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Analysis of the Conservation Status, Genetic Diversity and Population Structure of Endangered *Ostrya rehderiana* Resources Using SSR Markers

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Abstract: Climate change and anthropogenic habitat destruction have led to the extinction of many species. *Ostrya rehderiana* (Betulaceae) is a typical rare and endangered species, and only five wild individuals have survived. In the 1980s, the offspring of the five wild trees were planted for ex situ conservation and have grown into stable cultivated populations. To protect *O. rehderiana* resources, the genetic diversity and genetic structure of this species were analysed using SSR markers. A total of 167 alleles were detected among 116 individuals from the only wild population and five cultivated populations at 12 SSR loci. The genetic diversity level of *O. rehderiana* was He = 0.88. Genetic differentiations occurred among populations (Fst = 0.17), which was also validated via an analysis of molecular variance (AMOVA). The cultivated populations TM1, TM2 and WC showed considerable genetic differences from the wild population WP based on Bayesian clustering analysis, phylogenetic tree reconstruction and principal coordinate analysis (PCoA). The cultivated populations had more genetic diversity than the wild one. It is speculated that novel alleles may have emerged out of natural processes of evolution and adaptation. The cultivated population QY with the most unique alleles has begun to propagate seedlings naturally, and the small population size and geographical isolation may negatively influence the founding of this population. To weaken the effects of bottlenecks and genetic drift, anthropogenic gene flow among populations is necessary. In addition to the wild population, the cultivated population QY and six individuals from populations QY and WC were also found to be important for the conservation of *O. rehderiana*. The results of this study may guide the development of conservation policies for endangered *O. rehderiana*.

Keywords: *Ostrya rehderiana*; wild populations; rare alleles; genetic variation

Citation: Zhou, Q.; Wang, G.; Li, Y. Analysis of the Conservation Status, Genetic Diversity and Population Structure of Endangered *Ostrya rehderiana* Resources Using SSR Markers. *Forests* **2023**, *14*, 1519. https://doi.org/10.3390/f14081519

Academic Editor: Om P. Rajora

Received: 16 June 2023
Revised: 14 July 2023
Accepted: 24 July 2023
Published: 26 July 2023

1. Introduction

Biodiversity is the variety of life, including the variation among genes and species, and a loss of biodiversity will affect the functioning of ecosystems and human health [1]. Over the past few centuries, human activities and climate change have seriously affected biodiversity and have led to the extinction of many species [2]. The extinction of species will not only directly lead to a loss of biodiversity but also affect the development of human society [3,4]. As an important component of biodiversity, rare and endangered plant species are facing a high danger of extinction. China has abundant plant resources, and the areas in southwestern China are one of the 34 biodiversity hotspots in the world, with many rare and endangered species, such as *Taxus wallichiana* var. *chinensis*, *Cathaya argyrophylla*, and *Glyptostrobus pensilis* [5]. Approximately 10% of all native higher plants in China (3879 species) have been identified as rare and endangered plant species [6]. Hence, it is very urgent to study and protect rare and endangered plant species, which may be at risk of extinction due to artificial destruction and environmental change.

Ostrya rehderiana (Betulaceae) is a typical rare and endangered plant species in China. The species has undergone a historical process of a gradual decline in numbers. The effective population size of *O. rehderiana* began to decrease during the Quaternary glacial period and has continued to decrease in the last 10,000 years [7]. In 1927, this species was found only on Mt. Tianmu in northern China and was common in open woods [8]. Due to anthropogenic habitat destruction and natural disasters, the unique wild *O. rehderiana* population decreased, and only five wild trees of this species survived. Generally, when the habits of rare and endangered species are heavily degraded, ex situ conservation is essential [9]. In the 1980s, the offspring of the five wild trees were planted in different places for ex situ conservation. Many of the transplanted plants have now died, but the remaining individuals have grown into stable cultivated populations. A few of the cultivated populations already bore fruit, but few seedlings have grown naturally. In recent years, the five wild individuals were studied in terms of their ecology [9] and reproductive biology [10]. Moreover, four chloroplast DNA fragments (*trnH-psbA*, *trnL-trnF*, *rps16* and *trnG*), the nulcear internal transcribed spacer (ITS) region [11] and the nuclear genome [7] were sequenced for the five wild individuals, and the status and causes of the endangerment of *O. rehderiana* were analysed. The populations cultivated for ex situ conservation may produce new genetic variation, and thus, are important for the stability and recovery of endangered *O. rehderiana*. Understanding the genetic information of cultivated and wild populations is of significance for guiding the assessment of conservation status and the establishment of conservation programmes, which are lacking. Therefore, some questions remain to be clarified, such as the following: What is the genetic structure of wild and cultivated populations? In addition to the five wild individuals, which individuals need to be protected and utilised? These questions can be answered using molecular markers, which are important tools and have been used in some species [12–14]. The wild individuals of *O. rehderiana* were also analysed using random amplification polymorphic DNA (RAPD) [15]. However, RAPD was a dominant marker and was unable to distinguish alleles, which may limit the disclosure of genetic information. As codominant markers, simple sequence repeats (SSRs) have the characteristics of high polymorphism, easy use and stability, and are an effective molecular technique for studying the genetic diversity and genetic structure of plants [16,17].

In this study, SSR markers were developed, and the wild and cultivated populations of *O. rehderiana* were investigated to (i) analyse the genetic diversity in wild and cultivated populations of *O. rehderiana*, and (ii) develop technical guidelines for the conservation and utilisation of *O. rehderiana*.

2. Materials and Methods

2.1. Sample Information

Only five wild *O. rehderiana* individuals (population WP) have survived on Mt. Tianmu, Zhejiang, where they grow alongside other species such as *Cinnamomum japonicum*, *Boehmeria nivea* and *Camellia sinensis*. The area is characterised by a mean annual temperature of 14.8 °C, an annual rainfall of 1390 mm and an elevation above sea level of 251 m. One wild individual, approximately 300 years old (WP05), grows alongside the others at a distance of 400 m and can bear a small amount of fruit. The other four wild individuals, approximately 100 years old (WP01-WP04), grow 10~15 m apart and can bear fruit. The cultivated populations of *O. rehderiana* that were planted in the 1980s were also investigated. Due to mismanagement and climatic differences, there were fewer than three surviving individuals in the cultivated populations in Jiangsu, Yunnan and Fujian provinces. Five cultivated populations with more than four individuals were found in Zhejiang Province. Although the cultivated populations may be the offspring of the wild population, there was considerable differentiation between them. Three of them had large sample sizes, including populations TM1 (59), TM2 (91) and WC (35). Populations TM1 and TM2 were grown with species such as *Albizia julibrissin*, *Liquidambar formosana* and *Pseudolarix amabilis*, and population WC was grown with *Cunninghamia lanceolata*, *Quercus glauca* and *Castanopsis*

sclerophylla, among other species. The sample sizes of populations QY (13) and AF (5) were small, and natural-regeneration seedlings have appeared in population QY. Due to the small sample sizes, all mature individuals in populations QY, AF and WP were sampled. Some individuals were randomly selected from populations TM1, TM2 and WC. In total, 116 individuals from six populations were selected (Table 1; Figure 1). Young leaves were collected and stored at −80 °C.

Table 1. Information on the populations in this study.

Population Code	Location	Longitude (° E)	Latitude (° N)	Altitude (m)	Number	Origin
TM1	Mt. Tianmu, Zhejiang	119.4	30.4	310	25	Plantation
TM2	Mt. Tianmu, Zhejiang	119.4	30.3	430	37	Plantation
WC	Mt. Wuchao, Zhejiang	120.0	30.1	220	31	Plantation
QY	Qingyuan, Zhejiang	119.0	27.5	640	13	Plantation
AF	Academy of forestry, Zhejiang	120.0	30.2	20	5	Plantation
WP	Mt. Tianmu, Zhejiang	119.8	30.2	251	5	Nature
Total					116	

The population codes of the five plantations are based on the abbreviations of their locations, and the population code of the natural individuals is WP (wild population).

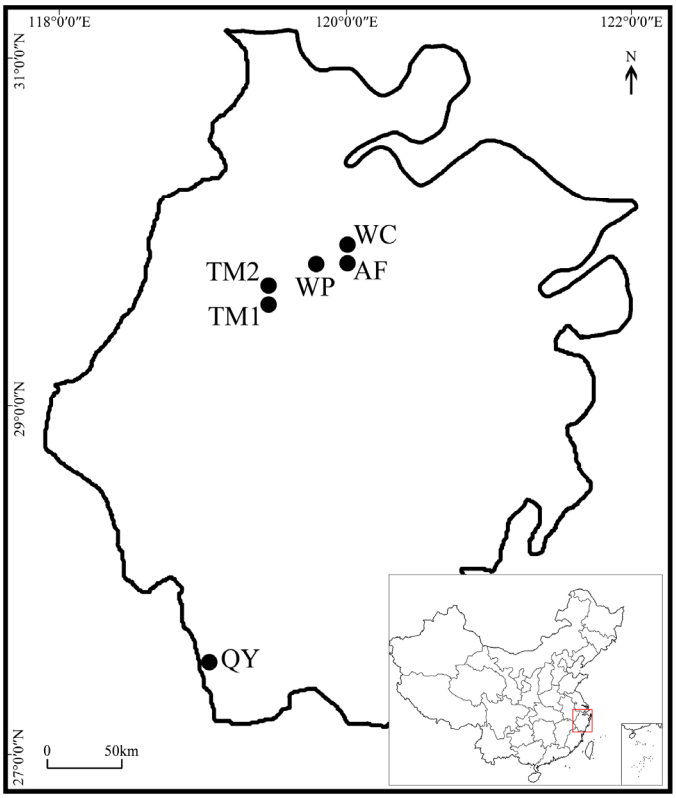

Figure 1. Geographic locations of the studied populations.

2.2. DNA Extraction and Genotyping with SSR Markers

Whole-genomic DNA was extracted using the cetyl trimethyl ammonium bromide (CTAB) method [18]. The concentrations and qualities of DNA were measured using a NanoDrop-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and 1% agarose gel electrophoresis. High-quality DNA was diluted to 50 ng/μL. Seventy-five SSR markers were developed [19] by our laboratory based on the transcriptome sequencing of *O. rehderiana* and tested by performing PCR amplification of the five wild individuals. Of these, 37 pairs yielded clear and reproducible amplicons, and the others were unstable or gave no product. In total, 12 SSR markers with more than one allele per locus were selected (Table 2). Polymerase chain reactions (PCRs) for all markers were carried out using an ABI Veriti 96 PCR system (Thermo Fisher Scientific, Waltham, MA, USA) after the upstream

primers were fluorescently labelled with FAM. The reaction mixtures of 10 µL contained 30 ng of DNA, 5 µL of 2 × TSINGKE Master Mix and 1 µL of each SSR forward and reverse primer. The PCR programme involved a pre-degeneration step of 5 min at 94 °C, followed by 35 cycles at 94 °C for 30 s, 60 °C for 35 s, and 72 °C for 90 s, and an extension step of 10 min at 72 °C. The PCR products were genotyped using an ABI 3730xl instrument (Thermo Fisher Scientific, Waltham, MA, USA).

Table 2. Information on the 12 SSR loci.

Locus Code	Repeat Motif	Primer Sequence (5′~3′)	TM (°C)
SSR01	(GGA) 6	GCAATAGCAACAGCAACAGC TGTCGCTCGTGTACTTCACC	60 °C
SSR02	(G) 16	CGCCCCCAAACTACTATCCT TGACCATGCATCCATTTGAC	60 °C
SSR03	(TAA) 6	CGAACAAGTCCCTCAAGCAT AATTTTGCAACTCCCACAGC	60 °C
SSR04	(AC) 7	CAAACACGAAAGCCAACAGA GGCAACCAAACAAAGCCTAA	60 °C
SSR05	(CTT) 7	TCCATTTTGAAGCAAATCCC GTGTATCAGGGGGAGGAGGT	60 °C
SSR06	(GTC) 8	TCGTCAAATCCTCGTTTTCC CTGACCATCGCGTTTACCTT	60 °C
SSR07	(ACC) 7	ATCGTCTTGCATGAGCCTCT ACAGGTCATTGGTGGAGGAG	60 °C
SSR08	(TAG) 5	GCTAGAGGGGAGGAGGAGAA ATGGTTGGCTCCATGACTTC	60 °C
SSR09	(ACT) 6	ACAACAATGGTGGTTGGGAT TAAGCTTGGTCCACTTGCCT	60 °C
SSR10	(AAT) 11	GATTGAGTTGGCTGGGATGT CAACCGCTTCAACACATTCA	60 °C
SSR11	(G) 15	GATGTGCGGTAGTAGGCGAT GTCGGTGGTCGTTTCAGTCT	60 °C
SSR12	(GTT) 7	AGGATCCAAATGACTCCGTG GAAGAGGAAGCGCAAGAGAA	60 °C

2.3. Data Analysis

The genotyping results were measured using a Peak Scanner v 1.0 (Thermo Fisher Scientific, Waltham, MA, USA) [20]. The number of observed alleles (Na), effective number of alleles (Ne), heterozygosity (He), genetic differentiation index (Fst) and Hardy–Weinberg equilibrium for all populations were determined using POPGENE version 1.32 (University of Alberta, Edmonton, AB, Canada) [21]. The polymorphism information content (PIC) of each SSR locus and a phylogenetic tree of all individuals constructed using the neighbour-joining method were analysed using PowerMarker version 3.25 (North Carolina State University, Raleigh, NC, USA) [22]. FSTAT version 2.9.3 (University of Lausanne, Lausanne, VD, Switzerland) was used to measure the allelic richness (AR) of each population [23]. With GenALEx version 6 (Rutgers University, New Brunswick, NJ, USA), an analysis of molecular variance (AMOVA) and principal coordinate analysis (PCoA) were performed [24]. The bottleneck effects were tested using BOTTLENECK version 1.2 at the population and species levels [25]. A two-phase mutation model (TPM) and a stepwise mutation model (SMM) were measured using Wilcoxon signed-rank tests. A 90% variance for SMM and 12% variance for TPM were selected with 1000 repeats [26].

To analyse the genetic structure of wild and cultivated populations, Bayesian clustering analysis was carried out using Structure version 2.3.1 (Stanford University, San Francisco,

CA, USA) [27]. For each value of K ranging from 1 to 7 (number of populations + 1), the Markov chain Monte Carlo (MCMC) method was used to estimate the genetic ancestry of each individual based on the admixture model. These 100,000 iterations were followed by a burn-in period of 100,000 iterations. The optimal K value was determined via the Evanno method using STRUCTURE HARVESTER [28]. Finally, CLUMPP version 1.1.2 (Stanford University, Stanford, CA, USA) [29] and DISTRUCT version 1.1 (Stanford University, San Francisco, CA, USA) [30] were also used.

3. Results

3.1. Genetic Diversity

The 116 individuals from six populations were successfully genotyped across 12 SSR loci, and a total of 167 alleles were detected (Figure 2; Table 3). The number of alleles per SSR locus ranged from 5 to 19, with an average of 13.92. SSR07 and SSR10 exhibited the most abundant genetic information and had the highest PIC value (0.90). SSR12 had the lowest PIC (0.73), He (0.77) and I (1.64) values. In general, the average values of Ne and He were 8.48 and 0.88, respectively. The average He of 111 individuals from five cultivated populations was 0.87. However, the frequencies of alleles at the 12 SSR loci all significantly deviated from Hardy–Weinberg equilibrium ($p < 0.01$).

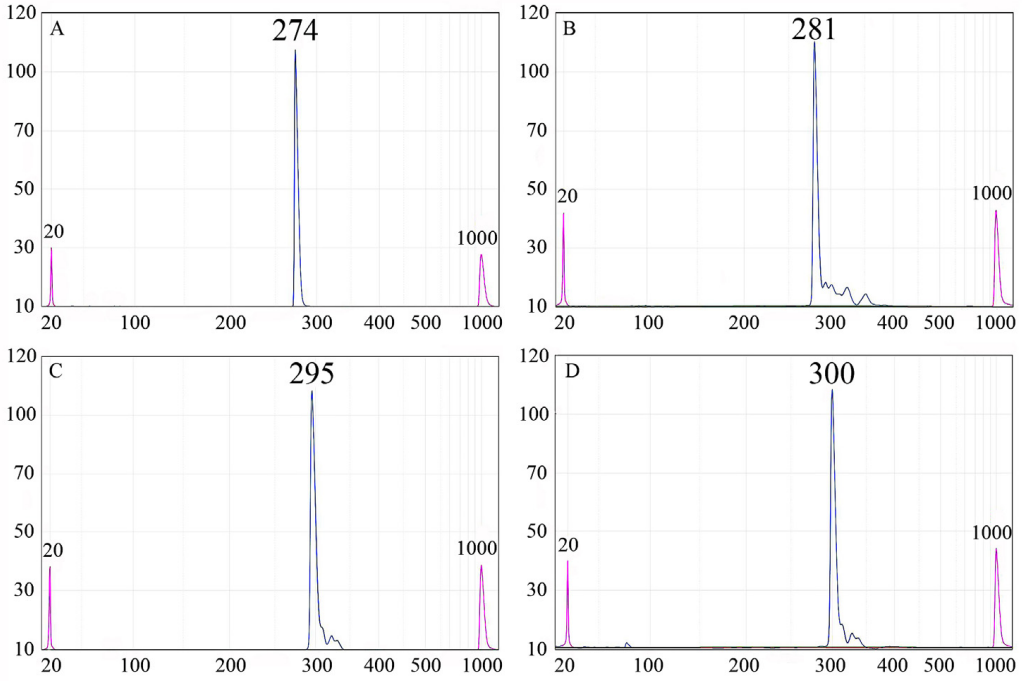

Figure 2. Genotyping results of four individuals from populations TM2 (**A**), TM1 (**B**) and WC (**C**,**D**) at SSR02 locus.

The wild population (WP) and cultivated populations (TM1, TM2, WC, QY and AF) showed different levels of genetic diversity (Table 4). The cultivated populations, except for population AF, had higher genetic diversity levels than the wild population. The genetic diversity level of population AF (He = 0.70) was lower than those of the other populations, while population QY showed the highest level of genetic diversity (He = 0.84). In addition, the alleles of population WP (Ne = 3.19; AR = 3.83) were less abundant than those of the other populations. The bottleneck test showed that bottleneck effects occurred in populations TM1 (TPM, $p < 0.05$), QY (TPM, $p < 0.05$) and WP (SMM, TPM, $p < 0.05$), which indicated that these populations experienced considerable declines in population size. The cultivated populations may be the free-mating offspring of the wild population. Hence, a bottleneck test was also carried out with all 116 individuals from the wild and

cultivated populations treated as one population. A bottleneck effect also occurred at the species level (TPM, $p < 0.05$).

Table 3. Polymorphism information of the 12 SSR loci.

Locus	Na	Ne	He	PIC	Fst	HWE
SSR01	14	10.18	0.91	0.89	0.26	**
SSR02	13	7.88	0.88	0.86	0.14	**
SSR03	12	5.58	0.82	0.80	0.18	**
SSR04	12	7.64	0.87	0.86	0.17	**
SSR05	15	6.98	0.86	0.84	0.11	**
SSR06	15	9.00	0.89	0.88	0.12	**
SSR07	16	10.50	0.91	0.90	0.06	**
SSR08	16	10.09	0.90	0.89	0.09	**
SSR09	19	9.85	0.90	0.89	0.23	**
SSR10	15	11.02	0.91	0.90	0.10	**
SSR11	12	8.77	0.89	0.88	0.14	**
SSR12	8	4.23	0.77	0.73	0.40	**
Mean	13.92	8.48	0.88	0.86	0.17	

Na: observed number of alleles, Ne: effective number of alleles, He: expected heterozygosity, PIC: polymorphism information content, Fst: genetic differentiation index, HWE: Hardy–Weinberg equilibrium, **: extremely significant ($p < 0.01$).

Table 4. Information on genetic diversity and bottleneck tests in the 6 populations.

Population Code	Na	Ne	AR	He	Population Level		Species Level	
					TPM	SMM	TPM	SMM
TM1	7.75	5.21	5.11	0.81	0.002 *	ns	0.003 *	ns
TM2	8.75	5.59	5.18	0.81	ns	ns		
WC	8.42	5.36	4.99	0.76	ns	ns		
QY	7.92	5.87	5.64	0.84	0.031 *	ns		
AF	4.00	3.31	4.00	0.70	ns	ns		
WP	3.83	3.19	3.83	0.72	0.007 *	0.046 *		

AR: allelic richness, TPM: a two-phase model of mutation, SMM: a stepwise mutation model, *: $p < 0.05$ (Wilcoxon signed-rank test), ns: not significant.

3.2. Genetic Variation and Genetic Structure

The average Fst value among the 12 SSR loci was 0.17 (Table 3), suggesting that there was genetic variation among the populations. To analyse the distributions of genetic variation, AMOVA was carried out and revealed significant genetic variation among populations, among individuals and within individuals ($p < 0.01$; Table 5). In general, 11% of genetic variation was detected among populations ($p < 0.01$), while 29% and 61% of genetic variation was detected within and among individuals ($p < 0.01$).

Table 5. AMOVA of 116 samples from 6 populations.

Source of Variance	Variance Component	Percentage of Total	*p* Value
Among populations	0.57	11%	<0.01
Among individuals	3.28	61%	<0.01
Within individuals	1.56	29%	<0.01
Total	5.41	100%	

To reveal the genetic structure of wild and cultivated populations, Bayesian clustering analysis was carried out. The K value was 4 when the ΔK value was highest, which indicated that the optimal K value was 4 (Figure 3A). When K was 4, 116 individuals from the studied populations were clustered into four groups (Figure 3B). At $K = 4$, two cultivated populations (QY and AF) showed similar genetic information to the wild population WP.

However, the genetic information of the other three cultivated populations (TM1, TM2 and WC) was different from that of the wild population (WP). Although most of the genetic information differed between populations TM1, TM2 and WC, a small part of the genetic information in populations TM1 and WC was similar to that of population TM2. Most of the genetic information in population WC came from a unique ancestral population, while that in populations QY, AF and WP came from a different ancestral population at $K = 2$. Moreover, the genetic information of populations TM1 and TM2 was a mixture of those two ancestral populations at $K = 2$. At $K = 3$, populations TM2 and WC showed different genetic information to the other populations. Populations TM1 and QY showed genetic variation from the wild population WP, while populations AF and WP were very similar in their genetic information. When K was 5, the cultivated populations TM1, TM2 and WC showed considerable genetic differences from the wild population WP.

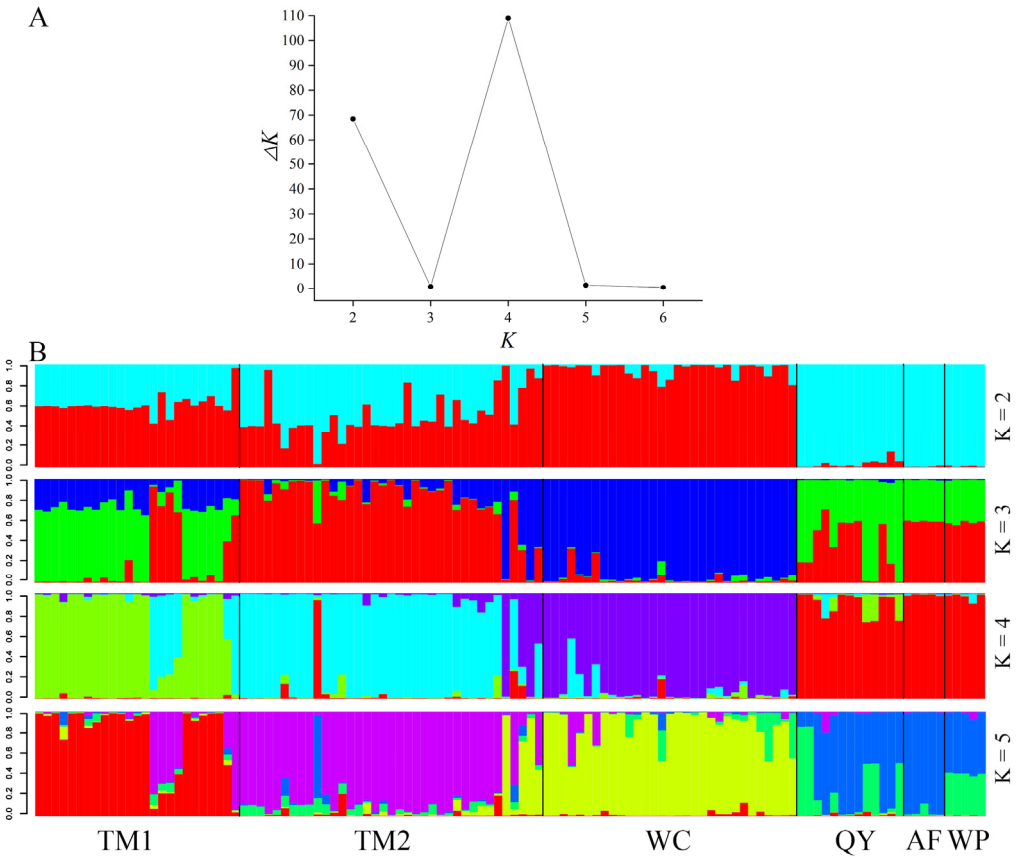

Figure 3. Relationships between the number of clusters (K) and the corresponding ΔK (**A**) and Bayesian cluster (**B**).

To further analyse the genetic relationships among the 116 individuals, a phylogenetic tree was constructed based on the neighbour-joining method (Figure 4A). The cultivated populations TM1, TM2 and WC also showed considerable genetic variation, and most individuals from the same population were placed into the same groups together. In addition, the cultivated populations (QY and AF) had a close genetic relationship with the wild population (WP). However, some individuals from population QY showed genetic differences from the wild individuals. A PCoA with the 116 individuals was also carried out, the results of which verified the Bayesian clustering analysis and phylogenetic tree reconstruction (Figure 4B). All the individuals in population WC were placed into one group. Most of the individuals from populations TM1 and TM2 were divided into two groups, while the individuals from populations QY, AF and WP were placed into one group.

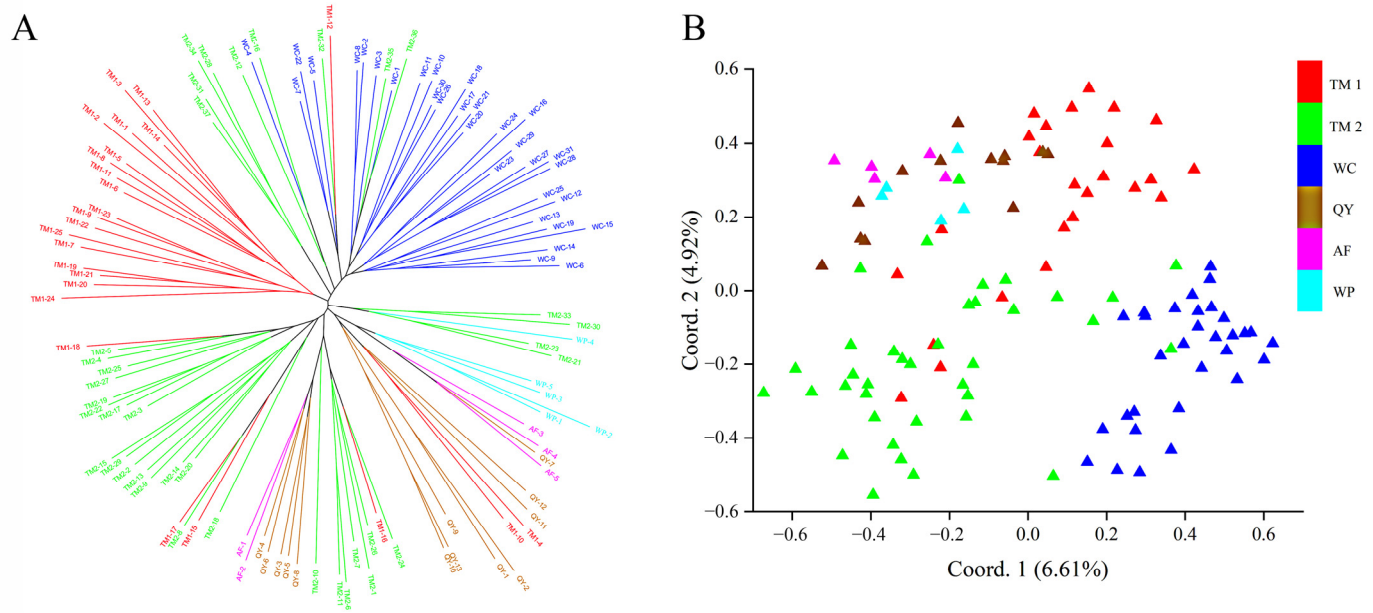

Figure 4. Phylogenetic tree based on neighbour-joining method (**A**) and scatter plot generated based on PCoA (**B**).

3.3. Unique Alleles

In general, individuals with some rare alleles should be given more attention in the conservation of rare and endangered species. Therefore, the unique alleles detected within only one population were analysed at 12 SSR loci (Table 6). A total of 30 unique alleles were detected, which also indicated that genetic variation may occur among the studied populations. There were six unique alleles detected at SSR09, while SSR04 and SSR10 had only one unique allele. The wild population had 4 unique alleles, while the cultivated populations had 26 unique alleles, accounting for 87% of the total. The QY population had the most unique alleles (11), while the AF population had the least (1). Twelve unique alleles were detected in populations TM1 (six) and WC (six). Moreover, six individuals from the cultivated populations QY (QY01, QY02, QY05 and QY10) and WC (WC19 and WC22) contained seven, three, three, three, three and three unique alleles, respectively, while most of the other individuals have no unique alleles.

Table 6. Frequencies of unique alleles within each of population at 12 SSR loci.

Allele	TM1	TM2	WC	QY	AF	WP
SRR01-B	-	-	0.03	-	-	-
SRR01-C	-	0.05	-	-	-	-
SRR01-N	-	-	-	0.15	-	-
SRR02-A	-	-	-	0.08	-	-
SRR02-J	-	-	0.06	-	-	-
SSR03-A	-	-	-	-	-	0.40
SSR03-H	-	-	-	0.15	-	-
SSR03-I	-	-	-	-	-	0.30
SSR04-L	-	-	-	0.15	-	-
SSR05-A	0.04	-	-	-	-	-
SSR05-B	-	0.03	-	-	-	-
SSR05-O	-	-	-	0.04	-	-
SSR06-A	-	-	-	0.15	-	-

Table 6. *Cont.*

Allele	TM1	TM2	WC	QY	AF	WP
SSR06-C	-	-	-	0.12	-	-
SSR06-O	0.04	-	-	-	-	-
SSR07-H	-	-	0.10	-	-	-
SSR07-P	-	-	0.10	-	-	-
SSR08-C	-	-	0.06	-	-	-
SSR08-D	-	-	0.13	-	-	-
SSR09-A	-	-	-	-	-	0.40
SSR09-E	-	-	-	0.15	-	-
SSR09-O	-	-	-	0.15	-	-
SSR09-Q	0.08	-	-	-	-	-
SSR09-R	0.08	-	-	-	-	-
SSR09-S	0.08	-	-	-	-	-
SSR10-O	-	-	-	-	-	0.10
SSR11-K	0.08	-	-	-	-	-
SSR11-L	-	-	-	-	0.20	-
SSR12-G	-	-	-	0.19	-	-
SSR12-H	-	-	-	0.04	-	-
Number	6	2	6	11	1	4

-: not detected in the population.

4. Discussion

4.1. Genetic Diversity

Genetic diversity is the basis of adaptation and evolution, and high levels of genetic diversity are conducive to the adaptation of species to new environments and climate change [31]. In this study, *O. rehderiana* showed a higher level of genetic diversity (He = 0.88; PIC = 0.86) than some endangered species, including *Cycas taiwaniana* [32], *Nuphar shimadai* [33], *Pisum sativum* [34] and *Semiliquidambar cathayensis* [35]. The ex situ conservation of *O. rehderiana* contributed to the preservation of genetic variation. Compared to rare and endangered *Pinus bungeana* (He = 0.91) [36], the genetic diversity level of *O. rehderiana* was lower. The wide distribution range and large number of wild individuals of *P. bungeana* produced a high level of genetic diversity, while for *O. rehderiana*, only one wild population containing five individuals survived. A genomic analysis of five wild individuals and nine cultivated individuals of *O. rehderiana* showed that the genetic diversity level of *O. rehderiana* ($\pi = 1.66 \times 10^{-3}$) was much lower than that of *Ostrya chinensis* ($\pi = 2.79 \times 10^{-3}$) and *Betula pendula* ($\pi = 8.84 \times 10^{-3}$) [7]. The small sample size may miss some genetic information of *O. rehderiana*, which may be a reason for the differences with the results of this study. In general, when the effective number of a population is sharply reduced, it experiences a bottleneck effect, which may lead to a lack of genetic diversity via inbreeding and genetic drift [37,38]. The effective population size of *O. rehderiana* has continued to decrease in the last 10,000 years [7], and only five wild trees of this species have survived [39]. The results of the bottleneck test showed that bottleneck effects occurred in the wild population WP. However, the genetic diversity of the cultivated populations was slightly higher than that of the wild population. There were limited generations in *O. rehderiana*, and the inbreeding and genetic drift caused by bottleneck effects did not significantly reduce the genetic diversity. The sample size of the cultivated populations (111) was 20 times greater than that of the wild population (5), and the cultivated populations may produce genetic variation (such as 26 unique alleles, accounting for 87% of the total), which led to a higher genetic diversity level of the cultivated populations than that of the wild population. In addition, SSR loci with abundant polymorphism and capillary electrophoresis may be beneficial for revealing abundant genetic variation.

4.2. Genetic Variation

Genetic variation and genetic structure are influenced by factors such as distribution range, evolutionary history and mating systems [40]. Understanding the genetic variation

and genetic structure of populations is essential to determining genetic characteristics and evolutionary history, which can guide the development of conservation policies for endangered species [41]. In this study, there was considerable genetic variation among the studied populations (Fst = 0.17). The results of AMOVA also detected 11% of genetic variation among populations. The cultivated populations TM1, TM2 and WC showed considerable genetic variation compared to the wild population WP based on the results of Bayesian cluster analysis, phylogenetic tree reconstruction and PCoA. Moreover, the cultivated populations contained 26 unique alleles, accounting for 87% of the total. It is speculated that novel alleles may have emerged out of natural processes of evolution and adaptation. Natural selection is important evolutionary forces, and genetic information, including allele frequencies, may be changed in response to environmental characteristics and climatic change [42,43]. *O. rehderiana* is very sensitive to climatic changes, including temperature and precipitation fluctuations, which may be one of the reasons for its endangered condition [39]. The QY population was located in the Baishanzu National Nature Reserve, where human activities are limited. Therefore, the reason for the disappearance of some individuals in population QY may be mainly natural selection, and the latitude and altitude of population QY were considerably different from those of the other populations. Moreover, the investigation showed that the QY population has begun to propagate seedlings naturally, which was not found in the other cultivated population. The highest AR value and the most unique alleles were detected in population QY, and it is speculated that this population may gradually develop into a new population with special genetic variation. However, recurrent bottlenecks occurring during population founding can lead to high genomic differentiation among populations and low genomic diversity within populations [44,45]. The low population size may increase genetic drift and genetic load [46], while mutation and gene flow can have the opposite effect [47]. In this study, bottleneck effects occurred in population QY, and the low population size and limited gene flow caused by geographical isolation may negatively affect the founding of population QY. Hence, increasing the population size and promoting genetic communication with other populations are necessary to reduce the effects of bottlenecks and genetic drift on population QY.

4.3. Conservation of *O. rehderiana*

For *O. rehderiana*, ex situ conservation was effective, and the offspring of the five wild trees did not reduce the genetic diversity level of this endangered species, which was beneficial for population reconstructions. An important part of the conservation of rare and endangered species is the conservation of genetic diversity. Therefore, individuals with rare genetic information should be focused on, and are recognized as an important source of variation in gene expression [48]. In this study, although the cultivated population QY had a small population size (13), which was much smaller than that of populations TM1 (25), TM2 (37) and WC (31), the QY population, with the highest AR value (5.64), contained the most unique alleles (11). Moreover, each of the six individuals from populations QY (QY01, QY02, QY05 and QY10) and WC (WC19 and WC22) contained more than three unique alleles. Therefore, these resources were not only related to the conservation of the genetic diversity of the endangered *O. rehderiana* but also valuable breeding materials. In addition, there were differences in genetic information and genetic structure among the cultivated and wild populations, and artificial gene flows between them may be beneficial for maintaining the genetic diversity of *O. rehderiana* [49]. The QY population has begun to propagate seedlings naturally, and a portion of the seedlings in this population should be planted in the other populations. However, the seeds of *O. rehderiana* are difficult to germinate naturally, and seeds from the wild (WP) and the large populations (TM1, TM2 and WC) should be artificially grown into seedlings that can be used to increase the population size of population QY.

5. Conclusions

The endangered *O. rehderiana* contained abundant genetic variation, and the cultivated populations showed slightly higher genetic diversity level than the wild population. Genetic differentiations were detected between the cultivated and wild populations. The cultivated population QY and six individuals from populations QY and WC have rare genetic information and should be focused on for the conservation of *O. rehderiana*. The exchange of genetic information between populations is necessary to reduce the effects of bottlenecks and genetic drift.

Author Contributions: Conceptualisation, Y.L. and Q.Z.; methodology, Q.Z.; software, Q.Z. and G.W.; validation, Q.Z., G.W. and Y.L.; investigation, G.W.; data curation, G.W.; writing—original draft preparation, Q.Z.; writing—review and editing, Q.Z.; supervision, Y.L.; project administration, Q.Z. and Y.L.; funding acquisition, Q.Z. and Y.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Cooperation Project between Chinese Academy of Forestry and Zhejiang Province (2023SY13) and the Special Project of the Research Institute of Zhejiang Province (2021F1065-1).

Data Availability Statement: Not applicable.

Acknowledgments: We are grateful to Haibo Li, Lifang Zhang and Mingshui Zhao for their help with sampling in the field and laboratory work.

Conflicts of Interest: The authors declare no conflict of interest.

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