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Letter to the editor Open Access

Conservation genomics of sibling grouse in boreal forests reveals introgression and adaptive population differentiation in genes controlling epigenetic variation

The Chinese Grouse (*Tetrastes sewerzowi*) and Hazel Grouse (*T. bonasia*) are sibling species that are well-adapted to harsh high-altitude and latitude habitats. In the current study, we sampled and sequenced 29 Chinese Grouse(*n*=16) and Hazel Grouse (*n*=13) from eight locations in China, Sweden, Germany, and northeast Poland to analyze population genetic diversity and structure, introgression, and local adaptation. Through selective sweep analysis, we found evidence that Chinese Grouse inhabiting the high-altitude Qinghai-Xizang (Tibet) Plateau (QTP) have evolved adaptations to hypobaric hypoxia and high ultraviolet radiation. We also found that many genes under positive selection in the two species were related to histone modification and chromatin structure. Thus, differentiation between the two species may be targeted at genes involved in epigenetic regulation, suggesting a role of phenotypic plasticity in species and population differentiation. Further analysis indicated a strong population structure for each species and evidence of local adaptation in the Chinese Grouse. Analysis also identified certain regions (Sweden and Qilian Mountains) with particularly low diversity. These results suggest a role of phenotypic plasticity in differentiation among and within species, which may explain local adaptation and may have profound implications for how to preserve threatened local populations.

The genetic basis for phenotypic plasticity has been debated for decades (West-Eberhard, 2003). While few doubt there is a genetic basis, the precise mechanisms and genes involved are still unknown (Oostra et al., 2018). Detecting such has profound importance for the role of phenotypic plasticity in local adaptation and whether populations can adapt to changing circumstances. This is of considerable importance for evolutionary ecology and conservation of biodiversity.

To investigate the genetic basis for local adaptation, we examined nuclear genomic diversity in a pair of sibling bird species, i.e., Chinese Grouse and Hazel Grouse, across their contemporary distribution while controlling for population structure within and between species (Figure 1; Supplementary Material). At the continental scale, we evaluated and compared genetic diversity, population structure, and introgression in populations and genes under divergent selection between the two taxa.

We performed whole-genome resequencing of 16 individuals representing three geographically distinct Chinese Grouse populations in the QTP, China, and 13 Hazel Grouse individuals from China, Sweden (SWE), Germany (GER), and northeast Poland (NEP). In total, 686.04 Gb of high-quality data were uniquely mapped to the 975 Mb Chinese grouse reference genome (Supplementary Table S1) (Song et al., 2021). The groups showed different nucleotide diversity (π) (Nei & Li, 1979), with higher diversity in the Hazel Grouse compared to the Chinese Grouse (Supplementary Table S2). Within the two species, lowest diversity was observed in an isolated Chinese Grouse population in the Qilian Mountains (QLS) and in the Scandinavian Hazel Grouse population (Supplementary Table S2). Tajima's *D* was positive in all populations, except in the SWE Hazel Grouse (Supplementary Table S2).

High F_{ST} values were observed among the different regional groups. For Chinese Grouse, the highest F_{ST} value was found between the northern QLS population and southern Lianhuashan National Nature Reserve (LHS) and Zhuoni County (ZN) populations, while the lowest value was found between the southern LHS and ZN populations. For Hazel Grouse, the F_{ST} value was high among the three locations, especially between the SWE and GER+NEP populations (Supplementary Table S3). Genome-wide linkage disequilibrium (LD) varied markedly within the sibling grouse species. For Chinese Grouse, the average distance over

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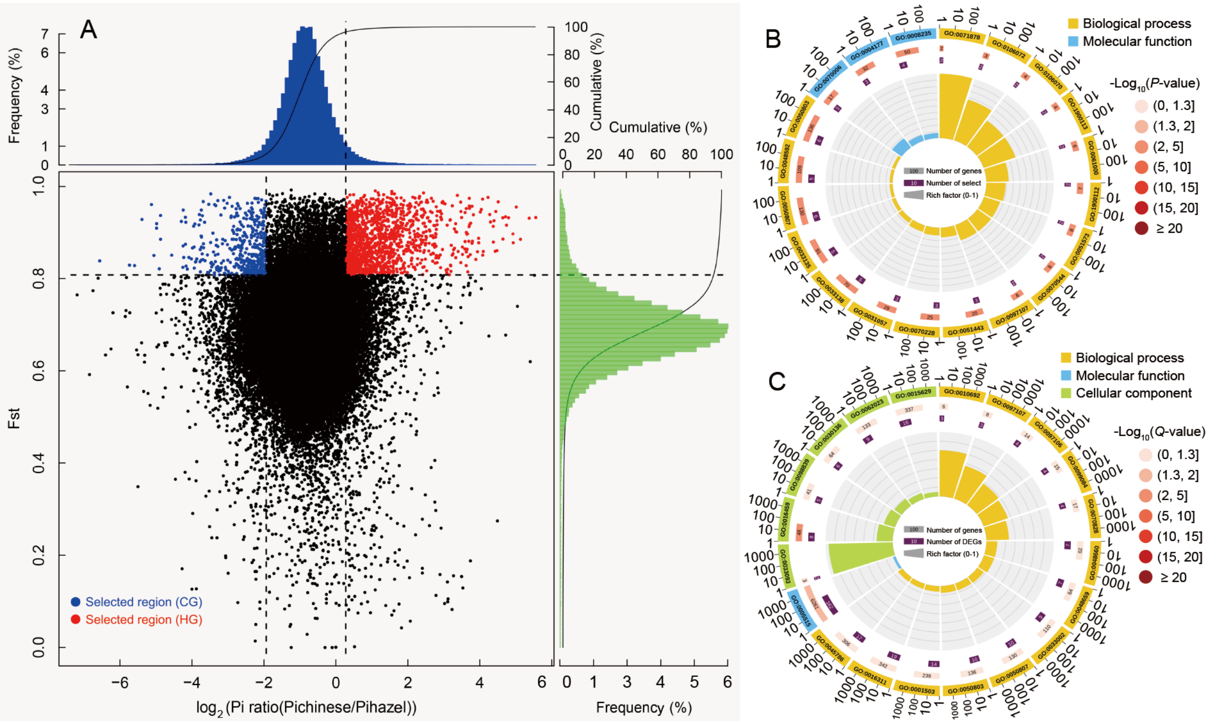

Figure 1 Analysis of genes under local adaptation

A: Genomic regions (blue points) located at top left of dashed lines are candidate positively selected regions for Chinese Grouse. Genomic regions (red points) located at top right of dashed lines are candidate positively selected regions for Hazel Grouse. B: Gene enrichment in Chinese Grouse in GO terms. C: Gene enrichment in Hazel Grouse in GO terms.

which LD decayed to half its maximum value was substantially longer in the QLS population (>20 kb) than in the ZN (−8 kb) and LHS populations (−2 kb). For Hazel Grouse, the average distance over which LD decayed to half its maximum value was longer in the SWE and GER populations than in the Chinese population (Supplementary Figure S1A). We applied principal component analysis (PCA) to determine genetic variation between the Chinese Grouse and Hazel Grouse populations using a stringent single nucleotide polymorphism (SNP) set after filtering based on minor allele frequency (-minMaf 0.05). The principal components (PCs) of the two species are shown in Supplementary Figure S1B. For the Chinese grouse, PC1, which accounted for 50.3% of the variation, separated the QLS population from the southern samples, whereas the distance between the LHS and ZN grouse populations was small. For the hazel grouse, PC1 separated the central European samples and accounted for 48.7% of the variation, while PC2 further separated the SWE samples and accounted for 35.5% of the variation.

To further explore differences among regions, we used ADMIXTURE, a maximum-likelihood estimation of individual ancestries based on multilocus SNP genotype datasets (Alexander et al., 2009), to identify subgroups within the sampling locations and assign samples without using prior information. Supplementary Figure S1C shows groupings when individuals were members of more than one of the K subgroups or clusters. For the Chinese Grouse, when K=2, the QLS birds were separated into one cluster and the LHS and ZN birds into the other; when K=3, the separate QLS cluster was retained, but the ZN birds formed a separate cluster with some LHS birds and some LHS birds showing evidence of admixture with ZN (Supplementary Figure S1C). For the Hazel Grouse, when K=2, the central European and SWE birds were separated from the Chinese birds; when K=3, the Chinese, central European (GER), and SWE birds formed separate clusters; when at K=4, the central European individuals were further separated into two birds from a captive breeding station (but with different original ancestry). We did not detect shared inbreeding by descent blocks in any population of the two species. However, the ABBA-BABA tests showed more introgression (in general) among the Hazel Grouse populations than in the Chinese Grouse (Supplementary Figure S1D).

To detect natural selection signatures associated with separation between the species, we explored genomic signatures of selection and local adaptation between the high-altitude Chinese Grouse and lowland (boreal forest) Hazel Grouse. Using F_{ST} and θ_{π} methods, we identified 141 genes within the top 5% of maximum F_{ST} and $\theta_{\pi1}/\theta_{\pi2}$ values suggesting divergent selection in the Chinese Grouse (Figure 1A). Gene Ontology (GO) analysis indicated that these genes were primarily involved in metabolic processes, high radiation, and hypoxia response (Figure 1B). Functional enrichment analysis based on Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways indicated that some genes were enriched in the MAPK signaling pathway (gga04010, P=2.80×10^{-4}), folate biosynthesis (gga00790, P=2.58×10^{-3}), porphyrin and chlorophyll metabolism (gga00860, P=8.84×10^{-}

^{3}), butanoate metabolism (gga00650, P=9.51×10^{-3}), and peroxisome (gga04146, P=9.65×10^{-3}), with GO enrichment in nervous system development or death (Supplementary Figure S3). Selection of these genes may be related to adaptation to hypobaric hypoxia and high ultraviolet radiation in the QTP habitat where the Chinese Grouse is found. In the Hazel Grouse, we identified 392 genes under selection (Figure 1A, C), which were partly enriched in postsynaptic density organization of the nervous system and muscle cell proliferation, ossification, and organ morphogenesis (Supplementary Figure S4).

Considering the recent divergence (1.76 million years ago (Ma)) between the Chinese Grouseand Hazel Grouse, their ancestor was likely adapted to a cold environment different from that experienced by the Chinese Grouse because the latest and most significant uplift of the QTP occurred 1.1 to 0.6 Ma, resulting in an altitudinal increase of ~3 000 m a.s.l. (Li & Fang, 1999). After their divergence, in contrast to Hazel Grouse, the Chinese Grouse adapted to the more environmentally challenging high-altitude plateau (e.g., hypobaric, hypoxic, high ultraviolet radiation, and poor food resources). After GO enrichment analysis of the selected genes, we found 239 significantly enriched functional categories for the 141 genes in the Chinese Grouse, and 78 significantly enriched functional categories for the 392 genes in the Hazel Grouse. Furthermore, we identified five enriched "Biological Function" categories in the Chinese Grouse and one in the Hazel Grouse, indicating that the selected genes are involved in histone methylation/heterochromatin organization, suggesting that gene expression and phenotypic plasticity played a role in the genomic divergence of the two species (Supplementary Figures S2, S3).

We found evidence of positively selected genes for adaptation to high altitude in the Chinese Grouse. Most previous studies on high-altitude adaptation have focused on species that dwell above the tree line on rocky steppes and grassland of the QTP, such as humans (Lorenzo et al., 2014) and Tibetan migratory locusts (Ding et al., 2018). Here, we showed high-altitude molecular adaptations in a species that resides below the tree line. In both species, GO enrichment analysis indicated that positively selected genes were involved in histone methylation/heterochromatin organization, suggesting a role for gene expression and phenotypic plasticity in the genomic divergence of the two species. The role of phenotypic plasticity in differentiation among and within species may explain the presence of specifically adapted phenotypes, even in the absence of genomic regions mapping to such phenotypes. This may have profound implications for our understanding of the genetic basis for phenotypic divergence in speciation as divergence due to phenotypic plasticity may be an example of genetic draft (Neher, 2013). Under such a scenario, phenotypically plastic associations to an environment may be the first stage in divergence among taxa and may predate any genetically determined local adaptations. We found a significant difference in the significance function ratio between the two species (X^2=203, df=1, P<0.001). We can infer that after divergence, the selected genes of Chinese Grouse to adapt to the harsh high-altitude environment play a specific role in a wide range of functions in the GO terms, while the more selected genes of the Hazel Grouse play a specific role in the narrow function.

The Chinese Grouse and Hazel Grouse both reside in extremely cold forests during winter, either in high-altitude coniferous forests in the QTP or boreal forests in the Eurasian sub-arctic. The environmental conditions in the QTP may be more extreme than those in the Eurasian boreal forests given the hypobaric hypoxia and high solar radiation. Considering the divergence time (1.76 Ma) between the Chinese Grouse and Hazel Grouse, their ancestor was likely cold-adapted before the divergence (Song, 2020). The most significant change in vegetation in the eastern Palearctic occurred from the Pleistocene (2.58 Ma) when deciduous forest proliferated and coniferous forest and tundra moved northward (Comins & Kaufmann, 2011). Thus, finding evidence for cold adaptations based on comparative population genomic data from the Chinese Grouse and Hazel Grouse is difficult. Instead, differences between the species based on functional enrichment analysis may reflect adaptations of the Chinese Grouse to higher altitudes. High latitude and altitude species are very sensitive to demographic events and ongoing climate warming (Fedorov et al., 2020; Höglund, 2009). Our results provide insight into the strong population structures of these sibling species as well as evidence of specific and local adaptation. We also identified certain geographic populations (SWE and QLS) with particularly low diversity, which could be used to set priorities for conservation efforts.

To the best of our knowledge, this is the first description of the population structure and genetic diversity of the Chinese Grouse and the first study to use whole-genome data to reveal the population structure of the Hazel Grouse. PCA and ADMIXTURE analyses showed a strong population genetic structure for both species. In the Chinese Grouse, the QLS population was clearly divergent from the other two populations, with the low genetic diversity, high pairwise F_{ST}, and high LD decay supporting its strong divergence and long-term isolation.

DATA AVAILABILITY

Sequencing data for the Chinese Grouse and Hazel Grouse have been deposited in Short Read Archive under NCBI BioProjectID PRJNA588719 and GSA BioProjectID PRJCA005913.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

K.S., J.H., and Y.H.S. designed and managed the project. K.S., B.G., and P.H. performed the analyses. K.S., Y.F., and Y.X.J. collected the samples. K.S., J.E.S., Z.M.H., J.H., and Y.H.S. wrote the paper. J.H., Y.H.S., J.E.S., B.G., P.H., Y.F., Z.M.H., and Y.X.J. revised the paper. All authors read and approved the final version of the manuscript.

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