

Genomic analysis of demographic history and ecological niche modeling in the endangered Chinese Grouse *Tetrastes sewerzowi*

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Abstract

Background: The Quaternary is characterized by marked climatic oscillations between glacial and interglacial periods that had worldwide consequences in forming the contemporary diversity of many populations, species and communities. The origin and evolution of biodiversity in mountainous areas are highly dependent on historical orogenesis and associated climatic changes. The Chinese grouse *Tetrastes sewerzowi* is a forest-dwelling species endemic to the mountains to the east of the Qinghai–Tibet Plateau, which has been listed as Near Threatened with a decreasing trend by the IUCN because of ongoing deforestation and fragmentation of coniferous forests. Understanding demographic history is important in placing current population status into a broader ecological and evolutionary context.

Results: Analyses of the Chinese Grouse genome revealed fluctuations in effective population size throughout the Pleistocene. Populations decreased during early to middle Pleistocene but showed an expansion during late Pleistocene which was then followed by a sharp decline during the last glacial maximum (LGM). Ecological niche modeling indicated that a suitable habitat shift between high altitude regions to low altitude regions was due to a changing climate. This result parallels patterns of population size change in Chinese Grouse estimated from PSMC modelling, which suggested an expansion in population size from the last interglacial period (LIG) and then a peak and a bottleneck occurring at the last glacial maximum (LGM). Furthermore, the present-day distribution of Chinese Grouse is greatly reduced and fragmented. It will likely become even more fragmented in the future since coniferous forest cover is threatened in the region of their distribution and the availability of such habitat restricts their ecological niche.

Conclusions: The Chinese Grouse have experienced substantial population size changes from the beginning to the LIG and reached a peak before the LGM. A sharp decrease and bottleneck occurred during the LGM, when the coniferous forests were subjected to extensive loss. The results inferred from the whole genome sequencing and species distribution models both support historical population fluctuations. The distribution of the Chinese Grouse is strongly dependent on the coniferous forest cover. To protect the fragmented coniferous forests is an essential action to protect the Chinese Grouse.

Background

The Chinese grouse is a forest-dwelling bird species endemic to the Qinghai–Tibetan Plateau (QTP) [1, 2], which has been listed as *Near Threatened* with a decreasing trend by the International Union for the Conservation of Nature (IUCN) because of ongoing deforestation and fragmentation of coniferous forests [3]. Throughout the range of the species, only the wetter northern slopes of mountain coniferous forests have vegetation that support grouse populations [1]. This results in ongoing significant reductions of population size and area of occupancy when such areas are cut [2]. In addition, large-scale deforestation and intensive livestock grazing exacerbates habitat loss and fragmentation [4]. Furthermore, climate change possibly threatens this species not only by influencing range shifts, but also by increasing habitat loss and fragmentation [5, 6].

The origin and evolution of biodiversity in mountainous areas are highly dependent on the historical orogenesis and associated climatic changes [7-9]. Complex geological environments and Pleistocene glacial events contributes to complicated refugial patterns, species distributions and the demographic history of populations [9-13]. Four significant glaciations have occurred in the QTP during the Pleistocene, the Xixiabangma (Early Pleistocene), Nyanyaxungla (Middle Pleistocene; 0.78-0.50 million years ago), Guxiang (late Middle Pleistocene) and the last glaciation, including two glacial stages with the last corresponding to the LGM [10, 14, 15]. The coniferous forests declines and expansions coincide with the glaciations. Cold and dry weather conditions happened in the glaciation period, which resulted in decline of coniferous forests. On the contrary, warm and wet weather conditions followed the retreat of the glaciers which were ideal for the spread of coniferous forests. Pollen data and analyses of past animal faunas show that the forest and forest steppe including spruce and fir connected the QTP and the Siberian taiga by the loess plateau in northern China during the Mid Pleistocene [16]. The distribution of existing Palearctic realm taiga birds and mammals, such as Black Woodpecker (*Dryocopus martius*), Spotted Nutcracker (*Nucifraga caryocatactes*), Eurasian Jay (*Garrulus glandarius*), Snowy-browed Nuthatch (*Sitta villosa*), Red Deer (*Cervus elaphus*) support this past connection. The uplift of the QTP created a large altitudinal gradient across the region spanning from 500 to 8848 m [17]. The eastern edge of the QTP are now associated with deep valleys and characterized mainly by a warm and wet climate [18].

The Quaternary is characterized by marked climatic oscillations between glacial and interglacial periods that had worldwide consequences in forming the contemporary diversity of many populations, species and communities [19-22]. The effect of the Pleistocene climate fluctuations on species distributions and diversity is well known in Europe [23] and North America [24]. The periodic uplift of the QTP before the Pleistocene has contributed to geologically induced complex environments where Pleistocene glaciations formed many relatively small alpine glaciers and refugia [10, 25, 26]. As a consequence, more complex glacial effects may have influenced biotic speciation, distribution and diversity in the QTP compared to Europe and North America [10, 11]. Before the LGM, the warm and wet weather during the “Greatest lake period” (25,000-40,000 years ago) in this region could have contributed to the expansion of the alpine coniferous forests [27-29]. The temperature was 2–4 °C higher and precipitation was 40–100% higher than today [27]. Furthermore, pollen-based vegetation reconstruction shows that at this time [28, 30] the alpine coniferous forest extended c. 400–800 km beyond its present western limit. This expansion of the alpine coniferous forests, the primary habitat for the Giant Panda (*Ailuropoda melanoleuca*) as well as the Chinese Grouse, have resulted in a second population expansion of Giant Pandas [1, 12]. The Chinese Grouse as well as the other typical forests-dwelling birds and mammals should have had similar demographic histories during this period.

The aim of the present study is to infer the past population dynamics of Chinese Grouse by applying Partial Sequential Markov Chain (PSMC) modelling on whole genome sequence data. The PSMC model use a hidden Markov framework and identifies historical recombination events across a single diploid genome to infer the time of the most recent common ancestor for each independent DNA segment and infer ancestral effective population size (N_e) at a given epoch [31]. Combined with glacial event data, we

aim at understanding the effects of past climatic changes on past distribution changes of Chinese Grouse via Species Distribution Modelling (SDM). The combination of population genomics and SDM also provides new insights to understand the impact of past climatic changes on population dynamics. At present, climate and habitats are significantly changed by human activities in the QTP of southwest China, which have already had, and will continue to cause, untold biodiversity loss [32]. We also performed a GAP analysis, a Geographic Approach to Protection of Biological diversity [33], between present forests and climate driven habitat to inspire future work in the conservation of Chinese Grouse and other forest species in the QTP.

Results

***De Novo* Assembly of the Chinese grouse genome**

The Illumina sequencing had total 184.34 G PE 150 bp reads and the average depth was 184 X. The Pacbio long-read Sequencing had total length of 36.7 GB and the depth was 36 X. The software DBG2OLC produced a reference de novo genome assembly which comprises 1,272 scaffolds that had a total length of 1,003,232,344 bp, corresponding to 91 % of the total genome length of the expected 1.1 G. Scaffold N50 was 2.16 Mb, and the longest scaffold measured 5.27 Mb. The BUSCO complete assessment score is 93%. For comparison, the well assembled Chicken (*G. gallus*) genome is 1,054.6 Mb [34], which is similar with the genome size of Chinese Grouse in the current project.

Demographic history

The simulated demographic history of Chinese Grouse was inferred by plotting the population size fluctuation curve against time with superimposed glacial periods [15]. Chinese Grouse effective population size increased from the time of the last interglacial (around 115 kya ago). Before this time, the effective population size of Chinese Grouse was kept at a high number (20×10^4) from the beginning of the simulations. The results indicated that Chinese Grouse population size reached a peak before the last glacialiation Maximum (40-100 kya ago), experienced a significant decrease during the last glacialiation Maximum (LGM; ~20 kya ago) (Fig. 1), when substantial alpine glaciations (such as Gongga glacial II) would likely have resulted in extensive loss of coniferous forests.

Ecological niche modeling

To assess the role of climate change, we used SDMs and palaeoclimatic data on temperature and precipitation to estimate the suitable habitat of Chinese Grouse during the time periods of 120-140 kya (LIG), 21 kya (LGM), 6 kya (Mid-Holocene), and the present (Fig. 2). All four SDMs suggest that past climate change may have contributed significantly to the distribution change and population dynamics of Chinese Grouse. The model revealed that suitable distribution during the colder LGM was more widely available than during the warmer LIG and warmer mid Holocene (Fig. 2). Specifically, compared with the LIG period, the area of suitable distribution during the colder LGM period had increased about 11.9375 thousand km² and decreased 2.3875 thousand km² (Fig. 2a, Fig. 3). The expansion of suitable

distribution was moved eastwards which was a low elevation area. On the contrary, the area of suitable distribution during LGM period was less compared with the Mid Holocene period (Fig. 2b). The suitable distribution during Mid Holocene moved to westwards which was at high elevation in the Tibetan plateau and the former eastern area was lost (Fig. 2b).

The population size change in Chinese Grouse estimated from the PSMC model suggested an expansion in population size during the LIG and a bottleneck occurring at the LGM. Strikingly, the suitable distribution of Chinese Grouse expanded toward warmer eastern areas during the LGM and moved to higher and western areas during Mid Holocene. Compared with the Mid Holocene period, the predicted present-day distributions is increased and concentrated predominantly in the model only conducted in climate change scenario (Fig. 2c, Fig. 3). But the present-day distribution of Chinese Grouse was greatly reduced and become highly fragmented after the GAP analysis was performed between the predicted present-day distributions and coniferous forest cover (Fig. 4).

Discussion

The demographic analyses showed the grouse population reached a peak ~ 40,000 years ago followed by sharp decrease with a bottleneck ~ 20,000 years ago. The population expansion can be explained by the warmer weather during the Greatest Lake Period (30,000 - 40,000 years ago) as the alpine conifer forests, the primary habitat for Chinese Grouse [1], reached their greatest extent at this time [10, 12]. The Palaeo-distribution of coniferous forests changes in the QTP and adjacent mountains inferred by pollen cores [35] support the demographic changes inferred of Chinese Grouse [35, 36]. The demographic results and palaeo coniferous forests data show that coniferous forests played a pivotal role in Chinese Grouse demographic history by providing permanent refugias in the eastern QTP during the Quaternary. The QTP with its special intricate geographical environment had a significant influence on biodiversity and changes in the geographic distribution of animals in east of Asia during the Pleistocene and may play a key role on potential responses and feedbacks to global change in the future [11, 37].

Our demographic analyses highlighted that a high N_e of Chinese grouse persisted from the Early Pleistocene (2.43 million years ago-0.73 million years ago) to the Mid Pleistocene (0.73 million years ago - 0.10 million years ago) and that a population expansion occurred after the retreat of the Penultimate Glaciation (0.30-0.13 million years ago). Three significant glaciations have occurred in QTP during this time, the Xixiabangma glaciation in Early Pleistocene, Nyanyaxungla glaciation in Middle Pleistocene and Guxiang glaciation in late Middle Pleistocene [14, 15]. Like previous work, our results suggest that the QTP had several Pleistocene refugia which have affected N_e in this grouse inhabiting coniferous forests [10]. During the Pleistocene, many avian species living in coniferous forest in the boreal taiga and the QTP have diverged and are classified as superspecies or different subspecies, as a result of allopatric divergence and local adaptation [8, 23, 38, 39]. A narrow high altitude boreal forests were preserved in the southeast edge of the QTP by the uplift which comprise one of the key high-altitude biodiversity hotspots in the world [40]. This region harbors one of the world's richest fauna and floras, because it harbored large and complicated refugial areas in different mountain regions, lowland, rivers and basins [10, 41, 42].

A population bottleneck occurred during the last glacial maximum (~20,000 years ago), when substantial alpine glaciations (for example, Gongga glacial II) likely resulted in extensive loss of coniferous forests [15]. Population bottlenecks in the same region coinciding with the LGM have also been identified in other animals such as the giant panda [12] and snub-nosed monkeys (*Rhinopithecus*) [13], suggesting that the Last Glaciation and palaeo coniferous forests changes had strong impacts on the population sizes of arboreal species. The sharp decline in N_e for Chinese Grouse, as in the Giant panda, snub-nosed monkeys, is consistent with the extreme cooling of the climate during the LGM.

Many previous studies have suggested that the LIG was a favorable period for other avian species, such as black grouse (*Tetrao tetrix*) because of increased seasonality in temperatures [43, 44]. During this period, black grouse and Greenland rock ptarmigan (*Lagopus muta*) populations expanded to a high N_e and reached their peaks [43, 45]. It is suggested that the severe reduction in avian population sizes approximately coincide with the beginning of the last glacial period (LGP, 110-12 kya) or occurring during this period [44]. In contrast to previous work, the demographical results of Chinese Grouse show no fluctuations associated with historical climatic changes during Early Pleistocene and Middle Pleistocene [44]. Instead a peak followed by a sharp decrease and a bottleneck occurred during this period. This pattern observed in Chinese Grouse may be related to the special geographical position, climate change and the mass accumulation rate (MAR) of Chinese loess, an index indicating cold and dry or warm and wet climatic periods in China [46, 47].

The SDM results showed that climate change had an influence on the amount and distribution shifts of suitable habitat of Chinese grouse. The model also revealed that suitable habitat during the LGM was more widely available than both during the warmer LIG and mid Holocene. Climate events during the LGM period resulted in an expansion of suitable habitat to low altitude regions in eastward areas compared with the LIG period and a shrink from this low altitude region to a more westward distribution during the mid-Holocene period. The result parallels patterns of population size change in Chinese Grouse estimated from the PSMC model, which suggested an expansion in population size from LIG period and then a peak and a bottleneck occurring at the LGM. Significantly, the suitable habitat of Chinese Grouse expanded toward lower altitudes in eastern areas during the LGM. In contrast, the population moved into high altitudes in the western areas during the mid-Holocene. It is of significance that the geographic distribution of Chinese Grouse populations shifted in an east-west direction from LIG to the LGM and the LGM to mid-Holocene. The reason is that the shift of coniferous forests was significantly affected by seasonal extremes in temperature. In addition, the formation of the loess plateau stopped the forest to spread to the north.

Alarmingly, our results suggest that although suitable areas for the Chinese Grouse in the present-day are larger than during the mid-Holocene, the LGM and the LIG, the N_e has been kept at a low size after the bottleneck during the LGM. Dense coniferous forests are of critical importance for the Chinese Grouse, which are affected by climate change and recent human actions [1]. Our GAP analysis between the present-day distribution of Chinese Grouse and extent of present day coniferous forest shows that the Chinese Grouse populations were severely affected by loss and fragmentation of suitable forests. Forest

fragmentation has profound and lasting influences around the globe, not only on the Earth's biological diversity, but on ecosystem function and numerous ecosystem services [48, 49]. Because of the increase in density of local people with the high demands for farmland, timber, firewood and roads, deforestation of Chinese grouse's mountain habitats continued until 1998 when a disastrous flooding happened, which motivated greater conservation of forests [1, 6]. Previous work on Chinese Grouse, blood pheasant (*Ithaginis cruentus*) and Sichuan jay (*Perisoreus internigrans*) have found that their distributions would be decreased and fragmented severely in the future [5, 6, 50]. The Chinese Grouse distributions under climate change scenarios is similar to other montane species [51], which would shift northward and upward under realistic climate change scenarios [6]. This long term fragmentation of coniferous forests may have effect on gene flow, speciation and divergence of these endemic isolated populations.

Conclusions

The Chinese Grouse have experienced substantial population size changes from the beginning to the LIG and reached a peak before the LGM. A sharp decrease and bottleneck happened during the LGM, when the conifer forests were subjected to extensive loss. The results inferred from the whole genome sequencing and species distribution models both support a history of population size fluctuations. The distribution of the Chinese Grouse were strongly dependent on the coniferous forest cover. To protect the fragmented such forests is an essential action to protect the Chinese Grouse.

Methods

Sampling information. A blood sample was collected from one individual from the Lianhuashan Nature Reserve, Gansu province, China. The blood of the bird was collected with permission from the Forestry Department of China and conform to the National Wildlife Conservation Law in China. Blood collection procedures also conform to the regulations of the animal experimental and medical ethics committee of the Institute of Zoology, Chinese Academy of Sciences.

***De novo* genome sequencing and data production.**

Genomic DNA was extracted from whole a blood sample using a Gentra Puregene Blood Kit (Qiagen) according to the manufacturer's instructions. The quality and quantity of DNA, respectively, were assessed by electrophoresis on 1% agarose gel and with a BioDrop mLITE pectrophotometer (a total of 15 mg of DNA was quantified using the spectrophotometer). For whole genome shotgun sequencing and draft genome assembly, we used two sequencing platforms: Illumina (San Diego, CA) Hiseq4000 and Pacific Biosciences (Menlo Park, CA) PacBio RSII. First, three paired-end libraries with insert size of 350 bp were constructed using Illumina DNA Sample Prep Kit. Pacific Biosciences long reads (>10 kb) were generated using SMRT bell library preparation protocol (10 SMRT cells were sequenced). Construction of sequencing libraries and sequencing analyses were performed at *Annoroad Gene Technology* (Beijing, China).

Assembly

We removed contaminated reads with Illumina paired-end adaptors using TRIMMOMATIC 0.36 [52] with the options ILLUMINACLIP: TRUSEQ3-PE.FA: 2: 30: 10 MINLEN: 150. Then bad reads were eliminated based on threshold of: base quality lower than Phred score of 20; N content above 10%; base quality below 20 proportion above 50%. This allowed us to only keep high-quality paired end reads for the assembly steps.

KmerGenie was used to estimate the best k-mer length for genome de novo assembly. (Chikhi R., Medvedev P. Informed and Automated k-Mer Size Selection for Genome Assembly) We used a hybrid assembly strategy following the protocol of DBG2OLC toolset [53]. The parameter: LD 0 k 57 g 25 NodeCovTh 2 EdgeCovTh 1 GS 4000000000 were employed during SparseAssembler step to process the illumina paired-end reads. K 57 was chosen according to k-mer estimation of KmerGenie. GS was set to about 4 folds of conventional avian genome size 1G for memory usage management. The preliminary contigs file was passed to DBG2OLC to make layout with Pacbio long SMRT reads. LD 1 k 49 AdaptiveTh 0.005 KmerCovTh 5 MinOverlap 30 RemoveChimera 1 were used to generate raw backbone. Finally, blasr and consensus modules were implemented to produce the genome assembly. The final assembled genome was polished by pilon-1.22 [54] to detect possible misassemblies. The final assembly was used for downstream analysis.

Demographic history inference using PSMC

To reconstruct the demographic history of Chinese Grouse, we used the Pairwise Sequentially Markovian Coalescent (PSMC 0.6.5) [31] model to infer the effective population sizes (N_e) of Chinese Grouse across genome sequences with SNP sites. We used the consensus autosomal sequence (in *fastq* format) for Chinese Grouse as the input for the PSMC modelling. To run the PSMC method, we used two parameters: the generation time (2.5 years) and the mutation rate per generation (0.3×10^{-8} per nucleotide per year) of Chinese Grouse. The mutation rate (per nucleotide per year, μ) for Chinese Grouse was selected from studies of willow grouse (0.299×10^{-8}) and rock ptarmigan (0.310×10^{-8}) [55]. PSMC was run for 100 iterations using an initial $h=q$ ratio of 5 and the default time patterning. Bootstrapping was performed as in Li and Durbin (2011) [31] by resampling 500000 bp chunks of the genome with replacement to perform 100 bootstrap replicates.

SDM model

We used the maximum-entropy approach (MaxEnt, ver. 3.3.3k) [56] to model Chinese Grouse habitat. A set of layers, or environmental variables, as well as a set of georeferenced occurrence data were used to produce a model of the ancient and present range of Chinese Grouse. We projected the present-day and three other periods Species Distribution Models (SDMs) based on the climate data during the current, mid-Holocene (6000 years BP), last glacial maximum (LGM, 21,000 years BP), and last interglacial (LIG, 120,000–140000 years BP). We obtained all the 19 bio-climatic variables from the WorldClim 1.4 database at a spatial resolution of 2.5 arc-minutes [57]. The current climatic data layers were based on

spatially interpolated values of temperature and precipitation gathered from weather stations around the world from 1960 to 1990, whereas the variables for both the period mid-Holocene and LGM were generated from the climate reconstructions based on CCSM4 [57], and the variables for LIG were downscaled from simulations with CCSM4 [58]. We used the same set of grouse location (observation) records as those used by Lu et al. [5, 6]. The location records were set in a spatial distance threshold of 0.083 decimal degrees (i.e. 5', about 8 km) which can reduced spatial autocorrelation [59]. The location record was included only if the spatial distances with all other location records was larger than the threshold. Using these filtering criteria, we had 41 location records, which we used to construct the SDMs. Further parameter setting refer to Lu *et al.* [6]. Furthermore, a 10% training presence logistic threshold was used to define the minimum probability of suitable habitat. A GAP [33] analysis between current distribution and coniferous forests were performed to assess conservation status. All data preparation and raster calculations were performed in ArcGIS 10.5.

List Of Abbreviations

QTP: Qinghai–Tibetan Plateau; SDM: Species Distribution Modelling; PSMC: Pairwise Sequentially Markovian Coalescent; MID: mid-Holocene; LGM: last glacial maximum; LIG: last interglacial

Declarations

Ethics approval and consent to participate

This study was approved by the Animal Ethics Committee of the Institute of Zoology, Chinese Academy of Sciences. The blood sample was collected from a wild Chinese Grouse at Lianhuashan National Nature Reserve which was released to the wild. The procedure of blood collection was in strict accordance with the Animal Ethics Procedures and Guidelines of the People's Republic of China.

Consent for publication

Not applicable

Availability of data and materials

Sequencing data for the Chinese Grouse have been deposited in Short Read Archive under project number PRJNA588719 and accession number SUB6534172. WorldClim – Global Climate Data is free climate data for ecological modeling and GIS at <http://worldclim.org/>. These data can be used for mapping and spatial modeling.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

KS, JH and YHS designed and managed the project. KS, BG and PH performed analyses. YF and YJ collected the samples. KS wrote the paper. JH, YHS, BG and PH revised the paper. All authors have read and approved the manuscript.

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Additional Files

Additional file 1: ROC Plot for classification accuracy of this model.

Additional file 2: Predicted distribution of Chinese Grouse in different periods: a, LIG; b, LGM; c, Mid-Holocene; d, Present day. The grey area represent the distribution in different time and the color bar represent altitude.

Figures

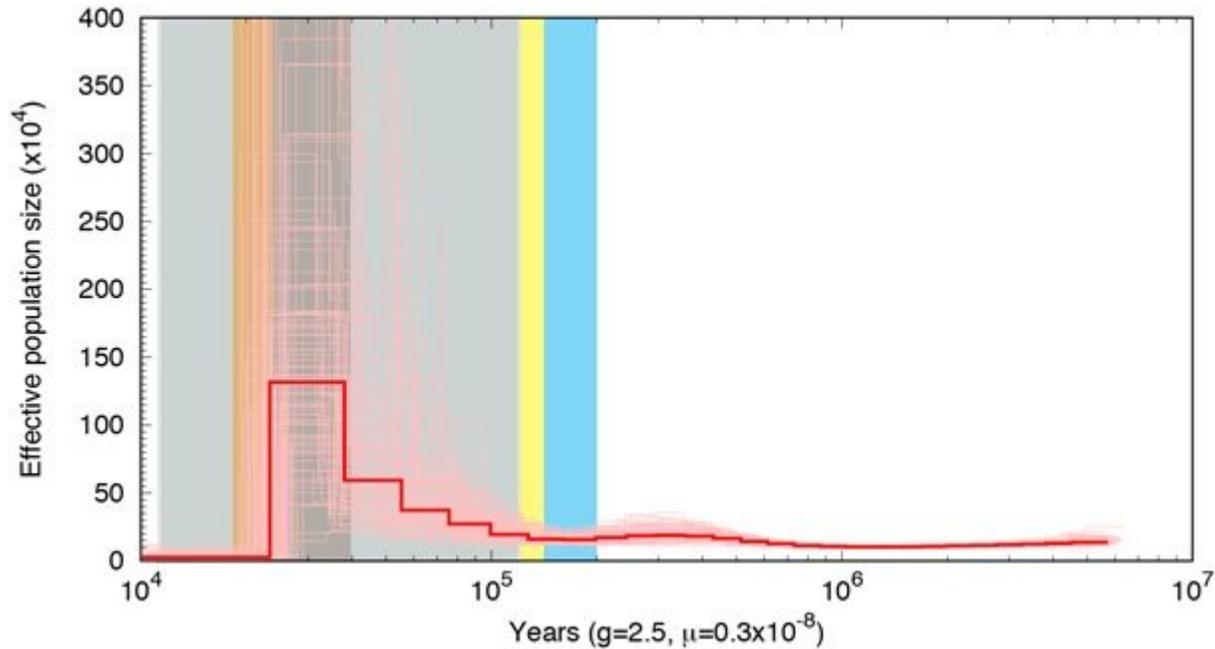


Figure 1

PSMC plot showing the demographic history of Chinese Grouse. The red line represent the estimated effective population size (N_e), and 100 thin curves represent the PSMC estimates for 100 sequences randomly resampled from the original sequence. Coloured bars correspond to the climatic periods. Blue: Penultimate Glacial Period, yellow: The last interglacial (Emain), light grey: last glacial period (LGP), dark grey: the last Greatest Lake Period (GLP), orange: LGM.

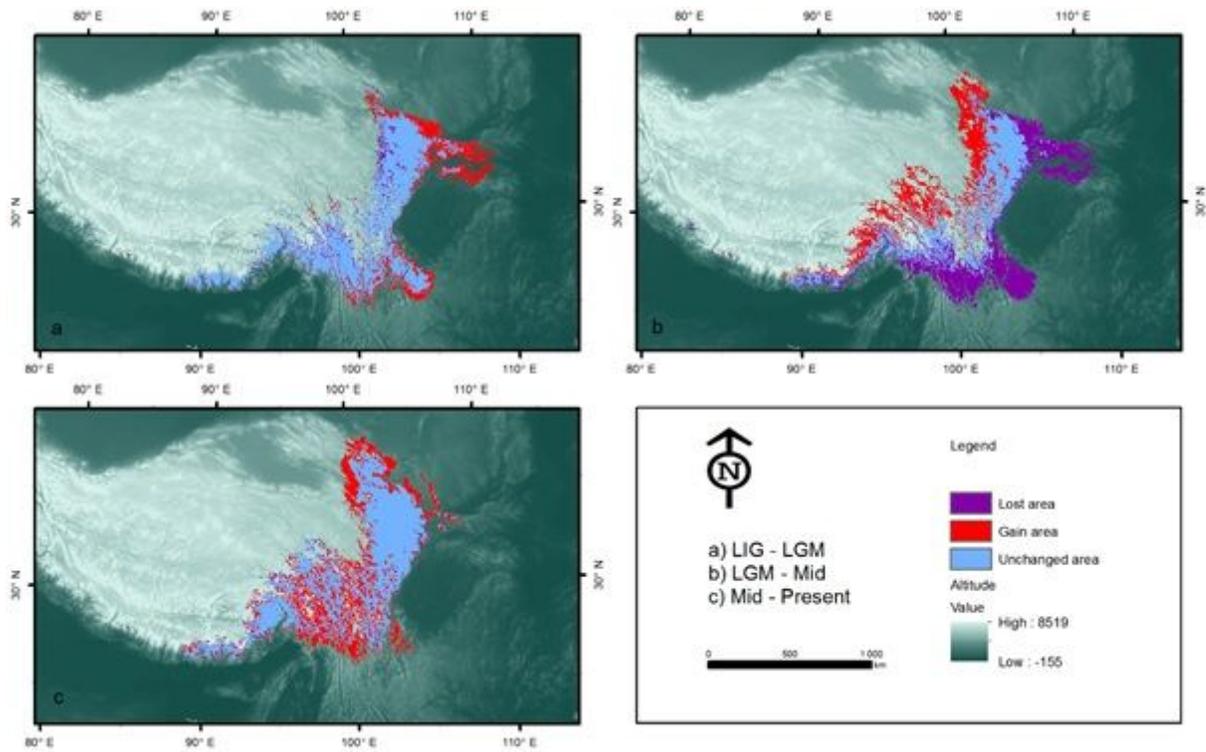


Figure 2

The change in predicted distributions of the Chinese Grouse: a) from LIG to LGM; b) from LGM to Mid-Holocene; c) predicted from Mid-Holocene to Present-day. Map data was from WorldClim-Global Climate Data, which is free data for ecological modeling and GIS.

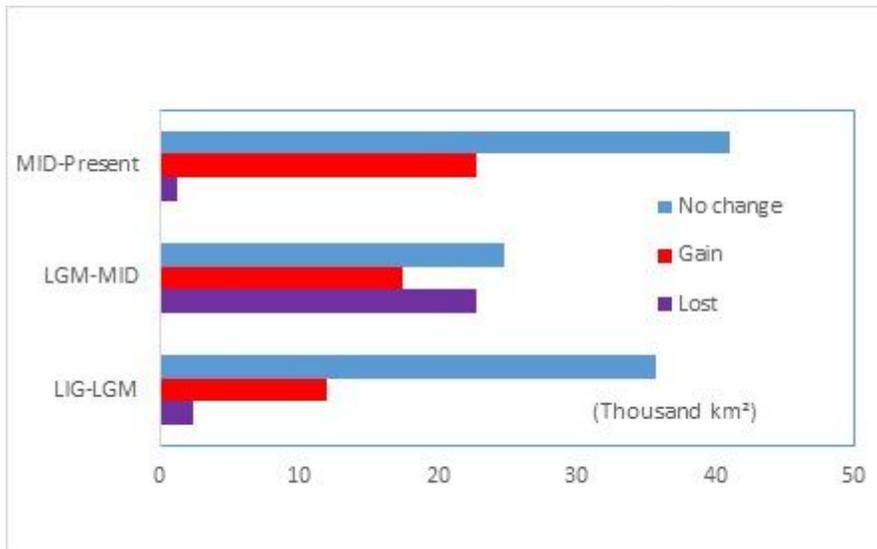


Figure 3

Predicted distribution changed from LIG to LGM, LGM to MID and MID to Present.

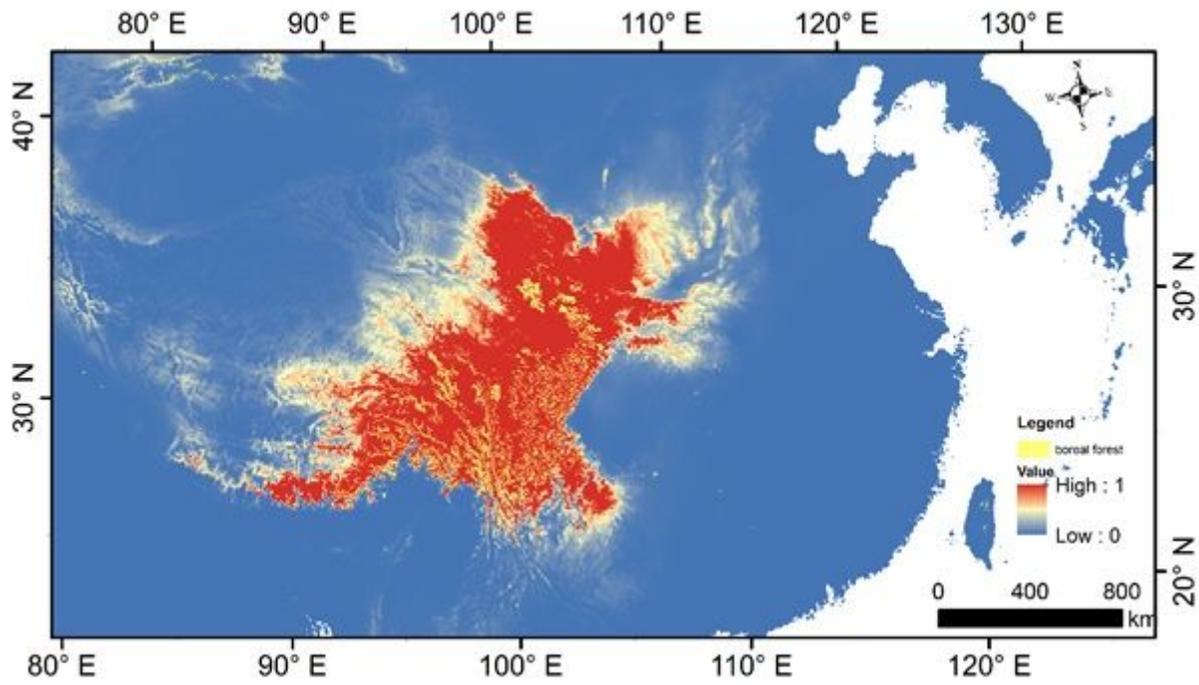


Figure 4

The GAP analysis of predicted distribution of the Chinese Grouse in the present-day (colour bar) and boreal forest cover (the yellow area). Colours represents the suitability, from low (blue) to high (red). Map data was from WorldClim-Global Climate Data, which is free data for ecological modeling and GIS. The map was created by ArcGIS 10.5, which URL link is <https://support.esri.com/zh-cn/products/desktop/arcgis-desktop/arcmap/10-5>.

Supplementary Files

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