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ORIGINAL ARTICLE

Z chromosome divergence, polymorphism and relative effective population size in a genus of lekking birds

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Sex chromosomes contribute disproportionately to species boundaries as they diverge faster than autosomes and often have reduced diversity. Their hemizygous nature contributes to faster divergence and reduced diversity, as do some types of selection. In birds, other factors (mating system and bottlenecks) can further decrease the effective population size of Z-linked loci and accelerate divergence (Fast-Z). We assessed Z-linked divergence and effective population sizes for two polygynous sage-grouse species and compared them to estimates from birds with various mating systems. We found lower diversity and higher F_{ST} for Z-linked loci than for autosomes, as expected. The π_Z/π_A ratio was 0.38 in *Centrocercus minimus*, 0.48 in *Centrocercus urophasianus* and 0.59 in a diverged, parapatric population of *C. urophasianus*, a broad range given the mating system among these groups is presumably equivalent. The full data set had unequal males and females across groups, so we compared an equally balanced reduced set of *C. minimus* and individuals pooled from both *C. urophasianus* subgroups recovering similar estimates: 0.54 for *C. urophasianus* and 0.38 for *C. minimus*. We provide further evidence that N_{eZ}/N_{eA} in birds is often lower than expected under random mating or monogamy. The lower ratio in *C. minimus* could be a consequence of stronger selection or drift acting on Z loci during speciation, as this species differs strongly from *C. urophasianus* in sexually selected characters with minimal mitochondrial divergence. As *C. minimus* also exhibited lower genomic diversity, it is possible that a more severe demographic history may contribute to its lower ratio.
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INTRODUCTION

The architecture of genetic divergence between sister species provides information on the mechanisms underlying the initiation and reinforcement of species boundaries. Sex chromosomes (X in most mammals and insects and Z in birds and some reptiles) are believed to play a fundamental role in speciation, housing a disproportionately large number of genes related to sexual selection, reproductive isolation and speciation (Charlesworth *et al.*, 1987; Coyne and Orr, 1989; Ritchie and Phillips, 1998; Presgraves, 2002). Species with chromosomal sex determination have shown two genomic patterns with varying degrees of generality: a disproportionate effect of sex-chromosome loci on the fitness of hybrids (the 'large-X' or 'large-Z' effect), and a 'fast-X' (or 'fast-Z') effect in which genes on sex chromosomes evolve faster than on autosomes (reviewed in the study by Presgraves, 2002). Evidence for a large-X/Z effect includes both direct investigation of the chromosomal locations of hybrid sterility/inviability factors (Jiggins *et al.*, 2001; Presgraves, 2008) and indirect evidence from the relative introgression of autosome vs sex-chromosome markers in hybrid zones (Payseur, 2010; Elgvin *et al.*, 2011). FastX/Z has been documented by comparing patterns of divergence on the sex chromosomes to divergence on autosomes (Counterman *et al.*, 2004; Vicoso and Charlesworth 2009; Mank *et al.*, 2010a,b; Sackton *et al.*, 2014).

Sex chromosomes hold distinctive properties (Vicoso and Charlesworth, 2006), which likely affect their rate of evolution (Rice, 1984; Charlesworth *et al.*, 1987). Unlike autosomes, new mutations occurring on the sex chromosome are exposed to selection in the hemizygous sex as there is no second allele present to mask that effect (Haldane, 1924). There are two possible explanations for fast-X/Z that are not mutually exclusive. First, selection can fix beneficial recessive or partially recessive mutations on the sex chromosomes more effectively than on the autosomes (Vicoso and Charlesworth, 2006). Alternatively, fast-X/Z may be the consequence of genetic drift and the fixation of mildly deleterious mutations, which is predicted to occur as the effective population size (N_e) of sex chromosomes relative to autosomes is 3/4 assuming equal reproductive success between males and females (Vicoso and Charlesworth, 2009). This ratio is based on the fact that there are three copies of sex chromosomes for every four copies of the autosomes. Reduced N_e of sex chromosomes broadens the range of selection coefficients for which allele frequency changes remain dominated by drift (Caballero, 1995; Laporte and Charlesworth, 2002; Mank *et al.*, 2010a).

Much of our knowledge of fast evolution on the sex chromosome stems from organisms with male heterogamety (males XY, females XX) such as mammals and *Drosophila* (Counterman *et al.*, 2004; Khaitovich *et al.*, 2005; Vicoso *et al.*, 2008; Xu *et al.*, 2012), yet several organisms with female heterogamety (males ZZ, females ZW) have also been studied including birds, insects and snakes (Sundström *et al.*, 2004; Borge *et al.*, 2005; Mank *et al.*, 2007, 2010a,b; Ellegren *et al.*, 2012; Vicoso *et al.*, 2013; Sackton *et al.*, 2014). Comparing rates of

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evolution on the X vs the Z allows for the separation of effects due to maleness from effects due to heterogamety (Mank *et al.*, 2007) and may provide clues as to the relative roles of selection and drift in fast-X/Z (Mank *et al.*, 2010a). Patterns of faster divergence on the Z are analogous to those predicted for the X: the N_e of the Z (N_{eZ}) is predicted to be 3/4 the N_e of the autosomes (N_{eA}) when reproductive success is equal between males and females. The ratio of N_e of the sex chromosome to that of the autosome (N_{eX}/N_{eA} or N_{eZ}/N_{eA}) is an important value as sex chromosomes with a reduced effective population size are more susceptible to genetic drift (Mank *et al.*, 2010a). In male heterogametic organisms, skewed male mating success will increase N_{eX}/N_{eA} above 3/4 thereby reducing the impact of genetic drift on fast-X evolution and favoring selection and adaptive evolution (Meisel and Connallon, 2013). The opposite is true in organisms with female heterogamety where skewed male mating success reduces N_{eZ}/N_{eA} and increases the influence of genetic drift and nearly neutral evolution over selection on fast-Z evolution (Vicoso and Charlesworth, 2009; Mank *et al.*, 2010a; Meisel and Connallon, 2013). Sexual selection and skewed reproductive success between males and females are common in nature, thus the ratio of N_{eX} or N_{eZ} to N_{eA} frequently deviates from 3/4 (Singh *et al.*, 2008; Ellegren, 2009a; Mank *et al.*, 2010b; Vicoso *et al.*, 2013; Miesel and Connallon, 2013). Birds vary widely in male reproductive success from strict monogamous to polygynous where only a few males breed with all females. Polygynous birds, therefore, provide an interesting system in which to examine the influence of mating systems on fast-Z evolution and, as such, can provide empirical evidence documenting such deviations in N_{eZ}/N_{eA}.

Sage-grouse (*Centrocercus* spp.) are North American galliforms that were recently split into two separate species (Young *et al.*, 2000), based on strong morphological (Hupp and Braun, 1991) and behavioral (Young *et al.*, 1994) differences. Examination of genetic differences between these species using mitochondrial and nuclear microsatellite loci revealed genetic patterns consistent with a lack of gene flow, although this divergence was modest and mitochondrial DNA haplotypes were not reciprocally monophyletic (Oyler-McCance *et al.*, 1999). Greater Sage-Grouse (*Centrocercus urophasianus*) occupy much of the North American West, while Gunnison Sage-Grouse (*Centrocercus minimus*) are restricted to southwestern Colorado and southeastern Utah (Figure 1). Within *C. urophasianus*, the most genetically divergent population sampled to date spans the border between California and Nevada ('Bi-State population' hereafter), and was found to be at least as divergent from other populations of *C. urophasianus* as *C. minimus* is from *C. urophasianus* at the same mitochondrial and microsatellite loci (Oyler-McCance *et al.*, 2005a), but is not distinct morphologically or behaviorally (Schroeder, 2008; Taylor and Young, 2006). Recent work with genomic methods, however, has shown that *C. minimus* is well differentiated from *C. urophasianus* and the Bi-State is also differentiated *albeit* more weakly (Oyler-McCance *et al.*, 2015). This system provides an interesting example where one group of sage-grouse (*C. minimus*) diverged in obvious morphological and behavioral ways while another group (Bi-State) did not.

Both species of sage-grouse are lekking birds with highly skewed mating systems (Wiley, 1974; Gibson and Bradbury, 1986; Gibson *et al.*, 1991), and males of the two species differ in plumage and display behavior, traits that are strongly sexually selected (Young *et al.*, 1994). Reduced effective population size and the fitness effects of sexually selected traits can potentially drive morphological and behavioral evolution faster than fixed differences accrue at unlinked neutral sites (Spaulding, 2007; Oyler-McCance *et al.*, 2010). The lack of reciprocal monophyly and strong genetic differentiation between *C. urophasianus* and *C. minimus* at the small number of neutral loci studied previously might reflect rapid, recent speciation or the restriction of divergence to a few genomic blocks of large effect. Examination of patterns of variation at the genomic level should offer

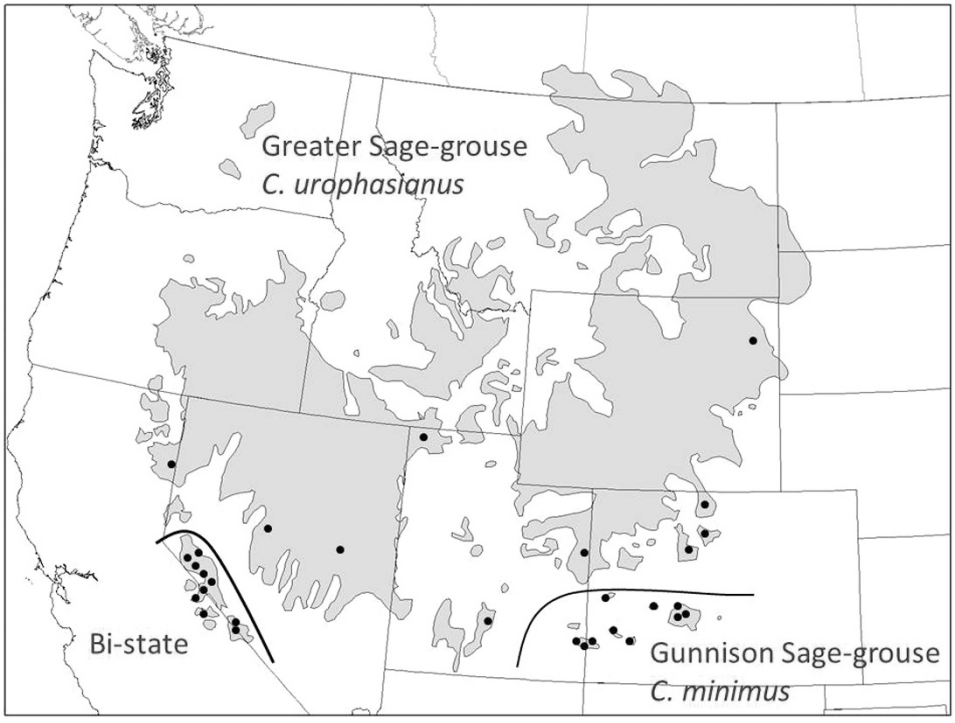

Figure 1 Current distribution of sage-grouse with approximate sampling locations of individuals included in the study. Map modified from the study by Schroeder *et al.* (2004).

answers to such questions. Further, the lekking behavior of sage-grouse provides an ideal system to examine how highly skewed reproductive success in males influences Z-linked diversity.

Most studies examining fast-Z in birds have used relative rates of nonsynonymous substitutions in protein-coding genes as the measure of evolutionary rate, which requires relatively deep-divergence times to detect significant differences (Mank *et al.*, 2007; Ellegren, 2009b). Analyzing change in the frequencies of single-nucleotide polymorphisms (SNPs) sampled randomly from the genome offers an alternative to protein substitution rates, one that is not limited to gene regions amplifiable with conserved primers and effective for recent divergence times. Here we employ the 'RAD-tag' approach of identifying SNPs from reduced-representation genomic libraries (Baird *et al.*, 2008) and use the genome sequences of two related model organisms, the chicken (*Gallus gallus*) and turkey (*Meleagris gallopavo*), as references to bin SNPs by chromosome. Our objectives were to determine the extent of divergence between *C. minimus* and *C. urophasianus*, using the outlying Bi-State population of *C. urophasianus* as a benchmark, as well as examine the short-term dynamics of Z chromosome evolution by comparing autosomal and Z-linked diversity and divergence within and between *C. minimus* and *C. urophasianus*.

MATERIALS AND METHODS

Sample collection and DNA extraction

We chose DNA samples from 30 sage-grouse (10 each from *C. minimus*, *C. urophasianus* and Bi-State) used in previous studies (Oyler-McCance *et al.*, 1999, 2005a). Bi-State *C. urophasianus* and *C. minimus* samples were chosen so that we covered the entire range of those groups. *C. urophasianus* samples (other than the Bi-State) were chosen to reflect the southern portion of the range most proximal to the other two sample groups (Figure 1). Sample tissue was either wing muscle from birds shot by hunters or blood taken from trapped birds. The number of males was five for the Bi-State group, eight for the *C. minimus* sample and two for the *C. urophasianus* sample. DNA extraction methods followed the study by Oyler-McCance *et al.* (1999, 2005b).

Because the number of Z chromosomes sampled in the full data set was not equal across the three groups (there were unequal numbers of males and females in each group), we used a subsample of the data to confirm that the different patterns of divergence between *C. minimus* and *C. urophasianus* at autosomal vs Z-linked loci were not impacted by these complications (Supplementary Table 1). Thus, we repeated our analysis on a subsample of nine *C. minimus* and nine *C. urophasianus* each consisting of seven males and two females. For each revised group 18 autosomes and 16 Z chromosomes were sampled. By combining the *C. urophasianus* and Bi-State samples, which are known to be somewhat diverged, our estimates of heterozygosity may be somewhat inflated since the divergence between these groups is counted as polymorphism. However, since these two groups have been shown to be much less differentiated compared with *C. minimus* (Oyler-McCance *et al.*, 2015) we feel that this potential inflation will be minimal.

Sequencing of reduced-representation genomic libraries

In ligase buffer, a 20 μl double digestion reaction was setup using 1 μg of whole genomic DNA (13 μl), and 1 μl each of the rare cutter SPEI and the common cutter Sau3AI. After digestion for 2 h, restriction enzymes were heat killed at 65 °C for 15 min and the reaction was cooled using a 37 °C hold. At 37 °C, 1 μl of each R1 and individually barcoded R2 RAD-tag adapters (Oyler-McCance *et al.*, 2015) were added into the reaction and allowed to equilibrate to dehybridize any self hybridization of the adapters. Once in equilibrium, 1 μl of T4 ligase was added, and the temperature reduced to 16 °C for 15 min to ligate the adapters to the double digested DNA. The ligation reaction was cleaned using AMPure bead (Beckman Coulter, Brea, CA, USA) procedure to assure the removal of any self-dimers that may have formed. PCR was performed on a Bio-Rad iCycler (Bio-Rad, Hercules, CA, USA), using IQ SYBR Green Supermix (Bio-Rad) to facilitate quantification and pooling of PCR products without individual clean up. After PCR and pooling, AMPure bead clean up procedure was performed on the pool to remove PCR dimers and small amplicons. Further size selection was done to 300–500 bp via Pippen Prep (Sage Science, Beverly, MA, USA). The pool of 30 libraries was sent to the University of Colorado Genomics and Microarray Facility for sequencing on the Illumina HiSeq 2000 platform (Illumina, San Diego, CA, USA). The resulting sequences were deposited in NCBI's Short Read Archive under BioProject PRJNA211878.

Data analysis

We used the FastX program (FastX-Toolkit; www.hannonlab.cshl.edu/fastx_toolkit/index.html) to trim reads at a Phred-equivalent quality score of 15 and purged reads that were shorter than 84 bp after trimming. We then used stacks (Catchen *et al.*, 2011) to collapse restriction-site-associated reads (RAD tags) into loci or 'stacks', using the combined reads from all populations and requiring at least identical three reads to designate an initial stack. We then made a catalog of shared loci among the two species, allowing up to three mismatches. These stacks were further clustered using cd-hit-est (Li and Godzik, 2006) at 85% identity and a word size of 7, to avoid competitively mapping reads to similar stacks. Reads from each individual sample were then mapped to stacks using bowtie2 (Langmead and Salzberg, 2012) in 'local' mode, using a seed length of 20 and tolerating 1 mismatch in the seed and 3 total mismatches. Reads with mapping quality of $\geqslant 15$ were retained in the alignment output, which was converted to variant-call format using samtools (Li *et al.*, 2009). Stacks with >100 reads mapped from any one population read pool were removed as potential genomic repeats. Any locus with GC content >2 s.d. from the mean GC content of 38.3% was excluded.

The total number of SNPs at this initial stage was 94 620, however a large majority of these had a minor allele frequency $<10\%$. As the total number of autosome sets sampled was 60, the biological frequency threshold for SNPs is ~1.67%, although lower values are expected to occur due to stochastic sampling and library-size variation. Furthermore, preliminary analysis showed no geographic structure of samples when the minor allele frequency in the pooled reads was $\leqslant 1\%$, and an alternative SNP prediction pipeline (CLC Genomics Workbench, CLC Bio, Cambridge, MA, USA) showed little overlap with these low-frequency SNPs, suggesting that these were due largely to sequencing and/or alignment error. Based on these considerations, we eliminated SNPs with minor allele frequency $<10\%$ in the total read pool and a variant-call quality <100. A small number of sites with more than two alleles were excluded for simplicity. Sites were further filtered to exclude loci within 3 bp of an indel and to retain loci with a combined coverage of 50–350×, using the vcfutils script of samtools. The coverage values were chosen based on the distribution of read counts around the mean coverage of ~150× for all individuals combined, or ~5× per individual.

After identifying 13 827 loci that met these criteria, we inferred individual genotypes by aligning each demultiplexed sample with bowtie2, using the same parameters given above. We used the default behavior of bowtie2, which is to report the best alignment and to provide a mapQ score. The mapQ score threshold we imposed was 15, which is a phred-scaled probability of error. The single most likely genotype was assigned for each locus-sample combination if the samtools-reported genotype quality was $\geqslant 10$ (68.2% of all individual-site combinations), otherwise the genotype was considered undetermined. Any loci exhibiting only heterozygous genotypes were removed. While a simple binomial test of equal read coverage by sex at $P>0.05$ was used to identify 11 630 probable autosomal loci for population-structure analyses reported elsewhere (Oyler-McCance *et al.*, 2015), for the present study we used stronger criteria to select subsets of loci that we could bin as 'autosomal' or 'Z-linked' with high confidence.

The first criterion was the *P*-value of a binomial test of equal frequency of reads in the pool of all males vs the pool of all females. For this comparison, loci with $P>0.50$ were retained as candidate autosomal loci and those with *P*-values <0.01 and fewer reads in females were considered candidate Z-linked loci. Given the modest total coverage, we believe these conservative *P*-values were warranted.

Next, candidate Z loci were examined for allele counts that were consistent with haploidy in females. This was implemented by removing loci that in any female had >1 count for a second allele if coverage was $<15\times$, or that had $<20\%$ of counts presenting a second allele if coverage was $\geqslant 15\times$ (to allow for

errors in sequencing or mapping). Candidate Z-linked loci passing this step were re-genotyped with samtools with the ploidy level specified as 1 for females and 2 for males. Candidate autosomal loci were excluded if none of the 15 sampled females were heterozygous.

Finally, we used the chicken genome (version WASHUC2, downloaded from Ensembl v.68) (Hillier *et al.*, 2004) and the turkey genome (version UMD2.69 (Dalloul *et al.*, 2010) downloaded from GenBank) as references to bin loci based on sequence homology. Given the strong conservation between the chicken Z and human chromosome 9 (Nanda *et al.*, 1999), an assumption of conservation between the Z chromosomes of sage-grouse, turkey and chicken is reasonable. Chicken and grouse are thought to have diverged roughly 49 million years ago (MYA), while turkey and grouse diverged roughly 32 MYA (Pereira and Baker, 2006). Further, it has been shown that that Z-linked synteny is highly conserved across avian genomes (Vicoso *et al.*, 2013; Skinner *et al.*, 2009). To maximize the sequence information available for BLAST searches, we did not limit ourselves to the RAD-tag 'stack' sequences but instead assembled the combined paired-end data with Abyss (Simpson *et al.*, 2009) using a hash length of 27. We also included whole-genome shotgun data from a previous 100-bp paired-end Illumina sequencing run of *C. minimus* that had been performed for microsatellite detection (Castoe *et al.*, 2012; Fike *et al.*, 2015). This run was assembled with CLC Genomics Workbench using automatic bubble sizes, a mismatch penalty of 2 and an indel penalty of 3. The two sets of contigs were combined with GSAssembler (Margulies *et al.*, 2005) using a seed step of 12, seed length of 16, a 90% identity and 35-bp match criterion, and a +2/−3 score for matches and mismatches, respectively. We were able to use 3982 contigs resulting from this assembly *in lieu* of the original stacks because they had MegaBLAST matches to the latter at >95% identity and were longer sequences, thereby providing greater power to detect homology with other bird genomes. These contigs and the remaining stacks were aligned to chromosomes with TBLASTX at a minimum e-value of 1e−5, provided there were no more than 10 total matches at that threshold (contigs with higher number of matches were considered potentially repetitive sequences and not included in this analysis) and all matches were to the same chromosome. Matches to each chromosomal scaffold or the associated 'unknown' (unscaffolded) bin associated with that chromosome were combined into a single count. We retained loci that had TBLASTX matches to either reference Z chromosomes, because requiring matches to both reduced the number of Z-binned SNPs by roughly one-half, which we believed was too conservative given the other, independent corroborating criteria used. We had only one high confidence SNP that assigned to the W chromosome so we disregarded it in this study.

As karyotypic variation in chromosome size is prevalent across the avian genome with corresponding differences in recombination rates and gene density that could affect diversity estimates (Ellegren, 2013), all population genetic parameters for autosomes were calculated using only SNPs matching to chicken macrochromosomes 1–10. Per-locus F_{ST} was inferred from read counts of pooled samples using PoPoolation2 (Kofler *et al.*, 2011), setting the 'pool size' parameter to the number of chromosomes appropriate for each comparison by analyzing Z-linked and autosomal loci separately. Expected heterozygosity and the proportion of pairwise differences (π) were computed with Arlequin v. 3.5.1.2 (Excoffier and Lischer, 2010) based on inferred genotypes, representing diploid loci as unphased pseudo-haplotypes and allowing up to 50% missing genotypes. We used the R (R Core Team, 2014) package diveRsity (Keenan *et al.*, 2013) to calculate pairwise F_{ST} and 95% confidence intervals. For comparison to N_e ratios inferred from π, we also used the F_{ST} approach by Holsinger and Weir (2009) to estimate the joint N_e of *C. urophasianus* and *C. minimus* Z chromosomes and autosomes since their divergence.

RESULTS

Using our filtering criteria, we identified 231 Z-linked and 2418 autosomal SNPs. F_{ST} at Z-linked loci was much higher than at autosomal loci for *C. minimus* relative to the other sampled groups. In the full data set, multilocus autosomal F_{ST} was much lower (0.08 (95% confidence interval (95% CI) 0.08–0.09)) between Bi-State and *C. urophasianus*, than between *C. minimus* and either *C. urophasianus* or the Bi-State (0.41 (95% CI 0.39–0.42) and 0.47 (95% CI 0.45–0.48), respectively, Table 1). Z-linked F_{ST} values showed similar patterns but were almost twice as high (0.15 (95% CI 0.11–0.20) between Bi-State and *C. urophasianus,* 0.77 (95% CI 0.74–0.81) between *C. urophasianus* and *C. minimus*, and 0.77 (95% CI 0.73–0.80) between Bi-State and *C. minimus*, Table 1). All pairwise multilocus F_{ST} values were significantly different from zero by permutation test for all comparisons at $P<0.001$. Mean expected heterozygosity at autosomal loci (0.167 for *C. minimus*, 0.353 for *C. urophasianus* and 0.324 for the Bi-State) was higher than Z-linked heterozygosity in all three sample groups (0.073 for *C. minimus*, 0.229 for *C. urophasianus* and 0.234 for the Bi-State) with the Bi-State and *C. urophasianus* having much higher heterozygosity than *C. minimus* (Table 1). Similarly, mean autosomal π was higher (0.15 (95% CI 0.15–0.16) for *C. minimus*, 0.31 (95% CI 0.30–0.31) for *C. urophasianus* and 0.28 (95% CI 0.28–0.29) for the Bi-State) than Z-linked π in all three groups (0.06 (95% CI 0.05–0.06) for *C. minimus*, 0.15 (95% CI 0.14–0.16) for *C. urophasianus* and 0.17 (95% CI 0.16–0.18) for the Bi-State), with *C. minimus* having the lowest values (Table 1). Ratios of π_Z/π_A were all lower than the predicted value of 0.75 with *C. minimus* having the lowest ratio of 0.38 (Table 1). Using the F_{ST} approach by Holsinger and Weir (2009) gave comparable values: the joint estimated N_{eZ}/N_{eA} between *C. minimus* and each group of *C. urophasianus* were lower (0.35 and 0.43 for *C. urophasianus* and

Table 1 Divergence between sampling groups as measured using F_{ST}

	F_{ST}			Heterozygosity		π		π_Z/π_A
	C. minimus	C. urphasianus	*Bi state*	*Autosomal*	*Z linked*	*Autosomal*	*Z linked*	
Full data								
C. minimus	—	**0.77 (0.74–0.81)**	**0.77 (0.73–0.80)**	0.167	0.073	0.15 (0.15–0.16)	0.06 (0.05–0.06)	0.38
C. urophasianus	0.41 (0.39–0.42)	—	**0.15 (0.11–0.20)**	0.353	0.229	0.31 (0.30–0.31)	0.15 (0.14–0.16)	0.48
Bi-State	0.47 (0.45–0.48)	0.08 (0.08–0.09)	—	0.324	0.234	0.28 (0.28–0.29)	0.17 (0.16–0.18)	0.59
Subsample								
C. minimus	—	**0.73 (0.69–0.77)**	—	0.168	0.075	0.15 (0.15–0.16)	0.06 (0.06–0.06)	0.38
C. urophasianus	0.43 (0.42–0.44)	—	—	0.347	0.254	0.32 (0.31–0.32)	0.17 (0.16–0.18)	0.54

95% confidence intervals are shown in parentheses. Values in bold above the diagonal represent Z-linked divergence, while those below the diagonal represent autosomal F_{ST}. Heterzygosity, proportion of pairwise differences (π), and ratio of Z-linked π to autosomal π are also given for each sample group. The full data set (top) includes 10 individuals from each of *C. minimus*, *C. urophasianus* and the Bi-State. The subsampled data set includes only a subsample of the individuals (seven males and two females) from each species.

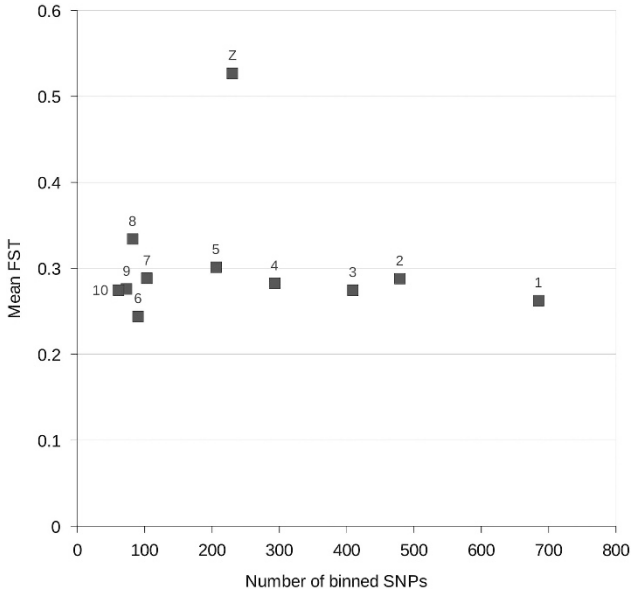

Figure 2 Mean F_{ST} values by chromosome between *C. urophasianus* and *C. minimus* in the reduced data set for SNPs that mapped to both chicken and turkey genomes.

Bi-State, respectively), than the ratio between *C. urophasianus* and the Bi-State (0.51).

In the subsampled data set (including 18 autosomes and 16 Z chromosomes each for both species), mean F_{ST} was higher on the Z than on any other chromosome (Figure 2). Z-linked multilocus F_{ST} remained higher between species (0.73 (95% CI 0.69–0.77)) than autosomal F_{ST} between species (0.43 (95% CI 0.42–0.44)). Both values were significantly different from zero by permutation test at $P<0.0001$ and their confidence intervals did not overlap. Expected heterozygosity was higher at autosomal loci than at Z-linked loci for both *C. urophasianus* (0.347 vs 0.254) and *C. minimus* (0.168 vs 0.075, Table 1). Mean autosomal π was 0.32 (95% CI 0.31–0.32) for *C. urophasianus* and 0.15 (95% CI 0.15–0.16) for C. *minimus*, whereas Z-linked π was 0.17 (95% CI 0.16–0.18) and 0.06 (95% CI 0.06–0.06), respectively (Table 1). The resulting Z-to-autosomal ratios were 0.54 for *C. urophasianus* and 0.38 for *C. minimus*. N_{eZ}/N_{eA} estimated with the F_{ST} method of Holsinger and Weir (2009) was 0.42. Overall patterns between the full data set (Table 1) and the subsampled data set (Table 1) were similar, yet for Z-linked diversity and divergence comparisons the subsampled data set provide a better representation as they are based on equal numbers of sex chromosomes between species.

DISCUSSION

In this study, we have used allele frequencies of random genomic SNPs to investigate the chromosomal patterns of diversity and divergence in sibling sage-grouse species, detecting faster evolution and smaller effective population size of the Z. Faster divergence of sex chromosomes relative to autosomes (Figure 2, Table 1) has been documented in a number of species, and is one factor that may contribute to the disproportionate role sex chromosomes play in adaptive divergence, particularly for traits associated with mate choice, reproductive isolation and speciation (Charlesworth *et al.*, 1987; Dobzhansky, 1974; Coyne and Orr, 1989; Reinhold, 1998; Presgraves, 2002, 2008; Qvarnström and Bailey, 2009) and may be further pronounced in organisms with female heterogamety (Iyengar *et al.*, 2002; Mank *et al.*, 2007; Ellegren, 2009a; Ellegren *et al.*, 2012; Wang *et al.*, 2014). Fast-Z is prevalent in most, if not all, birds examined in this manner (Sundström *et al.*, 2004; Borge *et al.*, 2005; Hogner *et al.*, 2012; Ruegg *et al.*, 2014; Wang *et al.*, 2014).

Several phenomena could drive fast evolution of the sex chromosome, and are not mutually exclusive. Fast-Z could be the result of an increased expression of beneficial recessive mutations on the Z that are directly exposed to selection in females due to their hemizygous nature in birds. Further, recessive mutations that are deleterious are purged more efficiently on the Z compared with autosomes and may reduce diversity (Charlesworth *et al.*, 1987; Borge *et al.*, 2005; Ellegren, 2011; Hogner *et al.*, 2012). Alternatively, fast-Z could be due to the smaller effective population size of the Z compared with autosomes, leading to the increased fixation of slightly deleterious mutations due to genetic drift (Mank *et al.*, 2010a,b; Ellegren, 2011). Purifying selection is weaker with lower N_e as genetic drift increases the number of alleles that behave neutrally, thereby fixing more slightly deleterious mutations on the Z than on autosomes (Caballero, 1995; Laporte and Charlesworth, 2002; Mank *et al.*, 2010a). Male polygyny in birds should reduce N_{eZ}/N_{eA} even further, making the impacts of drift even stronger in such species (Caballero, 1995; Laporte and Charlesworth, 2002; Ellegren, 2009a).

It is difficult to determine which of these two major factors is responsible for fast-Z in sage-grouse. On one hand, genes involved with male plumage traits, species recognition and hybrid incompatibility in flycatchers (*Ficedula hypoleuca* and *F. albicollis*) have been linked to the Z chromosome (Sætre *et al.*, 2003; Sæther *et al.*, 2007; Backström *et al.*, 2010) and theory predicts ZW systems to facilitate runaway selection (Kirkpatrick and Hall, 2004). If Z-linkage of sexually selected traits holds generally, then species with highly skewed reproductive success (such as lek-breeding sage-grouse) may exhibit particularly strong contributions of the Z to species boundaries. Indeed, strong sexual selection in lekking species can drive morphological and behavioral divergence much faster than can be tracked using neutral genetic markers and is a major force in speciation (Ellsworth *et al.*, 1994; Uy and Borgia, 2000; Panhuis *et al.*, 2001; Spaulding, 2007; Oyler-McCance *et al.*, 2010). *C. minimus* exhibit distinct morphological and behavioral characteristics (Hupp and Braun, 1991; Young, 1994; Young *et al.*, 1994) that ultimately contributed to the isolation of this group eventually leading to speciation (Young *et al.*, 2000). While it is compelling to believe that selection on the Z is responsible for fast evolution, empirical data supporting this theory are mixed and evidence of linkage of sexually selected traits to the Z chromosome is somewhat limited. Fast evolution of the Z chromosome is also attributable to genetic drift and a growing body of work has shown this empirically in birds (Vicoso and Charlesworth, 2009; Mank *et al.*, 2010a,b; Wang *et al.*, 2014). Behavioral observations of the lek mating system of sage-grouse have led to the suggestion that all females attending a given lek typically mate with one or two dominant males (Wiley, 1974; Gibson and Bradbury, 1986; Gibson *et al.*, 1991), although more recent molecular studies have shown that this reproductive skew may be too high and that females may mate with additional males off the lek (Semple *et al.*, 2001; Bush *et al.*, 2010). Either way, the mating system of sage-grouse provides a prime example of a species with extreme reproductive skew that likely is reflected in patterns of Z-linked divergence and may be the result of genetic drift.

Our data do not allow us to explicitly disentangle drift from selection. N_{eZ}/N_{eA} was lower in the presumably derived *C. minimus* than it was in *C. urophasianus* despite similar mating behavior, suggesting that divergence dynamics (differences in demographic

histories during and after speciation) may have contributed to this difference. Further investment in genomic sequencing and mapping of such plumage traits to chromosomes should be informative as would sequencing of plumage-trait loci identified from other species. Our data are not discordant, however, with evidence pointing toward the general influence of drift in fast-Z evolution, particularly in birds with reproductive skew (Vicoso and Charlesworth, 2009; Mank *et al.*, 2010a,b).

As predicted from theoretical models (Ellegren, 2009a), the diversity of *C. minimus* and *C. urophasianus* is higher on autosomes than on the Z chromosome. At equilibrium, the expected π for the Z is proportional N_e times the mutation rate and should be 3/4 the size of π for autosomes in systems where males and females have equal reproductive success ($\pi_Z/\pi_A = 0.75$). This ratio should theoretically reduce π_Z/π_A or θ_Z/θ_A below 0.75 in species with unequal reproductive success between males and females such as sage-grouse. Indeed, diversity ratios well below 0.75 have been reported in many birds with varying degrees of reproductive skew (Sundström *et al.*, 2004; Borge *et al.*, 2005; Balakrishnan and Edwards, 2009; Huynh *et al.*, 2010; Corl and Ellegren, 2012; Hogner *et al.*, 2012).

The Z/A diversity ratio should decline from 0.75 with increased reproductive skew expressed as the number of monopolized mates, and is predicted to decline to near 0.60 in highly polygynous systems (Corl and Ellegren, 2012). The study by Hedrick (2007) also predicts that a ratio close to 0.6 translates to effectively 10 times as many mating females than males. Such a ratio is a reasonable expectation for sage-grouse given behavioral and molecular studies documenting similar rates of reproductive skew in these species (Wiley, 1974; Gibson and Bradbury, 1986; Gibson *et al.*, 1991; Semple *et al.*, 2001; Bush *et al.*, 2010). Our ratios for the two species of sage-grouse, however, were even lower (0.54 for *C. urophasianus* and 0.38 for *C. minimus*) and similar to the joint estimate of N_{eZ}/N_{eA} (0.42) for both species based on divergence (F_{ST}). Even under extreme polygyny, where only one male breeds with all females, the ratio is expected to be 0.56 (Charlesworth, 2001,2009), which is close to what we found between *C. urophasianus* and Bi-State in the full data set.

The study by Corl and Ellegren (2012) examined the relationship between mating system and sex chromosome genetic diversity by comparing diversity ratios in six species of shorebirds with different mating systems (monogamous and polygynous). They found that most polygynous species had reduced Z diversity relative to their monogamous counterparts with diversity ratios ranging from 0.69 to 0.45. The study by Corl and Ellegren (2012) also summarizes diversity ratios expressed as θ_Z/θ_A for other birds, although direct comparisons may be problematic as mutation rates differ among loci. To make the ratios from the study by Corl and Ellegren (2012) directly comparable to our ratios, we calculated π_Z/π_A from the original publications where we could and report those values rather than θ_Z/θ_A (Table 2). In general, the monogamous species do show higher π_Z/π_A ratios compared with polygynous species, yet most values are still lower than what would be expected under extreme polygyny. Shorebird diversity ratios tended to be higher than passerines or galliforms, although domestication and the effects of assortative mating may influence this ratio.

Like the passerine and chicken ratios, the diversity ratios for both *C. urophasianus* and *C. minumus* are lower than would be predicted under extreme skew in mating success (Ellegren, 2009a) and may point to other factors such as selective sweeps that reduce diversity at linked loci, as it has been shown that Z chromosomes may be especially susceptible to such phenomena (Sundström *et al.*, 2004; Borge *et al.*, 2005). This is due to the fact that sexual selection at Z-linked loci should be expedited in female heterogametic systems like birds where fathers transmit a Z chromosome to their sons, thereby improving heritability and increasing the strength of selection of sexually antagonistic alleles that are beneficial to males (Sundström *et al.*, 2004; Ellegren, 2009a).

Other processes may also impact the relative diversity of autosome vs Z diversity in sage-grouse. Historical changes in population size such as bottlenecks and founder events have been shown to greatly affect diversity on sex chromosomes (Pool and Nielsen, 2007; Miesel and Connallon, 2013). It is possible that differences in historical demography between these two species may contribute to different N_{eZ}/N_{eA} ratios. The lower genetic diversity of *C. minimus* reported here and elsewhere (Oyler-McCance *et al.*, 1999, 2005b) is consistent with a genetic bottleneck or founder event and may inflate divergence estimates (F_{ST}) between species (Charlesworth, 1998; Cruickshank and Hahn, 2014). Both species have experienced significant population declines in recent history (Young *et al.*, 2000; Schroeder *et al.*, 2004) yet the demographic history of *C. minimus* may be much more severe with fewer than 5000 individuals remaining (Young *et al.*, 2000) compared with an estimated 100 000–500 000 *C. urophasianus* (USFWS, 2010). Unfortunately, a comprehensive understanding of the historical demographic history of *C. minimus* remains unclear and it is unknown whether this specie has lower long-standing N_e or has gone through a recent (possibly speciation related) bottleneck. Estimating the allele frequency spectrum of the two species with

Table 2 Comparison of published Z/A diversity ratios in various birds

Species	*Type*	*Mating system*	π_Z/π_A	*Citation*
Calidris minutilla	Shorebird	Monogamous	0.52	Corl and Ellegren (2012)
C. fuscicollis	Shorebird	Polygynous	0.45	Corl and Ellegren (2012)
C. mauri	Shorebird	Monogamous	0.58	Corl and Ellegren (2012)
C. melanotos	Shorebird	Polygynous	0.57	Corl and Ellegren (2012)
Phalaropus lobatus	Shorebird	Monogamous	0.69	Corl and Ellegren (2012)
Philomachus pugnax	Shorebird	Polygynous	0.52	Corl and Ellegren (2012)
Ficedula hypoleuca	Passerine	Polygynous	0.37	Borge *et al.* (2005)
F. albicollis	Passerine	Polygynous	0.42	Borge *et al.* (2005)
Taeniopygia guttata	Passerine	Polygynous with domestication	0.29	Balakrishnan and Edwards (2009)
Gallus gallus	Galliform	Polygynous with domestication	0.31	Sundström *et al.* (2004)
Centrocercus urophasianus	Galliform	Polygynous	0.54	This study
C. minimus	Galliform	Polygynous	0.38	This study

larger sample sizes could help infer past bottlenecks (Allendorf *et al.*, 2010). Male biased mutation may also impact N_{eZ}/N_{eA} ratios as the numbers of cell divisions in the male germline are much higher than in the female germline (Haldane, 1935; Miyata *et al.*, 1987; Axelsson *et al.*, 2004). While there is mixed evidence of an evolved compensatory effect for hemizygous sex chromosomes generally, the study by Axelsson *et al.* (2004) did not find this to be significant in galliform birds and estimated a male-to-female mutation rate ratio (α_m) of ~2-fold based on three different methods. As $\pi_Z/\ \pi_A$ is proportional to μ_Z/μ_A, allowing for higher male mutation rates will further lower N_{eZ}/N_{eA} ratios because Z chromosomes are more often found in males than females. This effect is moderate for a mutation rate ratio of 2 (that is, $\mu_Z/\mu_A = (2/3\alpha_m+1/3)/(1/2\alpha_m+1/2) = 1.11$) and reduces our ratios to 0.35 in *C. minimus* and 0.48 for *C. urophasianus* when we account for male mutational bias.

The significance of fast-Z evolution in *C. urophasianus* and *C. minimus* extends the observation of fast-Z as a phenomenon from deep-divergence comparisons of protein substitution rates (Mank *et al.*, 2007; Ellegren, 2009b) to very shallow divergence (not evident in mitochondrial DNA sequence or microsatellites) based on allele frequencies. While the current data cannot definitively determine the contributions of selection and drift to fast-Z, they highlight the utility of SNP data in investigating the generality of fast-Z mechanisms at time scales where individual components might be isolated, rather than aggregated or averaged effects. Judicious comparisons with genomic data, for example, might uncover whether fast-Z accrues uniformly or episodically through bottlenecks and selective sweeps. The application of genomic methods here has allowed us to better define patterns of genetic variation in sage-grouse and also begin to understand the mechanisms underlying speciation in these species.

DATA ARCHIVING

Arlequin files, text file: Dryad http://dx.doi.org/10.5061/dryad.75n5q.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on Heredity website (http://www.nature.com/hdy)