

Article

Spatial Genetic Patterns and Distribution Dynamics of the Rare Oak *Quercus chungii*: Implications for Biodiversity Conservation in Southeast China

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Abstract: A rapidly changing climate and frequent human activity influences the distribution and community structure of forests. Increasing our knowledge about the genetic diversity and distribution patterns of trees is helpful for forest conservation and management. In this study, nSSRs (nuclear simple sequence repeats) were integrated with a species distribution model (SDM) to investigate the spatial genetic patterns and distribution dynamics of *Quercus chungii* F.P.Metcalf, a rare oak in the subtropics of southeast China. A total of 188 individuals from 11 populations distributed across the natural range of *Q. chungii* were genotyped using nine nSSRs. The STRUCTURE analysis indicated that genetic admixture was present in all populations, but the population genetic variation and genetic differentiation were related to their geographical distributions. The SDM result indicated that *Q. chungii* retreated to the Nanling Mountains and adjacent areas during the Last Glacial Maximum (LGM) period, which corresponds to higher genetic diversity for populations in this region. Landscape genetic analysis showed that the Nanling Mountains served as a corridor for organism dispersal at the glacial and interglacial periods within the Quaternary. Based on these results, we propose that establishing nature reserves to protect the ecological corridor across the Nanling Mountains is necessary for the conservation of regional species genetic diversity, as well as the ecosystem of evergreen broadleaved forests in southern China. The study combines species distribution models and genetic diversity to provide new insight into biodiversity conservation and forest management under future climate change.

Keywords: climate change; species dispersal corridor; forests dynamics; genetic diversity; glacial refugia; oaks

1. Introduction

Forests play crucial roles in both the regional ecology and economy, including maintaining species diversity, regulating climate, conserving water and soil, and providing timber and food [1,2]. A great deal of research is still required to better understand and untangle the biotic and abiotic factors that drive speciation, genetic differentiation, and the distribution dynamics of trees [3–6]. The demographic history and distribution dynamics of trees can be reconstructed based on a species genetic diversity, paleontology data, and a species distribution model, or the combination of these data [7,8]. Understanding the historical distribution and divergence dynamics of trees is informative not only for untangling the species evolutionary histories, but also for predicting the responses of

forests to rapid climate change in the future. Even more importantly, this understanding can help to make forest conservation more efficient and management strategies more effective.

The *Quercus* genus is considered by some to be the most important woody genus in the Northern Hemisphere, making oaks model species to understand how evolutionary processes integrate with ecological processes [9]. Recent studies indicate that *Quercus* is comprised of two subgenera, subg. *Cerris* and subg. *Quercus*, with eight sections [10,11]. In the *Quercus* section *Cyclobalanopsis* is a dominant oak group in Asian subtropical evergreen broad-leaf forests (EBLFs) [10,12]. Southwest China and Indo-China are the centers of diversity for the section, containing a total of about 66 species [13,14]. Within the *Cyclobalanopsis* section alone, there are a total of two main lineages, CTB (compound trichome bases) and STB (singlecelled trichome bases), and six clades [14].

Quercus chungii belongs to the CTB lineage in the *Cyclobalanopsis* section, and is an evergreen oak that can grow to heights of 15 m. It is distributed mainly in the montane lowland forests of south China at elevations ranging from 200–800 m [12]. The species is clustered with *Quercus Rex* Hemsl., *Quercus delavayi* Franch., *Quercus Kerrii* Craib, *Quercus austrocochinchinensis* Hickel & A. Camus and *Quercus hypophaea* Hayata to form the Helferiana clade [14]. Most of the closely related species are distributed in Southwest China except for *Q. hypophaea*, which is endemically distributed in Taiwan Island. Interspecific hybridization and introgression frequently occur among the sympatric oaks, but most of the interspecific gene flow only exists between closely related species [15–18]. Several species in the STB lineage were sympatrically distributed with *Q. chungii*, e.g., *Quercus glauca* Thunb., *Quercus stewardiana* A. Camus, and *Quercus Myrsinifolia* Blume, however, those species diverged from *Q. chungii* during the Oligocene [14], which suggests that hybridization or introgression among *Q. chungii* and other sympatric oaks could be rather rare.

As a long lived tree with a valuable wood, *Q. chungii* is a specifically targeted species in regional logging practices, meaning that its population and area of extent underwent a rapid decrease in recent years. Meanwhile, the rapid expansion of artificial forests in recent decades has resulted in the replacement of much of the natural habitat of *Q. chungii* by *Cunninghamia lanceolata* (Lamb.) Hook., *Pinus massoniana* Lamb., and *Eucalyptus* spp. In its main range of distribution, Fujian Province of southeast China has listed this species as a rare and endangered tree [19]. A national reserve, the Huangchulin Nature Reserve of Fujian Province, was established to protect the natural forests of *Q. chungii*. While there is a relatively large amount of studies that investigate the community structure and forest restoration dynamics of *Q. chungii* [20–22], there are only a few that focus on its genetic diversity and demographic history [23]. A study on the population genetics of *Q. chungii* in seven plots in Huangchulin Nature reserve using RAPD (Random Amplified Polymorphic DNA) showed that the population had higher genetic diversity, and that the genetic differentiation within the species was correlated with environmental differences [23]. However, the spatial genetic patterns of *Q. chungii* at a large geographical scale remain unknown.

In this study, we examined the spatial genetic patterns, potential distribution dynamics, and dispersal corridor of *Q. chungii* using nSSRs (nuclear simple sequence repeat) and a species distribution model. A total of 11 populations with 188 individuals covering the natural range of *Q. chungii* were genotyped by nine nSSRs. The least cost path (LCP) method was used to infer the potential dispersal corridor of species based on the friction layer of habitat heterogeneity [24]. This method is based on the premise that species distribution within a suitable habitat would facilitate population connectivity, or in other words, would result in higher habitat suitability values in the species distribution model (SDM) at a lower cost of dispersal through the landscape matrix [24,25]. The aims of our study were to: 1) Reveal the spatial genetic structure and potential glacial refugia of *Q. chungii*; and 2) infer the potential dispersal corridor of *Q. chungii*. Our study can provide important guidelines to help conserve *Q. chungii* germplasm and increase and manage forest biodiversity in Southern China.

2. Materials and Methods

2.1. Species Distribution Model

A maximum entropy model was created in Maxent v3.3.3 [26] to estimate the potential distributions of *Q. chungii* at the LGM (Last Glacial Maximum) and during the present period. A total of 18 occurrence points of *Q. chungii* were obtained from our field collection and from the CVH (Chinese Virtual Herbarium; <http://www.cvh.org.cn/>). Nineteen climatic variables relating to temperature and precipitation were downloaded from WorldClim (<http://www.worldclim.org/>) at a 2.5 arc-min resolution for the LGM (MIROC-ESM and CCSM v4 scenarios) and present periods. To avoid multicollinearity, climatic variables for the present period that were highly correlated with the others ($r > 0.9$) were excluded.

As the available regional localities of *Q. chungii* were fewer than 25, the jackknife procedure [27] was used to estimate the species potential distributions. This method removed one locality from the total (n) each time, and the remaining localities ($n - 1$) were used to estimate the species potential distributions. The mean of the model values run n times were used as the potential distribution of *Q. chungii*. The area under curve (AUC) was calculated using the receiver operating characteristic (ROC) and was used to evaluate the model performance. As SDMs commonly over-predict the species distributions, a buffered minimum-convex-polygon (MCP) was used to clip the species range [28]. The suitable habitat outside the MCP was excluded. In this study, the MCP was generated using SDMtoolbox v1.1c [25] with 3 degrees beyond observed localities.

The potential migration corridors of *Q. chungii* in the past and present periods were calculated based on least-cost path analysis method using SDMtoolbox v1.1c [25] in ArcGis 10.5 (ESRI, Redlands, CA, USA). The detailed steps for this analysis were summarized as follows: 1) The SDM (1-SDM) was inverted as the dispersal cost layer (resistance layer); 2) the resistance layer of *Q. chungii* was used to create a cost distance raster for each sample locality; 3) the corridor layers were established between two localities based on the cost distance raster. To better depict habitat heterogeneity and its varying role in dispersal, the categorical LCP approach was used to calculate the least-cost corridor. The range of cost path values were divided into high, mid and low classes and reclassified as new values of 1, 2, and 5, respectively; and finally, 4) all of the pairwise reclassified corridor layers were summed and standardized from 0–1 as the eventual dispersal corridor for *Q. chungii*.

2.2. Population Sampling and Genotyping

A total of 188 individuals of *Q. chungii* were collected from 11 populations in south China, covering the representative distribution range. Fresh and healthy leaf samples were dried and preserved in silica gel immediately after being collected. The latitude and longitude of each population were recorded using a global positioning system (GPS; Etrex 201X, Garmin, Lenexa, USA), and are presented in Table 1. Voucher specimens for each individual are stored at the Herbarium of the Shanghai Chenshan Botanical Garden (CSH).

Total genomic DNA was extracted from the leaves following the modified cetyltrimethyl ammonium bromide (CTAB) method [29]. After screening about 200 pairs of universal primer sets that were previously published [30], nine nuclear microsatellite loci with higher alleles comparing with rest primers were selected to further examine species genetic diversity and structure (Table S1). The PCR reaction protocols followed the procedures as described by [31]. The PCR products were genotyped by a professional sequencing company. GENEMARKER version 2.2.0 (Soft Genetics LLC, State Collage, PA, USA) was used to analyze the allele sizes of each microsatellite marker. The genotyping errors and null alleles were checked by MicroChecker [32].

Table 1. Sampling sites and genetic diversity of *Quercus chungii*.

Code	Location	Long.	Lat.	<i>n</i>	<i>He</i>	<i>Ar</i>	<i>Ca</i>	<i>Cb</i>
1	Fengkai, Guangdong	111.90	23.48	8	0.67	3.32	0.93	0.07
2	Libo, Guizhou	107.94	25.29	11	0.50	2.74	0.89	0.11
3	Jiaoling, Guangdong	116.13	24.70	18	0.50	2.80	0.58	0.42
4	Minqing, Fujian	118.70	26.31	21	0.55	2.87	0.58	0.42
5	Jianou, Fujian	118.83	27.22	24	0.46	2.63	0.15	0.85
6	Yongan, Fujian	117.34	25.97	31	0.55	2.93	0.39	0.61
7	Anxi, Fujian	117.67	25.30	22	0.49	2.88	0.28	0.72
8	Yongtai, Fujian	118.53	25.75	23	0.49	2.84	0.22	0.78
9	Renhua, Guangdong	113.75	25.03	6	0.56	3.08	0.34	0.66
10	Xinning, Hunan	110.74	26.28	13	0.59	3.10	0.67	0.33
11	Xinfeng, Jiangxi	115.21	25.21	11	0.59	3.06	0.71	0.29

Long., Longitude; Lat., Latitude; *n*, Number of sample; *He*, expected heterozygosity; *Ar*, allelic richness; *Ca*, probability of population membership to cluster A; *Cb*, probability of population membership to cluster B.

2.3. Genetic Diversity and Structure

The species genetic diversity (*Ar*, allelic richness; *He*, expected heterozygosity) and genetic differentiation (*Fst*, Fixation index) indices were calculated. Pairwise *Fst* and *He* values were calculated using ARLEQUIN v3.5 [33]. To avoid the calculated bias for populations with different sample sizes, the *Ar* value was calculated by rarifying the allele data to be consistent with 12 gene copies using HP-RARE [34]. The relationship between pairwise genetic distance (*Fst*) and geographic distances was tested using a Mantel test in IBDWS v. 3.23 [35].

The genetic structure of *Q. chungii* was inferred by Bayesian clustering and Principal Coordinate Analyses (PCoA). Bayesian clustering was performed in Structure v2.3.1 [36] using the admixture model to deduce the genetic relationships of the population. We tested 10 replicates per cluster (*K*), from 1 to 8. Each replicate completed 100,000 Markov chain Monte Carlo (MCMC) iterations after a burn-in of 50,000 MCMC. We evaluated the optimal number of clusters using the ΔK method [37] performed by STRUCTURE HARVESTER [38]. Ten runs of STRUCTURE with the optimum *K* value were aligned using CLUMPP [39]. A PCoA based on the genetic distance among populations was performed in GenAlEx 6.5 [40]. To compare the relationship between geographic distribution and spatial genetic patterns (first two components of the PCoA), we performed Procrustes analysis using the “vegan” package [41] in R [42]. The similarity between geographic distribution and spatial genetic patterns was quantified using the formula $t_0 = \sqrt{1-D}$, where *D* is the minimum sum of the squared Euclidean distances between the two data.

2.4. Landscape Patterns of *Q. chungii*

The Landscape Genetics GIS Toolbox [43] in ArcGIS 10.5 was used to generate geographical landscapes of species genetic diversity (*Ar* and *He*) and genetic divergence (*Fst*). The inverse distance weighted interpolation method was used to generate a surface from the mapped genetic divergence or genetic diversity values. This analysis provided a more intuitive view for the spatial patterns of genetic diversity and genetic differentiation in a geographically continuous landscape.

3. Results

3.1. Species Potential Distribution

A total of eleven less-correlated climatic variables ($r < 0.9$) were used to estimate the potential distribution of *Q. chungii* (Table S2). The high AUC scores (AUC = 0.999) in the model evaluation indicated that the SDM analysis performed well. The predicted present potential distribution of *Q. chungii* is widespread in the Wuyi and Nanling Mountains, which is congruent with the natural distribution of the species (Figure 1). The Miroc and CCSM climate models for the LGM period

inferred a similar potential distribution, with the most suitable distribution range of *Q. chungii* located in the Nanling Mountains and adjacent area (Figure 1). Habitat suitability values of the sample sites range from 0.51–0.82, 0.03–0.62 and 0.01–0.64 for the present, LGM (CCSM) and LGM (Miroc) periods, respectively. The environmental factors that explained the most variation of the distribution of *Q. chungii* were maximum temperature of the warmest month, mean diurnal range, and precipitation of the driest month explaining 23.39%, 19.65% and 16.31% of the variation, respectively (Table S2). The remaining eight environmental factors explained about 40% of the variation.

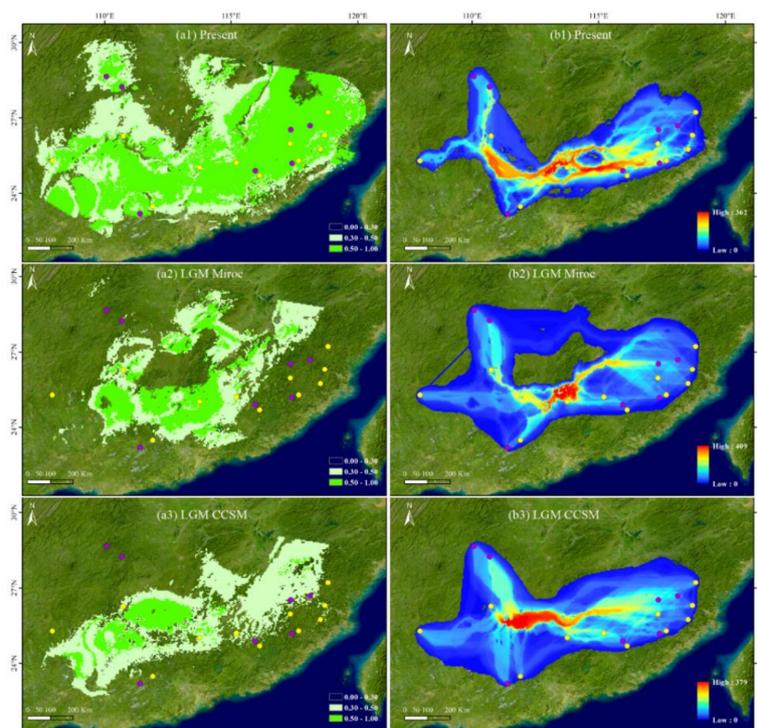


Figure 1. Potential distribution (left) and dispersal corridors (right) of *Quercus chungii* in the present and LGM periods. Left a1, a2 and a3 were the species potential distribution in the present, LGM period with MIROC-ESM scenarios and LGM period with CCSM v4 scenarios, respectively. Right b1, b2 and b3 were the potential dispersal corridor corresponding to a1, a2 and a3 respectively. Yellow and purple dots show the sampling sites in the fieldwork and occurrence data obtained from CVH (Chinese Virtual Herbarium; <http://www.cvh.org.cn/>), respectively. Colors in a1, a2 and a3 represent the values of species habitat suitability. Colors in b1, b2 and b3 from blue to red represent the possibility of species potential dispersal corridors from low to high.

The dispersal corridors of *Q. chungii* in the LGM and present periods were visualized using the SDM results. In the present period, the Wuyi and Nanling Mountains are the most important corridors for its dispersal (Figure 1). In the LGM, the dispersal corridors of *Q. chungii* using on the CCSM and Miroc climate models revealed a similar pattern that the Nanling Mountains formed a corridor connecting the east and the west and enabling its dispersal.

3.2. Genetic Diversity and Structure

A total of 56 alleles were detected from nine filtered nSSR markers. The number of alleles varied from 4 to 11 alleles per locus, with an average of approximately 6 (Table S1). The A_r and H_e values of populations ranged from 2.63–3.32 and 0.46–0.67, respectively (Table 1). The southernmost population (population 1) had the highest genetic diversity ($A_r = 3.32$, $H_e = 0.67$), while the easternmost population (population 5) showed the lowest genetic diversity ($A_r = 2.63$, $H_e = 0.46$; Table 1). The genetic differentiation among populations (F_{st}) ranged from 0.02–0.32 (Table S3), with the high difference

between populations 2 and 3. Overall, three populations in the west (populations 1, 2 and 10) had higher genetic differentiation than the other populations. The F_{st} was significantly correlated to the geographic distances ($r = 0.68$; Figure 2).

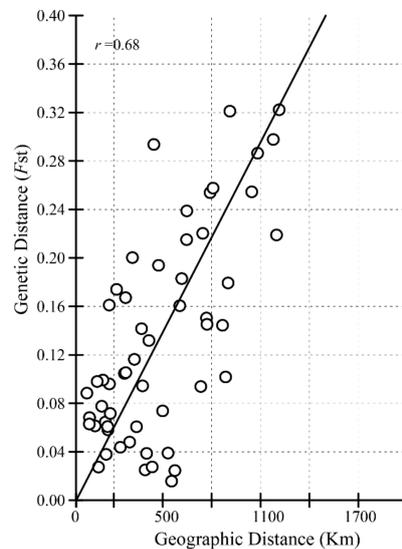


Figure 2. Relationship between geographic and genetic distances.

Bayesian clustering analysis using STRUCTURE showed that a higher ΔK was obtained when the populations were clustered into two or four groups ($K = 2$ or 4 ; Figures S1 and S2). When populations clustered in two groups, *Q. chungii* showed a common widespread genetic admixture (Figure 3a, Figure S2 and Table 1). PCoA for the *Q. chungii* populations revealed that the first two axes explained 67% of the total variability (PC1, 41.7% and PC2, 25.4%; Figure 3b). The geographical distribution of *Q. chungii* populations was highly consistent with the spatial genetic patterns ($t_0 = 0.78$).

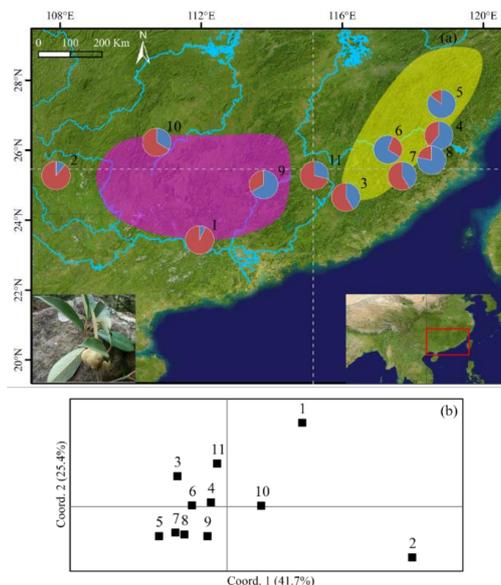


Figure 3. Spatial genetic patterns of *Quercus chungii*. (a) Pie charts represents the genetic structure of populations inferred from Bayesian cluster analysis. The red and blue colors represent the proportion of different genetic cluster respectively. The purple and yellow covered region represent the range of Nanling and Wuyi Mountains, respectively. (b) Plot of the first two coordinates of the principal coordinates analysis (PCoA) at population level based on the nSSR pairwise differentiation matrix for *Q. kerrii*.

3.3. Landscape Patterns

The genetic landscape interpolation showed that the population from the western area contained a relatively high genetic divergence, while divergence from the east was comparatively lower (Figure 4b). Nevertheless, the genetic diversity of populations from the Nanling Mountains was much higher than the eastern or the western regions of the species distribution range (Figure 4a and Figure S3).

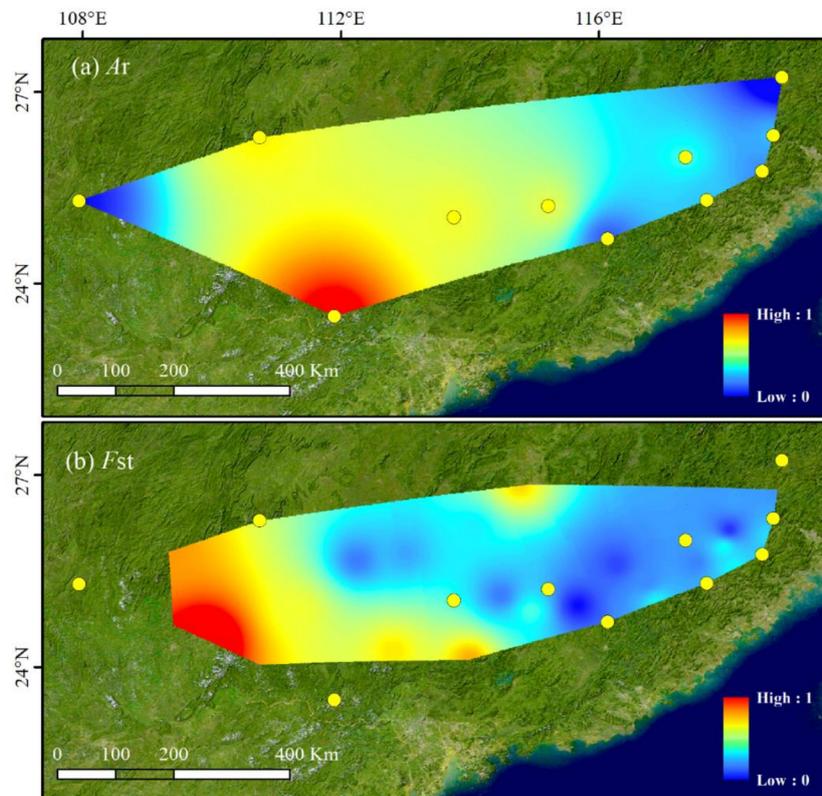


Figure 4. Spatial interpolation of genetic diversity (a) and genetic differentiation (b) of *Quercus chungii*.

4. Discussion

4.1. Potential Glacial Refugia

Glacial refugia, a source of postglacial recolonization for species, are geographic regions that maintained suitable habitats for a given organism's survival during a glacial period, especially in the LGM period [44]. The environment of refugia are relatively stable during glacial/interglacial cycles. Often, regions close to the equator can serve as species refugia [44], Mountain landscape with high environmental heterogeneity are also important climatic refugia, as species can shift the elevation of their distribution to targeting a suitable habitat during times of rapid climate change [45]. Over the long-term, steady distributions of organisms in refugia generally accumulate more mutations and maintains a higher genetic diversity [44]. However, the merger of two different lineages from different refugia in a contact zone can also boost the species diversity [46]. Therefore, combining the spatial genetic patterns and distribution dynamics of a species are a more reliable method of determining its glacial refugia and interglacial expansion route.

Spatial genetic patterns of *Q. chungii* showed that the species had a phylogeographic structure which suggested that multiple glacial refugia existed for *Q. chungii* in southern China. Populations of *Q. chungii* in the Nanling Mountains have had suitable and steady habitats since the LGM. Correspondingly, populations from the Nanling Mountains and the adjacent area (populations 1, 9 and 10) showed higher genetic diversity. Steady habitat since the LGM and a higher genetic diversity

suggested that the Nanling Mountains and the adjacent area were the glacial refugia of *Q. chungii* during the LGM period.

Although population 2 had lower genetic diversity and habitat suitability during the LGM period, it showed the highest genetic differentiation with respect to the rest of the populations, suggesting that this population contained unique germplasm and might have undergone long-term geographical isolation. The distribution region of population 2 might also therefore be classified as glacial refugium. The lower genetic diversity in population 2 may have resulted by selection from interactions over time with local conditions of environmental heterogeneity. In contrary, although the Wuyi Mountains are the main area where *Q. chungii* was distributed, population genetic diversity and genetic differentiation in this region were lower. Similarly, the habitat suitability of the species in these regions during the LGM period was also low. These results mean that the distribution of *Q. chungii* in the Wuyi Mountains was established by species dispersal from the Nanling Mountains and the adjacent areas when the climate was warming after the LGM.

The location of *Q. chungii* glacial refugia is consistent with others EBLFs in southern China. The Nanling Mountains and the adjacent area have previously been demonstrated to be an important glacial refugia for several EBLFs lineages, e.g., herbs (*Eomecon chionantha* Hance) [47], vines (*Tetrastigma hemsleyanum* Diels & Gilg) [48], conifers (*Pinus kwangtungensis* Chun ex Tsiang) [49], deciduous trees (*Eurycorymbus cavaleriei* (H.Lév.) Rehder & Hand.-Mazz.) [50], and evergreen trees (*Castanopsis eyrei* (Champ. ex Benth.) Hutch.) [51]. However, the location of plant refugia is also influenced by species niche and a given adaptive trait [52]. For example, although *Q. glauca* and *Q. chungii* are from the same section with slightly sympatric distribution in southeast China, their evolutionary histories differ, as populations of *Q. glauca* from the Nanling Mountains had higher genetic diversity but a lower habitat suitability during the LGM as determined by the species distribution model [53]. *Quercus chungii* and *Q. glauca* have very different physiological characteristics. The most likely evolutionary scenario of *Q. glauca* is that the Wuyi and Xuefeng Mountains were glacial refugia, and the present-day distribution of populations in the Nanling Mountains are the merger of the progenies from the Wuyi and Xuefeng Mountains via range expansion during the post-glacial period [53]. The most important climate factor that restricted the distribution of *Q. chungii* was growing season temperature (Table S2), and growing season precipitation for *Q. glauca* [53]. Moreover, seeds germinate instantly in *Q. chungii* once they are mature [54], but *Q. glauca* seed need physical dormancy of about one month to germinate [55]. The seed germination is the most vulnerable stage for the plant life cycle. These significant physiological differences could contribute to the different response to climate change found in *Q. chungii* and *Q. glauca*.

4.2. Dispersal Corridors of *Q. chungii*

The high environmental heterogeneity in montane environments is able to buffer climatic extremes, allowing them to serve as refugia for different organisms since the glacial period. Meanwhile, the rugged topography can also serve as a species dispersal corridor [56]. By integrating SDM and LCP approaches, we found that the Nanling Mountains was the dispersal corridors for *Q. chungii* in both the present and during the LGM. A recent phylogeographical study on *Eomecon chionantha*, an herbaceous plant in southern China indicated similar patterns, showing that the Nanling Mountains served dual roles: a dispersal corridor and a glacial refugium of the species during the Quaternary [47]. A dispersal corridor during the glacial and interglacial periods could have greatly impacted the biota exchange between the east and the west areas of the Nanling Mountains [57–59]. Compared to the Yungui Plateau of southwest China and the Wuyi Mountains and Central Range of southeast China, the mean elevation of the Nanling Mountains is the lowest. Therefore, the Nanling Mountains are very likely to serve as a geographical barrier for species with habitat at higher altitudes in the western and eastern mountains. However, species with disjunct distributions between the Yungui Plateau and the Wuyi Mountains/Central Range could establish a connection through the Nanling Mountains when species shifted to a lower altitude in response to climate cooling during glacial period [57].

Many discontinuously distributed species in southwest China and Taiwan Island may have been caused by this mechanism, such as *Rubus pinfaensis* H.Lév. & Vaniot, *Anemone hupehensis* (É.Lemoine) É.Lemoine, and *Pilea plataniiflora* C.H.Wright, genus *Whytockia* W.W.Sm., *Taiwania* Hayata and *Calathodes* Hook. f. & Thomson [58,59].

Furthermore, the dispersal from southwest China to Taiwan may undergone several waves. For example, Approximate Bayesian Computation (ABC) performed on *Q. championii* populations indicated that species dispersed from southwest to southeast China through the Nanling Mountains in the interglacial period, and then to Taiwan Island from southeast China in the glacial period [60]. To summarize, these results indicate that the Nanling Mountains constantly served as the dispersal corridor for subtropical organisms moving west to east in the Quaternary period at both interglacial and glacial period.

4.3. Implications for Conservation and Management

Quercus chungii can serve as an important native tree species for reforestation in Southern China, as suitable habitat for the species is widespread across the southern China subtropics. Populations from the Nanling Mountains and the adjacent area (the western area of the natural distribution of *Q. chungii*) contained higher genetic diversity with significant genetic differentiation. Seeds from the Nanling Mountains and adjacent areas could be very helpful in the optimization of collecting diverse regional germplasm and in facilitating the sustainable utilization of forest resources while considering both ecological and economic values. By managing the forest in this way, the genetic diversity of *Q. chungii* can be maintained or improved, which in turn would improve the ability of species to cope with future climate change.

The Nanling Mountains are a biodiversity hotspot and play an important role as a corridor that connects subtropical biota in southwest and the southeast China. As the economy and associated industry grew, the native EBLFs in southeast China suffered severe degradation and habitat loss during recent decades. Although several nature reserves have been established in the Nanling Mountains and adjacent areas, they remain isolated and highly fragmented, which presents an obstacle to gene flow and biota exchange between the western and eastern regions of southern China. To more effectively conserve and increase the existing biodiversity of south China, establishing nature reserves that function as an ecological corridor across the Nanling Mountains linking EBLFs from west to east is necessary [61]. A continuous corridor would be able to both optimize the local species diversity and also can boost the regional biota exchange.

5. Conclusions

Our results demonstrate that the western populations of *Q. chungii* contain high genetic diversity with significant differentiation and were likely the primary glacial refuges for EBLFs in southern China. The Nanling Mountains served as a dispersal corridor for organisms in the Quaternary, and effectively conserving its integrity is extremely important for maintaining biodiversity and ecosystem function in the region under the background of rapid global climate. To better understand the spatial genetic variations of forests in south China and their response to future climate change, it is necessary to compare a landscape genomics analysis for trees with different physiological characteristics in the future. By combining landscape genomics with genome-wide genetic variations, environmental data, and species biological traits, the precise response pattern of species to climate shift can be inferred, even if the species underwent frequent hybridization and introgression.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1999-4907/10/9/821/s1>. Figure S1: Distribution of delta K values in the STRUCTURE analysis. Figure S2. Bayesian cluster results of 188 *Quercus chungii* individuals for K as 2 (a) and 4 (b), respectively. Figure 3. Spatial interpolation of expected heterozygosity of *Quercus chungii*. Table S1: Primer information of nSSR using in this study. Table S2: Climatic variables and their percent contribution in constructing the potential distributions of *Quercus chungii*. Table S3: Pairwise *F*_{st}-values between the studied localities of *Quercus chungii*. *F*_{st}-values are below the diagonal, while above indicate *P*-values.

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