**Sorting out relationships among the grouse and ptarmigan using intron, mitochondrial,
and ultra-conserved element sequences**

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ABSTRACT

The Holarctic phasianid clade of the grouse and ptarmigan has received substantial attention in areas such as evolution of mating systems, display behavior, and population ecology related to their conservation and management as wild game species. There are multiple molecular phylogenetic studies that focus on grouse and ptarmigan. In spite of this, there is little consensus regarding historical relationships, particularly among genera, which has led to unstable and partial taxonomic revisions. We estimated the phylogeny of all currently recognized species using a combination of novel data from seven nuclear loci (largely intron sequences) and published data from one additional autosomal locus, two W-linked loci, and four mitochondrial regions. To explore relationships among genera and assess paraphyly of one genus more rigorously, we then added over 3000 ultra-conserved element (UCE) loci (over 1.7 million bp) gathered using Illumina sequencing. The UCE topology agreed with that of the combined nuclear intron and previously published sequence data with 100% bootstrap support for all relationships. These data strongly support previous studies separating *Bonasa* from *Tetrastes* and *Dendragapus* from *Falcapennis*. However, the placement of *Lagopus* differed from previous studies, and we found no support for *Falcapennis* monophyly. Biogeographic analysis suggests that the ancestors of grouse and ptarmigan were distributed in the New World and subsequently underwent at least four dispersal events between the Old and New Worlds. Divergence time estimates from maternally-inherited and autosomal markers show stark differences across this clade, with divergence time estimates from maternally-inherited markers being nearly half that of the autosomal markers at some nodes, and nearly twice that at other nodes.

Keywords: ultra-conserved elements; cyto-nuclear discordance; biogeography; species tree

1. INTRODUCTION

Galliformes are among the most well-studied and recognizable birds, including chicken, turkey, quail and pheasants. The majority of galliforms have Old World distributions, with each family being restricted (or largely restricted) to narrower regions. Phasianidae comprises more than 50% of all galliforms and, with the exception of the grouse, ptarmigan and turkey, phasianids are restricted to the Old World (Africa, Eurasia, and Australia). The two species of turkey are restricted to the New World, where they are widely distributed throughout North America and into Central America. The grouse and ptarmigan are sister to the turkeys (reviewed in Wang et al., 2013) and have a Holarctic distribution, being found in temperate and subarctic habitats in North America and Eurasia. Two species of circumpolar ptarmigans have populations in both the Old and New Worlds. Previous biogeographic reconstruction suggests that the grouse have dispersed multiple times among different regions, with at least three independent dispersals from the New World (Lucchini et al., 2001).

Despite their known dispersal history, extant grouse are generally non-migratory, even for species that live in subarctic habitats (Johnsgard, 1983). Grouse and ptarmigan are well adapted to cold environments, being medium to large-bodied and (atypically for birds) having feathered legs and nostrils. The grouse and ptarmigan are valued internationally as game birds throughout their distributions. Over-exploitation, habitat loss and other anthropogenic activities have led to declines and range contractions in many species (Blomberg et al., 2011; Hovick et al., 2014; Ludwig et al., 2009; Sim et al., 2008; Storch, 2007, 2013).

The grouse and ptarmigan have also been extensively studied due to their variation in mating systems and degrees of sexual dimorphism. In some species, there is a high degree of sexual dimorphism in body size (males may be twice the size of females) and plumage, with

males also having specialized structures such as brightly-colored erectile eye combs, and in some species, inflatable neck sacks capable of making acoustic signals (Johnsgard, 1983; Kimball and Braun, 2008; Madge and McGowan, 2002). Grouse do not maintain long-term pair bonds, being polygynous and/or promiscuous, with males displaying in dense leks, dispersed leks, or in solitary territories (e.g., Alatalo et al., 1991; Bird et al., 2013; Chamberlain et al., 2012; Gibson and Bradbury, 1985; Gratson, 1993; Höglund et al., 1997; Rintamäki et al., 1995; Rolstad et al., 2009; Rusch et al., 2000; Zwickel and Bendell, 2005). In contrast to grouse, the three ptarmigan species (*Lagopus*) exhibit little sexual dimorphism, are generally monogamous with bi-parental care, and frequently maintain long-term pair bonds (e.g., Benson, 2002; Bergerud and Mossop, 1984; Freeland et al., 1995; Hannon, 1984; Martin, 1987; Scherini et al., 2003). In addition to detailed studies of single species, the extensive variation in mating system and degree of dimorphism has led to their inclusion in studies examining the evolution or consequences of these traits (Drovetski et al., 2006; Höglund 1989; Lislevand et al., 2009; Oyler-McCance et al., 2010; Saether and Andersen, 1988; Spaulding, 2007).

Multiple molecular phylogenetic studies of the grouse and ptarmigan have provided the basis for some comparative studies and biogeographic reconstructions. However, there is substantial disagreement regarding relationships among genera, from studies using mitochondrial, autosomal, or sex-linked markers, or combinations of these markers (e.g., Fig. 1). Some studies used limited amounts of data [e.g., a short segment from a single nuclear locus (Butorina and Solovenchuk, 2004; Smith et al., 2005) or part of a single mitochondrial region (Ellsworth et al., 1996)] and might be expected to have limited ability to resolve relationships (e.g., in these cases well-accepted groups or species were not monophyletic). However differences persist even when considering only studies that have included multiple mitochondrial

gene regions and/or multiple nuclear loci, or that synthesized such data (Fig. 1; Burleigh et al., 2015; Dimcheff et al., 2002; Drovetski, 2002; Gutierrez et al., 2000; Jetz et al., 2012; Lucchini et al., 2001). These studies have helped identify non-monophyletic groups, and have led to several taxonomic changes, but the implementation of these changes depends upon which studies and authorities are favored. For example, the 19 extant species (Gill and Donsker, 2015) are split into varying numbers of genera [seven (Clements et al., 2014) up to 11 (Lepage and Warnier, 2014)], with many monotypic genera, reflecting the uncertainty of the phylogenetic relationships in this group.

A fully resolved and well-supported phylogeny of this group is critical for understanding the evolution of the diverse traits in this group, and will also aid in assigning priorities to conservation strategies in order to maximize phylogenetic diversity (Mooers et al., 2005). In this study, we collected DNA sequences of seven nuclear genes for all 19 tetraonine species, and combined these sequences with published data from one additional autosomal locus, two W-linked loci, and four mitochondrial regions. Finally, we used sequence capture and Illumina sequencing of ultra-conserved elements (UCE), which have been successful at resolving problematic relationships within galliforms (Sun et al., 2014), to estimate a generic phylogeny (sampling one member of all 11 genera that have been named, including some species placed in the same genus by the IOC taxonomy). Finally, we used the strongly supported phylogeny that resulted from our analyses to reconstruct biogeographic patterns of this group.

2. MATERIALS AND METHODS

2.1. Sequence Data

2.1.1. Introns and Mitochondrial Data

This analysis included 27 species (Supplementary Table S1), representing all 19 currently recognized species of grouse and ptarmigan. Eight were outgroup taxa representing other gallinaceous birds, including both species of turkey (the likely sister group of the grouse and ptarmigan; Wang et al., 2013), as well as several other taxa outside the clade. Genomic DNA was extracted from tissues following the standard protocol outlined for tissues in the PUREGENE® DNA Purification Kit (Gentra Systems).

We collected data from seven autosomal loci (EEF2, CLTC, CLTCL1, FGB, RHO, SERPIN, and TGFB2) using primers and PCR conditions described previously (Kimball et al., 2009; Wang et al., 2013). Wang et al. (2013) used six of the seven introns we included and we obtained sequences for some taxa from that study. We verified PCR product size and quality by gel electrophoresis, purified them by PEG:NaCl (20%:2.5 M) precipitation, and conducted capillary-based automatic sequencing for all of these markers at the University of Florida Interdisciplinary Center for Biotechnology Research. Raw sequence chromatograms were examined and edited using Geneious 6.1.6. Sequences have been deposited in GenBank (Accession KU094576- KU094676). We aligned nucleotide sequences for each locus in MacClade (Maddison and Maddison, 2000). We combined those nuclear intron data with sequences from published grouse and ptarmigan phylogenies (Dimcheff et al., 2002; Drovetski, 2002; Lucchini et al., 2001; Smith et al., 2005), which included four mitochondrial regions (CYB, ND2, 12S rRNA, and the control region) and three nuclear loci [two introns from aggrecan (ACAN) and two W-linked loci (HINTW and WPG)].

2.1.2. UCE Data

To increase sampling depth, we selected one representative of each genus (using all 11 of the genera described in various taxonomies) along with select outgroups for UCE sequencing (Faircloth et al., 2012). Some UCE data (sequences from *Bonasa umbellus*, *Centrocerus urophasianus*, *Falci pennis canadensis*, *Lagopus mutus*, *Tympanuchus cupido*, *T. phasianellus*, and the outgroups) were obtained from Hosner et al. (in revision) whereas those for other taxa (*Falci pennis falci pennis*, *Lyrurus mlokosi ewiczi*, *Tetrao urogalloides*, *Tetrastes bonasia*) were sequenced by RAPiD Genomics (Gainesville, FL) using protocols modified from Faircloth et al. (2012). Briefly, Illumina TruSeq libraries were prepared using the manufacturer's protocol (Illumina Inc., San Diego, CA, USA) modified to use primers with custom index tags (Faircloth and Glenn, 2012). Each library was enriched for 5060 UCE loci targeted using a set of 5472 probes (Mycroarray, Ann Arbor, MI; <http://www.mycroarray.com/mybait/mybait-UCes.html>) and 100nt paired-end reads were generated using an Illumina (San Diego, CA) HiSeq 2500. We de-multiplexed the raw reads, filtered for adaptor contamination and quality using Trimmomatic (Bolger et al., 2014), and assembled the data using Trinity r20131110 (Grabherr et al., 2011). Sequences have been deposited in the Short Read Archive (Bioproject: 301884). We extracted UCEs from resulting contigs using the PHYLUCE 1.5 pipeline (Faircloth et al., 2012) and aligned them using MAFFT 7 (Katoh et al., 2002; Katoh and Standley, 2013). We trimmed ends of alignments when 35% of cells in a 20 bp sliding window were missing and retained UCE loci containing > 75% of focal taxa for phylogenetic analysis.

2.2. Phylogenetic Analyses

2.2.1. Introns and Mitochondrial Data

We analyzed four separate concatenated alignments: 1) the seven nuclear introns collected for this study; 2) all autosomal sequences (the newly collected introns plus both published ACAN introns); 3) all maternally inherited sequences (mitochondrial sequences and W-linked loci); and 4) all traditional loci (the newly collected introns combined with published nuclear and mitochondrial regions). For each alignment, we performed maximum likelihood (ML) analysis with 10 randomized starting trees in RAxML 8.0.25 (Stamatakis, 2014) with the GTRGAMMA model (the general time reversible model with gamma-distributed rate variation among sites). We also performed 1000 bootstrap replicates in RAxML with the GTRGAMMA model to assess support at each node. We conducted both unpartitioned and partitioned analyses in RAxML, using each nuclear intron or mitochondrial gene region as a separate partition.

Concatenation is biased in some situations (Kubatko and Degnan, 2007; Roch and Steel, 2015), reflecting the existence of parts of parameter space where the majority of gene trees differ from the species tree (Degnan and Rosenberg, 2006). Therefore, we also performed analyses using a multi-species coalescent method (ASTRAL 4.4.7; ((Mirarab et al., 2014; Mirarab and Warnow, 2015)) that is consistent when gene tree topologies exhibit discordance due to incomplete lineage sorting. We used the ML gene trees (combining all maternally inherited loci into a single gene tree) and the ML bootstrap gene trees as input for ASTRAL.

2.2.2. UCE Data

We conducted ML analyses of concatenated UCE alignments in RAxML, similar to our concatenated analysis of intron and mitochondrial data. We assessed support using 500 ML bootstrap replicates for unpartitioned and partitioned analyses using all UCE loci, the latter using a partitioning scheme generated using the *hcluster* algorithm in PartitionFinder (Lanfear et al.,

2012). To estimate a multispecies coalescent tree from UCE alignments, we produced 500 ML bootstrap replicates for each gene tree using Garli 2.0 (Zwickl, 2006), implementing the best-fit model based on the AICc (MrAIC, Nylander, 2004). Meiklejohn et al. (in revision) showed that, as might be expected, the random resolution of zero or very near zero length branches that are common in the lowest information content UCEs can bias the results of species tree methods. We addressed this issue in two ways: 1) branches with near zero lengths were treated as polytomies (using the “collapsebranches = 1” option); and 2) only the 25% most informative loci (850 loci) were used (those with > 14 parsimony informative sites; based on Hosner et al., in revision) for the species tree analyses presented. The bootstrap gene trees were then used as input for reconciliation with ASTRAL 4.4.7.

2.3. Divergence time estimation and Biogeographic Reconstruction

We produced time-calibrated, ultrametric phylogenies with BEAST 2.2 (Bouckaert et al., 2014) using the combined autosomal and maternally inherited datasets (the concatenated Sanger data which included all species, unlike the UCE data) for biogeographical reconstructions. To explore discordance among estimates of divergence times we also analyzed the autosomal and maternally inherited (combined mitochondrial and W-linked) datasets separately. Preliminary analyses suffered from poor resolution among outgroups, and therefore we fixed outgroup topology according to ML analyses (since the Sanger and UCE datasets recovered identical outgroup topologies, we used that topology). We selected the fossil turkey *Rhegminornis* (18 Mya; Olson and Farrand, 1974; now known from more complete material; D. Steadman pers. comm., FLMNH specimens) to set the minimum age for the divergence of *Meleagris* and the grouse and ptarmigan (see also Stein et al. 2015), using the prior distribution: gamma; alpha =

2.0, beta = 5.0, offset = 18.0. We used a Birth-death tree prior, a separate uncorrelated lognormal relaxed clock for each gene, and a HKY+G substitution model for each partition (each gene and codon position). The maximum clade credibility tree inferred from the concatenated dataset was used as the input tree for reconstructing ancestral areas in the R package BioGeoBEARS (Matzke, 2013a; Matzke, 2013b; Matzke and Sidje, 2013) using the DEC, DEC+J, DIVA, and DIVA+J models (Matzke, 2014; Ree and Sanmartin, 2009; Ronquist, 1997). Of these models, we used AIC to select the best-fit biogeographic model. Tips were coded as New World (A), Old World (B), or Holarctic (A+B).

3. RESULTS

3.1. Analyses of Novel Nuclear Introns and Published Data

The seven nuclear loci we collected for this study resulted in an alignment of 4386 sites. Maximum likelihood analysis of this dataset resulted in a phylogeny that was largely well supported (most nodes had at least 70% bootstrap support; Fig. 2). The only two ingroup nodes that exhibited lower support was a relationship within *Tympanuchus* and the branch uniting the genus *Lagopus*. One genus, *Falciipennis*, was not monophyletic, and there was strong support for separating the two species into a grade rather than a clade. Adding the published ACAN introns (to estimate a phylogeny from the autosomal markers) or adding the ACAN introns and the W-linked data (to estimate a phylogeny using all nuclear sequences) resulted in a rearrangement for the poorly supported relationships within *Tympanuchus*, and increased the support to at least 70% for *Lagopus* monophyly (Supplementary Figs. S1 and S2).

The ML phylogeny estimated from the maternally inherited sequences (mitochondrial and W-linked) differed at several nodes from the autosomal phylogeny, though all with less than

70% bootstrap support (Supplementary Fig. 3). This was similar to the mitochondrial-only phylogeny (Supplementary Fig. S4). However, while the maternally inherited sequences (Supplementary Fig. S3) united *Bonasa* and *Tetrastes* with low support, the mitochondrial-only phylogeny placed *Tetrastes* as the earliest diverging lineage (Supplementary Fig. S4). In both ML trees, *Falcapennis* was not monophyletic, but the arrangement of the two species of *Falcapennis* differed from that of the nuclear and autosomal phylogenies (Fig. 2, Supplementary Figs. S1 and S2). Although most analyses found low support for relationships within *Tympanuchus*, the mitochondrial phylogeny gave 100% support for uniting *T. pallidicinctus* and *T. cupido* (Supplementary Fig. S4).

Combining all nuclear and mitochondrial sequences resulted in an alignment of 11504 bp, of which 38% of the sites were mitochondrial. With the exception of relationships within *Tympanuchus* (see above), all nodes received at least 75% bootstrap support (Fig. 3). The ML phylogeny we estimated from the new nuclear data (Fig. 2) was very similar to this phylogeny, including the non-monophyly of *Falcapennis*. However, the positions of *Falcapennis canadensis* and *F. falcapennis* differed between the two phylogenies; *F. canadensis* is sister to a clade comprising *F. falcapennis*, *Lyrurus*, and *Tetrao* in the nuclear phylogeny whereas the positions of the two *Falcapennis* species were switched in the combined nuclear and mitochondrial phylogeny (compare Figs. 2 and 3). The ASTRAL tree generated using the individual autosomal gene trees and the tree for maternally inherited sequences was topologically identical with analysis of the concatenated autosomal and maternally inherited data (Fig. 3), including the placement of the two *Falcapennis* species (and so in conflict with the nuclear phylogeny shown in Fig. 2).

3.2. Analyses of UCE Data

Target-capture and sequencing produced alignments for 3367 UCEs, yielding 1,725,726 aligned nucleotides. The topology estimated from UCEs was identical in partitioned and unpartitioned concatenated analyses (Fig. 4), and agreed with that of the combined nuclear and mitochondrial data for relationships among genera (e.g., compare Fig. 3 with Fig. 4). These results were well-supported, with 100% bootstrap support at all nodes (Fig. 4), and at all but one node in the ASTRAL gene tree summary analysis (calculated from 850 gene trees with > 14 informative sites; values below nodes, Fig. 4). The ML and ASTRAL UCE phylogenies showed strong conflict with the phylogeny estimated using nuclear introns (Fig. 2) regarding the positions of the two *Falciipennis* species. For the *Falciipennis* species, the UCE phylogenies were congruent with those obtained using the maternally-inherited data and combined autosomal and maternally-inherited data.

3.3. Autosomal vs. Maternally-Inherited Divergence Time Estimates

Bayesian ultrametric trees inferred with BEAST from the autosomal and maternally inherited datasets were similar to the ML inference of corresponding datasets with one exception—the maternally inherited BEAST tree suggested *Falciipennis* monophyly, albeit without strong support. In addition to topological differences between trees inferred from the autosomal and maternally inherited sequences, we observed substantial differences in terms of estimated divergence times for several nodes (Fig. 5). This was particularly striking within *Tympanuchus*, *Dendragapus*, *Centrocerus*, and *Tetrastes*. In the first three genera, divergence times estimated using the maternally inherited sequences were about half of that using autosomal sequences, although the reverse was true in *Tetrastes*.

3.4. Biogeography/Ancestral Range Reconstruction

The BEAST topology inferred from the concatenated nuclear and mtDNA dataset was identical to that of the corresponding ML topology for the ingroup taxa, and support values were similar. Biogeographic model selection using the BEAST maximum clade credibility tree chose DEC+J as the best-fit model, with 59% of the relative weight. DIVA+J was also a credible model, with 39% of the relative weight. Biogeographical reconstructions under both models were similar (e.g., Fig. 6), inferring an initial phasianid colonization of the New World by a *Meleagris*/grouse+ptarmigan ancestor *ca.* 20 MYA, the origin of crown grouse and ptarmigan *ca.* 12 MYA, and at least four transitions between the New and Old Worlds between *ca.* 11 MYA to recent (two species of ptarmigan are Holarctic). Recent speciation in the grouse and ptarmigan (5 MYA until present) has occurred within areas, rather than as result of dispersal between the New and Old Worlds, with the possible exception of the Holarctic ptarmigans.

4. DISCUSSION

Relationships among genera (and between species for *Falcipennis*) were identical in our autosomal plus maternally-inherited and UCE phylogenies. These independent datasets produced the same relationships when analyzed with concatenation or with a gene tree reconciliation method, supporting the hypothesis that they are robust and provide our best estimate of relationships among the grouse and ptarmigan. The topological and support differences between this and previous studies, are likely to reflect to the limited amounts of data included in those studies. Although mitochondrial sequences evolve more rapidly than nuclear introns they also exhibit substantially greater levels of homoplasy (e.g., Wang et al. 2013). Thus, while complete mitochondria can often yield well-supported phylogenies that are congruent with large nuclear

datasets (e.g., Meiklejohn et al., 2014), the previous grouse phylogenies have analyzed substantially smaller amounts of mitochondrial data (Gutierrez et al. 2000 included the largest amount of mitochondrial sequence data, but this was still less than 4000 bp). Similarly, single nuclear introns (e.g., Smith et al. 2005), or even small numbers of introns, are unlikely to be sufficient to resolve many questions (e.g., Chojnowski et al., 2008; Kimball and Braun, 2014). Large-scale supermatrix analyses of all birds (Burleigh et al., 2015; Jetz et al., 2012) that included much of the published data, also differed from our results with an expanded data matrix. The grouse and ptarmigan phylogeny from Burleigh et al. (2015) showed limited support for many of the nodes that differed with our study. However, the phylogeny from Jetz et al. (2012) featured posterior probabilities of 0.95 or greater at all but one node (Supplementary Fig. S5), including many that differed from our results. This strong support may be due to the priors used by Jetz et al. (2012) in their analyses (Pagel, 2012). Thus, despite their potential utility for some large-scale comparative analyses, supermatrix studies should be viewed with caution when one focuses on specific groups.

In agreement with published studies (Fig. 1), we found strong support for uniting the New World genera *Tympanuchus*, *Dendragapus*, and *Centrocercus* into a well-supported clade (Fig. 2) and for a sister relationship between *Lyrurus* and *Tetrao* (which are often combined into the single genus, *Tetrao*; e.g., Clements 2014). However, at other nodes, our phylogeny differed from published phylogenies (e.g., the position of *Lagopus*). Like several previous studies (Dimcheff et al., 2002; Gutierrez et al., 2000), we did not find a monophyletic *Falci pennis* (Fig. 2). *F. canadensis* has been classified into the monotypic genus *Canachites* (e.g., Lepage and Warnier, 2014); upholding this taxonomic classification would make all genera monophyletic.

Alternatively, *Falcipennis*, *Canachites*, and *Lyrurus* could be lumped into an enlarged *Tetrao*, reducing the number of monotypic genera in the grouse and ptarmigan.

There are two major hypotheses that have the potential to explain the conflicting placements of *F. falcipennis* and *F. canadensis* that we observed (e.g., Fig. 2 versus Fig. 3). It could reflect simple cyto-nuclear discordance. This hypothesis makes several predictions. First, the phylogeny estimated from maternally-inherited sequences (and the phylogeny estimated using only mitochondrial data) should have the same topology for these taxa as the combined analysis of autosomal and maternally-inherited loci. This reflects the potential for the large amount of mitochondrial data (38% of all sites and 46% of variable sites) to overwhelm the signal from the nuclear genome in the combined analysis. Second, the estimate of phylogeny obtained using ASTRAL is more likely to match the autosomal tree, because it treats the maternally-inherited sequences as a single gene tree (thus down-weighting the contribution of the mitochondrial data relative to the eight autosomal loci). Finally, the UCE phylogeny (Fig. 4), which broadly sampled the nuclear genome, should agree with the autosomal phylogeny (Fig. 2). The results of our analyses contradict the latter two of these predictions, falsifying the hypothesis of simple cyto-nuclear discordance. Alternatively, one or two of the autosomal loci might have a strong effect, swamping out the signal in the remainder of the loci (Kimball et al., 2013). We noted that no individual nuclear gene tree had a topology that matched either the nuclear-only or autosome plus maternally-inherited phylogeny, though one locus (FGB) did match the autosomal phylogeny regarding the relative positions of *F. falcipennis*, *F. canadensis*, *Tetrao*, and *Lyrurus* (though with low bootstrap support). We believe that error in gene tree estimation is likely to have played a major role in this failure of individual gene trees to match the concatenated

phylogeny given simulations showing that individual nuclear introns have little power to resolve avian relationships (e.g., Chojnowski et al., 2008; Patel et al., 2013).

We also found topological differences among datasets within *Tympanuchus*, although differences were not well supported. Mitochondrial data produced 100% bootstrap support for *T. cupido* + *T. pallidicinctus* (Supplementary Fig. S4). However, nuclear data produced short internodes among *Tympanuchus* (Fig. 2), and *T. cupido* was weakly united with *T. phasianellus* (which has been placed in a separate genus (Lepage and Warnier, 2014), rather than with *T. pallidicinctus*). Hybridization has been documented among all of the *Tympanuchus* species (e.g., Bain and Farley, 2002; Evans, 1966; Geiger, 1993; Lumsden, 2005), with up to 8% of hybrid individuals being reported in some areas of sympatry (Augustine and Trauba, 2015). This relatively high level of hybridization may have driven the very short internode and different relationships we obtained among different analysis. A detailed study of *Tympanuchus* exploring these issues (Galla and Johnson, 2015) suggests that the underlying species tree unites *T. pallidicinctus* and *T. cupido*, to the exclusion of *T. phasianellus*, in agreement with our estimates of phylogeny using the combined autosomal and maternally-inherited data (Fig. 3), as well as classifications that place *T. phasianellus* in its own genus (Lepage and Warnier, 2014).

Hybridization among *Tympanuchus* would also be consistent with the differences among estimated divergence dates we observed between the autosomal and maternally-inherited sequences (Fig. 5). If there has been mitochondrial introgression, this should lead to more recent divergences based on estimates of mitochondrial than nuclear data (as we see in *Tympanuchus*, *Dendragapus*, and *Centrocercus*). Our results might suggest recent introgression among *Tympanuchus*, although a more ancient introgression may have led to the large observed differences found within *Dendragapus* and *Centrocercus*. In grouse in general, hybridization

appears to be common both within and even among genera (e.g., Aldridge et al., 2001; Eng, 1971; Kohn and Kobriger, 1986; Ouellet, 1974; Porkert et al., 1997; Quintela et al., 2010; Rensel and White, 1988), making this a plausible explanation for the differences in divergence times observed in those taxa.

However, hybridization may not be the only explanation for our observed differences in divergence times, and multiple other factors may be involved (e.g., Galla and Johnson 2015). Different loci coalesce at different times and differently in independent lineages, which may explain much of the variation that we observe across grouse. Maternally-inherited DNA is, on average, likely to have a more recent coalescent than autosomal loci (e.g., Moore, 1995; Palumbi et al., 2001). This, however, is unlikely to lead to the large differences evident in some genera. Thus, hybridization is more likely for those cases, especially in cases where there is no overlap in the maternal and autosomal divergence times and the maternal times are more recent. Skews in male mating success have the potential to alter average coalescent times for maternal versus autosomal loci by reducing the effective population size of autosomal loci, and could even result in a more recent expected autosomal coalescent time than the expected maternal divergence time (e.g., Hoelzer, 1997). At first glance, this could explain the discordance among branch lengths observed in *Tetrastes*, especially when one considers the stochasticity of the coalescent time for any single gene region, including the mitochondrial genome (Hudson and Turelli, 2003). However, unlike many grouse species, both *Tetrastes* species appear to be monogamous (e.g., Sun and Fang, 1997; Swenson and Boag, 1993) and therefore would not be expected to have greatly reduced autosomal coalescent times. Instead, it may be that the stochastic variation in coalescent times is sufficiently large to lead to extreme differences in divergence time estimates without invoking any differences in the expected coalescent times for each data type. If so, this

has strong implications for studies that estimate divergence times using small numbers of loci where the coalescent variance is high.

Our biogeographic reconstruction is consistent with that of other studies (e.g., Wang et al. in review) and it suggests that the ancestor of the turkeys and grouse dispersed to the New World prior to diversification. Results suggested two possible scenarios to explain the current dispersal of the grouse and ptarmigan: 1) four dispersal events to the Old World (*Tetrastes*, within *Lagopus*, *F. falcipennis*, and *Tetrao/Lyrurus*; or 2) three dispersal events to the Old World, followed by a dispersal back to the New World in *F. canadensis*. These results are consistent with those of Lucchini et al (2001), who suggested at least three dispersal events between the New and Old Worlds. Unlike the more tropical/temperate galliformes lineages that have colonized the New World (Cracids, Pereira et al., 2002; New World quail, Hosner et al., 2015), frequent Nearctic/Palearctic transitions between continents have been a major historical factor in Tetraonine diversification, given that transitions are implied at most nodes prior to *ca.* 4 MYA. Transitions between continents in the boreal and temperate grouse (excluding the arctic ptarmigan) are estimated to have ceased by about 4–7 MYA, when the Beringian Land Bridge, and the continuous boreal forests across it were interrupted by increasing sea levels and periodic glaciations (Marincovich and Gladenkov, 1999). Unlike grouse, the arctic-adapted ptarmigan (*L. lagopus* and *L. mutus*) probably continued to disperse and/or maintained population connectively, at least periodically, until the Holocene. This is likely to have been facilitated by periodic sea level reductions and increased arctic sea ice associated with Pleistocene climate oscillations (Holder et al., 1999, 2000).

Overall, our results estimate the same relationships among genera using both the autosomal plus maternally-inherited dataset and the UCE dataset. Given that two independent

datasets were topologically identical and strongly supported lends confidence that future comparative studies can now be based upon a rigorously-estimated phylogeny. Our results also suggest that different parameters may be affecting divergence and coalescence of maternally-inherited and autosomal loci. Although the reasons for this are unclear, understanding the mechanisms of such differences are important to understanding how genomes evolve, and their influence on phylogenetic estimation.

ACKNOWLEDGEMENTS

We thank M. Kusnick and S. Hyde for assistance in the lab. Members of the Kimball-Braun lab provided valuable comments on earlier drafts of this manuscript. This study would not have been possible without generous loans of tissues from the following museums: Florida Museum of Natural History, Field Museum of Natural History, University of Kansas Biodiversity Institute, Louisiana State University Museum of Natural Science, Museum of Southwestern Biology, National Museum of Natural History Smithsonian Institute, University of Washington Burke Museum, and Zoological Natural History Museum of Denmark. Funding was provided by the U.S. National Science Foundation (DEB-1118823 to RTK and ELB), including an REU supplement to support NWP.

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FIGURE LEGENDS

Figure 1. Relationships among genera from studies that included multiple mitochondrial regions or loci, including two from analyses of all birds.

Figure 2. ML phylogeny using the seven newly sequenced introns. Values above nodes are bootstrap percentages from 1000 bootstrap replicates. * indicates 100% bootstrap support.

Figure 3. ML bootstrap consensus tree of the combined intron and mitochondrial data. Values above nodes are bootstrap percentages from 1000 bootstrap replicates from RAxML; values below nodes are bootstrap percentages from ASTRAL. * indicates 100% bootstrap support.

Figure 4. ML bootstrap consensus tree of the UCEs. Values above nodes are bootstrap percentages from 1000 bootstrap replicates from RAxML; values below nodes are bootstrap percentages from ASTRAL. * indicates 100% bootstrap support.

Figure 5. Branch lengths from maternal and autosomal markers. Graph in upper right indicates the confidence intervals around each time estimate for the two datasets.

Figure 6. Ancestral range reconstruction (DEC+J model) from BioGeoBears utilizing the BEAST maximum clade credibility tree. Polar projection map (inset upper left) showing grouse and ptarmigan distributions. Below the absolute time scale is the geologic time scale, and approximate timing of the presence of Beringia and globally warm temperatures (in gray) that may have influenced colonization and diversification.

Figure

Gutierrez et al. 2000

Lucchini et al. 2001; Drovetski et al. 2002

Dimcheff et al. 2002

Jetz et al. 2012

Burleigh et al. 2015

Figure2

Figure3

Figure4

Figure