EXPLORING THE EVOLUTIONARY HISTORY OF NORTH AMERICAN PRAIRIE GROUSE (GENUS: *Tympanuchus*) USING MULTI-LOCUS COALESCENT ANALYSES

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Conservation biologists are increasingly using phylogenetics as a tool to understand evolutionary relationships and taxonomic classification. The taxonomy of North American prairie grouse (sharp-tailed grouse, *T. phasianellus*; lesser prairie-chicken, *T. pallidicinctus*; greater prairie-chicken, *T. cupido*; including multiple subspecies) has been designated based on physical characteristics, geography, and behavior. However, previous studies have been inconclusive in determining the evolutionary history of prairie grouse based on genetic data. Therefore, additional research investigating the evolutionary history of prairie grouse is warranted. In this study, ten loci (including mitochondrial, autosomal, and Z-linked markers) were sequenced across multiple populations of prairie grouse, and both traditional and coalescent-based phylogenetic analyses were used to address the evolutionary history of this genus. Results from this study indicate that North American prairie grouse diverged in the last 200,000 years, with species-level taxa forming well-supported monophyletic clades in species tree analyses. With these results, managers of the critically endangered Attwater's prairie-chicken (*T. c. attwateri*) can better evaluate whether outcrossing Attwater's with greater prairie-chickens would be a viable management tool for Attwater's conservation.

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INTRODUCTION

Taxonomy plays an important role in how we manage species and populations (Agapow *et al.* 2004; Purvis *et al.* 2005). Not only is taxonomy used in prioritizing our efforts for protecting unique taxonomic groups (e.g., species or subspecies; Mace 2004), it also helps identify ways to prevent extinction by using information obtained from evolutionary relationships to improve conservation efforts (e.g., Bennett and Owens 1997; Johnson *et al.* 2007b; Purvis 2008). Therefore, taxonomy should reflect the evolutionary relationships among taxa with the timing of divergences among groups corresponding with general taxonomic organization (i.e., subspecies, species, genus, family, etc.) in order to be useful for informing management decisions. With that said, our ability to identify distinct taxonomic units relies heavily on our definition of species and the methods used for delineating taxonomic units.

While many biologists have debated species definitions over the last century, taxonomy is still largely based on characters such as morphology, behavior, and geography (Mayden 1997; Hey 2001; de Quieroz 2007). However, with improvements in obtaining DNA sequence data, reevaluating taxonomic limits using molecular approaches (i.e., the phylogenetic species concept; Cracraft 1983) has become standard practice. Indeed, genetic based phylogenetic trees helped to define limits among cryptic (Bickford *et al.* 2006), allopatric (Hung *et al.* 2013), and recently divergent groups (Baker *et al.* 2003; Milá *et al.* 2007; Jacobsen *et al.* 2010; Welch *et al.* 2011; McCormack *et al.* 2012) previously unknown to the scientific community.

The ability to use molecular data for identifying species groups is also controversial (Sites and Marshall 2004; Rubinoff *et al.* 2006; Knowles and Carstens 2007; Brito and Edwards 2009). Some biologists feel that reciprocal monophyly, or the forming of monophyletic clades based on a single-locus phylogenetic tree (i.e., gene tree), is appropriate for setting taxonomic limits (Zink 2004) while others question such practices (e.g., Haig *et al.* 2006; Carstens and Knowles 2007; Knowles and Carstens 2007; Rieppel 2010) and suggest that other criteria should also be evaluated. For example, in cases of recently divergent groups that possessed large effective historic population sizes, monophyly is unlikely given the amount of time that has passed, even if groups have been isolation for thousands of years (Hudson and Coyne 2002; Rosenberg 2003).

The genus *Tympanuchus* (i.e., prairie grouse) is one such species group where current taxonomy and evolutionary relationships remain unresolved. In this genus, a discord exists between the obvious differences among species (i.e. morphology and behavior) and the molecular sequence data used to construct phylogenies. In all gene trees published to date, *Tympanuchus* species are polyphyletic (Ellsworth *et al.* 1994; Gutiérrez *et al.* 2000; Lucchini *et al.* 2001; Dimcheff *et al.* 2002; Drovetski 2002 and 2003; Johnson 2008; Oyler-McCance *et al.* 2010). This disagreement between morphology and the DNA used to produce molecular phylogenies is largely due to recent diversification (<400,000 years) coupled with large historic effective population size (i.e., incomplete lineage sorting and retention of ancestral polymorphisms; see Johnson 2008). Strong sexual selection observed in prairie grouse (i.e., the lek breeding system; Höglund and Alatalo 2005; Nooker and Sandercock 2008; Behney *et al.* 2012)

may have contributed to the rapid diversification of morphological characteristics seen in prairie grouse today (Spaulding 2007; Oyler-McCance *et al.* 2010).

Currently there are three species within the genus *Tympanuchus* that are morphologically, behaviorally, and geographically distinct (Fig. 1, Table 1; Johnsgard 2002): the greater prairie-chicken (*T. cupido;* Johnson *et al.* 2011), lesser prairie-chicken (*T. pallidicinctus;,* Hagen and Giesen 2005), and sharp-tailed grouse (*T. phasianellus*; Connelly *et al.* 1998). Seven sharp-tailed grouse and three greater prairie-chicken subspecies are also recognized, with subspecies limits largely defined based on geography and slight differences in morphology (Johnsgard 2002).

Fig. 1 Male prairie grouse in breeding display. From left to right: greater prairie-chicken, lesser prairie-chicken, and sharp-tailed grouse.

Table 1 Phenotypic variation among species-level taxa in the genus *Tympanuchus* (Connelly *et al.* 1998; Hagen *et al.* 2005; Johnson *et al.* 2011).

Species	Habitat Type	Pinnae Feathers	Airsac Color	Tail Shape	Avg. Body Length	Avg. Body Weight
Greater prairie-chicken	Tall grass prairie	Yes	Yellow-orange	Square	43 cm	700-1200 g
Lesser prairie-chicken	Short-grass shrub prairie	Yes	Red	Square	38-41 cm	630-813 g
Sharp-tailed grouse	Steppe-prairie	No	Purple	Pointed	38-48 cm	548-1031 g

Prior to European settlement, prairie grouse distributions were widespread throughout prairie-like ecosystems in North America (Fig. 2; Johnson 2008). Sharp-tailed grouse have the largest and northernmost distribution, inhabiting steppe-prairie habitats of the intermountain west and north central Great Plains (Connelly *et al.* 1998; Johnsgard 2002; Spaulding *et al.* 2006). Lesser prairie-chickens are native to the drought-tolerant short-grass sandhills of south central United States (Hagen *et al.* 2004; Hagen and Giesen 2005). Greater prairie-chickens prefer the tall grass prairies in central North America, with the heath hen and Attwater's prairie-chicken inhabiting the scrub-oak savannahs of New England and the coastal prairies of Texas and Louisiana,

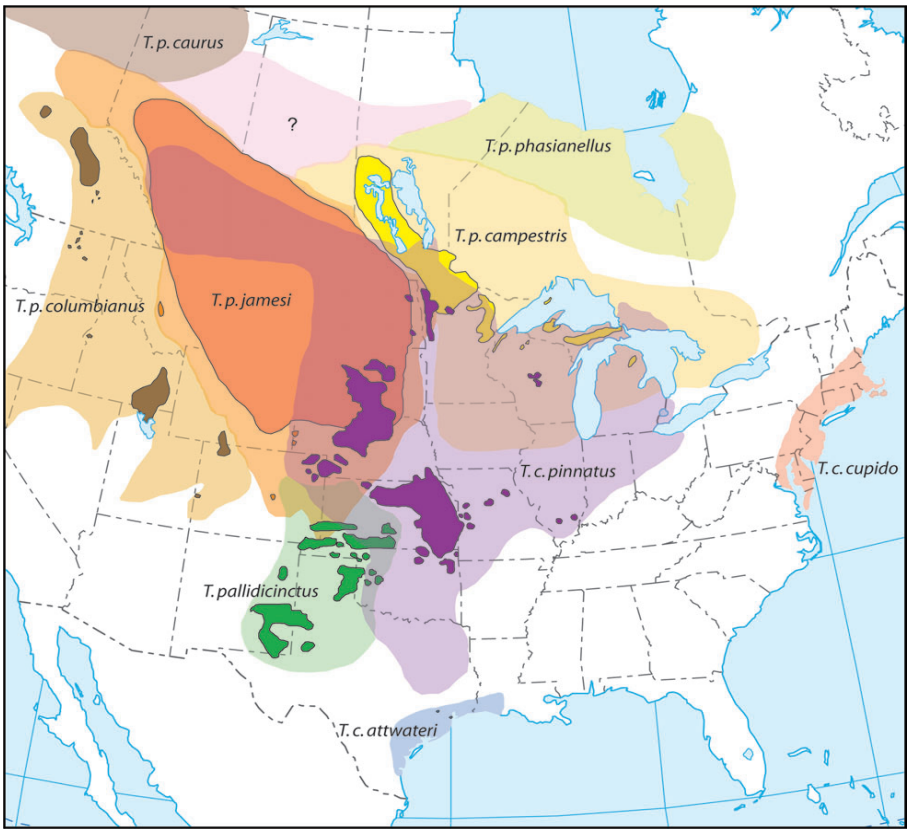


Fig. 2 Historic (transparent) and contemporary (solid) North American prairie grouse distribution (Johnson 2008). Northern Canadian Sharp-Tailed grouse contemporary distributions are not presented.

respectively (Johnsgard 2002; Johnson *et al.* 2011). In areas of geographic overlap, hybridization has been observed between greater prairie-chickens and sharp-tailed grouse (Johnsgard and Wood 1968; Lumsden 2005), greater and lesser prairie-chickens (Crawford 1978; Bain and Farley 2002), as well as sharp-tailed grouse and greater sage-grouse (*Centrocercus urophasianus*; Aldridge *et al.* 2001).

Due to human development and other anthropogenic factors, historic prairie grouse habitat has become highly fragmented and degraded, which has contributed to rapid declines in prairie grouse abundance (Johnsgard 2002). The south central range of sharp-tailed grouse has experienced decline, with the southernmost subspecies (New Mexico sharp-tailed grouse, *T. p. hueyi*) extinct and other populations (i.e., Columbian sharp-tailed grouse, *T. p. columbianus*) having experienced a 90% range contraction (Connelly *et al.* 1998; Johnsgard 2002; Silvy and Hagen 2004; Spaulding *et al.* 2006). Lesser prairie-chickens have also experienced a similar decline, with >90% reduction in their former habitat (Johnsgard 2002; Hagen *et al.* 2004; Hagen *et al.* 2005). Further, the U.S. Fish and Wildlife service has proposed threatened status for the lesser prairie-chicken (77 FR 73827 73888).

Habitat loss and fragmentation have also caused a significant decline in abundance among populations of greater prairie-chicken, with extirpation in nine U.S. states during the 20th century (Johnson *et al.* 2011). Furthermore, the heath hen became extinct in 1932 despite conservation efforts (Johnsgard 2002; Silvy *et al.* 2004; Johnson and Dunn 2006; Johnson *et al.* 2011) and the Attwater's prairie-chicken has experienced a significant decline in population size to warrant endangered status in 1967 (63 FR 31400 USFWS; Morrow *et al.* 2004). With less than 100 individuals

currently left in the wild, the critically endangered Attwater's prairie-chicken depends on supplementation from a captive breeding program that was initiated in the early 1990s from 19 founding lineages (USFWS 2010; M. Morrow, *pers. comm.*).

Maintaining genetic diversity is essential for prairie grouse conservation, especially in the case of small and isolated populations. Due to their high variance in male mating success (i.e., the lek breeding system; see also Höglund and Alatalo 1995), prairie grouse have a significantly smaller number of breeding individuals (i.e., effective population size, N_e) compared to census population size (N_c), making small and isolated populations of prairie grouse more susceptible to genetic erosion, inbreeding, and local extirpation (Bouzat *et al.* 1998; Johnson *et al.* 2003, 2004; Johnson and Dunn 2006; Pruett *et al.* 2011). In lesser prairie-chickens, for example, populations have a mean recorded N_e/N_c ratio of 0.359 while a neutral population under the Wright-Fischer model would have a ratio of 1.0 (Pruett *et al.* 2011), demonstrating the lower effective population size found in prairie grouse. With small and isolated populations, deleterious alleles can go to fixation leading to inbreeding depression (Charlesworth and Charlesworth 1987; Hedrick and Kalinowski 2000). A decrease in neutral genetic diversity has been observed in prairie grouse populations that have experienced significant declines in population size (i.e., bottlenecks; Bouzat *et al.* 1998; Bellinger *et al.* 2003; Johnson *et al.* 2003, 2004; Johnson and Dunn 2006), which in some instances have also been associated with a decline in fitness such as reduced hatching success (Westemeier *et al.* 1998; Bouzat *et al.* 2009) and juvenile survival (Hammerly *et al. in review*).

Translocating individuals from large contiguous to small isolated populations has been used as a management tool to increase genetic diversity and reduce inbreeding depression in declining prairie grouse populations. In the mid-1990's, for example, adult greater prairie-chickens from Minnesota, Kansas, and Nebraska were translocated to southern Illinois after the population had been reduced to less than 50 individuals (Westemeier *et al.* 1998). After the translocation event, the population experienced an increase in neutral genetic diversity and increased egg hatching success (Westemeier *et al.* 1998, Bouzat *et al.* 2009). The critically endangered Attwater's prairie-chicken has also experienced a decline in neutral genetic diversity and shows signs of inbreeding depression in reduced chick survival (Hammerly *et al*. in review). However, only a single population of wild Attwater's currently exists, with no wild source population available for supplementation.

As an alternative approach, the Attwater's Recovery Team is considering outcrossing Attwater's with its conspecific, the greater prairie-chicken, to increase genetic diversity and reduce inbreeding depression (USFWS 2010; M. Morrow *pers. comm.*) similar to other conservation programs such as the Florida panther (*Puma concolor coryi*; Johnson *et al.* 2010) and peregrine falcon (*Falco peregrinus*; Tordoff and Redig 2001). However, prior to implementing such an approach, a thorough assessment of the evolutionary history of Attwater's prairie-chicken relative to all other prairie grouse is warranted to evaluate the possibility of outbreeding depression (Edmands 2007; Frankham *et al.* 2011).

A previous study using single-locus mitochondrial control-region data attempted to evaluate the distinctiveness of the Attwater's prairie-chicken among prairie grouse

species using an isolation-with-migration model that accounts for gene flow and incomplete lineage sorting (Johnson 2008). Although only based on a single mitochondrial gene, Johnson (2008) suggested that the Attwater's prairie-chicken was as divergent from the greater prairie-chicken as it was from other prairie grouse species (i.e., lesser prairie-chicken and sharp-tailed grouse) and outbreeding depression may be of concern (Edmands 2007; Frankham *et al.* 2011). To verify these results, Johnson (2008) recommended that a multi-locus approach using additional nuclear loci was required (e.g., Brito and Edwards 2009) before crossing (or not crossing) Attwater's with greater prairie-chicken for management purposes.

Incomplete lineage sorting, contemporary introgression, and retention of ancestral polymorphisms (Johnson 2008) limit our ability to fully resolve the demographic history of Attwater's prairie-chicken relative to other *Tympanuchus* grouse based on single-locus analyses. However, recently developed methods have shown much promise in delimiting species relationships despite incomplete lineage sorting and lack of reciprocal monophyly (i.e., Knowles and Carstens 2007). These methods incorporate multiple loci and coalescent theory to identify a *species* tree, as opposed to single-locus or concatenated gene trees (Kubatko and Degnan 2007; Degnan and Rosenberg 2009; Knowles 2009). Species tree methods use multispecies coalescent theory to embed gene trees within a larger species tree, (Carstens and Knowles 2007a,b; Degnan and Rosenberg 2009; Knowles 2009; Liu *et al.* 2009), which utilizes the unique histories of each locus to identify overall species relationships.

The goal of this study is to resolve the evolutionary relationships among *Tympanuchus* grouse using a multi-locus phylogenetic approach with >6 sampled

individuals per population using both traditional (i.e., gene tree) and coalescent-based (i.e., species tree) methods. In addition to the mitochondrial control region, four autosomal and five Z-linked loci were sequenced across multiple prairie grouse populations per species. These data should provide the resolution necessary to identify species-level relationships within the genus *Tympanuchus* and give insight into the distinctiveness of subspecific taxa, particularly the Attwater's prairie-chicken.

METHODS

Taxon Sampling

The three species and the majority of extant subspecies in the genus *Tympanuchus* were sampled for this study (Table 2). The sampling for Attwater's, greater, and lesser prairie-chickens have been described elsewhere (Johnson *et al.* 2003, 2007; Hagen *et al.* 2010; Pruett *et al.* 2011). Sharp-tailed grouse samples were obtained from hunted birds provided by A. W. Spaulding (Appendix Table 1; see also Spaulding *et al.* 2006). Greater sage-grouse (*Centrocercus urophasianus*) samples were obtained from a concurrent project focused on greater sage-grouse population connectivity in Wyoming (see Appendix Table 2) conducted in the Johnson lab at the University of North Texas. Genomic DNA from sharp-tailed grouse and greater sage-grouse was extracted from

Table 2 *Tympanuchus* grouse samples and locations included in this study.

Taxa and Population Location	n	Period Collected
Tympanuchus cupido attwateri		
TX Wild Population	13	1990-1993
Tympanuchus cupido pinnatus		
Minnesota	9	1999
Kansas	9	1999
Oklahoma	6	1997-2001
Tympanuchus pallidicinctus		
New Mexico	10	2005
Oklahoma	9	2005
Kansas	10	2000-2002
Tympanuchus phasianellus		
Northwest Territories (*T. p. kennicotti*)	10	2002-2003
Wyoming (*T. p. jamesi*)	10	2005
Ontario (*T. p. phasianellus*)	7	1998
Wyoming (*T. p. columbianus*)	6	2004-2005
Centrocercus urophasianus		
Wyoming	12	2006-2010

muscle and blood samples, respectively, following manufacturer protocols using a Qiagen DNeasy extraction kit (Qiagen, Valencia, CA).

Within the genus *Tympanuchus*, 99 unrelated individuals representing six taxonomic groups (species and subspecies) were chosen for this study, and 12 greater sage-grouse individuals were included as an outgroup based on results from Lucchini *et al.* (2001), Dimcheff *et al.* (2002), and Drovetski (2003) (Table 2). Multiple populations of each species-level taxon were included, with samples collected in areas of known geographic overlap and areas distantly separated to address potential issues related to contemporary gene flow and introgression (or hybridization).

Marker Selection and Development

A total of 138 autosomal and Z-linked loci (Borge *et al.* 2005; Backström *et al.* 2006; Backström *et al.* 2008; Berlin *et al.* 2008; Kimball *et al.* 2009; Backström and Väli 2011; Elgvin *et al.* 2011) evenly spaced throughout the avian genome were screened to identify primer sets that consistently amplified DNA among *Tympanuchus* grouse. Genomic DNA from eight individuals (2 sharp-tailed grouse, 2 lesser prairie-chicken, 2 greater prairie-chicken, and 2 Attwater's prairie-chicken) were amplified using polymerase chain reaction (PCR) methods described elsewhere (Borge *et al.* 2005; Backström *et al.* 2006; Backström *et al.* 2008; Berlin *et al.* 2008; Kimball *et al.* 2009; Backström and Väli 2011; Elgvin *et al.* 2011).

Of the 138 loci, 57 marker-sets (41%) amplified DNA for selected *Tympanuchus* taxa without requiring additional optimization. A locus set of nine nuclear markers was selected based on sequence quality and the presence of phylogenetically informative

sites (Table 3). Each locus possessed at least four informative SNPs across all *Tympanuchus* taxa. In addition to autosomal loci, ~700 bp of the mitochondrial control region (control region I & II) was included in this study, as it has been shown to be highly polymorphic in *Tympanuchus* grouse based on previous studies (Johnson *et al.* 2007; Johnson 2008).

Table 3 Chosen loci in this study, location on the avian genome, and length.

Locus ID	Chromosome	Length
06419[4]	10	379bp
09300[4]	12	370bp
14726[4]	3	182bp
15506[4]	1	585bp
24105[2]	Z	440bp
25189[2]	Z	580bp
ALDOB[1,2,5]	Z	504bp
CHD1Z[1,7]	Z	402bp
NNT[2,6]	Z	587bp
CR-I and II[3]	mtDNA	684bp

[1]Borge *et al.* 2005
[2]Backström *et al.* 2006
[3]Johnson *et al.* 2007
[4] Backström *et al*. 2008
[5]Kimball *et al*. 2009
[6]Backström and Väli 2011
[7]Elgvin *et al*. 2011

A total of 111 samples were amplified using PCR for 10 different loci (Table 3 cc). PCR products were quantified using gel electrophoresis and purified using ExoSAP-IT (United States Biochemical, Cleveland, OH). Samples were sequenced using BigDye Terminator chemistry v 3.1 (Applied Biosystems) and an ABI 3130xl Genetic Analyzer. Sequences were initially aligned and edited manually using the program SEQUENCHER V.4.10.1 (Gene Codes, Ann Arbor, MI), with final alignments obtained including all samples using the program CLUSTAL-W (Larkin *et al.* 2007).

Haplotypes for the nuclear dataset were reconstructed using the PHASE algorithm (Stephens *et al.* 2001) in DNASP v 5.10 (Librado and Rozas 2009) with parameters set to 10,000 iterations, a thinning interval of 1, and a 1,000 iteration burn-in. Any haplotypes with phase probabilities below 0.9 were subjected to cloning using TOPO10 chemically competent cells (Invitrogen) to ensure correct haplotype inference.

Data Analysis

The assumptions of intra-locus non-recombination, linkage disequilibrium, and neutrality were tested for all sequence data prior to phylogenetic analysis. Sequences were tested for recombination using the program IMgc (Woerner *et al.* 2007), which finds the largest continuous block of non-recombinant DNA in each locus using the four-gamete rule for recombination. Multi-locus Hudson-Kreitman-Aguadé tests (HKA; Hudson *et al.* 1987) were performed using the HKA program (http://lifesci.rutgers.edu/~heylab/) to test for neutrality among loci between all populations of prairie grouse. Z-linked and autosomal loci were analyzed separately.

Linkage analysis among nuclear loci was performed by converting sequences to haplotypes using the program DNASP v 5.10 (Librado and Rosas 2009) and testing for linkage disequilibrium and Hardy-Weinberg equilibrium with the program GDA (available at: http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php). Summary statistics for each locus were also calculated using the program DNASP v 5.10. These statistics included: number of polymorphism sites, number of alleles or haplotypes, gene diversity (h) ±SE, nucleotide diversity (π) ±SE, and Tajima's D. Estimates of divergence, or mean number of nucleotide substitutions per site (D_{XY}), between Attwater's prairie-chicken and all other prairie grouse were also calculated using DNAsp v. 5.10.

Population Genetic Structure and Differentiation

Population genetic structure among sampled populations was assessed using multiple methods. First, pairwise estimates of Wright's F_{ST} was calculated using Weir and Cockerham's (1984) estimate q as calculated in ARLEQUIN v 3.11 (Excoffier *et al.* 2005). Differences in population structure between populations were tested using 10,000 permutations among populations with Fisher's exact test. *P*-values were adjusted to control for multiple comparisons using sequential Bonferroni correction (Rice 1989).

Two Bayesian methods were also used to identify population differentiation while accounting for admixture among the sampled populations. The method implemented in STRUCTURE v 2.3.4 (Prichard *et al.* 2000) uses a Bayesian Markov Chain Monte Carlo (MCMC) approach to identify genetic clusters (*K*) that minimize Hardy-Weinberg and linkage disequilibrium. Using STRUCTURE, multiple analyses were conducted using different datasets to investigate whether sampling affected the method's ability to identify differentiation between populations. Analyses using the compete dataset, including the outgroup, were compared to those from separate STRUCTURE runs for each species group. Each run was performed using the admixture model with correlated allele frequencies among populations and individual alpha parameters (Pritchard *et al.* 2000). After a burnin period of 100,000 replicates, an additional 500,000 replications were used to compute the posterior probabilities for identifying the number of distinct populations (*K*) given the data. A range of *K* values (1-8) was used for each analysis, including a total of four independent iterations for each *K*. The final determination of *K* was based on the greatest mean likelihood estimates (LnP[K]) and the Evanno method

(Evanno *et al.* 2005) as used in the program STRUCTURE HARVESTER (Earl and vonHoldt 2012). Iterations for each *K* were averaged and aligned using the program CLUMPP v 1.1.2 (Jacobsson and Rosenberg 2007), and visualized using DISTRUCT v 1.1 (Rosenberg 2004).

Although STRUCTURAMA v 2.0 (Huelsenbeck and Andolfatto 2007) is similar to the method implemented in STRUCTURE, it differs by including the number of theoretical populations as a parameter in the model and a posterior distribution of the probabilities of *K* is generated (Huelsenbeck and Andolfatto 2007), thereby making it less subjective for identifying the most likely number of populations (Huelsenbeck *et al.* 2011). Using a gamma prior with a shape of 2.5 and scale of 0.5, the program was run for 250,000 steps with four chains and sampled every 25 generations for a total of 10,000 samples. Prior number of populations and expected number of populations were set as random variables. Three population subsets (Table 4) including the complete dataset were analyzed with STRUCTURAMA to identify whether taxonomic sampling affected the results.

Table 4 Definitions of population subsets used in various analyses.

Subset ID	Populations Included	Known Hybridization
1	GRPC (Attw. *TX*), GRPC (*MN*), LEPC (*NM*), STGR (*NWT*), GRSG (*WY*)	GRPC MN in known hybrid zone with STGR
2	GRPC (Attw. *TX*), GRPC (*KS*), LEPC (*KS*), STGR (Jam. *WY*), GRSG WY	LEPC KS in known hybrid zone with GRPC
3	GRPC (Attw. *TX*), GRPC (*KS*), LEPC (*NM*), STGR (*NWT*), GRSG (*WY*)	Populations in known areas of allopatry

Haplotype Networks and Phylogenetic Analysis

To estimate phylogeographic structure among *Tympanuchus* grouse, median-joining haplotype networks were produced for each locus using the program NETWORK v.4.610 (available at: www.fluxus-engineering.com) with parameter e = 0. Both traditional gene-tree and coalescent species-tree approaches were also used to explore species-level relationships among *Tympanuchus* grouse. The best-fit model of evolution for each phased nuclear and mtDNA control region locus was determined by Akaike information criterion (AIC) in jModelTest v 0.1.1 (Posada 2008). Greater sage-grouse was used as the outgroup in all analyses.

Gene tree phylogenies were reconstructed using MRBAYES v 3.2 (Ronquist *et al.* 2012) for each of the 10 loci and a concatenated nuclear phased dataset. Individual gene trees for each locus were identified using the best-fit model, appropriate inheritance scalars, and a uniform clock since none of the loci rejected the global clock based on likelihood ratio tests ($p>0.05$). Two independent runs were obtained for each gene tree, each with 5 million generations, five chains, and a 25% burn-in. A uniform clock was also used on the nuclear concatenated dataset. In the concatenated analysis, each locus was unlinked and assigned its best-fit model and appropriate inheritance scalar (i.e., diploid or Z-linked). Two independent analyses were run for 60 million generations and a 25% burn-in. The program TRACER v 1.5 (Rambaut and Drummond 2009) was used to identify the appropriate burnin for each run and confirm MCMC convergence for each parameter with effective sample size (ESS) values > 200.

Species-tree analyses were implemented in the program *BEAST version 1.7.4 (Drummond *et al.* 2012). *BEAST is unique among species tree programs because it

simultaneously creates gene trees within a larger species tree phylogeny, with each gene tree following the coalescent in each extant and ancestral species increasing its overall computational efficiency (Drummond and Rambaut 2007, 2012). Three different species trees were constructed depending on the sequence data used in the analysis: only mitochondrial control region data, only nuclear data, and all sequence data. All *BEAST analyses used a Yule prior on the species tree and *a priori* population boundaries of greater and lesser prairie-chicken populations, Attwater's prairie-chicken, and all sharp-tailed grouse subspecies. The 95HPD of the posterior distribution of the standard deviation of the substitution rate parameter for each nuclear locus using a relaxed uncorrelated lognormal clock included zero during preliminary runs, so a strict clock was used for each of the nuclear loci in subsequent analyses. A relaxed uncorrelated lognormal clock was used with the mtDNA control region locus because the locus rejected the global molecular clock model using the likelihood ratio test ($p<0.01$) and the 95HPD of the posterior distribution of the standard deviation of the substitution rate parameter did not include zero.

A *Tympanuchus* spp. fossil from Jewell Co., Kansas was used as a calibration point for the *BEAST analyses. This fossil is the oldest known fossil assigned to *Tympanuchus* based on morphometric analyses, and this fossil dated to the middle Irvingtonian age (0.25-1.8 mya) using biochronology and fission-track age determination on an ash sample from the fossil site (Eshelman and Hager 1984). It is not known whether this fossil belongs to a specific species of *Tympanuchus* or an ancestor of the three extant species. Therefore, a conservative approach was applied to our dating procedure by placing this calibration at the stem of the *Tympanuchus* node with an

exponential prior (mean=2.0, offset=0.20, 95% CI 0.251-7.578 MA) on the lower bound. Therefore, an even later divergence date for *Tympanuchus* is possible if in fact the fossil was associated with one of the three extant species existing in Kansas at that time as opposed to the stem. Additional analyses with an exponential prior (mean=5.0, offset 0.12, 95% CI 0.247-18.56 MA) and a uniform prior (95% CI 0.25-100 MA) were used as fossil priors on the *Tympanuchus* stem to assess how the choice of calibration prior influenced the results. Nodal support, topology, and nodal dates were consistent across runs, and results presented here are based on the original exponential prior fossil calibration. A fossil-calibrated estimate of mtDNA control region substitution rate of 0.045 ± 0.012 substitutions/MY (mean ± S.D., or 2.27 - 6.25% substitutions/site/lineage/MA) was also used based on a normal distribution, representing the 95% CI substitution rate estimated across 21 species of grouse in Drovetski (2003).

The three datasets required differing sampling strategies to reach MCMC convergence. The mtDNA-only dataset was run once for 8×10^8 generations sampling every 1000 generations. The nuclear-only and the combined nuclear and mtDNA analyses were each run six or eight times, respectively, for 8×10^8 generations sampling every 4000 generations. In each run 10% for mtDNA-only and 50% for nuclear-only and combined trees were discarded as burnin, and final runs were assessed for convergence using TRACER v 1.5 with all ESS values >200 and similar posterior distribution curves between runs for each parameter. In each of the three analyses, independent runs were combined using LOGCOMBINER v1.7.4 (Rambault and Drummond 2012a) after excluding the appropriate burnin from each run, and the

maximum clade credibility tree was calculated using TREEANNOTATOR v1.7.4 (Rambault and Drummond 2012b).

RESULTS

Polymorphism, Divergence, and Signs of Selection

A total of 4,713 base pairs (bp) were obtained from the mitochondrial, autosomal, and Z-linked loci used in this study. PCR amplifications and their subsequent sequences were generated for most of the sampled individuals, with some exceptions. At locus 06419, non-specific binding was observed with three Attwater's, two lesser prairie-chicken, and all greater sage-grouse samples producing multiple overlapping sequences. Locus 09300 also experienced non-specific binding for one greater sage-grouse and one sharp-tailed grouse (Ontario) sample. Samples that produced multiple overlapping sequences were removed from the dataset. The entire dataset including the outgroup possessed 273 polymorphic sites (5.8%), or 175 (3.7%) after excluding the outgroup (Table 5).

Table 5 Number and percentage of polymorphic sites per locus.

Locus	Chromosome	Length	(n) Polymorphic Sites in All Individuals	%	(n) Polymorphic Sites in In-group	%
mtCRI-II	Mitochondria	684	82	0.12	40	0.058
NNT	Z	587	22	0.037	14	0.024
CHD1Z	Z	402	16	0.04	9	0.022
ALDOB	Z	504	22	0.044	18	0.036
25189	Z	580	24	0.041	13	0.022
24105	Z	440	17	0.039	14	0.032
15506	1	585	36	0.062	20	0.034
14726	3	182	16	0.088	13	0.071
09300	12	370	19	0.051	15	0.041
06419	10	379	19	0.05	19	0.05
Total		4713	273	0.058	175	0.037

Number of haplotypes, gene diversity, and nucleotide diversity were calculated on a per locus and per population basis (Appendix tables 3.1-3.5). Gene and nucleotide diversity values were similar across taxonomic groups and populations for each locus, but average values differed between types of loci. Z-linked loci showed significantly lower gene diversity ($\bar{x}$=0.4701; independent *t*-test *P*<0.0001) and nucleotide diversity ($\bar{x}$=0.001486; independent *t*-test *P*<0.0001) among populations compared to the autosomal loci (gene diversity $\bar{x}$=0.64687; nucleotide diversity $\bar{x}$=0.00660715). Furthermore, mitochondrial gene diversity was significantly higher than both autosomal and Z-linked loci ($\bar{x}$= 0.7978; *ANOVA P*<0.0001) and mitochondrial nucleotide diversity was significantly higher than Z-linked loci ($\bar{x}$=0.0059, *ANOVA P*<0.0001). Tajima's *D* values were significant in three cases: Columbian sharp-tailed grouse at locus 09300, greater prairie-chicken in Oklahoma at locus 14726, and lesser prairie-chicken in New Mexico at locus ALDOB. Given that each of the three populations only showed a significant Tajima's *D* values at one locus, significant values may be more indicative of demographic events (i.e., population expansion of contraction) than removal from neutrality (Tajima 1989). Multi-locus HKA tests that were performed with autosomal and Z-linked introns yielded no significant (*P*<0.05) results, suggesting neutral conditions for each of the locus-population comparisons.

To measure divergence between Attwater's prairie-chicken and other prairie grouse taxa and populations, the mean number of nucleotide substitutions per site (D_{XY}) was measured and averaged across three locus types (i.e., mitochondrial, autosomal, and Z-linked; Fig. 3). As expected, the outgroup (greater sage-grouse) showed the highest overall level of divergence with Attwater's prairie-chicken. Within the ingroup,

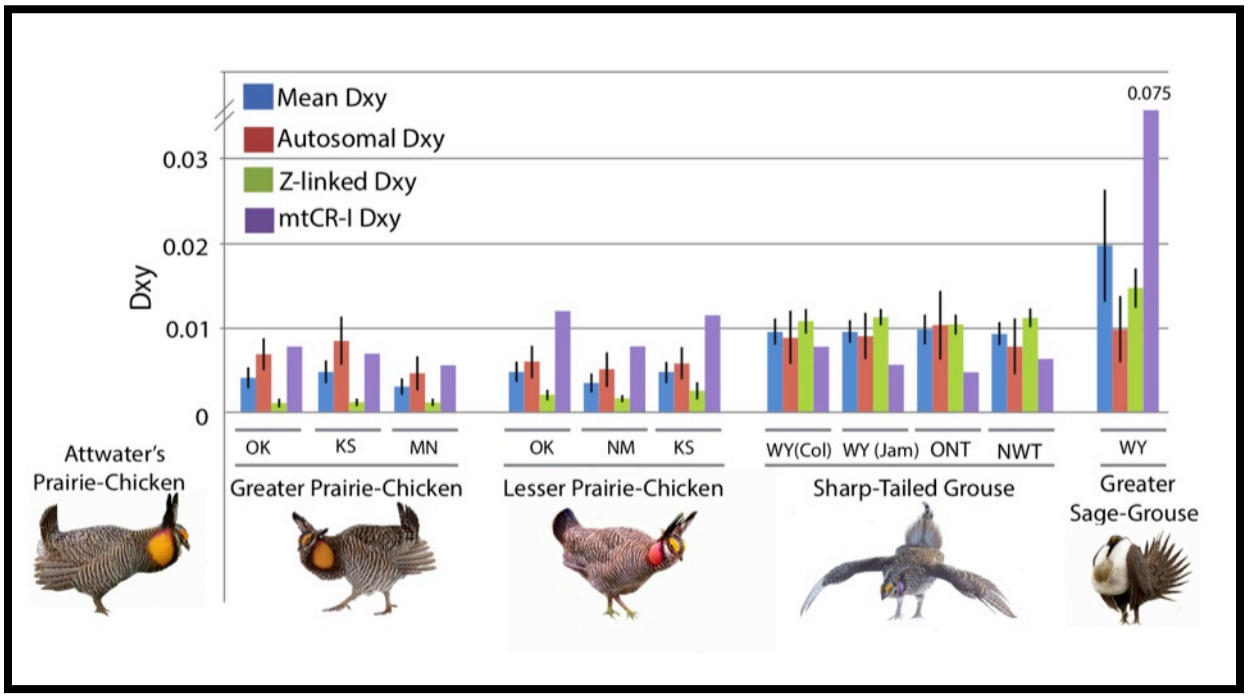


Fig. 3 *Dxy* divergence estimates between Attwater's prairie-chicken and all other populations of prairie grouse. Mean, autosomal, *Z*-linked, and mitochondrial *Dxy* levels are denoted by color.

mean D_{XY} estimates showed that Attwater's prairie-chicken was more divergent from sharp-tailed grouse than from pinnated grouse (i.e., greater and lesser prairie-chicken), with Attwater's showing the lowest divergence with its conspecific, the greater prairie-chicken. Overall, divergence patterns depended on the specific locus, but differed among taxonomic comparisons. For example, Z-linked loci showed little divergence between Attwater's prairie-chicken and greater and lesser prairie-chicken, but showed the highest level of divergence with sharp-tailed grouse and a similarly high level of divergence with greater sage-grouse. MtDNA control region indicated that Attwater's also had lower divergence with the greater prairie-chicken, but also with sharp-tailed grouse, yet higher divergence with lesser prairie-chicken (Fig. 3).

Population Genetic Structure and Differentiation

Mean pairwise F_{ST} values were calculated for each population based on haplotypes (Appendix Table 4). Intraspecific pairwise F_{ST} values were comparatively lower (F_{ST} 0.01606-0.29083) than interspecific pairwise F_{ST} values (F_{ST} 0.25433-0.57567). Sharp-tailed grouse showed moderate F_{ST} levels between recognized subspecies (F_{ST} 0.12061-0.29083), while subspecies within greater prairie-chickens showed comparatively lower levels of genetic subdivision (F_{ST} 0.01606-0.07148). Significant F_{ST} levels ($P<0.05$, denoted in bold in Appendix Table 3) were calculated after adjusting for multiple comparisons (Rice 1989). Most significant values correspond with interspecies comparisons, however some interspecies comparisons with large F_{ST} values were not significant, likely because of the effects of small sample size (i.e., $n \leq 7$).

Results from the Bayesian program STRUCTURE indicated four population clusters (K=4) when all populations, including the outgroup, were included in the analysis (Fig. 4a). After excluding the outgroup, three population clusters were identified corresponding with current species taxonomy (Fig. 4b). K=3 was also identified as the most probable number of populations when Attwater's prairie-chicken were analyzed with greater prairie-chicken, excluding all other populations (Fig. 4c). However, upon closer inspection, each of the populations (K), including that from K=2 (data not shown), possessed equal admixture among individuals suggesting a single population cluster for Attwater's and greater prairie-chicken with the given dataset. Similarly, when lesser prairie-chicken was analyzed without any other species, K=4 was identified as the most likely number of clusters, yet the results for individual assignment showed no

discernable pattern among populations (Fig. 4d). The lesser prairie-chicken population analysis for *K*=2 and 3 also showed a similar lack of obvious differentiation with each individual showing equal admixture to each cluster (data not shown). An analysis focused on sharp-tailed grouse suggested *K*=3 as the most probable number of populations (Fig. 4e). Unlike the greater and lesser prairie-chicken analyses, however, the identified structure observed among sharp-tailed grouse populations corresponded partially with current subspecies taxonomy with *T. p. columbianus and kennicotii* subspecies forming their own distinct cluster and *T. p. phasianellus* and *T. p. jamesi* forming a single cluster.

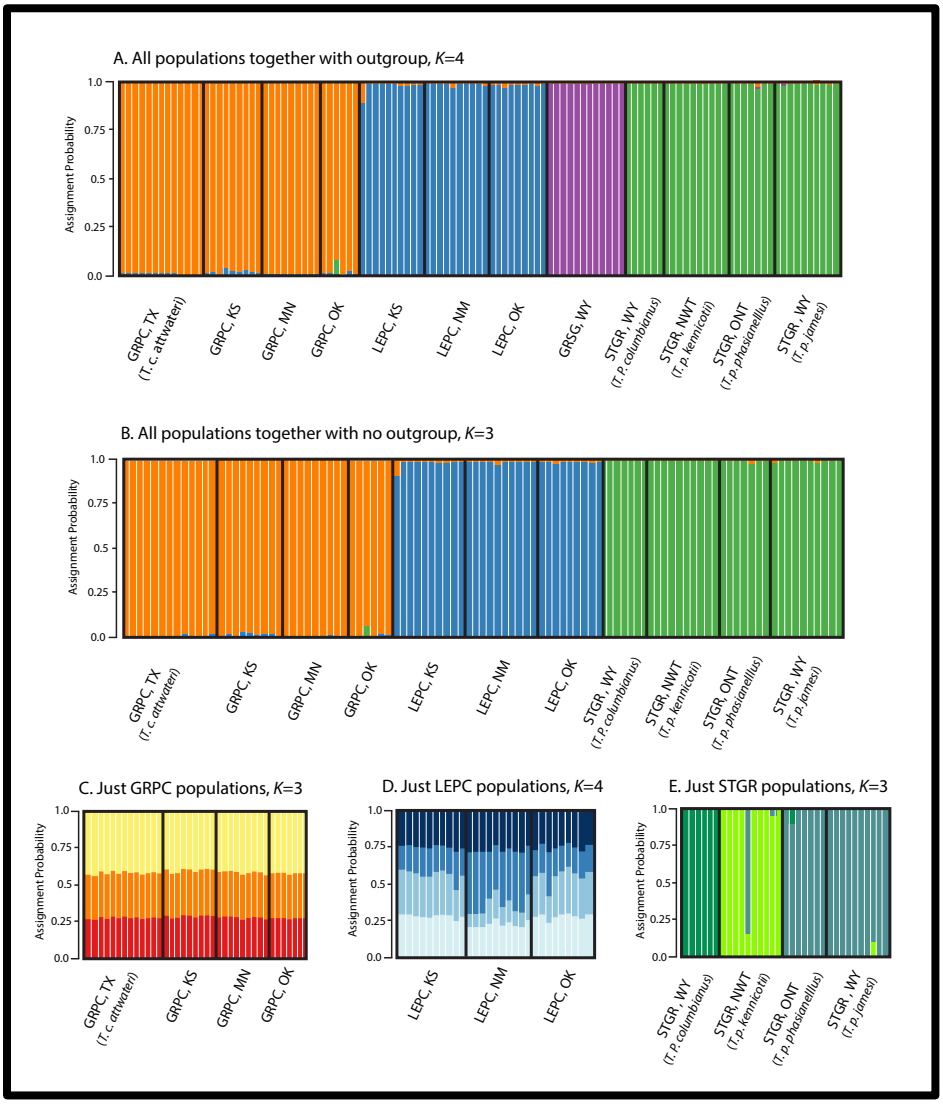


Fig. 4 STRUCTURE plots based on nuclear sequences using various taxa (A-E). Single vertical lines represent individuals within each population, clustering into groups (K) based on color.

The program STRUCTURAMA corroborates the results from STRUCTURE, with the exception of when all samples including the outgroup were analyzed as a single dataset. STRUCTURAMA estimated three populations (pinnated grouse, sharp-tailed grouse, and greater sage-grouse) where STRUCTURE estimated four. After excluding the outgroup, STRUCTURAMA identified three populations that correspond with current species taxonomy, suggesting that greater sage-grouse influenced the results relative to the ingroup. Results from all of the remaining runs from STRUCTURAMA with the data subdivided into taxonomic groups (Table 4) produced similar results to those obtained from STRUCTURE.

Phylogenetic Analyses

Median-joining haplotype networks were constructed for each locus. Autosomal loci showed extensive allele sharing among prairie grouse species and subspecies with minimal haplotype clustering corresponding with taxonomy (Fig. 5). In fact, two haplotypes for autosomal locus 09300 was shared among all species including the outgroup. In contrast, Z-linked loci showed comparatively less polymorphism, but increased divergence (i.e., haplotype clustering), that corresponded with current species-level taxonomy, particularly greater sage-grouse, sharp-tailed grouse and the pinnated grouse as separate groups. The mitochondrial control region haplotype network was characterized by a larger number of haplotypes compared to the autosomal and Z-linked loci, but with minimal haplotype clustering observed between taxa within the ingroup. In each network, with the exception of the Z-linked loci, the

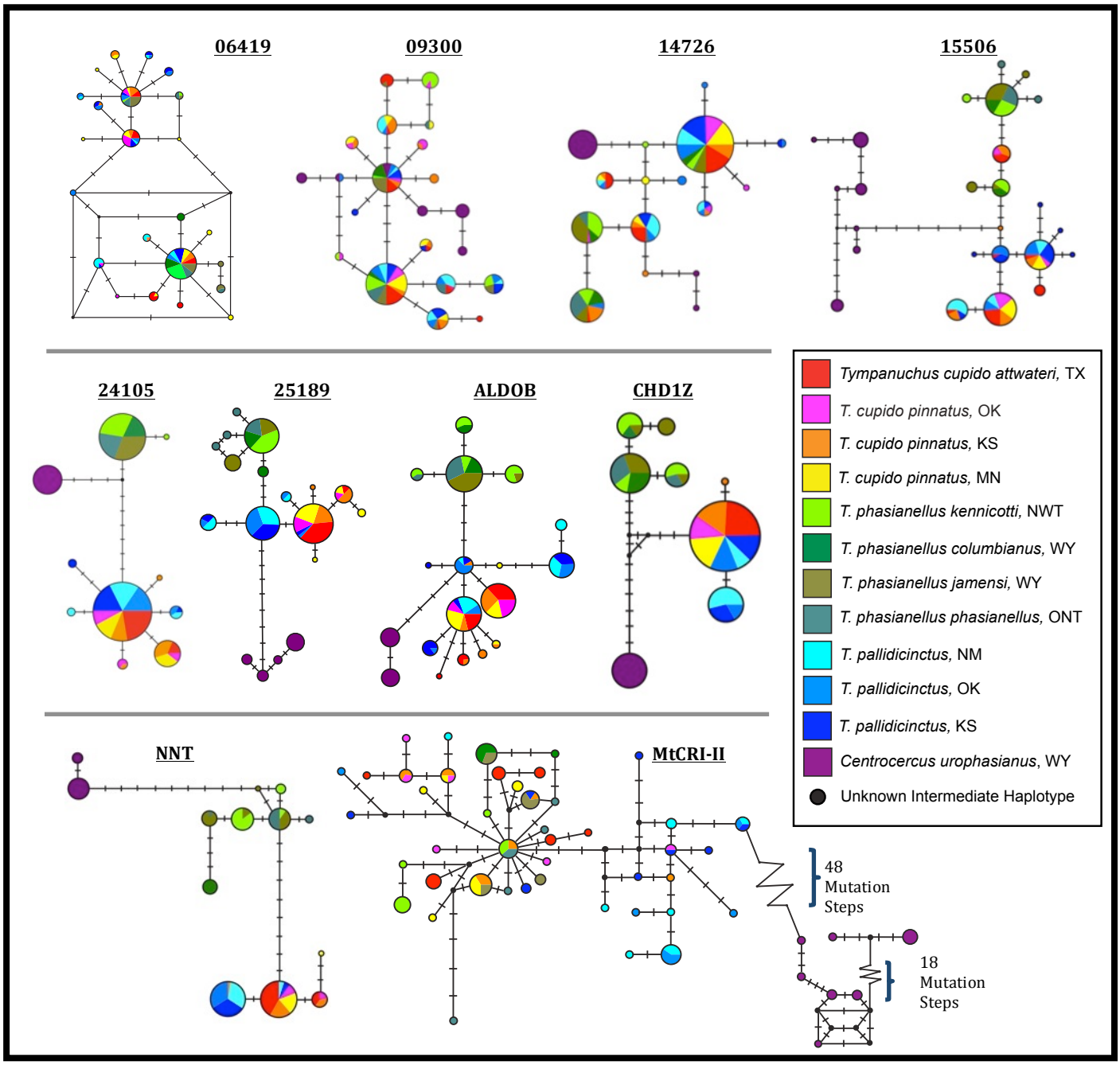


Fig. 5 Median-joining haplotype networks for each locus used in this study. Circles represent individual haplotypes (or alleles) with the diameter of the circle corresponding to the number of individuals that share a particular haplotype. Tick marks denote mutational steps between haplotypes.

Attwater's prairie-chicken shared haplotypes with all of the prairie grouse species, while no Z-linked haplotypes were shared with sharp-tailed grouse.

Single locus gene trees showed varying degrees of species monophyly, depending on the locus (Appendix Fig. 1- 10). No reciprocal monophyly among ingroup taxa was observed in the autosomal and the mitochondrial control region gene trees (Appendix Fig. 1- 5). However, sharp-tailed grouse do form a monophyletic clade in each of the Z-linked gene trees with relatively high posterior support (posterior = 0.81-1.0; Appendix Fig. 6- 10). While the Bayesian 50% majority rule consensus tree using the concatenated sequence data resulted in a well-supported monophyletic clade for

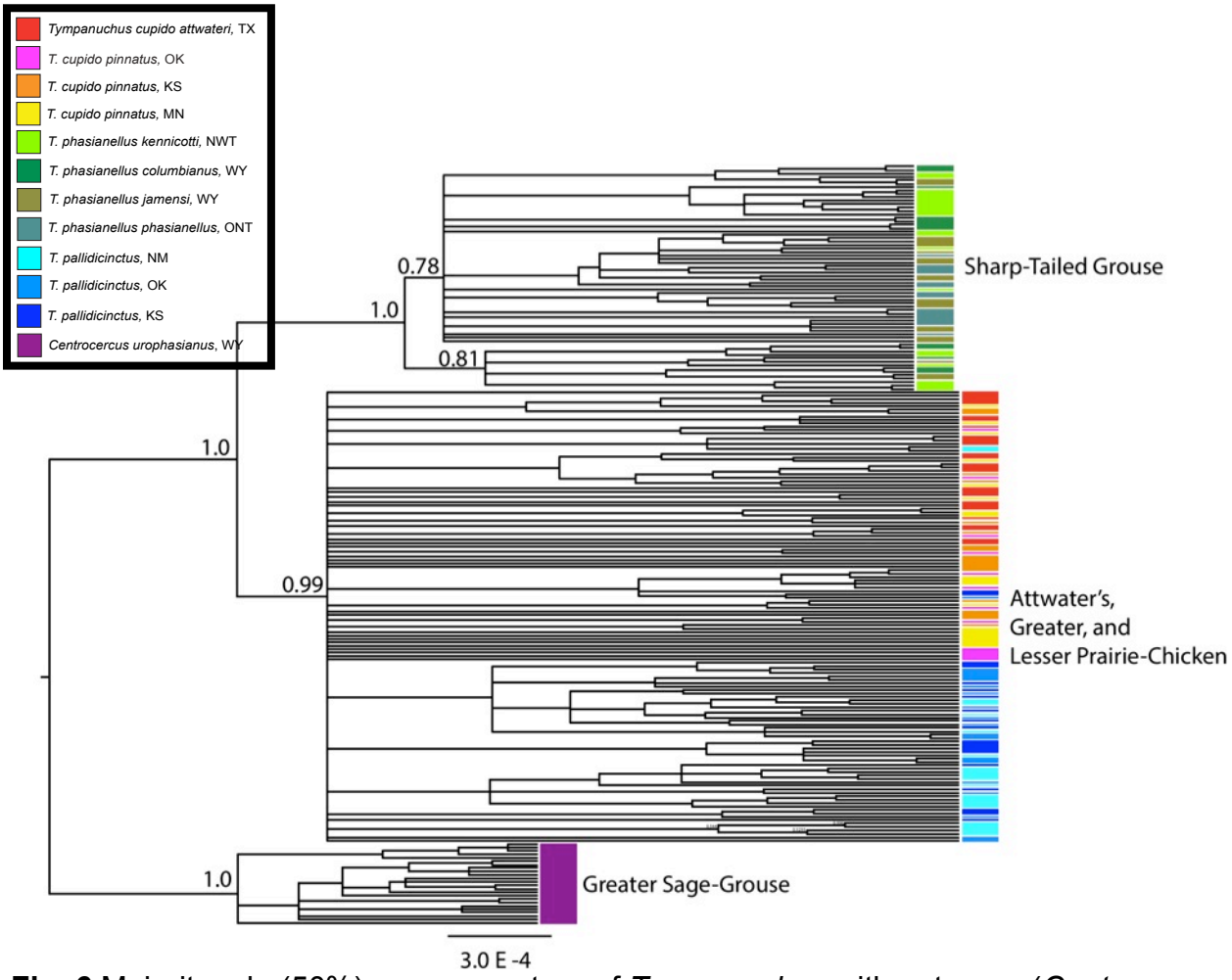


Fig. 6 Majority rule (50%) consensus tree of *Tympanuchus* with outgroup (*Centrocercus urophasianus*, greater sage-grouse) based on concatenated nuclear sequences. Posterior probability values are presented at the bases of larger nodes. Colors at the end of each tip correspond with the populations denoted in the legend.

sharp-tailed grouse sister to the pinnated grouse, sharp-tailed grouse subspecies were polyphyletic (Fig. 6). Lesser, greater and Attwater's prairie-chicken were also polyphyletic in the concatenated gene tree.

In contrast, each *Tympanuchus* species in the *BEAST species tree analysis was monophyletic using both the nuclear and combined datasets (Fig. 7 and 8). Greater and lesser prairie-chickens were sister taxa, with Sharp-tailed grouse being sister to pinnated grouse. The species tree based on the mtDNA control region sequence data, however, resulted in incongruent species-level support. While the sampled lesser prairie-chicken populations were monophyletic, sharp-tailed grouse were paraphyletic with greater and Attwater's prairie-chicken (Fig. 9).

No strong nodal support was given for any of the subspecies and/or population-level groups within the species tree regardless of dataset. Both Attwater's and each of the sharp-tailed grouse subspecies formed polytomies with each of their conspecific taxa for the nuclear and complete datasets (Fig. 7 and 8). For the mtDNA species tree, sharp-tailed grouse was paraphyletic with the two subspecies of sharp-tailed grouse that possess overlapping distributions with greater prairie-chickens embedded among greater and Attwater's prairie-chicken populations (Fig. 9), suggesting that female-mediated contemporary gene flow rather than incomplete lineage sorting may be a factor influencing these results.

The results of the *BEAST calibrated species tree agree with current taxonomy, suggesting that among *Tympanuchus* grouse, sharp-tailed grouse diverged before greater and lesser prairie-chickens diverged, with Attwater's diverging from greater prairie-chickens most recently. All divergence times (including the outgroup) occurred

within the past 2.5 million years (95% HPD), corresponding with the Pleistocene epoch in North America (Wisley *et al.* 2008). When considering the *BEAST analyses with the entire data set, sharp-tailed grouse were estimated to have diverged from pinnated grouse approximately 0.2-0.271 MYA, and greater prairie-chickens diverged from lesser prairie-chickens 0.032-0.088 MYA (Fig. 7). Subspecies and populations within each species-level taxa have low posterior support values, and 95% HPD intervals for divergence times within each species range from 0.001-0.056 MYA.

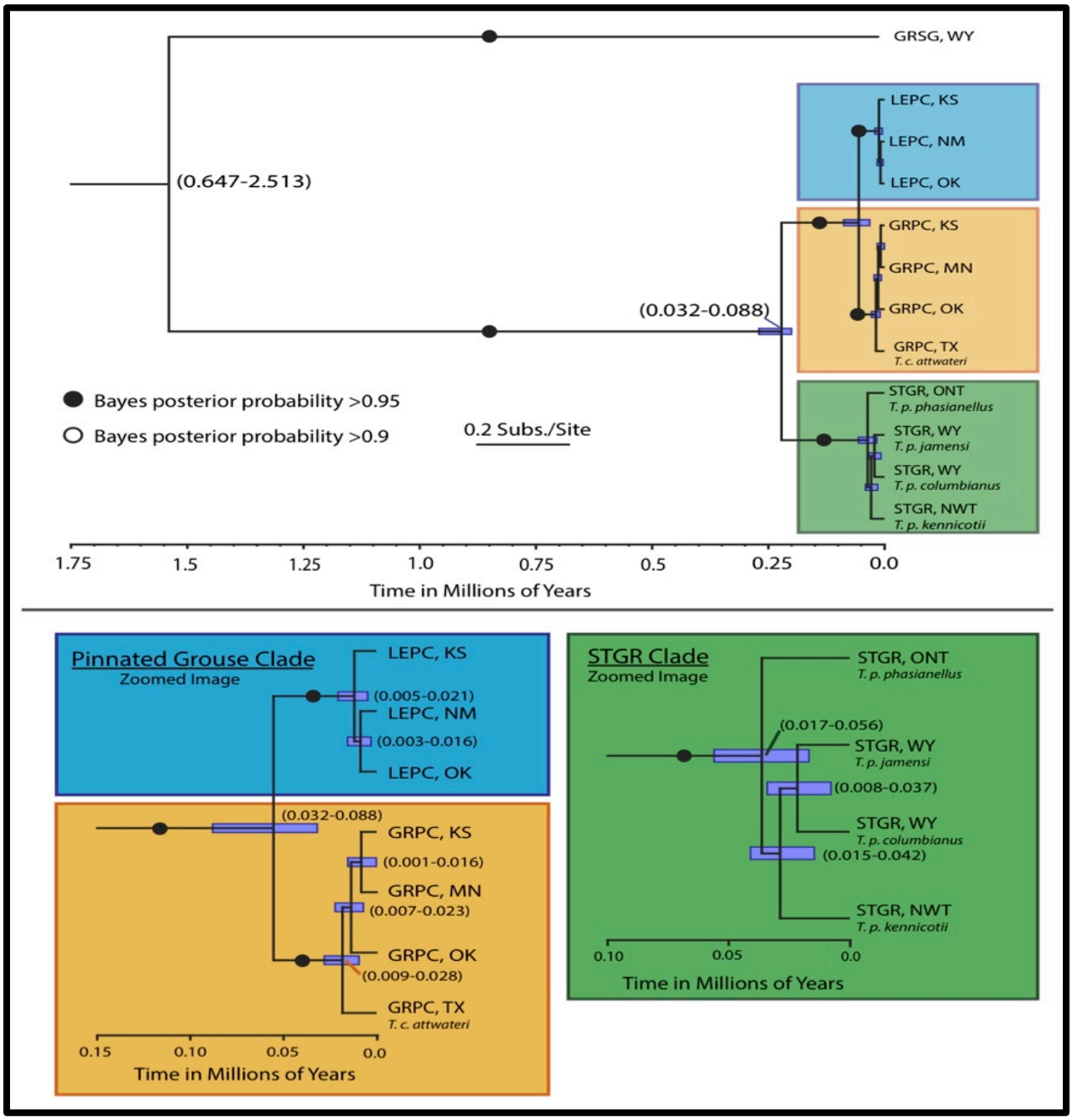


Fig. 7 *BEAST time-calibrated species tree based on all loci and individuals. High posterior probabilities are denoted with a black or white circle (see legend), and all probabilities below 0.9 are not denoted. Branch divergence times (in millions of years) are in parenthesis near the split of each node and blue 95%HPD confidence bars are provided around each node. To show finer detail on pinnated grouse and Sharp-tailed grouse clades, zoomed images corresponding to color are provided below the entire species tree. Species groups are denoted by AOU alpha code, with abbreviated state populations and subspecies detailed, when applicable.

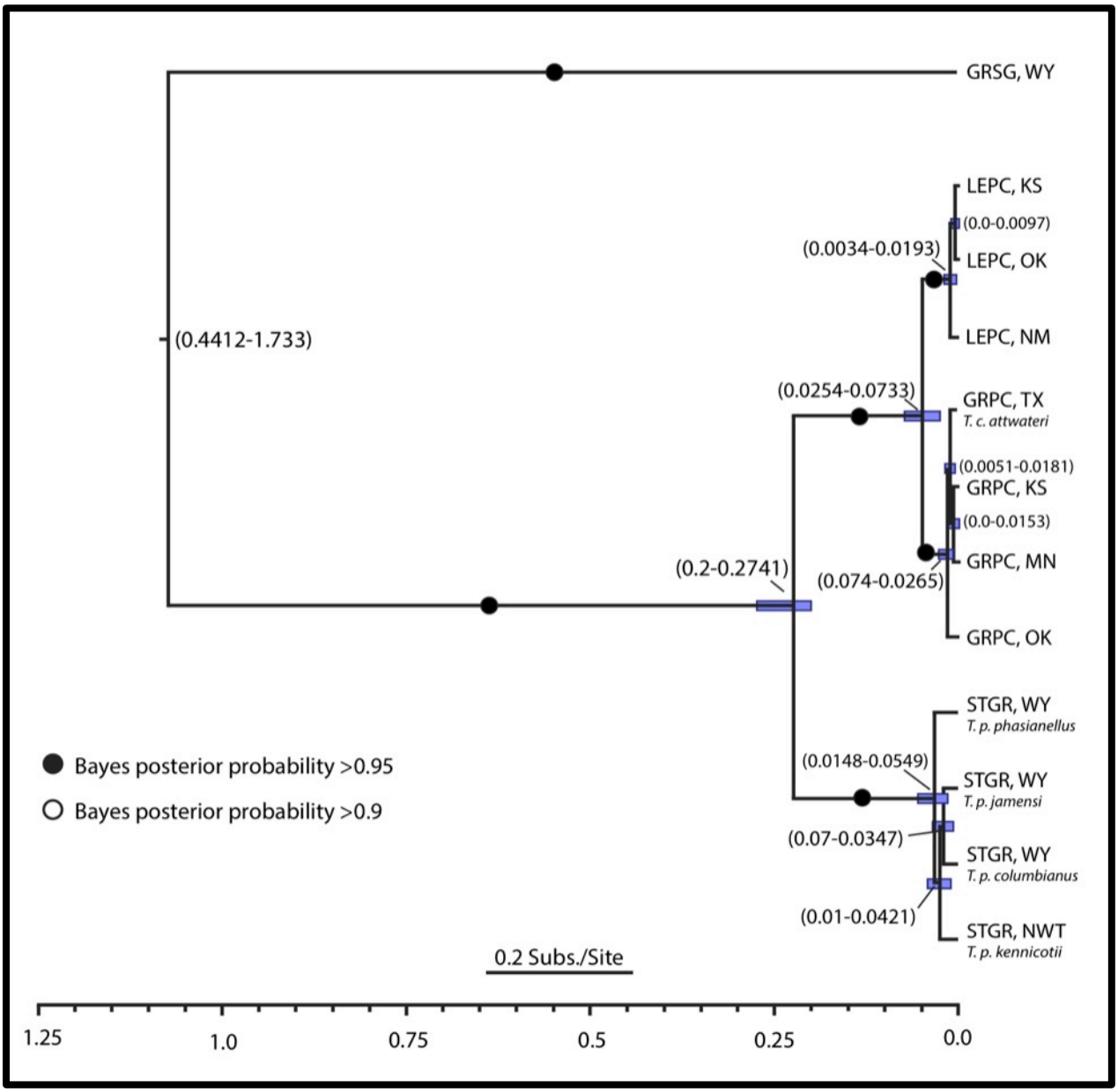


Fig. 8 *BEAST time-calibrated species tree based only nuclear loci and all individuals. High posterior probabilities are denoted with a black or white circle (see legend), and all probabilities below 0.9 are not denoted. Branch divergence times (in millions of years) are in parenthesis near the split of each node and blue 95%HPD confidence bars are provided around each node.

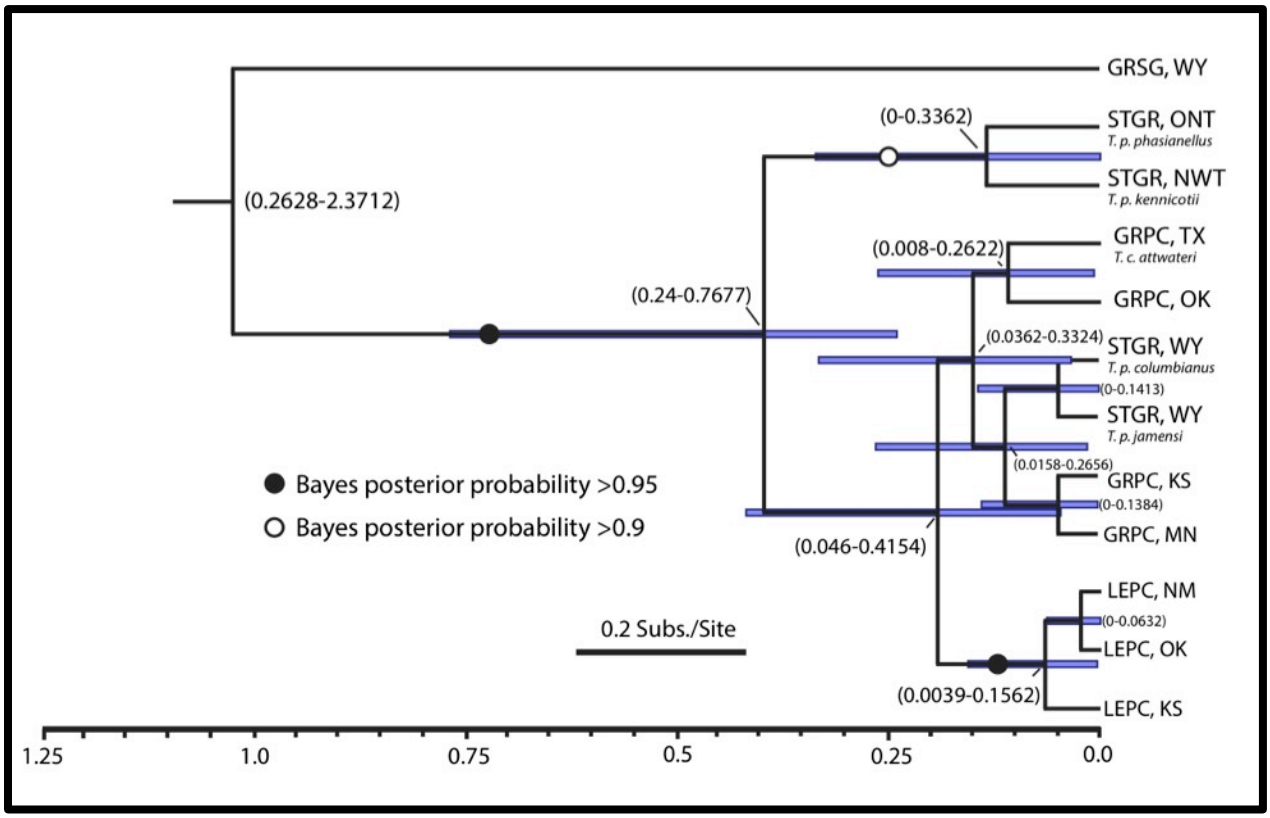


Fig. 9 *BEAST time-calibrated species tree based on a single mitochondrial locus, with all individuals represented. High posterior probabilities are denoted with a black or white circle (see legend), and all probabilities below 0.9 are not denoted. Branch divergence times (in millions of years) are in parenthesis near the split of node and blue 95%HPD confidence bars are provided around each node.

DISCUSSION

In this multi-locus coalescent species tree analysis, recognized species-level taxa within the genus *Tympanuchus* formed well-supported monophyletic clades. This level of resolution has not been documented before using traditional gene tree methods (i.e., Ellsworth *et al.* 1994; Gutiérrez *et al.* 2000; Lucchini *et al.* 2001; Dimcheff *et al.* 2002; Drovetski 2002 and 2003; Johnson 2008; Oyler-McCance *et al.* 2010). Therefore, this is the first study using molecular methods identifying reciprocal monophyly among *Tympanuchus* species, further supporting current taxonomy based on morphology and behavioral comparisons among prairie grouse taxa.

Results from this analysis suggest that North American prairie grouse diverged recently (<200,000 years), agreeing with previous phylogenetic studies on this genus (Drovetski 2003; Johnson 2008; Oyler-McCance *et al.* 2010). The time frame of *Tympanuchus* diversification corresponds to the expansion of tall-grass prairies in North America (3-8 MYA; Retallack 1997; Janis *et al.* 2002; Kelley and Rundel 2005; Stromberg 2005; Edwards *et al.* 2010) followed by the opening and closing of glacial refugia during the Pleistocene (0.0145-2.5 MYA; Wisely *et al.* 2008). While some biologists question importance of the Pleistocene in avian diversification (i.e., Klicka and Zink, 1997), these results suggest that this period was important for prairie grouse speciation, as it has been for other recently-divergent avian species in North America (e.g.., *Icterus spurius spp.,* Baker *et al.* 2003; *Juncos hyemalis spp.*, Milá *et al.* 2007; *Passerina spp*., Carling *et al.* 2010; *Aphelocoma spp.*, McCormack *et al.* 2010; *Zonotrichia leucophrys spp.* McCormack *et al.* 2012).

Phylogenetic Relationships: Gene Trees and Species Trees

A discord existed in this study among single-locus gene trees, with some loci supporting recognized species-level taxa based on monophyly, while other loci were uninformative with prairie grouse forming polytomies. In addition to the single-locus gene trees, the multi-locus species tree and concatenated gene tree analyses also differed in overall topology. With the exception of the mtDNA species tree, monophyly was supported among all recognized *Tympanuchus* species in the species tree analyses, while only sharp-tailed grouse formed a monophyletic clade in the concatenated gene tree analysis.

These differences in concatenated gene trees and species trees can be attributed to stochastic lineage sorting among loci. When individual loci are combined into a 'supergene', stochastic differences in gene topologies and the unique evolutionary histories of each locus are ignored and resulting phylogenies produce conflicting results. In contrast, species tree approaches use a multispecies coalescent to calculate gene trees within a larger species tree that minimizes deep coalescence, all while accounting for lineage sorting among geneologies (Carstens and Knowles 2007; Knowles and Carstens 2007; Kubatko and Degnan 2007; Degnan and Rosenberg 2009; Knowles 2009). Using multiple loci was also important in species tree analyses, as the single-locus mitochondrial species tree was also unable to recover evolutionary relationships among prairie grouse. Consequently, multi-locus species tree approaches are increasingly used to explore species-level relationships compared to single-locus or concatenated gene-tree approaches, particularly in cases with recently divergent taxa where stochastic lineage sorting among loci may be an issue limiting phylogenetic

resolution (e.g., Carstens and Knowles 2007; Brumfield *et al.* 2008; McCormick *et al.* 2010; Alström *et al.* 2011).

Differences in Utility Among Mitochondrial, Autosomal, and Z-linked Markers

Species tree and gene tree results were interesting given that the choice of genetic marker proved important for describing species-level resolution among *Tympanuchus* grouse. In this analysis, three types of markers were used (mitochondrial, autosomal, and Z-linked), each possessing different levels of polymorphism, divergence, and species-level topology. The mtDNA control region showed high polymorphism, but very little divergence among currently recognized taxa. Autosomal loci also showed a high degree of polymorphism, although less than that observed with the mtDNA control region, with limited resolution for designating taxonomic groups within the genus *Tympanuchus*. The Z-linked loci, however, were noticeably different, as these loci showed limited polymorphism, but a high degree of divergence among the sampled species-level taxa. Clearly, Z-linked loci provided resolution for species-level divergence in our study (see also Corl and Ellegren 2013), suggesting that Z-linked loci are behaving differently than nuclear autosomal and mtDNA loci in *Tympanuchus* grouse.

The effective size (N_e) of mtDNA markers is often referenced as being a quarter of the size of nuclear markers (i.e., 0.25 inheritance scalar in monogamous species), while Z-linked loci are 0.75 that of autosomal (Sundström *et al.* 2004; Mank *et al.* 2007; Ellegren 2009; Smith and Klicka 2013). Theoretically, lineage sorting among isolated

populations should take less time for mtDNA to reach reciprocal monophyly compared to Z-linked and autosomal loci due to differences in N_e among loci, with Z-linked also taking less time than that of autosomal (Moore 1995; Hudson and Coyne 2002; Funk and Omland 2003; Rosenberg 2003). However, in the case of populations with a polygynous mating system (i.e., lekking grouse) with female biased dispersal, the N_e of mitochondrial markers can be over three times greater than that of nuclear markers (Chesser and Baker 1996; see also Johnson *et al.* 2003) suggesting that the time required to establish reciprocal monophyly may take longer for mtDNA than nuclear loci in such species. Similarly, polygyny can also further reduce the N_e of Z-linked loci than expected based on monogamy. In the case of birds a highly skewed mating system (1 breeding male for every 10 breeding females) N_e has been estimated at 0.55 instead of 0.75 (Sundström *et al.* 2004), which will increase the overall speed at which Z-linked loci reach reciprocal monophyly.

This difference in effective population size between Z-linked and mitochondrial DNA may explain the differences in nuclear and mitochondrial species trees observed among *Tympanuchus* grouse. If the Z-linked loci have a smaller N_e compared to the mtDNA used in this study, the Z-linked loci should reach reciprocal monophyly before both the mtDNA and autosomal loci. This scenario agrees with our results, but selection may also be an important factor, in addition to drift, for explaining how the markers differ with respect to their ability to resolve species-level relationships among *Tympanuchus* grouse (see also Elgvin *et al*. 2011).

Since females are the heterogametic sex in birds, Haldane's Rule proposes that females are more likely to suffer fitness consequences following interspecific crosses

(Orr 1997). Deleterious fitness traits as a result of hybridization and incompatibilities associated with the Z-chromosome are expressed with the heterogametic sex, and therefore purged from the population through 'selective sweeps' (Charlesworth *et al.* 1987). Consequently, due to these factors including low recombination on the Z chromosome, many bird species possess low Z chromosome allelic variation, high allele fixation, and reduced introgression between species (Sundström *et al.* 2004; Borge *et al.* 2005; Storchová *et al.* 2009; Carling *et al.* 2010; Backström and Väli 2011; Elgvin *et al.* 2011).

Multiple studies comparing genomes among bird species suggest that the Z chromosome evolves faster than autosomes (i.e., the 'Fast-Z' effect), with this 'Fast-Z' effect being significantly more pronounced than the 'Fast-X' counterpart in mammals (Mank *et al.* 2007; Ellegren 2009). The fast evolutionary rate found on the Z chromosome in birds is faster than what would be expected by Haldane's Rule, which suggests that genes important for traits used for maintaining species boundaries or reinforcement are located on the Z chromosome. Indeed, studies have confirmed that genes found on the Z chromosome in birds are directly associated with sexually selected plumage traits, female mate preference behavior, and in some cases female hybrid sterility (Sætre *et al.* 2003; Sæther *et al.* 2007; Ellegren 2009). This 'Fast-Z effect' is supported by our data, in which Sharp-tailed grouse, in particular, show strong differentiation in Z-linked loci relative to other autosomal loci.

The results from this study also suggest that hybridization between *Tympanuchus* species, particularly sharp-tailed grouse and greater prairie-chickens may also account for the lack of resolution observed in the mtDNA species tree. While

some studies have found minimal or no mtDNA introgression among hybridizing bird species (e.g., Carling *et al.* 2010; Jacobsen and Omland 2012), others have documented introgression on mitochondrial markers (Funk and Omland 2003; Chan and Levin 2005; Bossu and Near 2009). Contemporary hybrid zones for sharp-tailed grouse and greater prairie-chickens extend from North Dakota and Minnesota south to Nebraska, while lesser and greater prairie-chicken distributions overlap only in northwest Kansas, with both groups known to hybridize (Johnsgard and Wood 1968; Crawford 1978; Bain and Farely 2002; Lumsden 2005). In the mtDNA species tree, two subspecies of sharp-tailed grouse in Wyoming (*T. p. jamesi and T. p. columbianus*) formed a paraphyletic clade with greater prairie-chickens, while two geographically distant subspecies (*T. p. kenicottii* and *T. p. phasianellus*) formed a separate monophyletic clade. Given the geographic proximity and overlapping distributions of *T. p. jamesi and T. p. columbianus* in contrast to the two allopatric subspecies, these results suggest that hybridization in combination with incomplete lineage sorting may account for the shared mtDNA haploytpes between these taxa. In contrast, no such patterns were observed with lesser and greater prairie-chickens despite including samples from there region of geographic overlap in northwest Kansas.

While hybridization may have affected the topology of the mitochondrial species tree, signals of introgression were not likely to affect Z-linked loci. As stated earlier, studies have shown that genes on the Z chromosome are important for maintaining reproductive isolation by influencing sexually-selected traits or mate preference behavior (Sætre *et al.* 2003; Sæther *et al.* 2007; Ellegren 2009). The high degree of sexual selection found in lekking grouse may act on the Z chromosome, preventing

introgression with sex-linked genes while some signal passes through to mitochondrial DNA (Chan and Levin 2005). Isolation-with-Migration analyses conducted between mitochondrial, autosomal, and Z-linked loci (e.g., Storchová *et al.* 2009; Carling *et al.* 2010; Backström and Väli 2011; Jacobsen *et al.* 2012) would be informative on how introgression acts on different marker types in prairie grouse.

Subspecific comparisons within *Tympanuchus* grouse

In both the species and gene tree analyses, recognized subspecies for sharp-tailed grouse and greater prairie-chicken were not supported. However, subspecies within sharp-tailed grouse were supported by the STRUCTURE analyses and pairwise F_{ST} estimates, while not supported for subspecies of greater prairie-chicken. Due to the slightly earlier divergence of sharp-tailed grouse from pinnated grouse seen in the species tree analysis, the time available to accumulate differences between populations through lineage sorting and/or the effects of genetic drift may have influenced the ability of STRUCTURE to detect subspecies.

It is important to consider that while the Attwater's prairie-chicken does not appear distinct using sequence data, previous work using 11 microsatellite loci show that Attwater's and greater prairie-chickens form two distinct populations based on the methods implemented in STRUCTURE (J. Johnson, unpublished data). Similarly, subspecific distinctions among sharp-tailed grouse subspecies have been resolved using microsatellite loci (Spaulding *et al.* 2006). Microsatellites have also been able to detect distinct population clusters in lesser prairie-chickens from New Mexico and Oklahoma (Pruett *et al.* 2011). Clearly, structure on a population level has been

detected previously in prairie grouse populations, while sequence data in our analysis were unable to recover population-level structure in greater and lesser prairie-chickens.

In a previous microsatellite simulation study investigating F_{ST} and population structure, Latch and colleagues (2006) showed that an F_{ST} value of at least 0.03-0.05 is required before STRUCTURE is able to detect distinct populations. Our F_{ST} values between Attwater's and greater prairie-chickens are within this range (0.055-0.07), however, the differences in polymorphism between microsatellites and sequence data may cause disparity between in our results and other microsatellite studies. Furthermore, to what degree our small sample sizes (<10 individuas per population) are influencing these results are not known (e.g., Kalinowski 2005; Hale *et al.* 2012). In addition to differences in markers and sample size, recent declines in population size and the effects of genetic drift may have affected these results (e.g., Smith and Klicka 2013). Indeed, differentiation at microsatellite loci has also been observed between populations of both greater (Johnson *et al.* 2003, 2004) and lesser (Pruett *et al.* 2011) prairie-chickens that have experienced recent habitat fragmentation and increased isolation within the past 50 years. To what degree the differentiation between Attwater's and greater prairie-chicken populations exist deserves further study.

Conservation implications

Using a single mtDNA control region locus and coalescent-based analyses, Johnson (2008) suggested that the Attwater's prairie-chicken was as divergent from greater prairie-chicken as other *Tympanuchus* grouse, such as lesser prairie-chicken

and sharp-tailed grouse. Based on those results, Johnson (2008) cautioned against outcrossing Attwater's with greater prairie-chickens and suggested a multi-locus coalescent-based analysis was required to eliminate any uncertainty associated with stochastic lineage sorting, or a single-locus approach (Brito and Edwards 2009) for discerning the evolutionary relationships among *Tympanuchus* taxa. The results from our multi-locus coalescent analysis, however, do not corroborate the results obtained previously (Johnson 2008), and suggest that the Attwater's prairie-chicken is more closely related to the greater prairie-chicken than any other prairie grouse.

Inbreeding depression has been documented in both the wild and captive populations of the critically endangered Attwater's prairie-chicken (Hammerly *et al. in review*). Managers have considered outcrossing Attwater's with greater prairie-chickens to improve fitness (Mike Morrow, *pers. comm.*) similar to other conservation programs where threatened species also experienced inbreeding depression (Westemeier *et al.* 1998; Johnson *et al.* 2010). While inbreeding depression has been detected in this subspecies, the risk of outbreeding depression is still not known.

Using an extended form of the breeders' equation, Frankham *et al.* (2011) recommend that populations that have been isolated for at least 500 years or inhabit different environments should not be allowed to breed with each other due to an increased probability of outbreeding depression. In the case of the Attwater's prairie-chicken, however, it is still unclear as to when Attwater's and greater prairie-chicken diverged, as low support was observed for the node separating these two taxa in the species tree analysis. In our study, a Bayesian isolation-with-migration model (Pinho and Hey 2010) was attempted to ascertain the timing of population divergence between

taxa while accounting for migration and changes in population size, but unfortunately, due to limited information provided with the data (i.e., limited polymorphism), runs failed to converge and multiple runs resulted in contradictory results. Therefore, more sequence data are necessary to explore the timing of divergence between Attwater's and greater prairie-chickens using an isolation-with-migration model of population divergence.

While Attwater's were not shown to be distinct relative to the greater prairie-chicken based on the generated genetic dataset used in this study, these results do not necessarily negate that Attwater's are distinct. Habitat characteristics of the Attwater's home range differ from those of greater prairie-chickens. Attwater's are native to the Gulf coastal prairies, which are dominated by tall-grass species and also marsh species, like rushes (Family: Juncaceae), sedges (Family: Cyperaceae), and cordgrass (*Spartina spp.*; Johnsgard 2002). Besides living in a different habitat type, Attwater's differ from greater prairie-chickens in plumage and air sac color, number of tarsal feathers, and number and width of pinnae feathers (Lehmann 1942; Johnsgard 2002; Johnson *et al.* 2011). Although species (and subspecies) concepts are debated among biologists, distinct morphological characteristics found in Attwater's, along with unpublished microsatellite data confirming structure between Attwater's and greater prairie-chicken (J. Johnson *unpublished data*), would support distinction as a subspecies according to prominent sources (Haig *et al.* 2006; Helbig *et al.* 2002).

Our multi-locus analyses provided helpful insight into the evolutionary relationships among prairie grouse. More research is warranted to obtain more precise estimates of divergence and historical inferences of gene flow between populations and

subspecies. One way that this can be accomplished is by utilizing new methodologies for generating sequence data. Next generation sequencing (NGS), for example, is a new method for generating large amounts of sequence data at a fairly low cost (e.g., Carstens *et al.* 2012). In recent literature, NGS has provided sufficient sequence data to increase the resolution necessary for discerning taxonomic relationships among recently divergent taxa (Lerner and Fleischer 2010; McCormick *et al.* 2012) that were previously not possible to determine using similar methods as employed in this study. Therefore, it is recommended that further analyses are needed for discerning the timing of divergence between Attwater's and greater prairie-chicken. This can be accomplished by combining additional sequence data generated with NGS methodologies and those obtained from this study to provide further resolution to discern the timing of divergence between these two taxa.

APPENDIX

TABLES AND FIGURES

Appendix Table 1 Sharp-tailed grouse (*Tympanuchus phasianellus*) samples used in this study. All samples provided by Allan Spaulding.

DNA ID	Species	Province/State, Country	Location	Date Collected	Tissue
NWT-1	*T. p. kennicotti*	NWT, Canada	Norman Wells	Nov 2002	Wing-Muscle
NWT-2	*T. p. kennicotti*	NWT, Canada	Norman Wells	Feb 2003	Wing-Muscle
NWT-3	*T. p. kennicotti*	NWT, Canada	Unknown	Jan 2002	Wing-Muscle
NWT-4	*T. p. kennicotti*	NWT, Canada	Norman Wells	Feb 2003	Wing-Muscle
NWT-5	*T. p. kennicotti*	NWT, Canada	Norman Wells	Unknown	Wing-Muscle
NWT-6	*T. p. kennicotti*	NWT, Canada	Norman Wells	Jan 2003	Wing-Muscle
NWT-7	*T. p. kennicotti*	NWT, Canada	Turton Lake	Jan 2003	Wing-Muscle
NWT-8	*T. p. kennicotti*	NWT, Canada	Norman Wells	Dec 2002	Wing-Muscle
NWT-9	*T. p. kennicotti*	NWT, Canada	Norman Wells	Jan 2003	Wing-Muscle
NWT-10	*T. p. kennicotti*	NWT, Canada	Norman Wells	Dec 2002	Wing-Muscle
Plains-1	*T. p. jamesi*	WY, United States	Campbell/Western Co.	Sept 2005	Wing-Muscle
Plains-2	*T. p. jamesi*	WY, United States	Campbell/Western Co.	Sept 2005	Wing-Muscle
Plains-3	*T. p. jamesi*	WY, United States	Campbell/Western Co.	Sept 2005	Wing-Muscle
Plains-4	*T. p. jamesi*	WY, United States	Campbell/Western Co.	Sept 2005	Wing-Muscle
Plains-5	*T. p. jamesi*	WY, United States	Campbell/Western Co.	Sept 2005	Wing-Muscle
Plains-6	*T. p. jamesi*	WY, United States	Campbell/Western Co.	Sept 2005	Wing-Muscle
Plains-7	*T. p. jamesi*	WY, United States	Campbell/Western Co.	Sept 2005	Wing-Muscle
Plains-8	*T. p. jamesi*	WY, United States	Campbell/Western Co.	Sept 2005	Wing-Muscle
Plains-9	*T. p. jamesi*	WY, United States	Campbell/Western Co.	Sept 2005	Wing-Muscle
Plains-10	*T. p. jamesi*	WY, United States	Campbell/Western Co.	Sept 2005	Wing-Muscle
Col-1	*T. p. columbianus*	WY, United States	Carbon Co.	Sept 2005	Wing-Muscle
Col-2	*T. p. columbianus*	WY, United States	Carbon Co.	Sept 2005	Wing-Muscle
Col-3	*T. p. columbianus*	WY, United States	Carbon Co.	Sept 2005	Wing-Muscle
Col-4	*T. p. columbianus*	WY, United States	Carbon Co.	2004	Wing-Muscle
Col-5	*T. p. columbianus*	WY, United States	Carbon Co.	2004	Wing-Muscle
Col-6	*T. p. columbianus*	WY, United States	Carbon Co.	2004	Wing-Muscle
ONT/NE-1	*T. p. phasianellus*	ONT, Canada	Near Fort Albany	Spring 1998	Freeze-dried Muscle
ONT/NE-2	*T. p. phasianellus*	ONT, Canada	Near Fort Albany	Spring 1998	Freeze-dried Muscle
ONT/NE-3	*T. p. phasianellus*	ONT, Canada	Near Fort Albany	Spring 1998	Freeze-dried Muscle
ONT/NE-4	*T. p. phasianellus*	ONT, Canada	Near Fort Albany	Spring 1998	Freeze-dried Muscle
ONT/NE-5	*T. p. phasianellus*	ONT, Canada	Near Fort Albany	Spring 1998	Freeze-dried Muscle
ONT/NE-6	*T. p. phasianellus*	ONT, Canada	Near Fort Albany	Spring 1998	Freeze-dried Muscle
ONT/NE-7	*T. p. phasianellus*	ONT, Canada	Near Fort Albany	Spring 1998	Freeze-dried Muscle

Appendix Table 2 Greater Sage-grouse (*Centrocercus urophasianus*) samples used in this study.

DNA ID	Species	Province/State, Country	Location	Date Collected	Tissue
SG-15	*Centrocercus urophasianus*	WY, United States	Teton Co.	Apr 2007	Blood
SG-86	*Centrocercus urophasianus*	WY, United States	Teton Co.	Jun 2008	Blood
SG-104	*Centrocercus urophasianus*	WY, United States	Teton Co.	May 2009	Blood
SG-105	*Centrocercus urophasianus*	WY, United States	Teton Co.	May 2009	Blood
SG-408	*Centrocercus urophasianus*	WY, United States	Sublette Co.	Apr 2006	Blood
SG-410	*Centrocercus urophasianus*	WY, United States	Sublette Co.	Apr 2006	Blood
SG-426	*Centrocercus urophasianus*	WY, United States	Sublette Co.	Apr 2006	Blood
SG-600	*Centrocercus urophasianus*	WY, United States	Johnson Co.	Mar 2008	Blood
SG-622	*Centrocercus urophasianus*	WY, United States	Johnson Co.	Mar 2008	Blood
SG-623	*Centrocercus urophasianus*	WY, United States	Johnson Co.	Mar 2008	Blood
SG-740	*Centrocercus urophasianus*	WY, United States	Johnson Co.	Mar 2010	Blood
SG-747	*Centrocercus urophasianus*	WY, United States	Johnson Co.	Mar 2010	Blood

Appendix Table 3.1 Descriptive statistics for each population studied across two loci: 06419 and 09300. Statistically significant figures are denoted with a double asterix **.

	06419 (379 bp) Autosomal					09300 (370 bp) Autosomal				
Pop.	n	# Alleles/ Haplotypes	Gene Diversity ± SE	π ± SE	Tajima's D	n	# Alleles/ Haplotypes	Gene Diversity ± SE	π ± SE	Tajima's D
GRPC (ATTW) TX	20	5	0.821± 0.038	0.00892± 0.00084	1.68	26	8	0.797± 0.0556	0.0072± 0.000707	0.417
GRPC KS	18	7	0.843± 0.228	0.00799± 0.00076	1.0366	18	8	0.889± 0.0465	0.00749± 0.000775	0.209
GRPC MN	18	6	0.627± 0.124	0.00202± 0.000548	-1.529	18	6	0.739± 0.0978	0.00624± 0.00126	-0.0279
GRPC OK	12	4	0.636± 0.128	0.00472± 0.00176	-0.382	12	7	0.833± 0.1	0.00782± 0.001	-0.519
LEPC KS	20	4	0.563± 0.116	0.00174± 0.000447	-0.626	20	6	0.832± 0.0390	0.00663± 0.000837	0.295
LEPC NM	16	5	0.750± 0.078	0.00356± 0.00078	-0.379	20	6	0.811± 0.0497	0.00737± 0.000707	1.255
LEPC OK	18	7	0.882± 0.0394	0.00818± 0.00114	0.643	18	7	0.850± 0.0566	0.00631± 0.0011	0.0111
GRSG WY	0	N/A	N/A	N/A	N/A	22	3	0.544± 0.0967	0.00217± 0.000447	1.0237
STGR (COL) WY	12	2	0.485± 0.106	0.00128± 0.000316	1.0659	12	3	0.667± 0.0910	0.00704± 0.000837	2.116**
STGR NWT	20	3	0.195± 0.114	0.00276± 0.00158	-1.213	20	3	0.574± 0.0552	0.00317± 0.000316	0.97
STGR ONT	14	4	0.747± 0.0779	0.00814± 0.001	1.468	12	3	0.591± 0.108	0.00356± 0.00179	-1.283
STGR (PLN) WY	20	5	0.758± 0.0575	0.00837± 0.000775	1.983	20	6	0.758± 0.0645	0.00676± 0.000775	-0.0499

Appendix Table 3.2 Descriptive statistics for each population studied across two loci: 14726 and 15506. Statistically significant figures are denoted with a double asterix **.

	14726 (182 bp) Autosomal					15506 (585 bp) Autosomal				
Pop.	n	# Alleles/ Haplotypes	Gene Diversity ± SE	π ± SE	Tajima's D	n	# Alleles/ Haplotypes	Gene Diversity ± SE	π ± SE	Tajima's D
GRPC (ATTW) TX	26	4	0.612± 0.0806	0.0106± 0.00164	0.153	26	3	0.428± 0.0949	0.00141 ±0.0003 16	0.0912
GRPC KS	18	6	0.758± 0.0647	0.0182± 0.00152	0.953	18	5	0.791± 0.0519	0.00465 ±0.0008 37	0.587
GRPC MN	18	4	0.477± 0.134	0.00643± 0.00173	0.018	18	3	0.647± 0.0691	0.00463 ±0.0008 37	1.118
GRPC OK	12	4	0.455± 0.17	0.00549± 0.00298	** -1.894	12	3	0.545± 0.144	0.00425 ±0.0012 6	0.282
LEPC KS	20	4	0.489± 0.117	0.0072± 0.00195	-0.717	20	7	0.753± 0.0787	0.00269 ±0.0006 32	-1.306
LEPC NM	20	4	0.658± 0.0647	0.0101± 0.000949	0.928	20	3	0.626± 0.0787	0.00291 ±0.0005 48	1.484
LEPC OK	18	7	0.771± 0.0834	0.00716± 0.00145	-1.279	18	3	0.686± 0.0499	0.00211 ±0.0003 16	1.154
GRSG WY	24	3	0.163± 0.099	0.00309± 0.00187	-1.301	24	4	0.572± 0.0954	0.00347 ±0.0007 75	0.221
STGR (COL) WY	12	3	0.712± 0.0698	0.0165± 0.00232	1.945	12	2	0.53± 0.0764	0.00272 ±0.0004 47	1.973
STGR NWT	20	4	0.679± 0.0742	0.0127± 0.00214	1.167	20	3	0.616± 0.0769	0.00273 ±0.0001 41	1.199
STGR ONT	14	4	0.571± 0.132	0.00924± 0.00303	-0.391	14	3	0.484± 0.142	0.0009± 0.00031 6	-0.438
STGR (PLN) WY	20	3	0.626± 0.0787	0.0124± 0.00221	1.0579	20	4	0.553± 0.111	0.00292 ±0.0007 07	0.654

Appendix Table 3.3 Descriptive statistics for each population studied across two loci: 24105 and 25189. Statistically significant figures are denoted with a double asterix **.

	24105 (440 bp) Z-linked					25189 (580 bp) Z-linked				
Pop.	n	# Alleles/ Haplotypes	Gene Diversity ± SE	π ± SE	Tajima's D	n	# Alleles/ Haplotypes	Gene Diversity ± SE	π ± SE	Tajima's D
GRPC (ATTW) TX	26	2	0.271± 0.099	0.00062± 0.000316	0.0544	26	2	0.148± 0.0888	0.00025± 0.0	-0.714
GRPC KS	18	4	0.627± 0.0733	0.00165± 0.000316	-0.466	18	2	0.542± 0.0861	0.001± 0.0	0.00096
GRPC MN	18	2	0.503± 0.0639	0.00114± 0.0	1.378	18	3	0.307± 0.132	0.00055± 0.000316	-1.0963
GRPC OK	12	3	0.545± 0.144	0.00138± 0.000447	-0.248	12	3	0.545± 0.144	0.00136± 0.000447	0.554
LEPC KS	20	3	0.279± 0.123	0.00109± 0.000548	-1.158	20	3	0.426± 0.122	0.00125± 0.000447	-0.377
LEPC NM	20	3	0.358± 0.127	0.00086± 0.000316	-0.769	20	3	0.532± 0.1	0.00201± 0.000447	0.105
LEPC OK	18	3	0.392± 0.133	0.00143± 0.000548	-0.778	18	4	0.477± 0.134	0.00159± 0.000548	-0.623
GRSG WY	24	1	NP	NP	NP	24	4	0.656± 0.0803	0.00224± 0.000316	1.546
STGR (COL) WY	12	1	NP	NP	NP	12	2	0.485± 0.106	0.00084± 0.0	1.0659
STGR NWT	20	2	0.1± 0.088	0.00023± 0.0	-1.164	20	1	NP	NP	NP
STGR ONT	14	1	NP	NP	NP	14	4	0.659± 0.12	0.00167± 0.000447	0.0768
STGR (PLN) WY	20	1	NP	NP	NP	20	2	0.526± 0.0363	0.00091± 0.0	1.565

Appendix Table 3.4 Descriptive statistics for each population studied across two loci: ALDOB and CHD1Z. Statistically significant figures are denoted with a double asterix **.

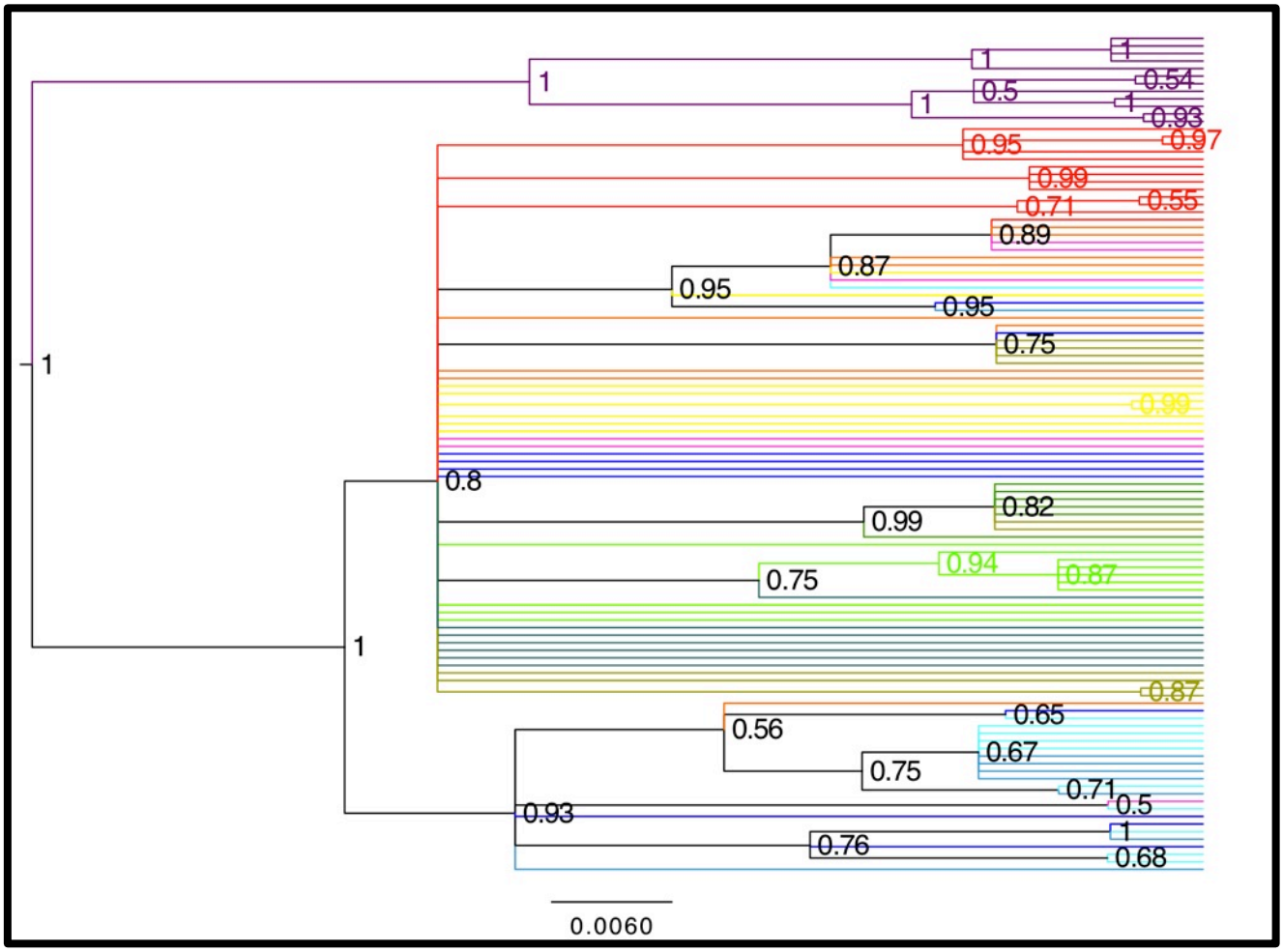
	ALDOB (504 bp) Z-linked					CHD1Z (402 bp) Z-linked				
Pop.	n	# Alleles/ Haplotypes	Gene Diversity ± SE	π ± SE	Tajima's D	n	# Alleles/ Haplotypes	Gene Diversity ± SE	π ± SE	Tajima's D
GRPC (ATTW) TX	26	4	0.625± 0.0691	0.00278± 0.000316	0.194	26	1	NP	NP	NP
GRPC KS	18	6	0.680± 0.109	0.00289± 0.000447	0.00836	20	2	0.1± 0.0880	0.00025± 0.0	-1.164
GRPC MN	18	4	0.608± 0.0857	0.00237± 0.000316	0.0855	18	1	NP	NP	NP
GRPC OK	12	2	0.485± 0.106	0.00192± 0.000447	1.356	12	1	NP	NP	NP
LEPC KS	20	3	0.574± 0.0552	0.00124± 0.0	0.238	20	2	0.479± 0.0720	0.0012± 0.0	1.262
LEPC NM	20	4	0.711± 0.0541	0.00477± 0.000316	**2.162	20	2	0.479± 0.0720	0.0012± 0.0	1.262
LEPC OK	18	4	0.725± 0.0553	0.00386± 0.000447	1.0757	20	2	0.337± 0.11	0.00084± 0.000316	0.352
GRSG WY	24	2	0.518± 0.0344	0.00103± 0.0	1.573	24	1	NP	NP	NP
STGR (COL) WY	12	2	0.485± 0.106	0.00096± 0.0	1.0659	12	2	0.409± 0.133	0.00102± 0.000316	0.541
STGR NWT	20	4	0.753± 0.0482	0.00232± 0.000316	1.0196	20	3	0.668± 0.0537	0.00249± 0.0	1.814
STGR ONT	14	2	0.264± 0.136	0.00052± 0.000316	-0.341	16	2	0.4± 0.114	0.001± 0.000316	0.65
STGR (PLN) WY	20	2	0.189± 0.108	0.00038± 0.0	-0.592	20	4	0.674± 0.0764	0.00283± 0.000316	0.92

Appendix Table 3.5 Descriptive statistics for each population studied across two loci: 06419 and 09300. Statistically significant figures are denoted with a double asterix **.

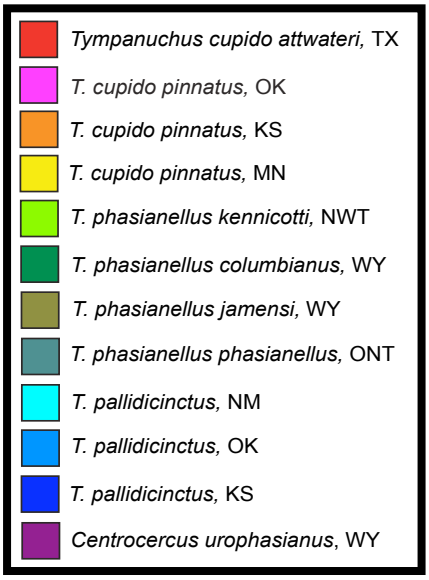
	NNT (587 bp) Z-linked					mtCR-I and II (684 bp) Mitochondrial				
Pop.	n	# Alleles/ Haplotypes	Gene Diversity ± SE	π ± SE	Tajima's D	n	# Alleles/ Haplotypes	Gene Diversity ± SE	π ± SE	Tajima's D
GRPC (ATTW) TX	26	2	0.271± 0.099	0.00046± 0.0	0.0544	13	6	0.859± 0.0632	0.00520± 0.000837	-0.347
GRPC KS	18	4	0.595± 0.109	0.00162± 0.000316	0.24	9	5	0.861± 0.0872	0.000636 ±0.00141	-0.449
GRPC MN	18	3	0.542± 0.0861	0.00191± 0.000316	0.78	9	5	0.806± 0.12	0.00441± 0.000894	-0.433
GRPC OK	12	3	0.712± 0.0691	0.00161± 0.000316	1.223	6	6	1.0± 0.0962	0.00783± 0.00167	-0.387
LEPC KS	20	2	0.268± 0.113	0.00046± 0.0	-0.0861	10	7	0.867± 0.107	0.00772± 0.00141	-0.821
LEPC NM	20	2	0.268± 0.113	0.00046± 0.0	-0.0861	10	7	0.911± 0.0773	0.00643± 0.00155	-0.528
LEPC OK	18	1	NP	NP	NP	9	6	0.833± 0.126	0.00719± 0.00202	-1.063
GRSG WY	24	3	0.420± 0.11	0.00377± 0.00192	-1.274	12	7	0.879± 0.0752	0.01992± 0.00232	1.635
STGR (COL) WY	12	2	0.485± 0.106	0.00249± 0.000548	1.523	6	2	0.333± 0.215	0.00098± 0.000632	-1.132
STGR NWT	20	2	0.189± 0.108	0.00032± 0.0	-0.592	10	4	0.711± 0.117	0.00346± 0.000548	1.334
STGR ONT	14	3	0.484± 0.142	0.00135± 0.000447	-0.494	7	4	0.714± 0.181	0.00294± 0.00141	-1.553
STGR (PLN) WY	20	4	0.689± 0.0596	0.00192± 0.0	0.887	10	4	0.8± 0.0888	0.00418± 0.000632	0.633

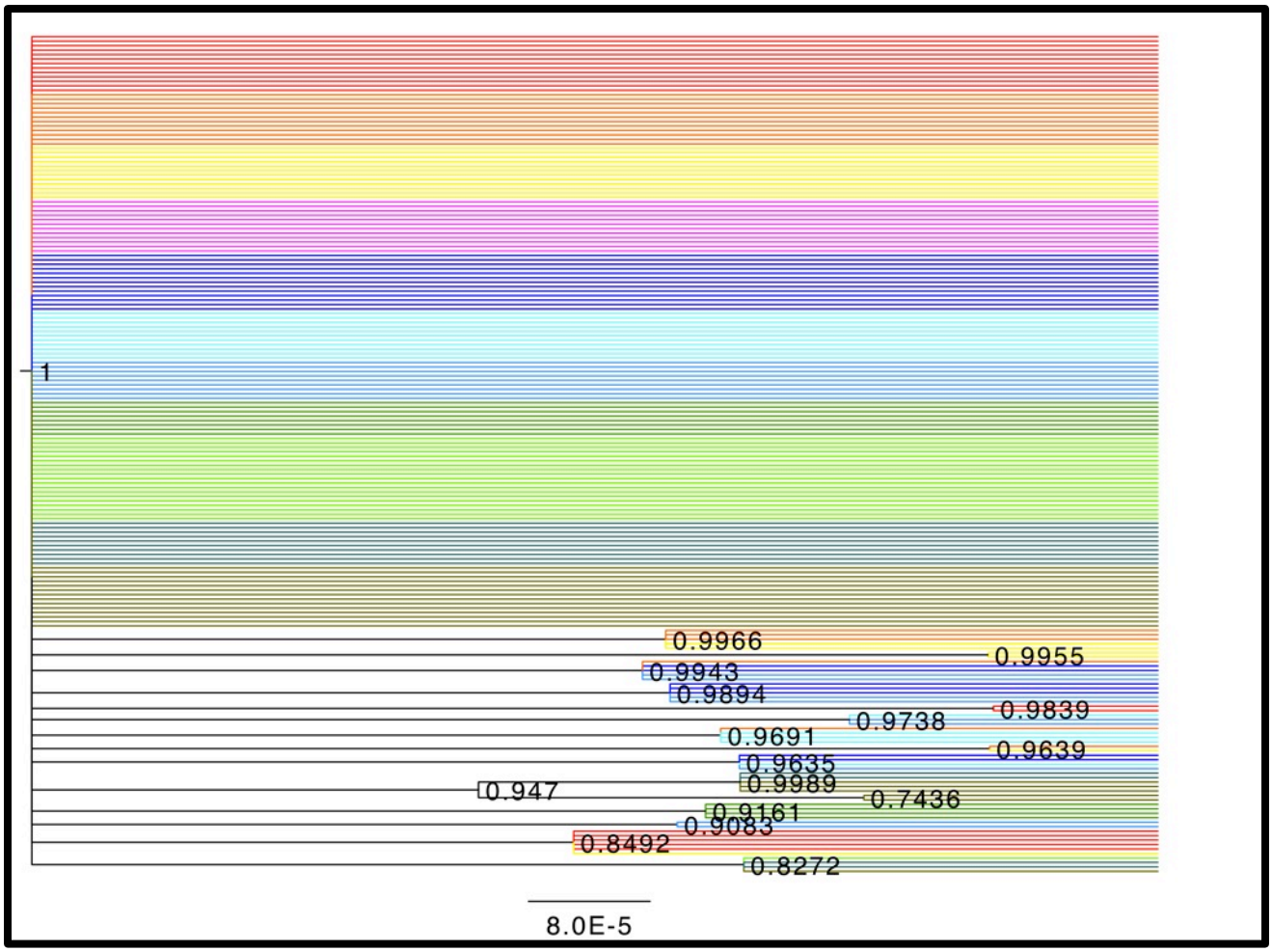
Appendix Table 4 Mean pairwise Fst values for nuclear loci. Significant values are denoted in bold.

	GRPC (Attw) TX	GRPC KS	GRPC MN	GRPC OK	LEPC KS	LEPC NM	LEPC OK	STGR (Col) WY	STGR NWT	STGR ONT	STGR (Jam) WY	GRSG WY
GRPC (Attw) TX	0											
GRPC KS	0.07148	0										
GRPC MN	0.05565	0.05093	0									
GRPC OK	0.05542	0.04004	0.01606	0								
LEPC KS	**0.32011**	**0.25433**	**0.29493**	**0.2848**	0							
LEPC NM	**0.3541**	**0.26685**	**0.32871**	**0.31851**	**0.08226**	0						
LEPC OK	**0.34498**	**0.26352**	**0.3136**	0.29522	0.02633	0.10416	0					
STGR (Col) WY	**0.52296**	0.41911	0.50478	0.48189	**0.46143**	0.46614	**0.4761**	0				
STGR NWT	**0.53105**	**0.44429**	**0.51012**	**0.49028**	**0.4847**	**0.48139**	**0.49165**	**0.26449**	0			
STGR ONT	**0.54176**	0.44142	**0.52334**	0.50934	**0.49628**	**0.48365**	**0.49545**	**0.21636**	**0.29083**	0		
STGR (Jam) WY	**0.50693**	**0.42044**	**0.48818**	**0.46625**	**0.45523**	**0.45835**	**0.46674**	0.16289	**0.2434**	0.12061	0	
GRSG WY	**0.57567**	**0.49562**	**0.56478**	**0.55486**	**0.5336**	**0.52584**	**0.53996**	**0.56952**	**0.57004**	**0.58738**	**0.54895**	0

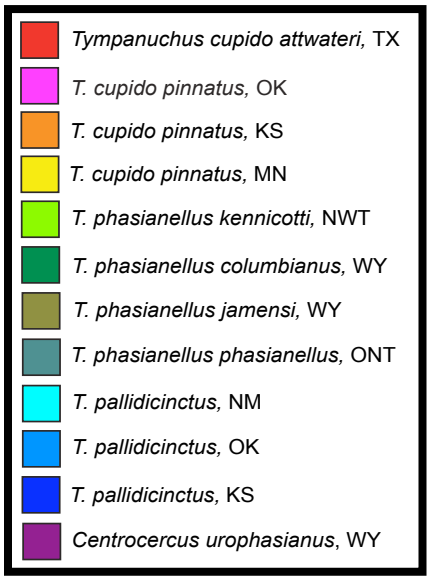


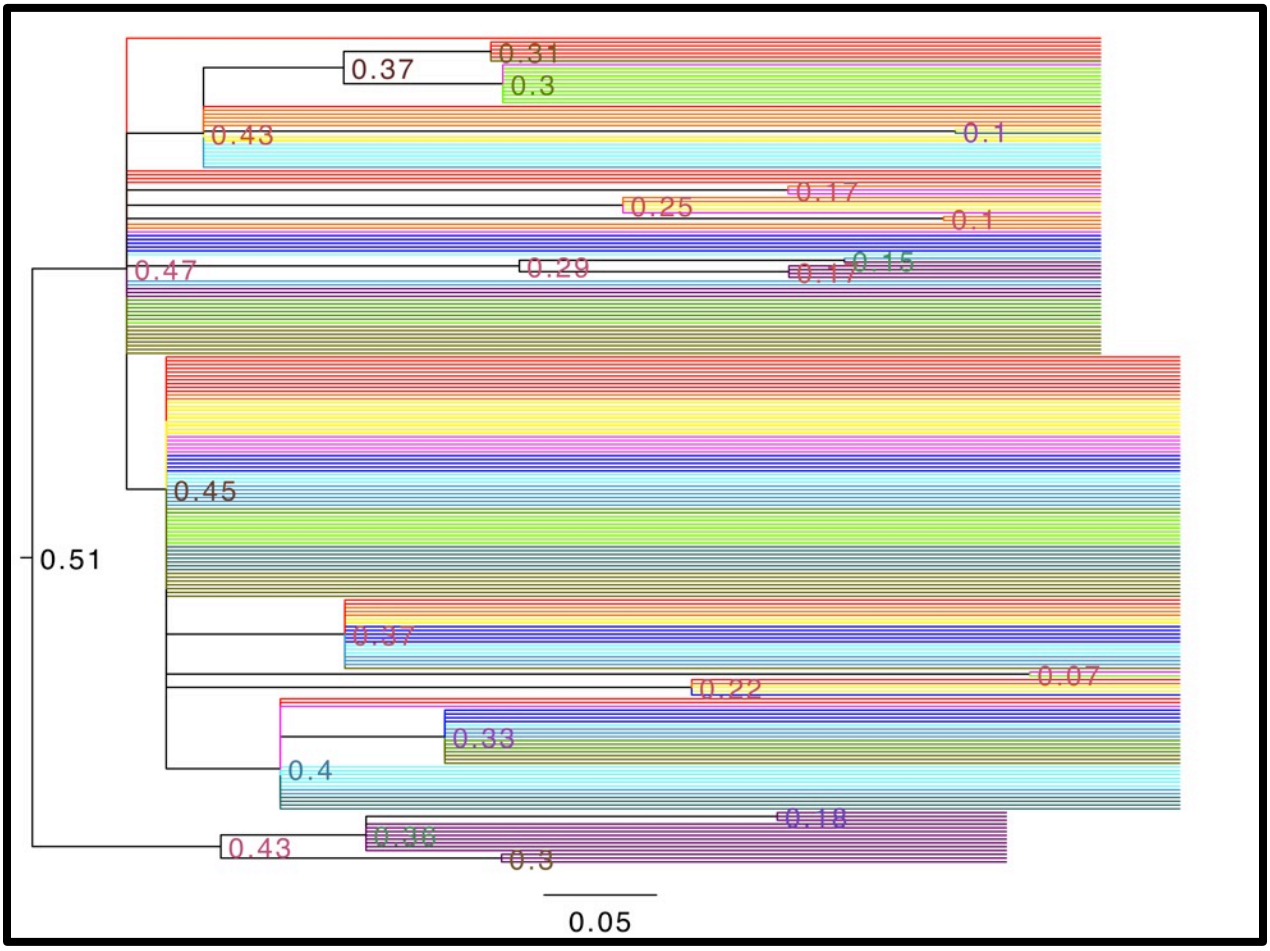
Appendix Fig. 1 MrBayes 50% majority rule consensus tree for 684 base pairs of the mitochondrial control region (I & II). Posterior probability values are denoted next to each node. Tip colors correspond to different populations of prairie grouse (see legend).



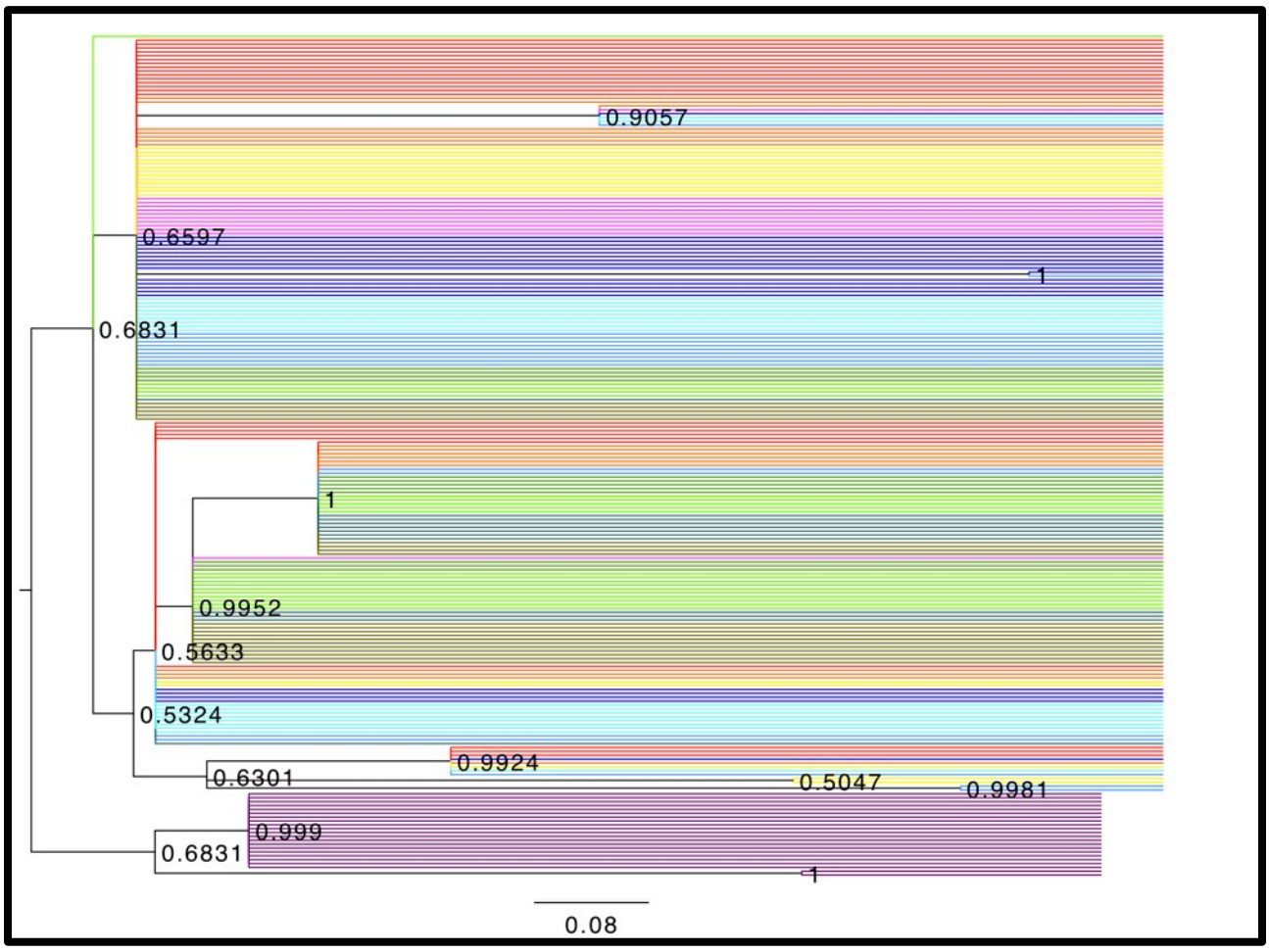


Appendix Fig. 2 MrBayes 50% majority rule consensus tree for 379 base pairs of autosomal locus 06419. Posterior probability values are denoted next to each node. Tip colors correspond to different populations of prairie grouse (see legend). The outgroup (greater sage-grouse) did not amplify for this locus.

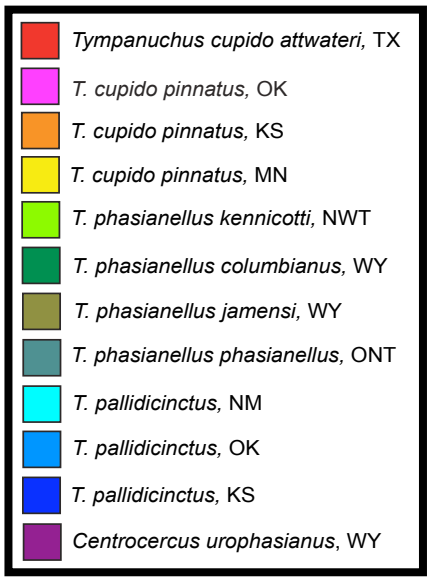


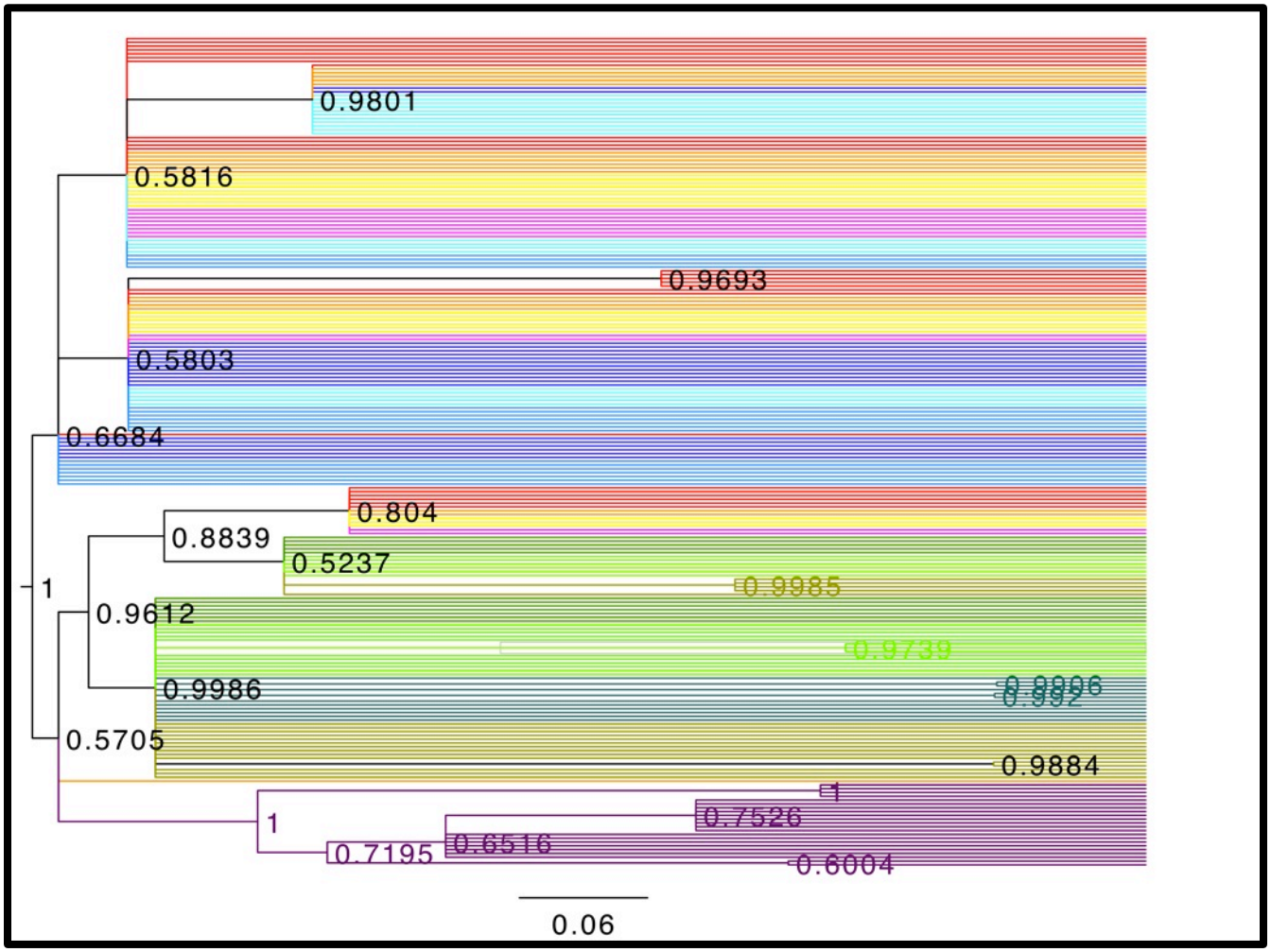


Appendix Fig. 3 MrBayes 50% majority rule consensus tree for 370 base pairs of autosomal locus 09300. Posterior probability values are denoted next to each node. Tip colors correspond to different populations of prairie grouse (see legend).

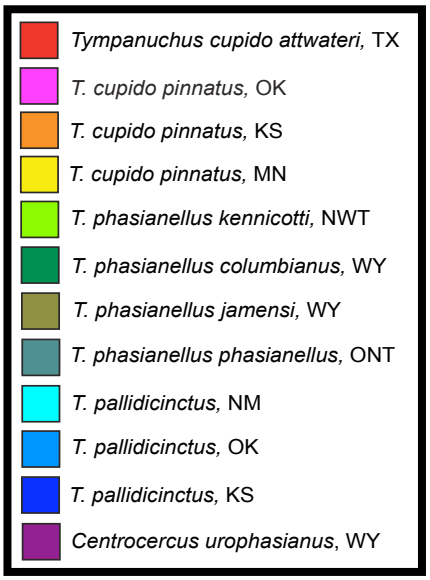


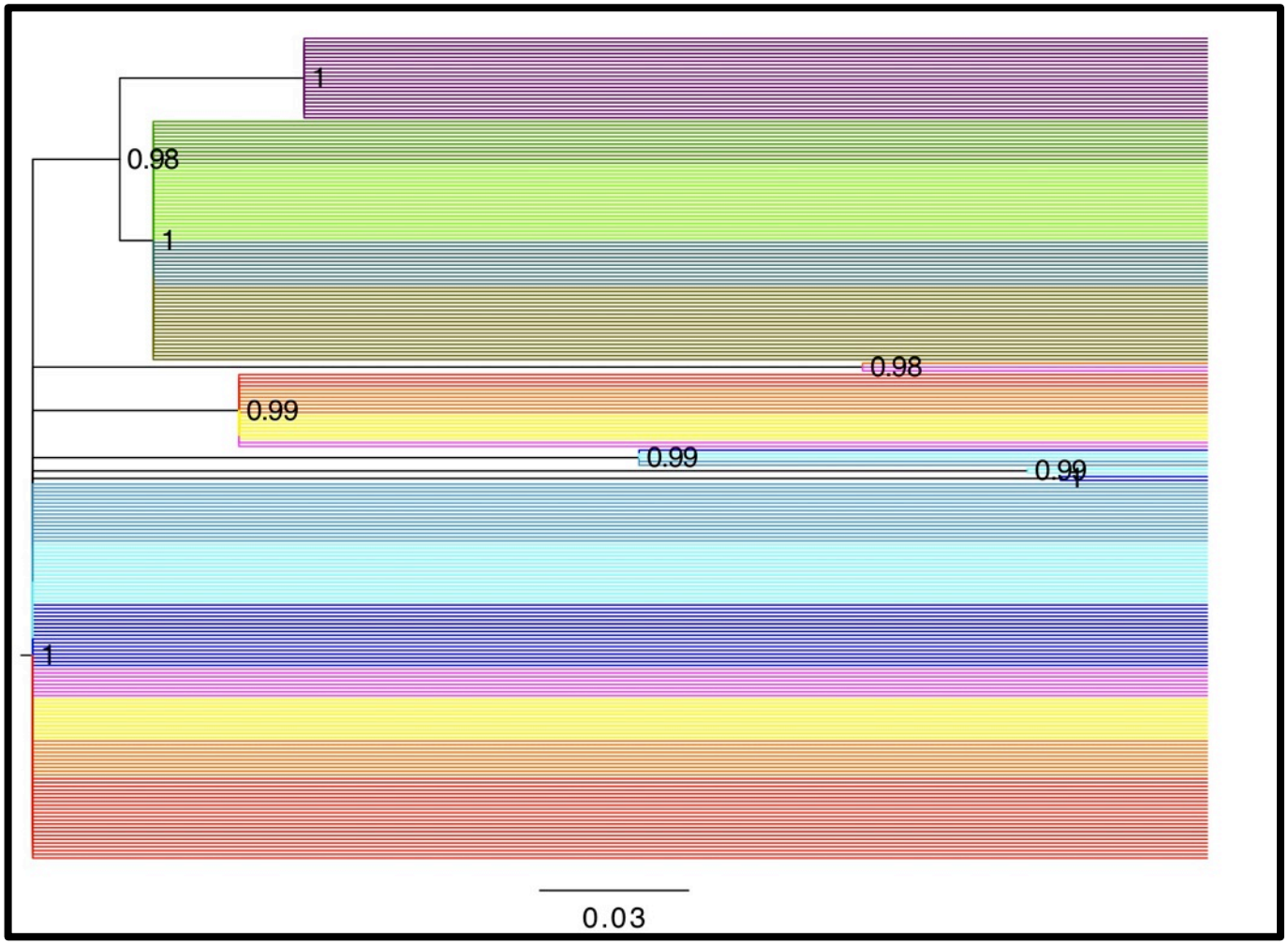
Appendix Fig. 4 MrBayes 50% majority rule consensus tree for 182 base pairs of autosomal locus 14726. Posterior probability values are denoted next to each node. Tip colors correspond to different populations of prairie grouse (see legend).



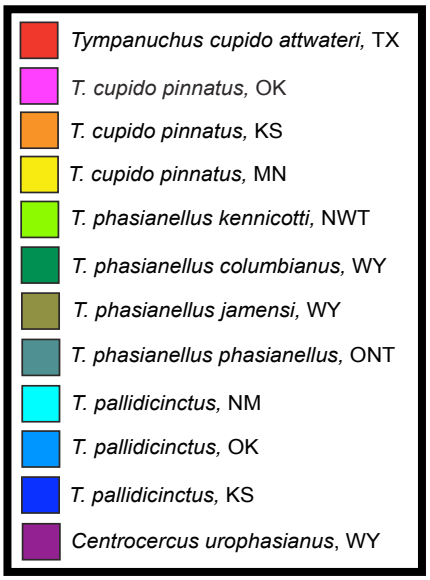


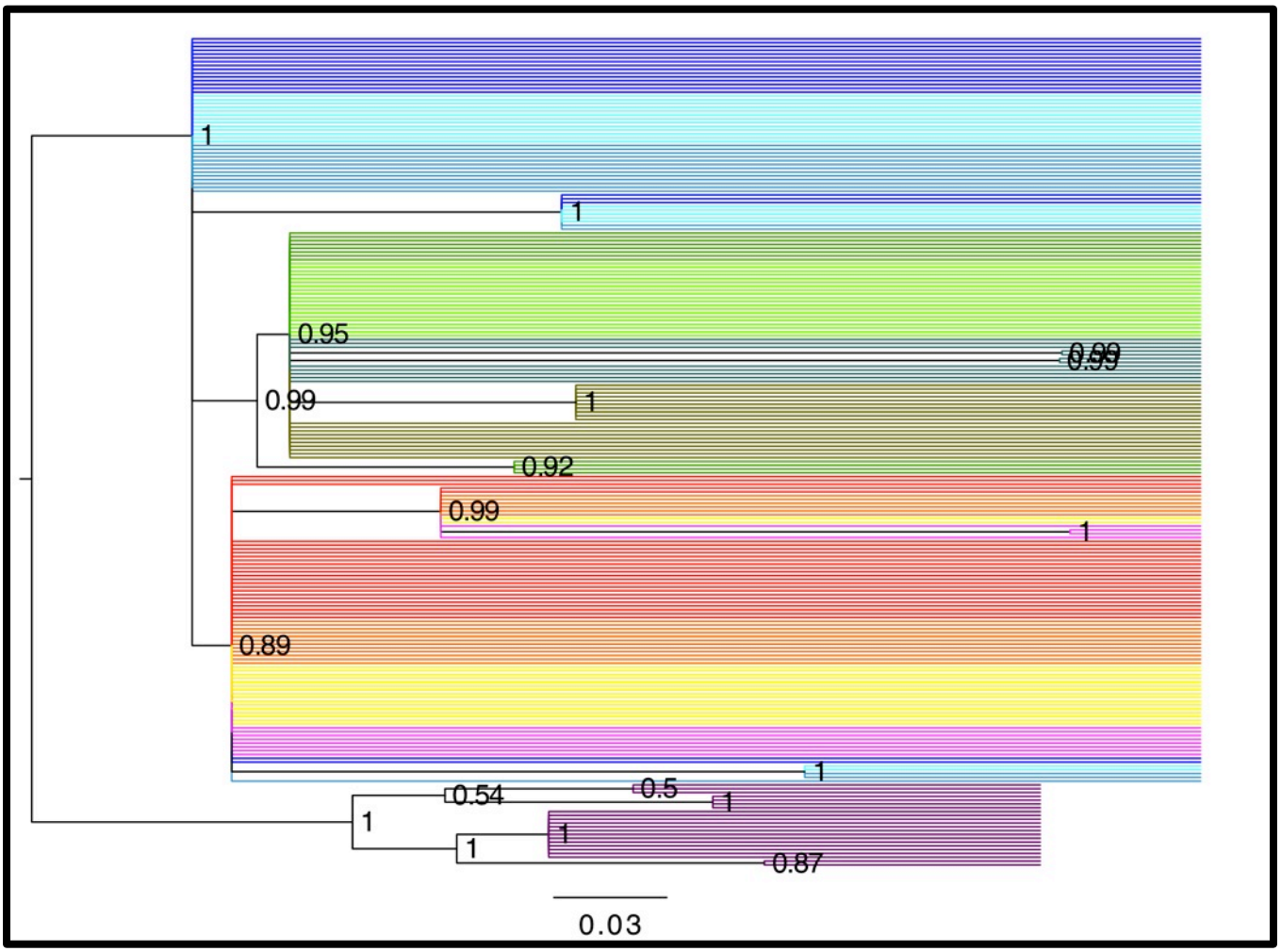
Appendix Fig. 5 MrBayes 50% majority rule consensus tree for 585 base pairs of autosomal locus 15506. Posterior probability values are denoted next to each node. Tip colors correspond to different populations of prairie grouse (see legend).



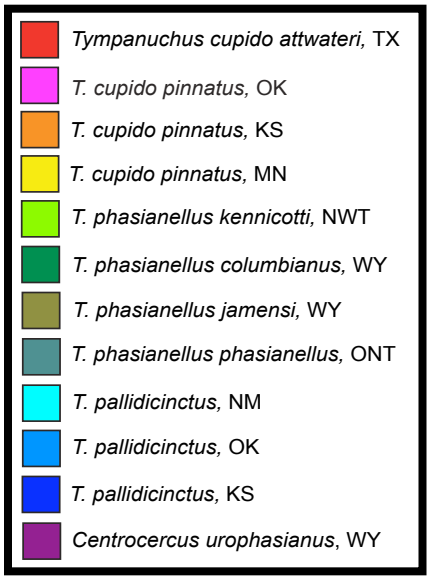


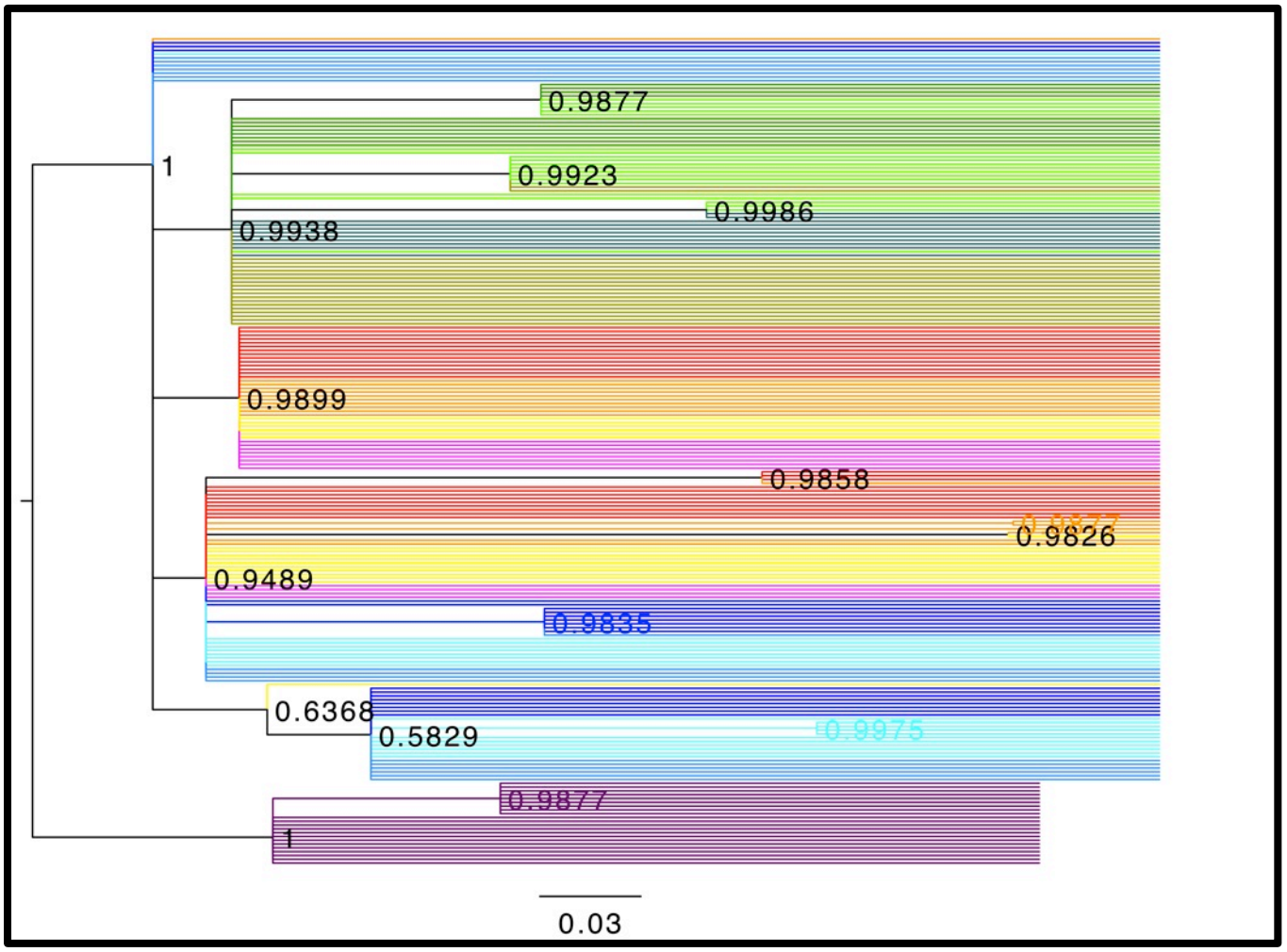
Appendix Fig. 6 MrBayes 50% majority rule consensus tree for 440 base pairs of the Z-linked locus 24105. Posterior probability values are denoted next to each node. Tip colors correspond to different populations of prairie grouse (see legend).



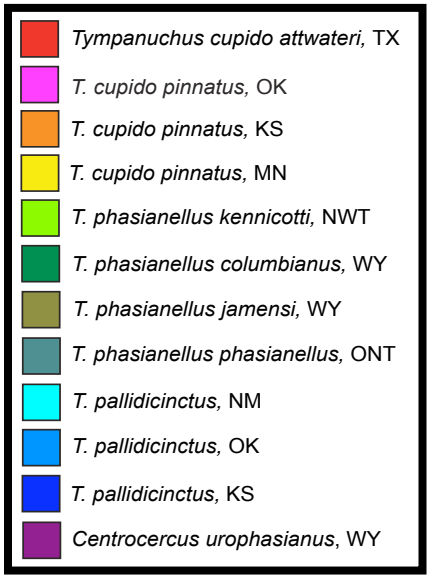


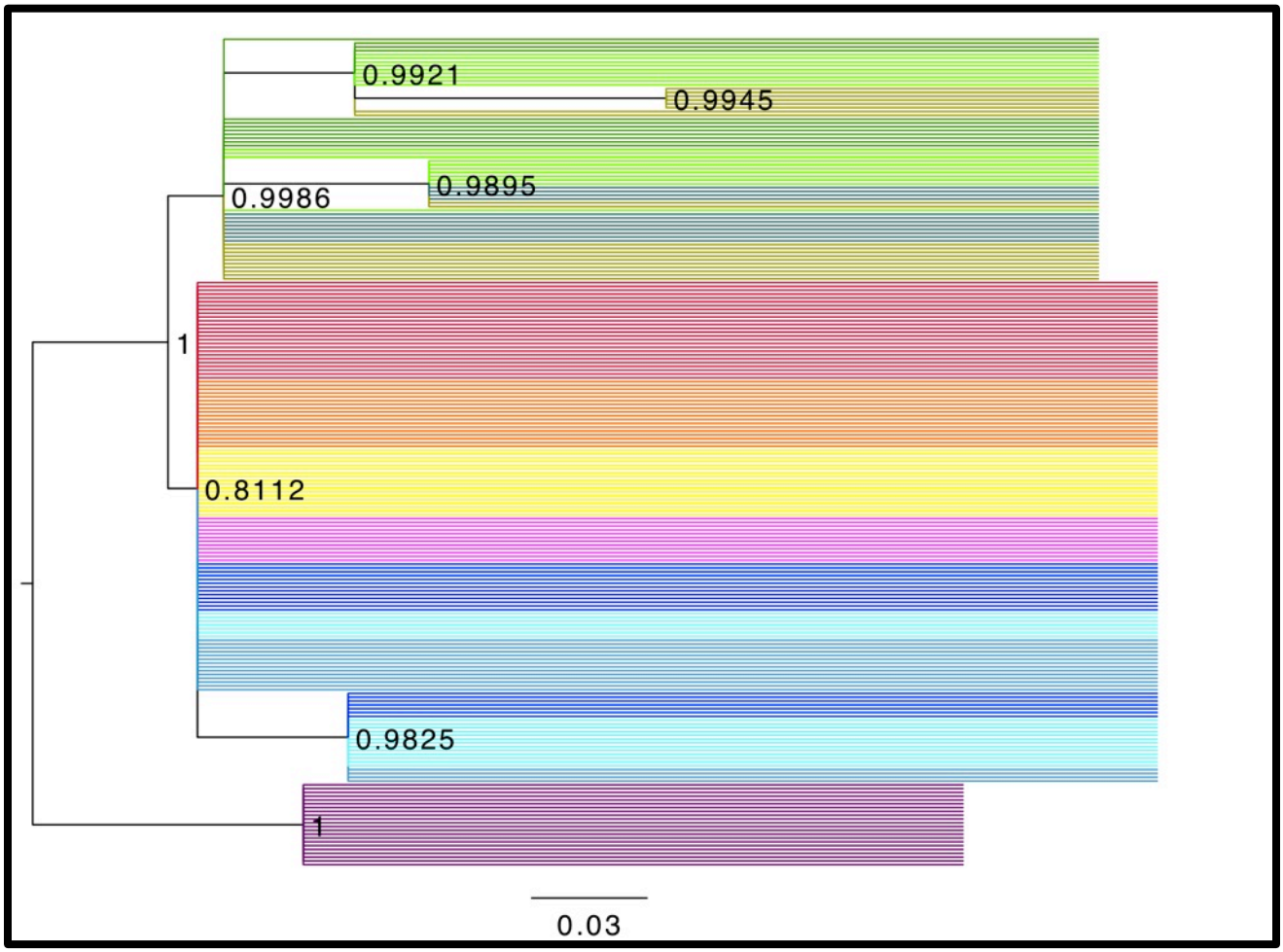
Appendix Fig. 7 MrBayes 50% majority rule consensus tree for 580 base pairs of the Z-linked locus 25189. Posterior probability values are denoted next to each node. Tip colors correspond to different populations of prairie grouse (see legend).



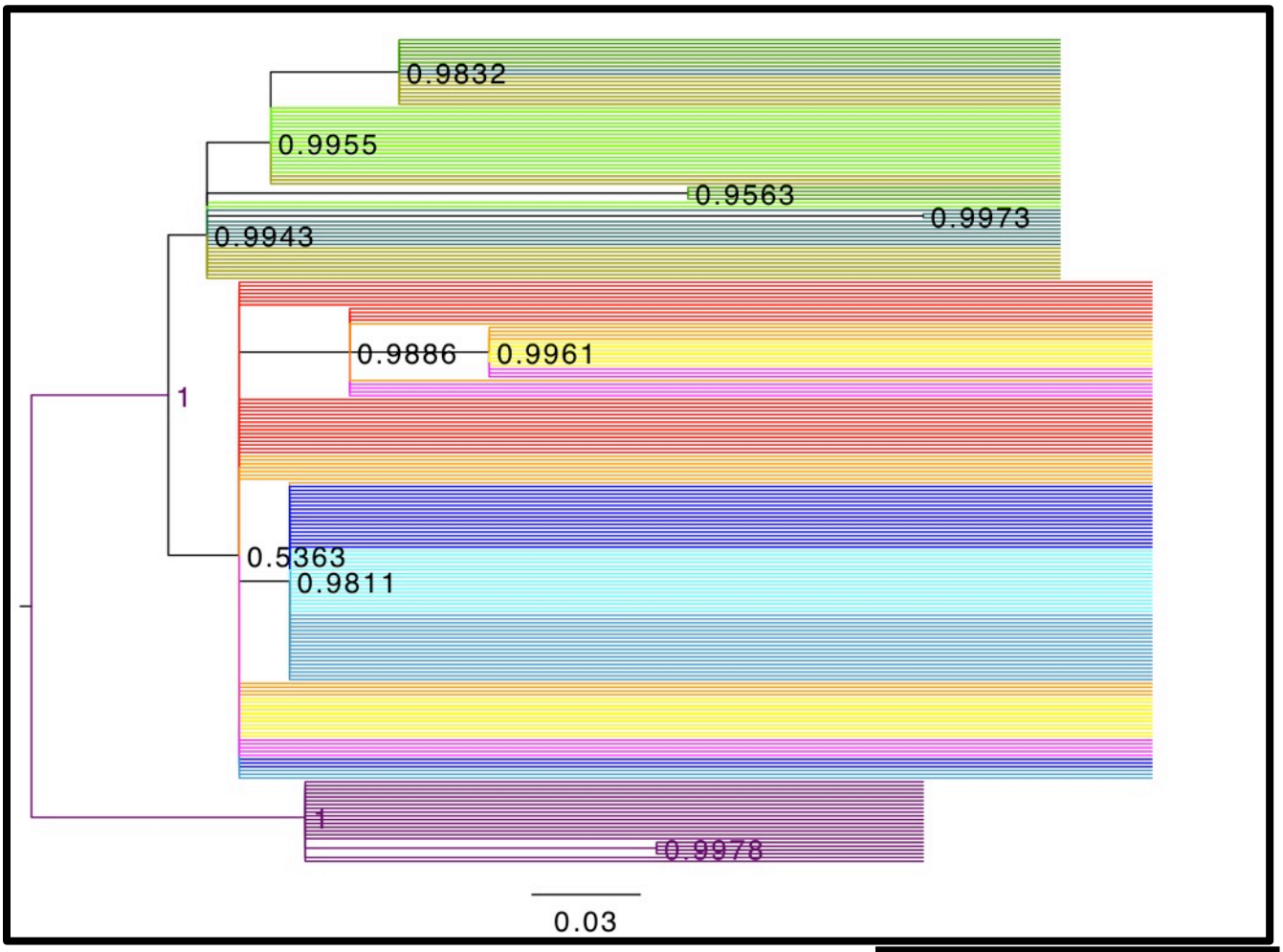


Appendix Fig. 8 MrBayes 50% majority rule consensus tree for 504 base pairs of the Z-linked locus ALDOB. Posterior probability values are denoted next to each node. Tip colors correspond to different populations of prairie grouse (see legend).

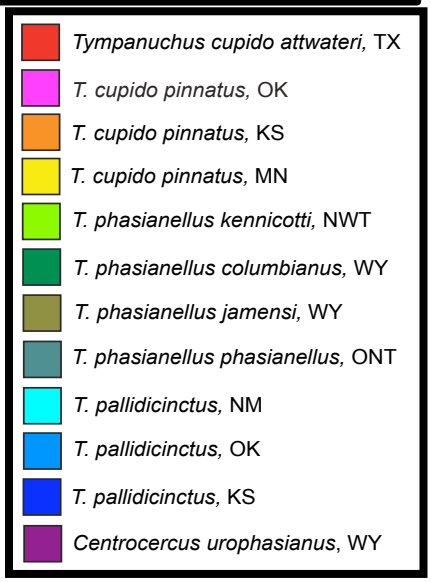




Appendix Fig. 9 MrBayes 50% majority rule consensus tree for 402 base pairs of the Z-linked locus CHD1Z. Posterior probability values are denoted next to each node. Tip colors correspond to different populations of prairie grouse (see legend).



Appendix Fig. 10 MrBayes 50% majority rule consensus tree for 587 base pairs of the Z-linked locus NNT. Posterior probability values are denoted next to each node. Tip colors correspond to different populations of prairie grouse (see legend).



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