

Determining evolutionary relationships among populations and their association with taxonomy have important implications in conservation (Purvis et al. 2005; Haig et al. 2006). In avian systematics, in particular, the identification of unique morphological (e.g., plumage) and behavioral characters has played a predominant role historically in delimiting species level taxonomy (Watson 2005). In the absence of any substantial differences in these characters, however, we can only assume that species share a more recent common ancestry, and in these cases, molecular data can improve our ability to identify independently evolving lineages. A common approach in molecular phylogenetics is to use the criterion of monophyly to describe evolutionary distinct groups or species (i.e., genealogical species concept), by which sufficient time has passed for all individuals to share a common ancestor before any coalescent events with individuals from a different species (Baum and Shaw 1995; Avise 2004). The absence of reciprocal monophyly, however, does not necessarily imply the lack of divergence between populations because the rate of lineage sorting is dependent on the population size (N_e) with time to complete sorting expected to take $4N_e$ generations for neutral mitochondrial loci (Hudson and Coyne 2002; Rosenberg 2003; see also Omland et al. 2006).

Our understanding of the evolutionary relationships among North American prairie grouse (*Tympanuchus*) based on molecular sequence data remains unclear due to the lack of reciprocal monophyly among recognized species (Lucchini et al. 2001; Dimcheff et al. 2002; Drovetski 2002, 2003). This uncertainty may hinder conservation efforts. Three species of prairie grouse are recognized and once existed throughout much of North America but have recently experienced significant population declines, isolation, and extinction (Johnsgard 2002; Figure 1). Sharp-tailed grouse (*Tympanuchus phasianellus*) possess the largest geographic range in this genus and consist of 7 subspecies with recent declines largely in central and southern portions of its range (Connelly et al. 1998). The greater prairie chicken (*Tympanuchus cupido pinnatus*) including 2 additional subspecies, the federally endangered Attwater's prairie chicken (*Tympanuchus cupido attwateri*) and the extinct heath hen (*Tympanuchus cupido cupido*), currently occupy a small

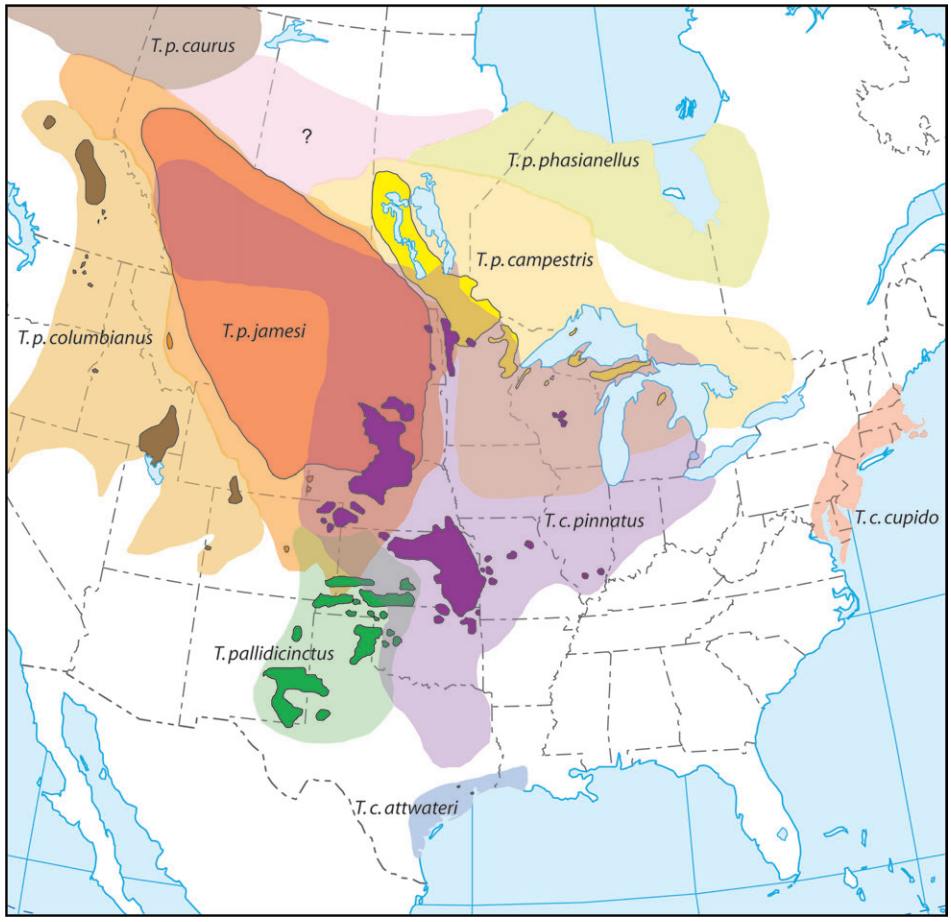

Figure 1. Historic and contemporary (outlined in black) distributions of *Tympanuchus* grouse (sharp-tailed grouse, *Tympanuchus phasianellus*; greater prairie chicken, *Tympanuchus cupido*; and lesser prairie chicken, *Tympanuchus pallidicinctus*). All identified species and subspecies taxa in this figure were included in this study, with the exception of the 2 *T. phasianellus* subspecies, *Tympanuchus phasianellus caurus*, and *Tympanuchus phasianellus phasianellus*. The question mark (?) identified in the northern distribution of *T. phasianellus* indicates an area that has not been assigned subspecific status.

fraction of their historical range (Schroeder and Robb 1993). Similarly, the lesser prairie chicken (*Tympanuchus pallidicinctus*, Giesen 2005) has declined in abundance and has been listed since 1998 as "warranted but precluded" federally threatened species status (63 FR 31400; United States Fish and Wildlife Service). All 3 *Tympanuchus* species have distinct morphological (plumage and body size), behavioral, and ecological characteristics; whereas designations for subspecies have been largely based on geography (Johnsgard 2002).

Despite their morphological and behavioral differences, accurate inferences of evolutionary relationships among *Tympanuchus* taxa have not been obtained based on single-gene tree approximations (Ellsworth et al. 1994; Gutiérrez et al. 2000; Lucchini et al. 2001; Dimcheff et al. 2002; Drovetski 2002, 2003). In fact, multiple studies have identified shared mitochondrial DNA (mtDNA) control region haplotypes among *Tympanuchus* species (Palkovacs et al. 2004; Johnson and Dunn 2006; Spaulding et al. 2006; see Supplementary Material). This incongruence among studies and the lack of reciprocal monophyly among taxa is likely due to a recent diversification coupled with large ancestral effective population sizes and the retention of ancestral polymorphisms (Arbogast et al. 2002; Hudson and Coyne 2002; Rosenberg 2003). Therefore, approaches based on coalescent theory should provide more robust estimates of divergence time between *Tympanuchus* taxa because they do not require that lineage sorting has reached completion (Hey and Machado 2003).

Here, I estimate the timing of geographic expansion and population divergence within *Tympanuchus* based on the distribution of pairwise mtDNA control region nucleotide differences among individuals (Rogers and Harpending 1992) and by using a coalescent method that accounts for factors associated with lineage sorting and gene flow (Nielsen and Wakeley 2001). Given the significant decline and extinction of populations among all recognized *Tympanuchus* grouse, estimates of population divergence

Table 1. mtDNA control region haplotype (*h*) and nucleotide (π) diversity estimates for *Tympanuchus* grouse populations

Population	*n*	Number of haplotypes	*h* ± SE	π ± SE
Tympanuchus cupido pinnatus[a,b]				
Nebraska	20	15	0.963 ± 0.006	0.010 ± 0.000
Kansas	20	11	0.858 ± 0.015	0.010 ± 0.000
Oklahoma	10	6	0.889 ± 0.024	0.012 ± 0.001
South Dakota	20	14	0.958 ± 0.006	0.012 ± 0.000
Tympanuchus cupido attwateri[b]				
Texas	19	11	0.912 ± 0.011	0.009 ± 0.000
Tympanuchus cupido cupido[c]				
Martha's Vineyard	19	3	0.205 ± 0.027	0.002 ± 0.000
Tympanuchus palladicinctus[d]				
New Mexico	63	9	0.828 ± 0.003	0.009 ± 0.000
Oklahoma	62	22	0.945 ± 0.001	0.014 ± 0.000
Tympanuchus phasianellus campestris[e]				
Minnesota	11	9	0.964 ± 0.015	0.008 ± 0.000
Tympanuchus phasianellus jamesi[e]				
South Dakota/ Nebraska	15	10	0.933 ± 0.012	0.007 ± 0.000
Tympanuchus phasianellus columbianus[e]				
B.C./Washington	15	7	0.724 ± 0.031	0.005 ± 0.000

SE, standard error.

[a] Johnson et al. (2003).

[b] Johnson et al. (2007).

[c] Johnson and Dunn (2006).

[d] Van Den Bussche et al. (2003).

[e] Spaulding et al. (2006).

time and gene flow are important for conservation and management purposes. In particular, the Attwater's prairie chicken is on the verge of extinction with less than 50 birds currently in the wild. Since being listed as federally endangered (32 FR 4001) in 1967, there has been no improvement on levels of abundance and managers are considering outcrossing Attwater's with the greater prairie chicken, its conspecific, in hopes to improve survivorship (Silvy et al. 2004; Morrow M and Rossignol T, personal communication). Therefore, the information obtained by resolving the demographic history among *Tympanuchus* grouse is necessary to better inform those involved in their conservation.

Materials and Methods

Samples

A 384 base-pair fragment from Domain I of the mtDNA control region was analyzed in 274 individuals representing all species and the majority of subspecies within the genus *Tympanuchus* (Table 1; 3 subspecies of *T. phasianellus* were not included in this study). DNA sequences were either obtained through GenBank (see Supplementary Material for accession numbers) or directly from the primary author of each study. For *T. c. attwateri* and *T. c. cupido* populations, DNA sequences were used from birds sampled prior to their decline in abundance (1854–1948) in Texas and 30–40 years prior to their extinction in 1932 on Martha's Vineyard, Massachusetts, respectively (Johnson and Dunn 2006; Johnson et al. 2007). Two heath hen samples that were included in Johnson and Dunn (2006) were excluded from these analyses because results from the previous study suggested that the 2 museum specimens were either misidentified or the result of introgression with translocated greater prairie chickens to the East Coast in the late 1800s (see also Palkovacs et al. 2004). DNA sequences were used from 4 greater prairie-chicken populations in the core of their distribution with samples from Oklahoma (Osage County), Kansas (Wabaunsee County), Nebraska (Garfield County), and South Dakota (Fort Pierre National Grasslands; Johnson et al. 2003, 2007). For *T. pallidicinctus*, DNA sequences were obtained from 2 populations from New Mexico (Roosevelt County) and Oklahoma (Ellis County; Van Den Bussche et al. 2003). DNA sequences were also used from 3 populations of *T. phasianellus*, each representing a separate subspecies (*Tympanuchus phasianellus columbianus*, British Columbia and Washington; *Tympanuchus phasianellus jamesi*, South Dakota and Nebraska; and *Tympanuchus phasianellus campestris*, Minnesota; Spaulding et al. 2006).

Statistical Analyses

Levels of mitochondrial haplotype diversity (*h*) and nucleotide diversity (π) were calculated using the program DnaSP v. 4.10.4 (Rozas et al. 2003). Standard errors were estimated for both haplotype and nucleotide diversity measures for each population. To test for geographic expansion within each population, I used both Fu's (1997) test of neutrality (F_s), where large negative values of F_s indicated an excess of rare alleles often observed in expanding populations, and compared the observed distribution of pairwise nucleotide differences among individuals with that expected from a sudden population expansion model (mismatch distribution; Rogers and Harpending 1992). Both approaches were conducted using the program Arlequin v. 3.0 (Excoffier et al. 2005), with significance of F_s values and mismatch distributions evaluated with either 1000 random permutations or the proportion of simulations producing a larger sum-of-squared deviation (SSD) than the observed SSD, respectively. The raggedness index of the observed mismatch distribution was also estimated for each population and its significance determined similar to SSD as implemented in Arlequin.

The coalescent-based program MDIV was used to determine whether 2 populations possessed shared polymorphisms due to recent gene flow or incomplete lineage sorting following gene coalescence (see Nielsen and Wakeley 2001; Hey and Nielsen 2004). Using the basic isolation with migration model, the program jointly

estimates theta or the female effective population size scaled by the neutral mutation rate ($\theta = 2N_{ef}\mu$, where N_{ef} is the female effective population size and μ is the neutral mutation rate), symmetric gene flow ($M = N_{ef}m$, where m is the fraction of effective migrants per generation), population divergence time ($T = t_1/N_{ef}$, where t_1 is the divergence time in years before present [y.b.p.]), and time to most recent common ancestor (TMRCA = t_2/N_{ef}, where t_2 is the gene coalescence time in y.b.p.) for each pairwise comparisons between populations. A minimum of 2 independent runs (length of Markov chain = 3 000 000 cycles and burn-in = 200 000 cycles) with different random seeds were performed for each pairwise population comparison to check for convergence while using the finite sites mutation model (HKY) (Hasegawa et al. 1985). Values for M_{max} (5, 15, or 50) and T_{max} (1, 3, or 10) for each pairwise comparison were selected as those that generated a bell-shaped posterior distribution but minimized the relative number of data points in the upper portion of the curve (i.e., tail). Final point estimates of θ, M, and T were identified as the mode of their respective posterior distributions (Nielsen and Wakeley 2001), and credibility intervals (CIs) were calculated for θ and M but not for T because the upper portion of the curve for T slowly decreases to zero and, therefore, accurate CI estimates for this measure could not be determined. Furthermore, 10 of 38 population pairwise estimates of M, while using the maximum prior (M_{max}=50), never reached zero, and consequently, their upper credibility limits were undefined ($M > 50$), suggesting comparably higher levels of gene flow than those with defined upper CIs. Pairwise estimates of T and TMRCA were converted to y.b.p. since population divergence and gene coalescence, respectively, using an estimate for mutation rate per locus per year ($\mu = 5.635 \times 10^{-5}$; see Johnson et al. 2007) and a 1-year generation time for female prairie chickens.

The recently developed program IM (Hey and Nielsen 2004; Hey 2005) also uses the basic isolation with migration model similar to MDIV. However, IM differs by allowing separate estimates of θ for each population, including the ancestral population, and 2 estimates of direction gene flow to investigate asymmetric migration for each pairwise comparison (see Hey and Nielsen 2004). In contrast, these estimates with MDIV are assumed to be equal (e.g., $m_1 = m_2$), thereby reducing the number of parameters in the model (Nielsen and Wakeley 2001). Although, I recognize the importance of including additional parameters to investigate potentially complex demographic histories, the single-locus dataset used in this study limited the resolution by which the model could reflect the species' histories (see Knowles 2004). For example, not only did I have difficulty with convergence of parameters across multiple runs while using IM, in many cases, the posterior distributions for particular parameter estimates were flat and difficult to interpret or the CIs were much wider than those obtained using MDIV (data not shown). Given that the main difference between MDIV and IM pertains to latter's ability to include multiple independent loci, each with specific mutation scalars (see Hey and Nielsen 2004), this approach may be more appropriate with additional loci and, therefore, unsuitable with this particular dataset. For these reasons, I have reported only the results obtained using the program MDIV.

Results and Discussion

Estimates of population divergence among *Tympanuchus* grouse indicate this genus experienced a rapid expansion (Table 2) and diversification (Tables 3 and 4) in the late Pleistocene 10 000–80 000 y.b.p. (see also Ellsworth et al. 1994; Ross et al. 2006; Spaulding et al. 2006; Johnson et al. 2007). Given the similarity of population divergence time (T) and gene coalescence (TMRCA), along with low estimates of gene flow (M) observed between species pairs (Tables 3 and 4), these results support the majority of currently recognized species-level designations for *Tympanuchus*, with much of the observed genetic diversity in this genus present prior to the divergence of these taxa. Therefore, incomplete lineage sorting rather than recent gene flow is a more likely scenario for describing the association of the majority of haplotypes among *Tympanuchus* species and further explains why previous phylogenetic studies based on single-gene tree approximations were unable to identify monophyletic relationships despite morphological and behavioral differences between taxa (Ellsworth et al. 1994; Gutiérrez et al. 2000; Lucchini et al. 2001; Dimcheff et al. 2002; Drovetski 2002, 2003).

When ancestral haplotypes persist, it is difficult to determine whether populations differ because of differences in the levels of isolation or migration. A given level of population differentiation may be due to an ancient divergence followed by more recent exchange of genes, or it may simply reflect a recent divergence with little subsequent gene flow. The overall influence of recent glacial cycles on avian speciation during the late Pleistocene varies among studies (Klicka and Zink 1997; Avise and Walker 1998; Johnson and Cicero 2004; Weir and Schluter 2004; Lovette 2005); yet, an increasing number of studies has documented recent divergences (since the last glacial maximum) with rapid phenotypic diversification among avian taxa: juncos (*Junco* spp.; Milá, McCormack, et al. 2007), orioles (*Icterus* spp.; Baker et al. 2003; Kondo et al. 2004), yellow-rumped warblers (*Dendroica coronoata*; Milá, Smith, and Wayne 2007), redpolls (*Carduelis flammea*; Ottvall et al. 2002), and crossbills (*Loxia* spp.; Piertney et al. 2001; Parchman et al. 2006).

Following glacial retreat as populations expanded northward, a variety of unoccupied habitats with differing characteristics (e.g., Williams et al. 2004) would have allowed rapid diversification depending on the relative degree of isolation and selection (Hewitt 2001, 2004; Lessa et al. 2003). In a recent study, Spaulding (2007) indicated an increased evolutionary rate in the divergence of secondary sexually selected traits among *Tympanuchus* species likely influenced by a high variance in male mating success (i.e., lek

Table 2. Mismatch distribution and Fu's neutrality test (F_s) results for prairie grouse populations

Population	*n*	*Tau* (95% CI)[a]	Y.b.p.	Raggedness index[a]	F_s
Tympanuchus cupido pinnatus					
Nebraska	20	3.711 (1.643–5.350)	33 000	0.022	−9.337***
Kansas	20	5.389 (1.119–9.588)	48 000	0.038	−2.697 n.s.
Oklahoma	10	6.881 (1.447–11.295)	61 000	0.034	0.270 n.s.
South Dakota	20	4.957 (2.418–6.666)	44 000	0.023	−5.235**
Tympanuchus cupido attwateri					
Texas	19	4.236 (1.674–6.443)	38 000	0.036	−3.358*
Tympanuchus cupido cupido					
Martha's Vineyard	19	3.000 (0.346–3.000)	27 000	0.513	0.197 n.s.
Tympanuchus palladicinctus					
New Mexico	63	4.424 (0.969–7.783)	39 000	0.036	1.218 n.s.
Oklahoma	62	2.646 (0.541–14.051)	24 000	0.018	−4.465 n.s.
Tympanuchus phasianellus campestris					
Minnesota	11	3.230 (1.264–5.078)	29 000	0.140	−4.391**
Tympanuchus phasianellus jamesi					
South Dakota/Nebraska	15	2.188 (0.914–3.508)	19 000	0.168	−4.793**
Tympanuchus phasianellus columbianus					
B.C./Washington	15	2.172 (0.000–7.783)	19 000	0.046	−2.230*

Tau (τ) was converted to y.b.p. assuming female generation time equals 1 year. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; n.s., not significant.

[a] In all cases, the sudden expansion model could not be rejected, SSD and Raggedness indices $P > 0.05$.

breeding; see also Prum 1997; Panhuis et al. 2001; Svensson and Gosden 2007). The results reported in this study further support the role of recent postglacial range expansion and sexual selection in driving diversification and speciation among *Tympanuchus* grouse rather than due to the development of such differences while in separate glacial refugia (see also Milá, McCormack, et al. 2007).

Among subspecific comparisons within *Tympanuchus*, differing levels of differentiation and gene flow were also observed. All 3 subspecies of *T. cupido* (*T. c. cupido*, *T. c. attwateri*, and *T. c. pinnatus*) are as divergent from each other as they are from other *Tympanuchus* taxa (Tables 3 and 4; see also Palkovacs et al. 2004; Johnson and Dunn 2006). This suggests that despite their apparent morphological similarities, the timing of divergence among *T. cupido* subspecific groups also occurred at approximately the same time as that between other diagnostically distinct species, such as *T. phasianellus*, which until recently was placed in a monotypic genus (*Pedioecetes*; Connelly et al. 1998). The 3 subspecies of *T. cupido* are difficult to differentiate based on morphology; however, a few characters (e.g., tarsi plumage coverage and the number of pinnae feathers) have been suggested to differ between subspecies (Gross 1928; Johnsgard 2002), but additional work is needed to investigate these characters in more detail.

In addition, habitat characteristics differ among the 3 *T. cupido* subspecies. For example, *T. c. cupido* once occupied scrub oak barrens of historic East Coast prairie habitat, whereas *T. c. attwateri* and *T. c. pinnatus* prefer bluestem dominated tallgrass prairie in Midwestern states and the Great Plains, with *T. c. attwateri* historically restricted to the sandy costal plain of Texas and Louisiana (Schroeder and Robb 1993; Johnsgard 2002). Early attempts to establish *T. c. pinnatus* on the East Coast following the extinction of *T. c. cupido* on the mainland were unsuccessful despite thousands of birds being released as early as 1852 (Gross 1928; Phillips 1928). Whether this failure was due entirely to inadequate habitat availability or hunting pressures is not known. Based on estimates from MDIV, the *T. c. cupido* population from Martha's Vineyard was distinct and possessed a unique demographic history with minimal or no gene flow observed since the timing of population divergence $\geq$67 000 y.b.p. (Table 3; see also Palkovacs et al. 2004; Johnson and Dunn 2006), suggesting that this extinct taxon may warrant species status.

For *T. c. attwateri*, estimates of population divergence time appear to increase with geographic distance from populations of *T. c. pinnatus* in Oklahoma to South Dakota (Table 4). Despite overlapping CIs in some cases, gene flow estimates between these 2 taxa (M = 0.8–1.4) are consistently lower than those obtained among *T. c. pinnatus* population pairwise comparisons (M = 2.6–22.1), all of which had undefined upper 95% CIs for M (upper CI >50; Table 4). In contrast, levels of gene flow between *T. c. pinnatus* and 2 subspecies of *T. phasianellus* (*T. p. jamesi* and *T. p. campestris*) were similar, if not greater, than those estimated between *T. c. pinnatus* and *T. c. attwateri* populations. Hybrid *T. c. pinnatus* and *T. phasianellus* individuals have been documented (see Schroeder and Robb 1993; Johnsgard 2002, p. 96) in areas where their geographic distributions overlap, suggesting that the higher estimates of M between these taxa may be influenced by recent gene flow. Similarly, hybrid *T. c. pinnatus* and *T. pallidicinctus* individuals have also been identified in

Table 3. Population pairwise estimates of θ ($2N_{ef}\mu$; upper number) and gene flow ($N_{ef}m$; lower numbers in bold) above diagonal, and population divergence time ($N_{ef}T$; upper numbers in bold) and gene coalescent time (N_{ef}TMRCA; lower number) in y.b.p. below diagonal

	Tympanuchus cupido pinnatus (NE)[a,b]	*Tympanuchus cupido attwateri (TX)*[a,b]	*Tympanuchus cupido cupido* (MA)[a,b]	*Tympanuchus pallidicinctus* (OK)[a,c]	*T. pallidicinctus* (NM)[a,c]	*Tympanuchus phasianellus campestris* (MN)[a,d]	*Tympanuchus phasianellus jamesi* (SD)[a,d]	*Tympanuchus phasianellus columbianus* (BC)[a,d]
pinnatus (NE)[a,b]	—	5.64 (3.84–9.38)	4.77 (3.11–7.99)	9.59 (7.18–13.81)	4.65 (3.06–7.68)	7.25 (4.28–13.04)	6.50 (3.85–11.47)	5.57 (3.44–10.64)
		1.4(0.9–23.4)	**0.1(0.0–0.5)**	**0.7(0.4–2.0)**	**0.1(0.0–0.6)**	**3.3(1.9–udf)**	**3.3(1.65–udf)**	**0.5(0.1–4.6)**
attwateri (TX)[a,b]	**34 000**	—	3.37 (2.29–6.07)	8.33 (6.20–12.05)	3.88 (2.47–6.43)	9.91 (6.73–15.11)	5.39 (3.18–9.83)	4.17 (2.51–8.14)
	63 000		**0.1(0.0–0.8)**	**0.5(0.2–1.4)**	**0.2(0.0–0.8)**	**0.6(0.2–7.3)**	**1.6(0.6–25.6)**	**0.3(0.1–3.6)**
cupido (MA)[a,b]	**78 000**	**71 000**	—	7.33 (5.62–11.12)	2.93 (1.81–5.01)	3.30 (1.94–6.90)	3.21 (1.93–6.66)	2.06 (1.19–4.52)
	115 000	99 000		**0.1(0.0–1.1)**	**0.0(0.0–0.6)**	**0.0(0.0–0.7)**	**0.0(0.0–0.6)**	**0.0(0.0–0.5)**
pallidicinctus (OK)[a,c]	**68 000**	**37 000**	**70 000**	—	5.40 (3.77–8.39)	9.91 (6.73–15.11)	8.79 (6.22–13.91)	8.71 (6.14–13.71)
	105 000	97 000	109 000		**1.7(0.6–4.5)**	**0.5(0.2–1.7)**	**0.8(0.3–2.8)**	**0.6(0.2–2.1)**
pallidicinctus (NM)[a,c]	**66 000**	**24 000**	**69 000**	**8 000**	—	4.25 (2.74–7.39)	4.00 (2.53–6.68)	3.15 (2.02–5.67)
	94 000	80 000	107 000	90 000		**0.1(0.0–0.6)**	**0.1(0.0–0.6)**	**0.1(0.0–0.6)**
campestris (MN)[a,d]	**14 000**	**25 000**	**74 000**	**46 000**	**51 000**	—	6.02 (3.49–11.62)	4.61 (2.74–9.67)
	67 000	57 000	102 000	104 000	91 000		**24.4(2.8–udf)**	**0.3(0.1–5.6)**
jamesi (SD)[a,d]	**21 000**	**17 000**	**77 000**	**41 000**	**61 000**	**9 000**	—	4.23 (2.39–8.21)
	67 000	66 000	107 000	98 000	92 000	66 000		**1.8(0.8–udf)**
columbianus (BC)[a,d]	**24 000**	**21 000**	**67 000**	**40 000**	**50 000**	**25 000**	**15 000**	—
	54 000	51 000	94 000	99 000	80 000	49 000	63 000	

The numbers in parentheses are 95% CIs for the estimates of θ and gene flow; udf, undefined ($M > 50$).

[a] NE, Nebraska; TX, Texas; MA, Massachusetts; OK, Oklahoma; NM, New Mexico; MN, Minnesota; SD, South Dakota; and BC, British Columbia.

[b] *T. c. pinnatus*, greater prairie chicken; *T. c. attwateri*, Attwater's prairie chicken; and *T. c. cupido*, heath hen.

[c] *T. pallidicinctus*, lesser prairie chicken.

[d] *T. phasianellus*, sharp-tailed grouse and *T. p. columbianus*, Columbian sharp-tailed grouse.

Table 4. Attwater's (*Tympanuchus cupido attwateri*) and greater (*Tympanuchus cupido pinnatus*) prairie chicken pairwise estimates of θ (upper number) and gene flow (lower numbers in bold) above diagonal and estimates of population divergence (upper numbers in bold) and gene coalescent time (lower number) in y.b.p. below diagonal

	T. c. attwateri (TX)[a]	*T. c. pinnatus* (OK)[a]	*T. c. pinnatus* (KS)[a]	*T. c. pinnatus* (NE)[a]	*T. c. pinnatus* (SD)[a]
T. c. attwateri (TX)	—	4.19 (2.83–7.96) **0.8 (0.4–22.5)**	5.03 (3.23–8.15) **1.0(0.4–4.8)**	5.64 (3.84–9.38) **1.4(0.9–23.4)**	5.88 (4.14–9.98) **1.3(0.6–9.2)**
T. c. pinnatus (OK)	**14 000** 64 000	—	3.94 (2.40–6.76) **22.1 (5.9-udf)**	5.85 (3.91–10.18) **3.8(3.0-udf)**	5.66 (3.75–9.81) **2.6(1.4-udf)**
T. c. pinnatus (KS)	**18 000** 67 000	**400** 69 000	—	6.74 (4.75–11.23) **9.7(4.5-udf)**	5.62 (3.90–9.37) **7.6(5.9-udf)**
T. c. pinnatus (NE)	**34 000** 63 000	**2000** 70 000	**900** 72 000	—	7.55 (4.94-11.31) **4.0(3.3-udf)**
T. c. pinnatus (SD)	**39 000** 70 000	**7000** 73 000	**2000** 73 000	**9000** 82 000	—

The numbers in parentheses are 95% CIs for the estimates of θ and gene flow; udf, undefined ($M > 50$).

[a] TX, Texas; OK, Oklahoma; KS, Kansas; NE, Nebraska; and SD, South Dakota.

overlapping distributions in Kansas (Bain and Farley 2002); yet, estimates of gene flow between these 2 taxa are quite low ($M = 0.1–0.7$) and comparable with estimates obtained between subspecific *T. c. attwateri* and *T. c. pinnatus* populations ($M = 0.8–1.4$; Table 3).

Although gene flow between *T. c. attwateri* and *T. c. pinnatus* cannot be ruled out completely given the upper 95% CIs observed between populations (upper CI = 4.8–23.4; Table 4), the results do suggest that the historic rate of gene flow between these 2 taxa has been relatively low and similar, if not less, than that observed between populations from morphologically diagnosable species. Further, the *T. c. attwateri* samples used in this study were collected prior to their population decline in the latter half of the 20th century; thus, the effect of recent bottleneck events on reduced estimates of gene flow do not explain this difference. Comparable demographic estimates were obtained when using samples collected from predecline *T. c. pinnatus* populations (data not shown; see Johnson et al. 2007).

Among *T. phasianellus* population pairwise comparisons, differing divergence and migration estimates were obtained between subspecies. Population divergence times for *T. p. columbianus* relative to other *T. phasianellus* subspecies are 15 000 and 25 000 y.b.p., comparable with the timing of population divergence observed with *T. c. pinnatus* (24 000 y.b.p.; Table 3). In contrast, population divergence time for the 2 subspecies, *T. p. jamesi* and *T. p. campestris*, in the south central portion of the species' range is more recent (9000 y.b.p.) with much higher migration rates between populations ($M = 24.4$; Table 3), suggesting that separate subspecies designations may not be warranted for these 2 taxa (see also Spaulding et al. 2006).

Populations of *T. p. columbianus* have declined significantly over the past century (Connelly et al. 1998; Schroeder et al. 2000), and estimates of haplotype diversity are lower than other surveyed *Tympanuchus* populations (Table 1). Results of this study agree with those given by Spaulding et al. (2006) and suggest that this subspecies is distinct from other *T. phasianellus* subspecies (Table 3). However, the low gene flow estimates for these comparisons ($M = 0.3$ and 1.8) may be due to recent fragmentation and increased genetic drift similar to the sampled *T. pallidicinctus* populations in New Mexico and Oklahoma (see below). In a recent study using the program MDIV, Johnson et al. (2007) documented reduced levels of gene flow between *T. c. pinnatus* subpopulations in Wisconsin while using a temporal dataset collected before and after habitat fragmentation and population decline. Although, *T. p. columbianus* has witnessed a recent reduction in overall geographic distribution (see Figure 1), historical genetic diversity measures are not available to assess whether this population has experienced a recent bottleneck similar to Wisconsin's *T. c. pinnatus* population.

Population divergence time (8,000 y.b.p.) is also similar for the 2 *T. pallidicinctus* populations from New Mexico and Oklahoma to that observed between *T. c. pinnatus* populations (200–9,000 y.b.p.); however, gene flow was lower ($M = 1.7$ and 2.6–22.1, respectively) with non-overlapping 95% CIs in 3 of the 6 pairwise comparisons (Tables 3 and 4). This lower gene flow estimate may be due to the effects of recent genetic drift (see Johnson et al. 2007) as significant habitat fragmentation and increased isolation have developed between these 2 sampled populations. This species' historic distribution once extended through much of northwestern Texas into southeastern Colorado and western Oklahoma and Kansas (Figure 1); yet today, very few birds are observed between the 2 sampled locations (Van Den Bussche et al. 2003; Giesen 2005). These results suggest that gene flow is currently restricted between New Mexico and Oklahoma and concern exists that levels of genetic variability may decline due to reduced population size similar to *T. c. pinnatus* populations in Illinois (Bouzat et al. 1998) and Wisconsin (Johnson et al. 2003, 2004).

In conclusion, by taking into account the demographic processes that may have led to the distribution of haplotypes among sampled populations, inferences of demographic

history can be obtained to help in conservation efforts. This approach could be especially useful among taxonomic groups that have recently diverged since the last glacial maximum where ancestral polymorphisms exist and evolutionary relationships among taxa remain equivocal. For example, despite phenotypic similarities, *T. c. attwateri* and *T. c. cupido* appear as divergent from their conspecific, *T. c. pinnatus*, as they do from other *Tympanuchus* species. Therefore, given the overall morphological and behavioral differences observed between species, other adaptive characteristics may exist among *T. cupido* subspecies. Although it is too late to aid conservation efforts for extinct *T. c. cupido*, these results do caution against outcrossing *T. c. attwateri* with *T. c. pinnatus* without fully investigating additional differences that may exist between these 2 taxa (see Kawecki and Ebert 2004; Rader et al. 2005; Edmands 2007). Future studies should also incorporate multiple nuclear loci to investigate the demographic history of prairie grouse and thereby help alleviate any uncertainty (e.g., Carstens and Knowles 2007) due to the inherent stochasticity associated with both lineage sorting and sampling effects.

Supplementary Material

Supplementary materials can be found at http://www.jhered.oxfordjournals.org/.

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