Genome Assembly of the Common Pheasant *Phasianus colchicus*: A Model for Speciation and Ecological Genomics

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Abstract

The common pheasant (*Phasianus colchicus*) in the order Galliformes and the family Phasianidae, has 30 subspecies distributed across its native range in the Palearctic realm and has been introduced to Europe, North America, and Australia. It is an important game bird often subjected to wildlife management as well as a model species to study speciation, biogeography, and local adaptation. However, the genomic resources for the common pheasant are generally lacking. We sequenced a male individual of the subspecies *torquatus* of the common pheasant with the Illumina HiSeq platform. We obtained 94.88 Gb of usable sequences by filtering out low-quality reads of the raw data generated. This resulted in a 1.02 Gb final assembly, which equals the estimated genome size. BUSCO analysis using chicken as a model showed that 93.3% of genes were complete. The contig N50 and scaffold N50 sizes were 178 kb and 10.2 Mb, respectively. All these indicate that we obtained a high-quality genome assembly. We annotated 16,485 protein-coding genes and 123.3 Mb (12.05% of the genome) of repetitive sequences by ab initio and homology-based prediction. Furthermore, we applied a RAD-sequencing approach for another 45 individuals of seven representative subspecies in China and identified 4,376,351 novel single nucleotide polymorphism (SNPs) markers. Using this unprecedented data set, we uncovered the geographic population structure and genetic introgression among common pheasants in China. Our results provide the first high-quality reference genome for the common pheasant and a valuable genome-wide SNP database for studying population genomics and demographic history.

Key words: common pheasant, *Phasianus colchicus*, genome sequencing, RAD-sequencing, SNP, population genomics.

Introduction

The common pheasant (*Phasianus colchicus*), belonging to the order Galliformes in the family Phasianidae, is a common gamebird with a worldwide distribution (Hill and Robertson 1988; Pfarr 2012). It is a well-known game bird with global importance for both research and wildlife management. First, as one of the world's most widespread resident species, the common pheasant is native to the temperate zones in the

Palearctic region, from the Russian Far East to eastern-southeastern Europe (east of the Black Sea), and southwards to Indochina and Afghanistan (Cramp 1980; Johnsgard 1999). Second, it is among the most subspecies-rich bird species with thirty described subspecies defined mainly by plumage characters in males and geographical range (Madge et al. 2002). These subspecies occupy substantially different environments and climatic zones, from isolated oases in

semideserts, to montane regions, displaying unique phenotypes and genotypes (Hill and Robertson 1988; Pfarr 2012). The common pheasant also has great economic values, and have long history of being released for hunting or kept captive in bird farms in Western Europe, North America and Australia (Johnsgard 1999; Madge et al. 2002). This makes the common pheasant a promising model to investigate important questions about speciation, trait evolution, biogeography and local adaptation to various climatic conditions, as well as in wildlife management and conservation genetics.

Previous studies of the common pheasant have mainly focused on its ecology and biology. Several aspects of the species' life history and reproduction, such as survival rate (Draycott et al. 2008), habitat selection (Long et al. 2007), and breeding biology (Robertson 1996) has been well quantified, which provide useful knowledge for sustainable management. The taxonomy and systematics of common pheasant have long been actively studied. For example, the thirty-recognized subspecies were hypothesized to be clustered into five subspecies-groups based on morphological resemblance and biogeographical affinity (Madge et al. 2002). However, given that clinal variation may explain connectivity and contiguous distributions, the validity of some subspecies has been questioned (Cramp 1980; Liu and Sun 1992; Johnsgard 1999; Madge et al. 2002). Molecular phylogenetic approaches have been applied to resolve these puzzles since the subspecies-groups have been identified as independent evolutionary lineages by mitochondrial fragments (Qu et al. 2009; Liu et al. 2010; Zhang et al. 2014). Recent works using multi-locus nuclear markers corroborate previous findings, and further showed that viscous boundaries between subspecies are probably due to extensive gene flow among contiguous populations (Kayvanfar et al. 2017; Wang et al. 2017). These works used a small number of genetic markers, which are capable of delineating population structure and subspecies relationships. However, due to the lack of genomic-level data, many questions related to demographic histories, for example (Nadachowska-Brzyska et al. 2013), population admixture and molecular genetic basis of phenotypes, for example, plumage color and pattern (Toews et al. 2016), remain undetermined.

Taking advantage of next-generation sequencing (NGS), it is now feasible to obtain unprecedented genomic-scale data, allowing direct quantification of genomic variation and identification of the genomic landscape associated with specific phenotypes in nonmodel organisms (Ellegren 2014; Toews et al. 2016; Bosse et al. 2017). Particularly in the study of speciation, it is of interest to understand the size and extent of regions of divergence across the genome among the related species (Wu 2001). Recent empirical studies have been uncovering a notable pattern that genomic regions with accumulated genetic differentiation between closely related avian species/subspecies pairs contain genes involved in divergence whereas gene flow and genetic drift homogenize other

regions (Ellegren et al. 2012; Poelstra et al. 2014). Divergent heterogeneous regions have been coined "islands of divergence" (Turner et al. 2005; Harr 2006; Nosil et al. 2009) and divergent selection is considered to contribute to such differentiation islands, though it is not necessarily the only evolutionary force explaining this phenomenon (Cruickshank and Hahn 2014). Other processes, such as linked selection are evident to contribute divergent genomic landscapes as shown by some recent studies (Burri et al. 2015, Vijay et al. 2017). The common pheasant is distributed in a vast geographical range and provide several evolutionary contrasts to investigate complex evolutionary processes and demographic history that could contribute intraspecific divergence. Obviously, the availability of genomic information will help facilitate finer-scale characterization of the genomic-wide regions involved in divergence and adaptation of common pheasant.

In this study, we present the first genome assembly of the common pheasant with detailed description of its genetic architecture and population-level genomic polymorphisms in order to provide genomic resources toward studies of speciation, local adaptation, and conservation genomics of an ecologically important species.

Materials and Methods

Sampling and Sequencing

The sequenced sample was fresh blood from a male common pheasant, (the subspecies *P. colchicus torquatus*: NCBI taxonomy ID 9054; BioProject ID PRJNA449162; BioSample ID SAMN08888528). This individual was a captive individual with a wild origin from Beijing Wild Animal Park (Daxing) sampled in August 2015. All sampling procedures were performed in accordance with Chinese wildlife regulations and protocols.

Genomic DNA was extracted using a Qiagen DNA purification kit following the manufacturer's instruction and the quality of extracted DNA was checked using gel electrophoresis (1% agarose gel/40 ng loading). We built four short insert libraries (two for 250 bp and two for 450 bp) and three mate pair libraries (2 kbp, 5 kbp, 10 kbp) following Illumina's standard protocol. Briefly, the qualified genomic DNA was randomly sheared into short fragments by hydrodynamic shearing system (Covaris, Massachusetts, USA). Then, followed by end repairing, dA-tailing and further ligation with Illumina adapter, the required fragments (in 300–500 bp size) with both P5 and P7 sequences were PCR selected and amplified. After gel electrophoresis and subsequent purification, the required fragments were obtained. The constructed libraries were loaded on the Illumina HiSeq platform for paired-end sequencing, with the read length of 150 bp at each end.

Raw data obtained from sequencing also contain adapter contamination and low-quality reads. These sequence artifacts may complicate the downstream processing analysis.

The raw data were thus filtered to reduce low quality bases and reads by the following strategies: 1) filtering our reads with adapters; 2) reads with N bases >5%; 3) the paired reads when single end sequencing reads contain low quality (<5) bases that exceed 10% of the read length.

Evaluation of Genome Size

The genome size was estimated according to a k-mer analysis with the formula: $G = $ k-mer_number/k-mer_depth, where G is the genome size, k-mer_number is the total counts of kmers and k-mer_depth refers to the main peak in the k-mer distribution. In this study, we collected all reads in a short-insert library to conduct the 19-mer analysis with Jellyfish 2.0 (Marçais and Kingsford 2011). A total of 39,151,211,661 k-mers were produced and the peak k-mer depth was 38 (supplementary fig. S1, Supplementary Material online).

Genome Assembly and Assessment

To assemble the common pheasant genome, we firstly evaluated genome-wide heterozygosity using the above k-mer analysis. Double peaks suggested that this diploid genome was highly heterozygous. We therefore employed Platanus v1.2.4 (Kajitani et al. 2014), which is particularly designed for highly heterozygous genomes, to assemble the common pheasant genome. The first round included three steps: Contig assembly, scaffolding, and gap closing. Firstly, all filtered reads in short-insert libraries (250 bp, 450 bp) were input for contig assembly. After constructing de Brujin graphs, clipping tips, merging bubbles, and removing low coverage links with default parameters, assembled contigs and bubbles in the graphs were obtained in this step. In the scaffolding steps, the bubbles and reads from both short-insert library (250 bp, 450 bp) and long-insert library (2 kbp, 5 kbp, 10 kbp) were mapped onto contig sequences to build scaffolds with default parameters. Finally, the intrascaffold gaps were filled with reads from all libraries. After five rounds of gap closing, the gap rate in the scaffolds reached a plateau. To evaluate the completeness of the genome, we performed BUSCO v3 (Simão et al. 2015) with the representative chicken gene data set aves_odb9.

Genome Annotation

To identify genomic repeat elements in the assembly, both ab initio and homolog-based methods were used. For the homolog-based methods, we used RepeatMasker (www.repeatmasker.org; last accessed November 22, 2019) (Smit et al. 2016) to search against the Repbase library version 22.12 (Jurka 1998). In the ab initio method, a custom repeat library was constructed using RepeatModeler (www.repeatmasker.org) (Smit and Hubley 2008) with RECON (Bao and Eddy 2002), RepeatScout (Price et al. 2005), and Tandem

repeats finder (TRF) (Benson 1999), which was then used in RepeatMasker to annotate repeats.

Gene annotation for the common pheasant genome assembly were conducted with the MAKER2 pipeline (Holt and Yandell 2011), which incorporates ab initio prediction and homology-based prediction. For the ab initio method, repeat regions were first masked based on the previous results of repeat annotation, and then Augustus (Stanke and Waack 2003) and GeneMark_ES (Lomsadze et al. 2005) were employed to generate gene structures. In addition, FGENESH (Salamov and Solovyev 2000) was also used to for ab initio prediction. For homology-based prediction, protein sequences from three different species, Chicken (*Gallus gallus*), Zebra finch (*Taeniopygia guttata*), Turkey (*Meleagris gallopavo*) (downloaded from Ensemble database 9.1 release), were mapped onto the genome assembly using tBlastN of the NCBI BLAST suite v2.7.1 (Madden 2013; Coordinators 2017) and Exonerate v2.2.0 (Slater and Birney 2005) was used to polish BLAST hits to get exact intron/exon position.

Population Genomics Analysis Using the Reference Genome of the Common Pheasant

To facilitate future population genomics studies of the common pheasant, we further sequenced an additional 45 male individuals from seven subspecies across China (fig. 1A) using restriction site-associated DNA sequencing (RAD sequencing) (Miller et al. 2007). The de novo common pheasant genome was used as a reference to facilitate mapping and SNP calling. We analyzed population structure among 45 individuals using ADMIXTURE 1.3 (Alexander et al. 2009) and principal component analysis (PCA). The detailed lab and sequencing procedures, and analyses are available in the supplementary appendix, Supplementary Material online.

Results and Discussion

We sequenced and assembled a reference genome of a male common pheasant. We obtained 94.88 Gb clean paired reads (supplementary table S1, Supplementary Material online). The assembled genome size of is 1.02 Gb (1,021,360,992 bp) in length with a genomic coverage $= 93\times$. The assembled genome contains 58,369 contigs (contig N50 of 178 kb) and 39,677 scaffolds (scaffold N50 of 10.2 Mb) (table 1). The completeness of the common pheasant draft genome is high: We totally identified 4790 BUSCOs (97.5%) including 4,585 complete (93.3%) and 205 fragmented (4.2%) BUSCOs (supplementary table S2, Supplementary Material online).

We found that a total of 12.05% (123.3 Mb) repeats elements were identified in the genome assembly of common pheasant, with unclassified elements constituting the greatest proportion (supplementary table S3, Supplementary Material online). We used different prediction methods to produce a consensus gene set. In total 16,485 protein-coding genes
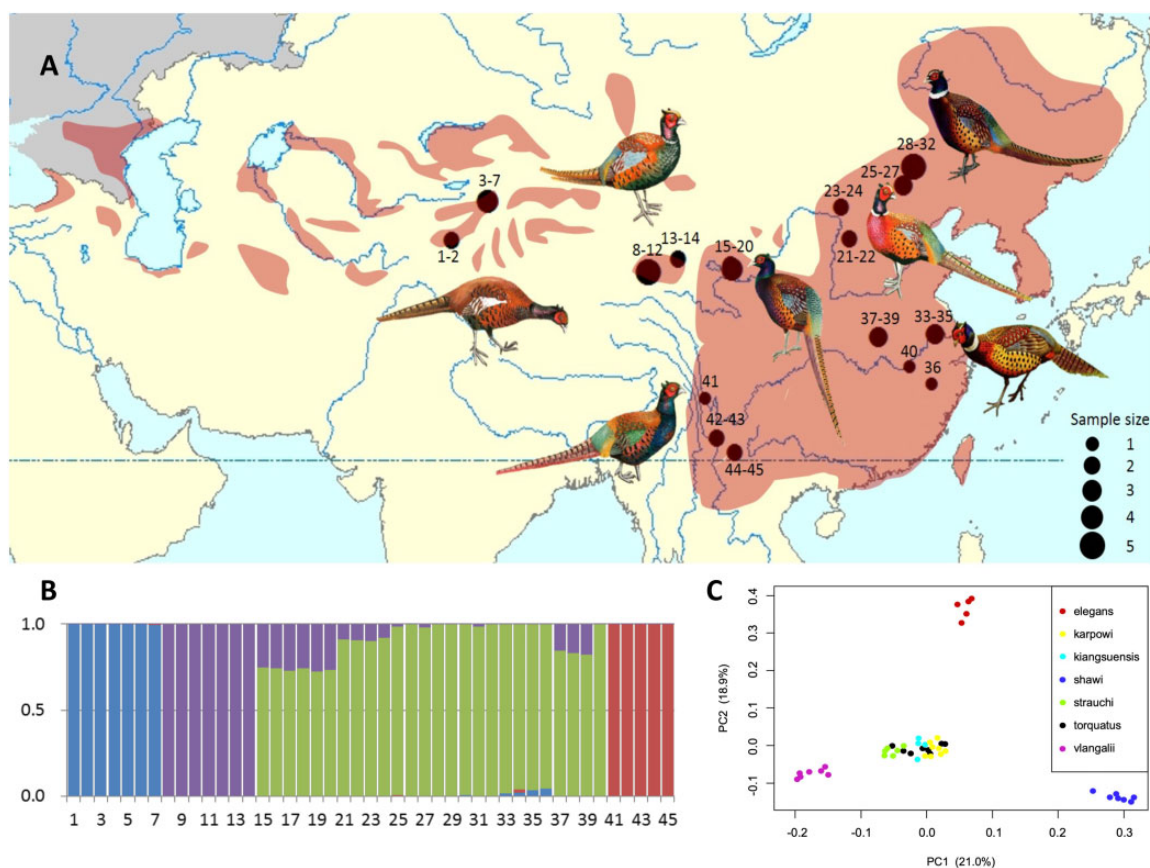
Fig. 1.—Population genetic structure of common pheasant in China. (*A*) The distributions of samples of seven subspecies of common pheasant. Shown are males with divergent phenotypes (pheasant artworks were modified from Pfarr 2012). The size of the circles is proportional to the number of individuals. (*B*) Four genetic clusters were inferred by ADMIXTURE using 4,376,351 SNPs. The *Y*-axis represents the proportion of each ancestral genetic component and numbers in the *X*-axis represent sample locations (details in supplementary table S5, Supplementary Material online) and seven subspecies affinities: 1–7: *shawii*; 8–14: *vlangalii*; 15–20: *strauchi*; 21–24: *kiangsuensis*; 25–32: *karpowi*; 33–40: *torquatus*; 41–45: *elegans*. (*C*) Principal component analysis (PCA) of 45 individuals from seven subspecies of common pheasant using the same SNP data set with ADMIXTURE analysis. Color dots represent individuals of different subspecies.

Table 1

Summary statistics of the genome assembly for the common pheasant

Common Pheasant Genome	
Total length	1,021,360,992 bp
Number of contigs	58,369
N50 of contigs	178,013 bp
Number of scaffolds	39,677
N50 of scaffolds	10,186,719 bp
Longest scaffolds	42,030,034 bp
GC level	41.24%
Number of BUSCOs	4790 (97.5%)
Number of CDS	165,367
Number of mRNA	16,485
length of repeat elements	123,323,121 bp

were identified in the common pheasant genome using our described prediction methods (supplementary table S4, Supplementary Material online).

In addition, ~114.91 Gb were generated (clean data) for all 45 samples (supplementary table S5, Supplementary Material online). After filtering and SNP callings, we obtained 4,376,351 SNPs overall, and we established a database including 328,473–999,733 SNPs for each individual (supplementary table S5, Supplementary Material online). We managed to identify 59,453 SNPs in exon regions, UTR regions and splice sites. 602,747 SNPs were identified in 5 kbp upstream/downstream regions, which can also be associated with phenotypes and functions. In addition, 1,260,753 SNPs and 3,092,463 SNPs were found in intron regions and intergenic regions, respectively. Intron regions are suggested to have genomic functions (Cech 1990) and we can further test this hypothesis using the common pheasant model. Since populations of common pheasants dwelling in contrasting environments and climatic zones, for example, monsoon regions, basins in the Qinghai-Tibetan plateau, semiarid zones, and deserts. These resources can be used to

investigate population demography and genomic architectures associated with local adaptation of the common pheasant in the future (supplementary table S6, Supplementary Material online).

Our population structure results by ADMIXTURE clearly show that when four groups were inferred, the cross-validation error has the lowest value among the alternatives (supplementary fig. S2, Supplementary Material online). Given this, populations in western Xinjiang (subspecies *shawii*), Qinghai (subspecies *vlanglii*), Yunnan (subspecies *elegans*) and the remaining four populations in central and eastern China (subspecies *strauchi, kiangsuensis, karpowi,* and *torquatus*) form distinct genetic groups, respectively (fig. 1*B*). The results of PCA using the similar SNP data set were consistent with ADMIXTURE results, showing four genetic clusters, that is, Yunnan, western Xinjiang Qinghai, and the remaining subspecies (fig. 1*C*). Clearly, the subspecies of *karpowi, kiangsuensis*, and *torquatus* show varied magnitudes of genetic introgression with the subspecies of *vlanglii* from Qinghai, which corroborates previous results as shown by multilocus phylogeography studies (Liu et al. 2010; Kayvanfar et al. 2017). The resulting population structuring pattern is likely due to genetic drift of small and isolated populations in those regions, but consistent with genetic introgression caused by population expansion in contiguous populations in northern China (Liu et al. 2010; Kayvanfar et al. 2017). Obviously, the derived genomic-level polymorphism provides unprecedented power, which allows exclusive tests of specific hypotheses demographic history in common pheasant using model-based population genomic analysis (Luikart et al. 2003).

In conclusion, we report the first high quality assembled and annotated common pheasant genome. This reference genome facilitated us to obtain a high-resolution population genomic data. Such valuable resources allow unbiased downstream population genetic analysis that might be more robust than SNP sets called from de novo approaches (Shafer et al. 2017). Overall, our effort will offer an opportunity to future deep investigation of questions in evolutionary biology and wildlife management in the common pheasant, a bird species of long-standing interest in ecology, evolution, and economics.

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online.

Acknowledgments

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