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Population Survey Combined with Genomic-Wide Genetic Variation Unravels the Endangered Status of *Quercus gilva*

Yi-Gang Song [1,*,†], Tian-Rui Wang [1,†], Zi-Jia Lu [1,2], Bin-Jie Ge [1], Xin Zhong [1], Xiao-Chen Li [1], Dong-Mei Jin [1], Quan Yuan [1,2], Yu Li [1,3], Yi-Xin Kang [1,4], Xin Ning [1], Si-Si Zheng [1], Li-Ta Yi [3], Xi-Ling Dai [2], Jian-Guo Cao [2], Jung-Hyun Lee [5] and Gregor Kozlowski [1,6,7]

1 Eastern China Conservation Centre for Wild Endangered Plant Resources, Shanghai Chenshan Botanical Garden, Shanghai 201602, China
2 College of Life Sciences, Shanghai Normal University, 100 Guilin Rd., Shanghai 200234, China
3 College of Forestry and Biotechnology, Zhejiang A&F University, Hangzhou 311300, China
4 School of Ecology and Nature Conservation, Beijing Forestry University, 35 Qinghua East Road, Beijing 100080, China
5 Department of Biology Education, Chonnam National University, Gwangju 61186, Republic of Korea
6 Department of Biology and Botanic Garden, University of Fribourg, Chemin du Musée 10, 1700 Fribourg, Switzerland
7 Natural History Museum Fribourg, Chemin du Musée 6, 1700 Fribourg, Switzerland
* Correspondence: ygsong@cemps.ac.cn
† These authors contributed equally to this work.

Abstract: Since the Anthropocene, biodiversity loss owing to human activity and climate change has worsened. *Quercus gilva* is an evergreen oak species native to China, Japan, and South Korea and is threatened by a long history of human impact. The purpose of this study was to (1) reassess the threatened category of *Q. gilva* based on a detailed survey, and (2) identify the genetic structure and diversity of *Q. gilva* based on genomic data. First, we conducted a detailed survey of the populations in China. Second, we collated all the literature and information. Finally, genome-wide genetic variation was analyzed based on 65 individuals from 22 populations. We found that *Q. gilva* has suffered rapid population decline, and at present, most populations are very small. The evolutionary path of *Q. gilva* was from the southwest to east of China and then to Japan and South Korea. *Quercus gilva* showed no distinct genetic structure and had a relatively low genetic diversity. Among the 22 populations, most populations in southwestern China, South Korea, and Japan had high genetic diversity. The populations in Jingning (Zhejiang province; ZJN), Wuyuan (Jinaxi province; JWY), and Zherong (Fujian province; FZR) suffered a strong bottleneck. In conclusion, *Q. gilva* is an endangered species native to East Asia. Because of the very low genetic diversity of *Q. gilva* and most populations are small, we need to (1) strengthen the protection of this species, (2) conduct conservation actions with *in-situ* reinforcement populations, and (3) select populations with high genetic diversity as provenances for afforestation efforts. Finally, we suggest that in the future, genetic diversity should be considered as the sixth criterion for IUCN to evaluate the threatened category.

Keywords: biodiversity loss; conservation genomics; endangered species; Fengshui/shrine/temple forests; genetic diversity; human impact

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1. Introduction

Trees form the principal components of forests and serve as immense support for terrestrial ecosystems and are of vital importance ecologically, economically, and culturally [1–3]. *Quercus* (oaks), predominantly in the Northern Hemisphere, is the largest genus of the family Fagaceae and one of the largest genera of all tree families [4]. Unquestionably, oaks are among the most successful, widely distributed, and valuable hardwood trees ecologically, economically, and culturally [5]. As keystone species in many ecosystems,

oaks play pivotal roles in shaping biodiversity, creating healthy ecosystems, and carbon sequestration [6,7]. During the Anthropocene, oaks have also been a valuable source of food, housing components, and materials [7].

Since the Anthropocene, biodiversity loss owing to human activity and climate change has worsened, and more attention should be paid to biodiversity conservation [8]. Through the global tree assessment, we know that currently, 30% of tree species are threatened with extinction [1]. Forty-one percent of oaks are of conservation concern, and 31% are estimated to be threatened with extinction [4]. Although the percentage of threatened species is already high, the assessment of many species of least concern (LC) is very rough (with only the area of occupancy (AOO) and extent of occurrence (EOO) calculated based on the occurrence data). A detailed population survey and genetic diversity estimation can help us to reassess the conservation status of these species of LC.

Genetic diversity is recognized as one of the three basic elements of biodiversity [9]. Current approaches to biodiversity conservation are largely based on geographic areas, ecosystems, ecological communities, and species, with less attention paid to genetic diversity and the evolutionary continuum from population to species [10,11]. Genetic diversity within all species, not just domesticated species and their wild relatives, must be conserved and monitored using appropriate metrics [11]. Thus, genetic diversity should be recognized as one of the main targets for biodiversity conservation under the international agreements on the "post-2020" framework [12].

Quercus gilva Blume is an ecologically important large tree of evergreen broad-leaved forests in China, Japan, and South Korea [13,14]. It is a precious tree species with hard and reddish-brown timber [15]. Because of the long history of large-scale regional development and excessive logging, many populations of *Q. gilva* have limited habitats and a small population size in their entire distribution range [14,16,17]. Most of the natural populations of *Q. gilva* are threatened with extinction, and local governments have classified this species as endangered or critically endangered [13–15,18]. *Quercus gilva* has recently been assessed as LC by the Botanical Gardens Conservation International (BGCI) and IUCN SSC Global Tree Specialist Group [19]. Thus, the reassessment of this species based on detailed population survey data is urgently needed.

Forest and landscape restoration are approaches that aim to regain ecological functionality and enhance human well-being in deforested or degraded landscapes [20]. Well planned and executed for reforestation with selected species and populations of selected provenances could maximize carbon sequestration, biodiversity, and livelihood benefits [21]. During the last 10 years, *Q. gilva* has been recognized as an important tree species and has been used for forest restoration in Zhejiang, Fujian, Jiangxi, and Hunan province of China. In the future, focus needs to be on the conservation of natural populations, germplasm evaluation, and utilization of excellent germplasm. Genetic diversity has been recognized as an important criterion to consider the prioritizing populations for protection [22] and as the basis for excellent germplasm selection [23]. The rapid expansion of genomic information will transform our understanding of the amount, distribution, and functional significance of genome-wide genetic variation in natural populations to guide conservation and reforestation [24,25].

The main aim of this study was to understand the endangered and conservation status of *Q. gilva* based on a detailed population survey and genetic diversity. The following specific aspects were explored: (1) the size, age composition, and main threats to the natural populations of *Q. gilva*, (2) the phylogeny and population structure of *Q. gilva* based on the genomic data, and (3) the patterns of genetic diversity at the genomic level.

2. Materials and Methods

2.1. Data Collection and Population Survey

Occurrence data with geographical coordinates of *Q. gilva* were compiled from the Chinese Virtual Herbarium [26], IUCN Red List of Threatened Species 2019 [19], and other publications related to *Q. gilva*. We then collected the population status (size, age

composition, and main threats, if possible) of *Q. gilva* in Japan and South Korea based on the publications. Additionally, between 2020 and 2022, an intensive field survey was conducted to explore the size, age composition, and main threats to each population in China. Finally, we surveyed 40 mainland Chinese populations. The populations were then divided into four categories based on the number of individuals in each population: large (>500 individuals), medium (100–500 individuals), small (30–100 individuals), and very small (<30 individuals) populations. The AOO and EOO were calculated using the GeoCAT online browser (http://geocat.kew.org/ (accessed on 23 November 2022)) [27]. We also collected the main threats, population trends within three generations, and habitats for each population of *Q. gilva*. Finally, we reassessed the status of *Q. gilva* across its distribution, following the "IUCN Red List Categories" [28].

2.2. Plant Material Samples, Resequencing, Control, and Mapping

A total of 65 individuals from 22 populations (three individuals for each population, except one population (only two individuals for the population of Jingning, Zhejiang province (ZJN)) were carefully selected to represent most of the natural populations of *Q. gilva* in East Asia (Figure 1 and Table S1). For each sample, genomic DNA was extracted from mature leaves using a cetyltrimethylammonium bromide (CTAB)-based protocol [29]. The concentration and quality of the total genomic DNA were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA libraries (350 bp) for Illumina sequencing were constructed for each accession according to the manufacturer's specifications. After DNA library construction, sequencing was performed on an Illumina NovaSeq 6000 platform by a commercial service (Biomarker Technologies, Beijing, China) with 150 bp paired-end reads. Raw reads were filtered based on the following criteria: paired-end reads with >10% 'N' bases, reads on which more than 50% of the bases had a quality score of less than 20 (Phred-like score), and sequencing adapter. Finally, high-quality clean reads were obtained for subsequent analysis.

2.3. SNP and Insertion/Deletion (InDels) Calling

All clean reads for each individual were mapped to the reference genome using the MEM algorithm of the Burrows–Wheeler Aligner (bwa-mem2 v2.2). The average mapping rate was 89.5%, and the average coverage rate was 10-fold for the reference genome. The mapping results were sorted, and duplicate reads were removed using SAMtools rmdup (version 1.9) [30]. SNPs and InDels were called using the HaplotypeCaller module in the Genome Analysis Toolkit (GATK) (version 3.8) [31] and were filtered with the following parameters: QD < 2.0 || MQ < 40.0 || FS > 60.0 || QUAL < 30.0 || MQrankSum < −12.5 || ReadPosRankSum < −8.0-clusterSize 2-clusterWindowSize 5. The SNPs identified above were subjected to a second round of filtering to improve the accuracy and efficiency of subsequent analyses. Only SNPs with a minor allele frequency greater than 5% and less than 20% of missing data were considered as high-quality SNPs. Transition (Ti), transversion (Tv), Ti/Tv, heterozygosity, homozygosity, and heterozygosity ratio were further identified using GATK. We used *Fagus sylvatica* [32] as an outgroup for phylogenetic analysis. Finally, 4,020,695 SNPs containing outgroup and 2,993,608 SNPs without outgroup was identified and used for subsequent downstream analysis.

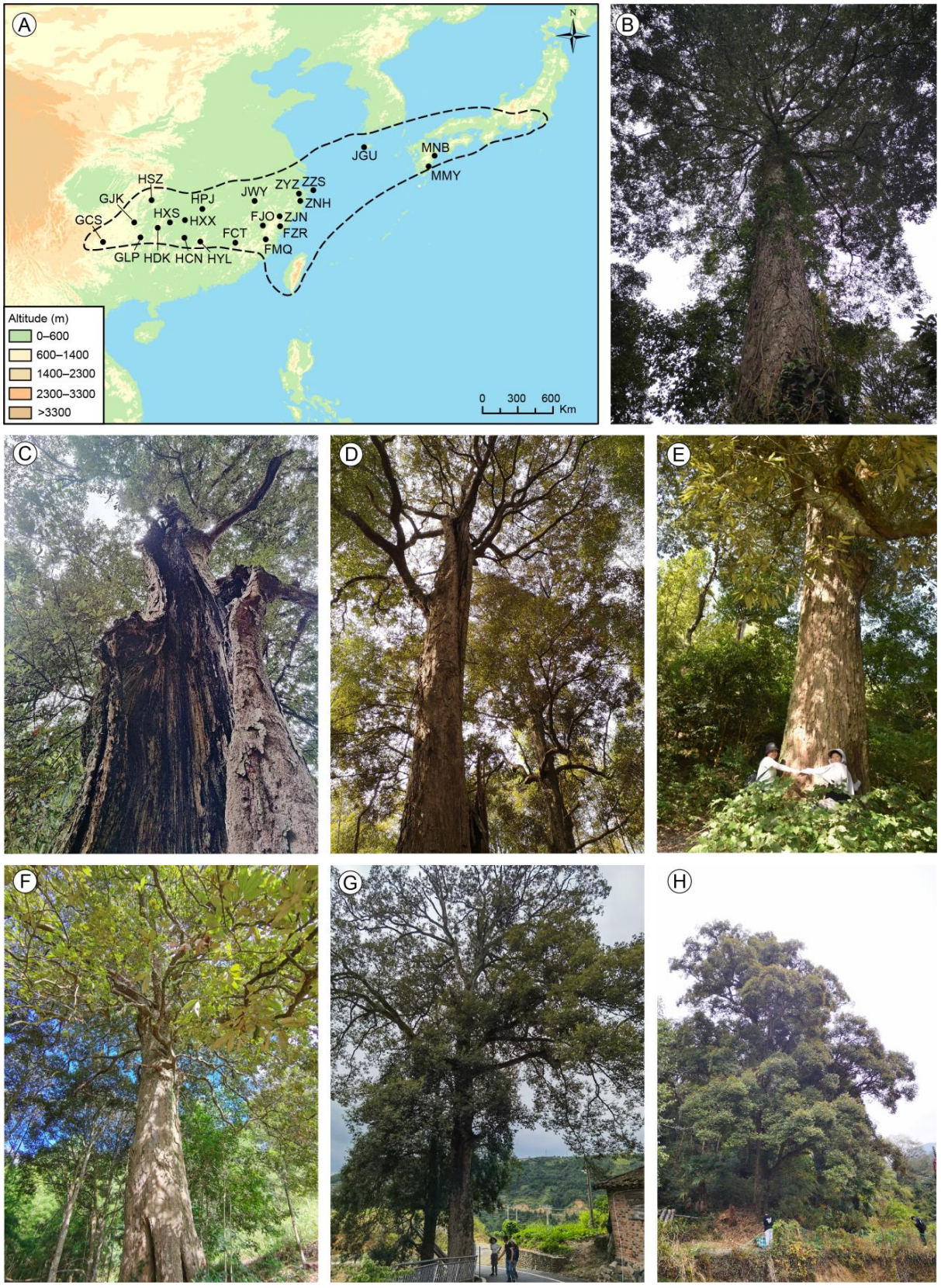

Figure 1. Geographic distribution (black dotted line) and the sampling locations (black dots) of *Quercus gilva* (**A**). The forests and selected old trees of *Q. gilva* (**B**–**H**). Population code abbreviations in Figure 1A are the same as in Table S1.

2.4. Phylogenetic Inference and Population Genomic Analysis

A neighbor-joining (NJ) phylogenetic tree was constructed using MEGAX [33] under the p-distances model with the 4,020,695 SNPs. We also used IQ-TREE [34] with self-estimated best substitution models to generate a maximum likelihood (ML) phylogenetic tree. The two phylogenetic trees were run with 1,000 bootstrap repetitions, using *Fagus sylvatica* as the outgroup.

To visualize the genetic relationships among the samples, principal component analysis (PCA) was performed using the smartpca program in EIGENSOFT version 6.0 based on 2,993,608 SNPs [35]. The initial three eigenvectors were plotted in three dimensions. ADMIXTURE version 1.22 [36] was used to infer historical ancestor clusters showing clusters of similar genotypes. The membership of each genotype was run for a range of genetic clusters from a value of K = 1 to 10 by using the admixture model.

2.5. Population Genetic Diversity and Linkage Disequilibrium (LD) Analyses

The observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphism information content (*PIC*), Nei diversity index (*H*), and Shannon–Wiener index (*I*) were calculated using the "PopGenome" package in the R project [37,38]. Nucleotide diversity (π) was calculated within a non-overlapping 100-kb window using VCFtools (version 0.1.13) [39]. The LD was calculated using PLINK version 1.9, within a 1000 kb window, and a maximum of 999,999 SNPs for each window [40]. The squared correlation coefficient (r^2) of each chromosome was calculated using SNP pairs only from the corresponding chromosome. Pairwise r^2 values within and between different chromosomes were averaged across the entire genome. We compared the LD patterns among different populations using the LD decay distance, indicated by the r^2 decreased to half of the maximum.

3. Results

3.1. Reassessment of Q. gilva

After collating all the distribution data of *Q. gilva* from different resources, there were a total of 108 known populations in East Asia (68 populations in China, 35 in Japan, and 5 in South Korea). According to the information gathered from indigenous people, almost all old trees (except individuals located in Fengshui forests and temples) have been deforested during the last 100 years. Based on this information, *Q. gilva* can be listed as endangered as per the EN-A4ad criteria.

Although the EOO of *Q. gilva* was very high, the area in South Korea was very small. The AOO of *Q. gilva* was less than 500 km^2 with the largest being in China (272 km^2) and the smallest in South Korea (20 km^2). More than half of the known populations have been surveyed in China. Only one large population had more than 500 individuals, and most of the surveyed populations were very small, with fewer than 30 individuals. There were even some occurrences with only one individual (Tables 1 and S2). Based on the population status, we estimated that more than 40% of the AOO after three generations (future-AOO) would be lost. Considering the populations without information, we inferred that more than 50% of the AOO would be lost in the next three generations. Finally, we estimated that there were fewer than 10,000 individuals within the distribution area of *Q. gilva*. During the last 100 years, the main threat to *Q. gilva* in natural populations has been logging and wood harvesting, as it is used as a biological resource. To support the rapid development of society, expansion of land under agriculture, residential use, and transportation infrastructure has also led to the destruction of natural *Q. gilva* populations. According to our survey, most of the current populations were conserved in the Fengshui forests near the villages, and forests surrounding shrines and temples with severe fragmentation. According to the IUCN Red List categories and criteria, the conservation status of *Q. gilva* is also determined to be endangered as per the EN-A4c criteria.

Table 1. Summary of the current status of *Quercus gilva*.

Country	China	Japan	South Korea	Total/Summary
Number of populations	68	35	5	108
AOO (km^2)	272	140	20	432
Future-AOO (km^2)	148	92	8	248
EOO (km^2)	873,462	161,420	84	1,921,293
NLP	1	0	0	1
NMP	2	0	0	2
NSP	12	0	1	13
NVSP	31	12	4	47
NISS	23	23	0	46
Total individuals	<5000	<2000	<600	<10,000
Main threats	Logging and wood harvesting; Agriculture and development	Logging; Agriculture and development	Human-mediated disturbance	Agriculture and Biological resource use
PTTG	Decrease noticeable	Decrease noticeable	No information	Decrease noticeable
Main area conserved	Fengshui forests and temples	Forests surrounding shrines and temples	Gotjawal (conserved area)	Protected Trees

AOO, area of occupancy; Future-AOO, AOO after three generations; NLP, Number of large populations: >500 individuals; NMP, Number of medium populations: 100–500 individuals; NSP, Number of small populations: 30–100 individuals; NVSP, Number of very small populations: <30 individuals; PTTG, Population trends within Three Generations; NISS, No information about population size and structure.

3.2. Detection of Genome-Wide Variant

We re-sequenced 65 individuals (22 populations) of *Q. gilva* collected from its main distribution area in East Asia: 19 populations from China, one population from South Korea, and two populations from Japan. A total of 706 Gb of high-quality clean reads were obtained. Among the 65 individuals, seven of them had 20 Gb clean reads and for all other individuals, clean reads were between 8.9 Gb and 11.2 Gb. We obtained an average of 36,206,470 reads, with an average Q20 value of 95.72%, Q30 of 89.46%, and average GC content of 37.01%. The average sequencing depth was 10.23. The 1× coverage of all individuals was higher than 80% with an average of 84.72%, except for one individual with a coverage of 56.75%. These high-quality sequences were aligned to the chromosome-level high-precision genome with the average mapping rate of 91.25%; alignment and proper mapping reached 83.17% (Table S1).

Among the 65 individuals of *Q. gilva*, 15,377,234 SNPs and 4,405,966 InDels were identified. The number of SNPs for each population was between 2,172,504 and 4,293,739, while for each individual, the number of SNPs for each individual was between 1,477,213 and 2,560,913 (Tables 2 and S2). Transitions and transversions accounted for 71.87% and 28.12% of the total number of SNPs, respectively, with an average transition/transversion (Ti/Tv) ratio of 2.56. The number of heterozygosities in different samples varied from a lowest of 712,570 to a highest of 1,513,312, with an average of 1,097,305 (Tables 2 and S2). The number of homozygosities in different samples varied between 761,543 and 2,136,876, with an average of 906,786 (Tables 2 and S1).

Table 2. Summary of genetic variation in *Quercus gilva* populations.

Population Code (Location)	SNPs	Indels	Transition	Transversion	Ti/Tv	Heterozygosity	Homozygosity	Het-Ratio
GLP (Liping, Guizhou)	3,507,918	1,115,687	1,403,828	547,870	2.56	1,106,329	845,370	0.5666
GJK (Jiangkou, Guizhou)	3,470,289	1,150,966	1,510,648	589,730	2.56	1,194,235	906,143	0.5636
GCS (Changshun, Guizhou)	3,448,941	1,055,047	1,507,376	579,442	2.597	1,132,727	954,091	0.5425
HXX (Xiangxiang, Hunan)	4,293,739	1,378,636	1,596,453	626,624	2.54	1,343,567	879,510	0.6023
HXS (Xinshao, Hunan)	3,561,533	1,162,826	1,514,130	589,450	2.56	1,247,186	856,394	0.5928
HDK (Dongkou, Hunan)	3,932,356	1,236,055	1,451,795	562,065	2.58	1,159,165	854,695	0.5755
HCN (Changning, Hunan)	3,819,985	1,222,766	1,477,122	574,678	2.57	1,179,174	872,625	0.5746
HSZ (Sangzhi, Hunan)	3,083,571	983,796	1,351,905	522,997	2.58	1,027,765	847,137	0.5453
HPJ (Pingjiang, Hunan)	3,149,545	1,019,904	1,324,570	516,136	2.56	931,328	909,378	0.5036
HYL (Yanling, Hunan)	2,445,579	850,489	1,370,498	535,637	2.56	1,046,839	859,296	0.5485
JWY (Wuyuan, Jiangxi)	3,129,945	1,056,778	1,451,178	566,446	2.56	1,043,045	974,579	0.5157
FCT (Changting, Fujian)	3,581,645	1,144,872	1,448,578	563,974	2.56	1,123,022	889,529	0.5525
FZR (Zherong, Fujian)	5,183,429	1,750,582	1,501,902	719,470	2.21	908,988	1,312,384	0.4290
FMQ (Minqing, Fujian)	3,486,120	1,157,688	1,578,398	614,521	2.56	1,296,886	896,033	0.5890
FJO (Jian'ou, Fujian)	3,361,907	1,112,365	1,471,386	573,316	2.56	1,128,122	916,580	0.5518
ZYZ (Yinzhou, Zhejiang)	2,428,260	858,412	1,382,278	544,668	2.54	986,717	940,225	0.5115
ZZS (Zhoushan, Zhejiang)	3,229,541	1,053,082	1,416,464	550,877	2.57	1,088,961	878,380	0.5522
ZNH (Ninghai, Zhejiang)	3,732,811	1,179,976	1,421,104	540,260	2.63	1,075,951	885,413	0.5442
ZJN (Jingning, Zhejiang)	2,172,504	699,612	1,112,899	413,417	2.69	714,120	812,196	0.4684
JGU (Gueok-ri, Jeju)	3,876,609	1,232,391	1,518,845	590,571	2.57	1,218,079	891,338	0.5774
MMY (Miyakonojo-shi, Miyazaki)	3,852,953	1,223,121	1,477,301	572,304	2.58	1,146,487	903,117	0.5564
MNB (Nobeoka-shi, Miyazaki)	3,551,342	1,109,444	1,400,624	533,912	2.62	1,069,663	864,873	0.5527
Total/Average	15,377,234	4,405,966	1,440,422	533,912	2.56	1,097,305	906,786	0.5462

3.3. Phylogenetic and Population Structure Analyses of Q. gilva

The NJ and ML phylogenetic trees were constructed using 4,020,695 SNPs in the single-copy genes. The NJ and ML trees consistently showed that individuals from the Zherong, Fujian (FZR), Dongkou, Hunan (HDK), and Xiangxiang, Hunan (HXX) populations did not cluster into one lineage. According to the NJ and ML trees, all the *Q. gilva* individuals could be divided into three major groups: West, Central, and East groups. Generally, the populations from Guizhou and western Hunan provinces comprised the western group. The populations from Eastern Hunan, Jiangxi, Fujian, and most of Zhejiang provinces formed the central group. Populations from South Korea, Japan, and ZZS (Zhoushan, Zhejiang) formed the main part of eastern group (Figure 2). There were three main differences in the phylogenetic structures between the NJ and ML trees of *Q. gilva* populations (Figures 2 and S1). First, the GLP population (Liping, Guizhou) was nested into the central group on the ML tree, whereas the western group was nested in the NJ tree. Second, the FMQ population (Minqing, Fujian) was nested into the central group on the ML tree, whereas the eastern group was nested in the NJ tree. Finally, compared to the NJ tree, the ML tree had four clear clades for the Central and East groups (Figures 2 and S1).

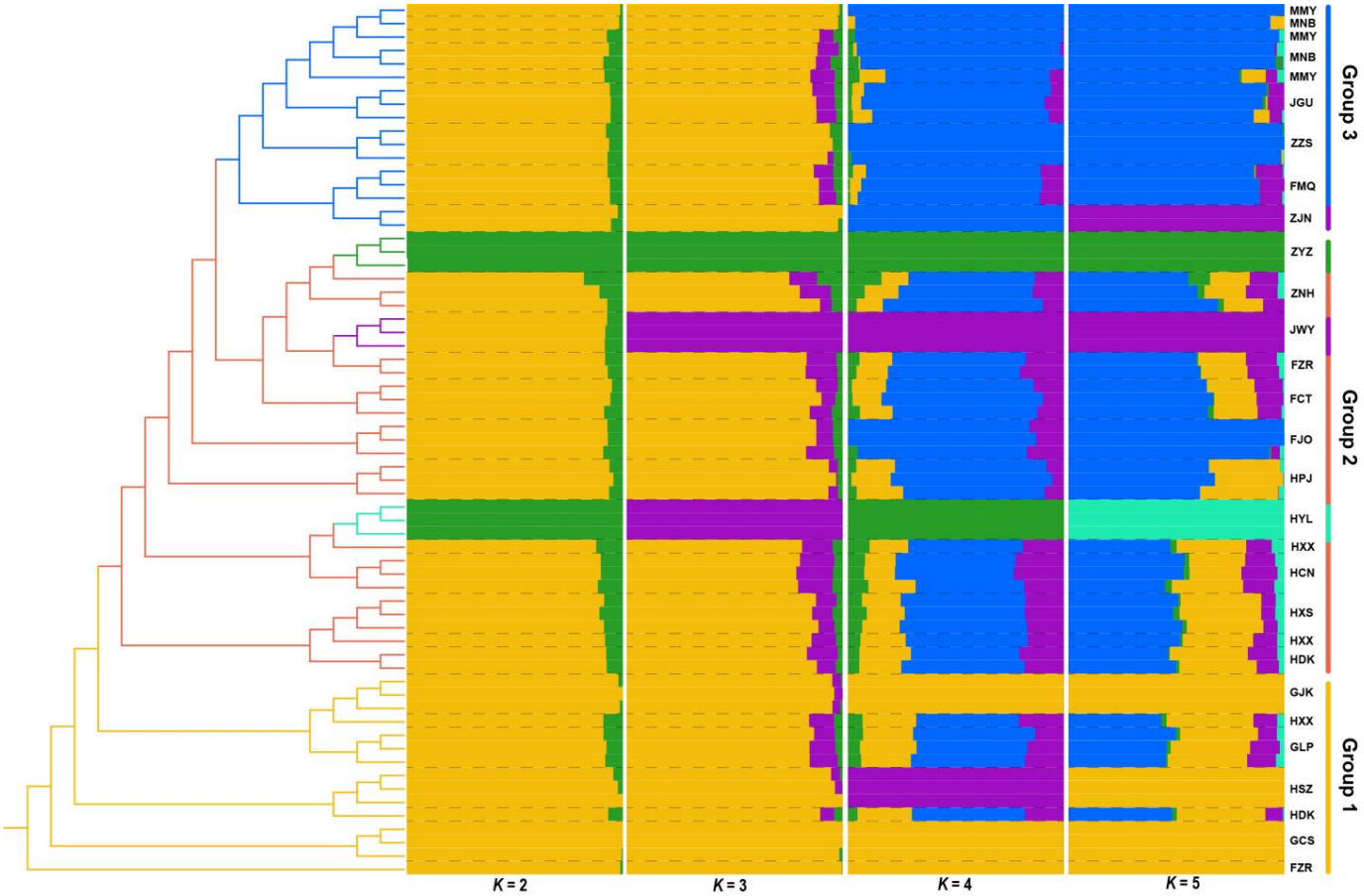

Figure 2. Neighbor-joining phylogenetic tree and population structure of *Quercus gilva*. *Fagus sylvatica* was used as the outgroup for the phylogenetic analysis. The figure does not show the outgroup. Population codes abbreviations are the same as in Table 2.

The results of the cross-validation (CV) provided by admixture analysis showed that the CV error rate had a minimum value when K = 1. The CV error rate was relatively low value when K = 2–5 (Figure S2). When K = 2, the populations of ZYZ and HYL formed one group, and the remaining populations formed the second group. When K = 3, the two populations in Jiangxi province formed one group, the ZYZ population was identified as the second group, and the remaining populations were classified into the third group.

When K = 4, the two most western populations (GCS and GJK) formed the first group, the HSZ and JWY populations formed the second group, the ZYZ and HYL populations formed the third group, and the remaining populations were classified into the fourth group. When K = 5, the minor change observed as that the HSZ population merged into the western group and the HYL population separated again (Figure 2). Based on the PCA results, we found that the ZYZ, HYL, and JWY populations were the most distinct. The remaining populations were clustered together (Figure S3).

3.4. Genome-Wide Patterns of Nucleotide Diversity and LD Analyses

Among the 22 populations, the values for observed heterozygosity (H_O) and expected heterozygosity (H_E) ranged between 0.1506 and 0.2441 and between 0.1156 and 0.2199, respectively. The polymorphism information content (*PIC*) values were between 0.089 and 0.1765, indicating that all the *Q. gilva* populations had a low level of polymorphism. Moreover, the Nei diversity index (*H*: ranged between 0.1399 and 0.265), Shannon–Wiener index (*I*: between 0.1646 and 0.328), and nucleotide diversity ($\pi \times 10^{-3}$: between 0.522 and 0.973) were calculated to evaluate the genetic diversity of different populations. The nucleotide diversity of *Q. gilva* was found to be 0.994. The ZYZ, FZR, ZJN, and HYL populations showed substantially lower diversity than the HXX, JGU, HDK, HXS, and MMY populations (Table 3).

Table 3. Genetic diversity of *Quercus gilva* populations.

Population	H_O	H_E	*PIC*	*H*	*I*	$\pi \times 10^{-3}$
GLP	0.2083	0.1876	0.15	0.2269	0.2787	0.863
GJK	0.2197	0.1807	0.1437	0.2182	0.2666	0.834
GCS	0.1867	0.165	0.131	0.1999	0.2431	0.735
HXX	0.2401	0.2199	0.1765	0.265	0.328	0.887
HXS	0.2322	0.1904	0.1516	0.2297	0.2815	0.964
HDK	0.216	0.2096	0.168	0.2534	0.3122	0.965
HCN	0.2192	0.2048	0.1639	0.2472	0.3045	0.727
HSZ	0.1965	0.1641	0.1301	0.1994	0.2413	0.727
HPJ	0.1793	0.1681	0.1337	0.2038	0.248	0.757
HYL	0.2077	0.1256	0.0968	0.1521	0.179	0.568
JWY	0.1975	0.1651	0.1307	0.1992	0.2425	0.762
FCT	0.2132	0.1945	0.1553	0.2354	0.2884	0.892
FZR	0.1913	0.1761	0.1389	0.2248	0.257	0.546
FMQ	0.2441	0.1897	0.1506	0.2285	0.2795	0.884
FJO	0.2126	0.1764	0.1409	0.2128	0.2618	0.815
ZYZ	0.1936	0.1156	0.089	0.1399	0.1646	0.522
ZZS	0.2113	0.1773	0.1408	0.2144	0.2612	0.812
ZNH	0.205	0.2021	0.1616	0.2449	0.3003	0.905
ZJN	0.1506	0.1207	0.0948	0.1609	0.175	0.549
JGU	0.2294	0.21	0.168	0.2532	0.3122	0.973
MMY	0.2161	0.2068	0.1655	0.25	0.3075	0.956
MNB	0.2079	0.1973	0.1576	0.2386	0.2928	0.891
Total						0.994

H_O, observed heterozygosity; H_E, expected heterozygosity; *PIC*, polymorphism information content; *H*, Nei diversity index; *I*, Shannon-Wiener index; π, nucleotide diversity. Population code abbreviations are the same as in Table 2.

Half of the maximum squared correlation coefficients (r^2) between pairwise SNPs ranged from 0.319 to 0.463. Linkage disequilibrium decayed to half among different populations in the range of 0.26 to 685.23 kb. The LD decay measured by physical distance, at which the pairwise correlation dropped to half of its maximum value, occurred at 685.23 kb in the GJK population (r^2 = 0.368) and 0.27 kb in the HYL population (r^2 = 0.451). There are three populations (ZJN, FZR, and JWY) that did not reach the half of the maximum r^2 (Figure 3 and Table S3).

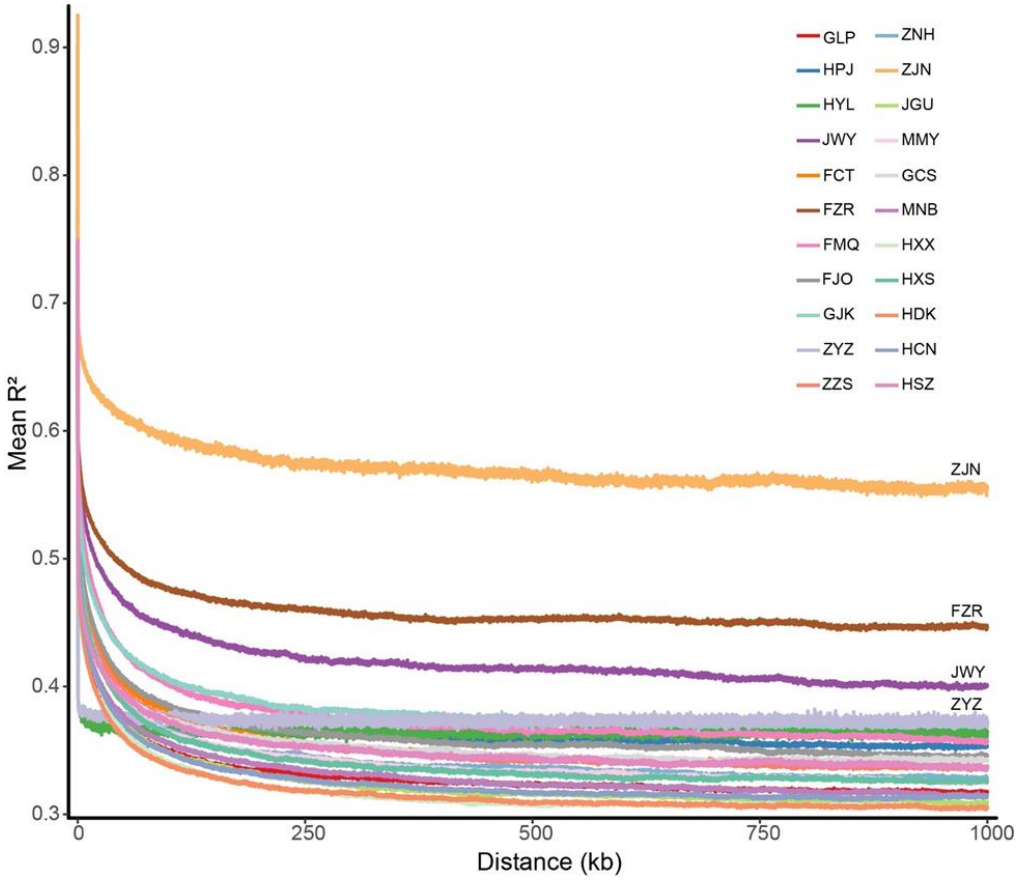

Figure 3. Linkage disequilibrium decay measured by r^2 in *Quercus gilva* species and each of the 22 populations. Population code abbreviations are the same as in Table 2.

4. Discussion

Our assessment showed that *Q. gilva* is an endangered (EN) species as per the EN-A4acd criteria. According to our extensive field survey and more than 30 literature sources on *Q. gilva*, we found that this species has suffered massive population decline and will be facing accelerated declines in the future. During the last 100 years, many natural populations have been logged for industrial timber, agriculture, and economic development. Currently, natural communities dominated by *Q. gilva* are rare, and most of the existing *Q. gilva* are scattered in other forest communities with ancient trees. More than 80% of *Q. gilva* populations occurred in the Fengshui forests or forests surrounding shrines and temples. Most of these populations were very small, or even just individual ancient trees. These populations have no natural regeneration of young adults and seedlings and thus seem to have no future. Therefore, legislation is required to protect this endangered species, and actively assist in the restoration of small populations. To date, *Q. gilva* has been listed as vulnerable (VU) in the Korea Red Data Book [41], endangered (EN) or critically endangered (CR) in several districts of Japan [14] and has also been described as a rare and endangered tree species in China [15]. The assessment results show a large disparity between the local government and the IUCN. Based on our global study, we suggested that the IUCN elevates the threatened category of *Q. gilva* from LC to EN.

In this study, we analyzed the genome sequences of 65 individuals representing the entire distributional range of *Q. gilva*. More than 15 million SNPs were identified, from which we determined the phylogeny, population structure, and genetic diversity of *Q. gilva*. Although the NJ and ML analyses showed considerable differences, both phylogenetic trees showed that *Q. gilva* has a strong evolutionary path from southwestern China to Central China, then to East China, and finally from the east coast of China to Japan and/or South Korea (Figures 2 and S1). The same pattern has been detected in many taxa native

to the Sino-Japanese Forest sub-kingdom, such as *Cercidiphyllum japonicum* [42], *Quercus glauca* [43], and Asian butternuts (*Juglans* section *Cardiocaryon*) [44]. The characterized genetic relationships among all individuals based on structure and PCA showed that the populations of Yinzhou, Zhejiang (ZYZ), Yanling, Hunan (HYL), and Wuyuan, Jiangxi (JWY) had the most distinctive genetic composition.

Quercus gilva exhibited a substantially lower genetic diversity (0.994×10^{-3}) than *Q. acutissima* ($\pi = 8.7 \times 10^{-3}$), *Q. variabilis* ($\pi = 9.0 \times 10^{-3}$), and *Q. chenii* ($\pi = 7.2 \times 10^{-3}$) at the genome-wide level, which are species that belong to *Quercus* in East Asia [45]. Compared with tree species from other genera in East Asia, *Q. gilva* exhibited genetic diversity of a level similar to that of *C. japonicum* (mean $\pi = 1.00 \times 10^{-3}$) [42], and two or three times lower than the living fossil *Ginkgo biloba* ($\pi = 2.11 \times 10^{-3}$) [46] and an endangered maple *Acer yangbiense* ($\pi = 3.13 \times 10^{-3}$) [47].

Among the 22 populations, the very low level of genetic diversity in the populations of Yinzhou, Zhejiang (ZYZ), Yanling, Hunan (HYL), Zherong, Fujian (FZR), and Jingning, Zhejiang (ZJN) indicates a possibility of different demographical dynamics. The LD decay was very slow for the FZR and ZJN populations, which did not decay to half of their maximum value at the end of the distance. In contrast, the HYL and ZYZ populations exhibited the fastest decay rates. The highest r^2 of the HYL and ZYZ populations ($r^2 = 0.9$) suggested that these two populations are artificial cultivation populations, and the seeds maybe from one individual. According to the genetic diversity and LD, a strong bottleneck was detected in the small populations of FZR, ZJN, and JWY. Overall, the populations with relatively high genetic diversity and large populations are suggested as the provenance of seeds for artificial breeding, such as the populations from southwest China, Jeju Island of South Korea, and Kyushu in Japan. It is important to highlight the limitations and risks of using seeds from areas with different environmental conditions for restoration purposes. Thus, we will continue to study the adaptive evolution of *Q. gilva* under the climate change in the future to provide more detailed guidance on provenance applications.

5. Conclusions

Genetic diversity is the basis for evolutionary change and is critical for species to adapt to changing climates and biotic interactions, including novel diseases [11]. Human-mediated destruction and environmental changes disrupt population and community dynamics, resulting in the loss of population genetic diversity and species extinction [48,49]. Based on this study, we confirmed that *Q. gilva* is an endangered (EN) species, regardless of population survey or genetic evidence. In the future, we need to uncover the evolutionary history, population vulnerability, and adaptive capacity under climate change for *Q. gilva*.

Based on a detailed survey of population status and the study of genetic diversity, we could provide a more accurate assessment of the endangered status of species. This study helps initiate the assessment of threatened categories of species combined with population field survey data on genetic diversity. We suggested that in the future, a sixth criterion regarding genetic diversity should be added to the IUCN criteria used to evaluate the threatened category.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/d15020230/s1, Figure S1: The maximum-likelihood (ML) phylogenetic tree of *Quercus gilva*; Figure S2: PCA (principal component analysis) of 65 individuals of *Q. gilva*. The cycles with different colors represent the different populations. The details of abbreviation codes for populations showed in Tables 2 and S1; Table S1: Information of each individual and population used in our study, and the quality of sequencing. Table S2: All the information of population status of *Q. gilva*; Table S3: Linkage disequilibrium decay measured by r^2 in each population and their position when LD decayed to half of their maximum value. References [14,19,50] are cited in the Supplementary Materials.

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Y.-G.S., T.-R.W., Z.-J.L., B.-J.G., X.Z., X.-C.L., D.-M.J., Q.Y., Y.L., Y.-X.K. and X.N.; resources, Y.-G.S. and X.Z.; writing—original draft preparation, Y.-G.S., T.-R.W., S.-S.Z., L.-T.Y., X.-L.D., J.-G.C., J.-H.L. and G.K.; writing—review and editing, L.-T.Y., X.-L.D., J.-G.C., J.-H.L. and G.K.; visualization, T.-R.W., X.Z., D.-M.J. and B.-J.G.; supervision, G.K.; project administration, Y.-G.S.; funding acquisition, Y.-G.S. and X.Z. All authors have read and agreed to the published version of the manuscript.

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