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Analysis of Genetic Structure of the Endangered Plant *Torreya jackii* Population Based on SSR Markers

Jianhui Li [1], Lili Liu [2], Rumin Liu [3], Haixiang Cheng [1], Jianhua Zhao [1], Rui Fan [1]
[1]College of Chemistry and Materials Engineering of Quzhou University, Quzhou 324000, China
[2]Quzhou Academy of Agricultural and Forestry Sciences, Quzhou 324000, China
[3]Forestry Technology Extension Center, Kecheng District of Quzhou City, Quzhou 324000, China

Abstract:

Torreya jackii (Taxaceae) is a rare and endangered plant endemic to China. The study of the genetic structure of the population of *T. jackii* can provide a basis for its scientific management and the development of reasonable and effective protection strategies. Genetic structure of seven subpopulations of *T. jackii* in Baiyunyuan Forest Park of Tonglu County of Zhejiang Province was analyzed based on pre-developed SSR markers. The results showed that 41 alleles were found in the seven subpopulations using 8 pairs of SSR primers. The observed heterozygosity were 0.4688~0.6027 with the average of 0.5427. The expected heterozygosity ranged from 0.4453 to 0.5471 with the average of 0.5060. AMOVA analysis showed that the genetic variation was mainly found within subpopulation, the genetic differentiation among subpopulations was low (FST =0.064, RST = 0.089). STRUCTURE cluster analysis showed that there has a comparatively high mixing ratio among subpopulations. Mantel test showed that there was no significant relationship between genetic distance and geographic distance. BOTTLENECK analysis showed that recent bottleneck effects were found in the subpopulation of HongQi Forestry Centre. There is a significant distance isolation effect in the seed diffusion of *T. jackii*, while the dioecious and wind-borne pollen characteristics to a certain extent cause it to be able to maintain a more stable genetic diversity and genetic structure in relatively isolated plaques.

Keywords: *Torreya jackii, Endangered plant, Genetic structure, SSR markers, Protection strategy*

I. INTRODUCTION

Understanding the spatial distribution of species genetic variation and population genetic structure is an important method to effectively manage their genetic resources[1]. The analysis of the genetic structure of endangered species can provide the basis for scientific management and reasonable

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and effective protection strategies[2]. At present, most of the researches on the genetic structure of species are focused on a large geographical scale[3-5], while the analysis of subpopulations in a small geographical scale is relatively less[6-8]. However, for a certain species, its genetic structure will show different contents and forms on different scales, and different scales will also reflect different information[9]. Therefore, if we want to have a comprehensive understanding of the spatial distribution of genetic variation of a species, we must study the genetic structure of populations at different scales[10].

Torreya jackii, belonging to *Torreya* of *Taxaceae*, is a unique Neogene Gymnospermum plant in China, with a history of about 200 million years. *T. jackii* has excellent wood, beautiful tree shape, edible seeds and the efficacy of expelling intestinal parasites, and is of great significance in studying the distribution of *Torreya*, ancient flora and the climate of Quaternary Ice Age[11]. However, with the continuous deterioration of the ecological environment and the continuous influence of human activities in recent years, the survival of *T. jackii* is being seriously threatened. Now the plants are scarce, and the distribution range is very narrow. It only remains in some remote areas in the mountainous areas of Zhejiang and Fujian. It has been listed in the national second-class key protected plants[12] and the IUCN red list of endangered species[13], and also included in the "China Species Red List"[14], which needs to be determined reasonably at present.

To protect and achieve sustainable use of the wild resources of *T. jackii*, in recent years, scholars at home and abroad have made a preliminary study on the population genetics of *T. jackii*. These studies provide an important theoretical basis for the analysis and protection of the genetic structure of *T. jackii*. However, these studies all use dominant molecular markers such as RAPD or ISSR[5, 15-18], which have poor stability and repeatability, certain defects in methods and insufficient available effective information, thus affecting the accurate understanding of the genetic structure of *T. jackii* population. Thus, it is necessary to carry out further research.

Simple sequence repeat (SSR) is a kind of tandem repeat DNA sequence consisting of several nucleotides (1-6 bp)[19]. Because the number of repeat sequences may be different in different individuals, it shows polymorphism. Microsatellite markers have high polymorphism and good repeatability[20]. At the same time, it also has the characteristics of low selection pressure for evolution, codominant inheritance and easy analysis[21-22]. It is an ideal marker for studying population genetic structure[23] and has been widely used in population genetic structure analysis[24-26] in recent years.

Therefore, in this study, *T. jackii* was selected as the object, and the polymorphic microsatellite markers developed and screened in the early stage were applied to the genetic structure analysis of different subpopulations in Baiyunyuan Forest Park, Tonglu County, Zhejiang Province, so as to reveal the genetic differentiation level and genetic structure characteristics of *T. jackii* in a small geographical range, and provide scientific basis for targeted rescue and protection measures for *T. jackii* and similar endangered species.

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II. MATERIALS AND METHODS

2.1 Research Site and Sample Collection

The research site is located in Baiyunyuan Forest Park, Tonglu County, Zhejiang Province. It is in the northern edge of the middle subtropical zone, with the central position of 29°42′N and 119°44′E. The zonal vegetation is evergreen broad-leaved forest, and the main dominant species are *Cyclobalanopsis glauca*, *Schima superba*, *Rhododendron ovatum* and *Eurya muricata*. Due to the fragmentation of habitat caused by tourism development, artificial felling and destruction, *T. jackii* has formed seven relatively isolated subpopulations in this area, and the distance between every two of the seven subpopulations is about 0.4 km-4.0 km. Among them, *T. jackii* in one subpopulation grows on the cliff. A plank road was built on the cliff during tourism development. The basic situation of the seven subpopulations is shown in Table I, and their distribution locations are shown in Fig 1.

According to the size of the population, the number of individual samples to be collected was determined. The sample collection followed the principle of randomness. About 30 adult individuals samples were collected as much as possible, while the distance between these individuals was more than 30m. Healthy young leaves of each plant were collected, wiped and put into envelopes, numbered and then put into self-sealing bags to be quickly dried with color-changing silica gel. The color-changing silica gel was changed every once in a while until the color of the silica gel did not change.

Table I. Basic conditions of 7 *T. jackii* subpopulations' plot

Subpopulation location and code	Geographical coordinate	Altitude (m)	Slope aspect	Subpopulation size
Kuowu (KW)	119°42′39″ E, 29°42′21″ N	412	NE15°	79
Wuyan (WY)	119°42′50″ E, 29°41′56″ N	360	SE43°	59
Dache (DC)	119°43′22″ E, 29°41′42″ N	428	NW28°	189
Qinglonxia (QL)	119°43′55″ E, 29°41′38″ N	559	SE46°	75
Dalonmen (DL)	119°44′13″ E, 29°41′20″ N	547	NE24°	292
Xuanya (XY)	119°44′24″ E, 29°41′34″ N	689	SE42°	72
HongQi Forestry Centre (HQ)	119°44′49″ E, 29°41′42″ N	703	NE73°	63

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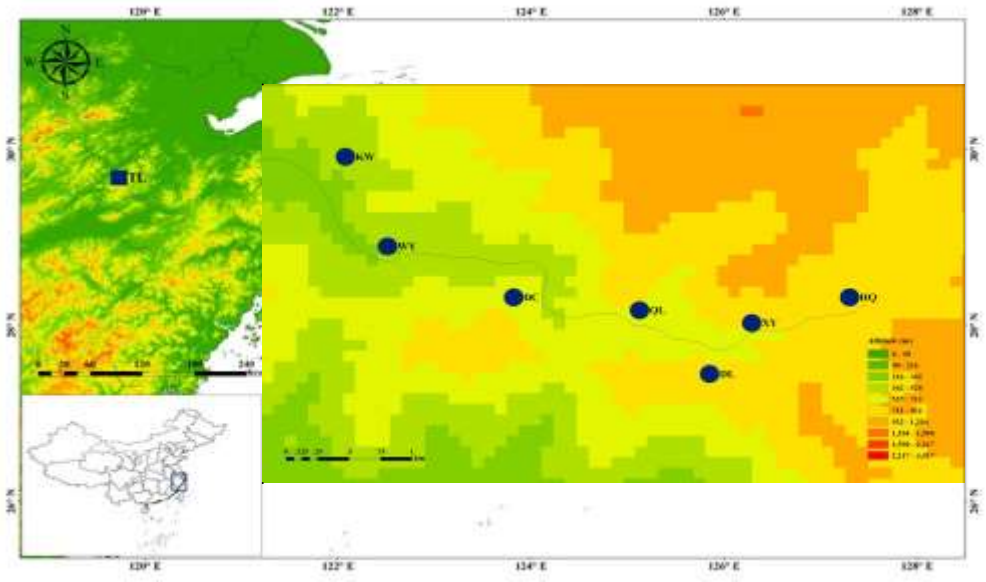

Fig 1: Map of sampling sites of T. jackii subpopulations

2.2 Extraction of Genomic DNA

The new plant genomic DNA extraction kit (centrifugal column type) made by TIANGEN Company was used to extract the genomic DNA of *T. jackii*. The purity of plant genomic DNA extracted by this kit is high, which is beneficial for subsequent microsatellite marker analysis.

2.3 Selection of Microsatellite Markers

In the early stage of this study, eight pairs of polymorphic microsatellite primers were isolated and screened from the genome of *T. jackii* by improved biotin streptavidin capture method[22], which provided an effective tool for studying the genetic structure of *T. jackii* subpopulation[27]. The forward primers of these eight pairs of microsatellite primers were fluorescently labeled (FAM in blue, ROX in red and HEX in green) (Shanghai Shenggong Bioengineering Company), and then the eight pairs of primers were divided into three combinations considering the fragment length: TJ10+TJ28+TJ45; TJ21+TJ62+TJ75; TJ55+TJ79.

2.4 PCR Amplification and Product Identification

PCR amplification reaction was completed on the Mastercycler ep gradient PCR instrument (Eppendorf, Hamburg, Germany). The reagents used in PCR amplification were purchased from Shanghai Shenggong Bioengineering Company.

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The optimized optimal reaction system for SSR amplification of *T. jackii* is: 15 μL PCR reaction volume, 1×Taq enzyme matching buffer (10 mmol L^{-1} Tris-HCl, pH 9.0, 50 mmol L^{-1} KCl, 0.1% Triton X-100), 0.15 mmol L^{-1} 4 ×dNTP, 1 U Taq DNA polymerase, 25 ng template DNA, 0.1 μM primer, 1.5 mM Mg^{2+}. The optimized PCR amplification procedure of *T. jackii* is: 94 °C 5 min; 94 °C 30 s, 54.2°C-63.9°C 30 s (see Li Jianhui[27] for details of different primer annealing temperatures), 72 °C 30 s, totally 35 cycles; 72 °C for 10 min. The product was stored at 4 °C.

The amplified products were electrophoresed in 1.2% agarose gel (containing 0.5 μg·mL^{-1} ethidium bromide) with 0.5×TBE electrophoresis buffer, and recorded on Gel Doc 2000TM gel imaging system (Bio-RAD, California, U.S.A.). pUC19 DNA/*Msp*I (*Hpa*II) (Fermentas, Vilnius, Lithuania) was taken as the standard molecular weight reference. The amplified products with bright target bands and no stray bands were selected and sent to Shanghai Sangon Bitotech Company for fragment analysis. The instrument used was 3730xl DNA Analyzer (Applied Biosystems, USA), and the sequencing reagent was BigDye terminator V3.1. The software GeneMapper v4.0 and Peak Scanner v1.0 (Applied Biosystems, USA) were used to analyze the results of fragment scanning and determine the genotype.

2.5 Data Processing

The expected heterozygosity and observed heterozygosity were calculated by the software TFPA1.3[28]; the average allele number, allele richness and inbreeding coefficient were calculated by FSTAT 2.9.3[29]. The genetic differentiation coefficient was calculated by the software GENEPOP v4.0[30], FSTAT2.9.3[29] and RECODEDATA[31]; the genetic structure of the subpopulation was analyzed by Bayesian method with software STRUCTURE 2.3.3[32, 33]; the AMOVA analysis in the software GenAlEx 6.5[34, 35] was used to test the source of genetic variation in subpopulations, and Mantel test was used to analyze whether there was a correlation between genetic distance and spatial distance among subpopulations. Bottleneck effect was detected by the software BOTTLENECK v1.2.02[36]; SPSS13.0 software was used to make regression analysis on the geographical distance and genetic distance between HQ subpopulation and other subpopulations.

III. RESULTS AND ANALYSIS

3.1 Genetic Diversity of Subpopulation

A total of 41 alleles were detected by 8 pairs of microsatellite primers in seven subpopulations of *T. jackii*, and the number of alleles detected at each locus ranged from 3 to 8, with an average of 5.125. See Table II for each genetic diversity parameter. Among the seven subpopulations, the observed heterozygosity (H_O) ranged from 0.4688 to 0.6027, with an average of 0.5427. The highest was DL subpopulation and the lowest was XY subpopulation. The expected heterozygosity (H_E) ranged from 0.4453 to 0.5471, with an average of 0.5060. The highest was DL subpopulation and the

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lowest was XY subpopulation. Among the seven subpopulations, the average number of alleles ranged from 3.1 to 4.1. The DL subpopulation was the most, while DC and HQ subpopulation were the least. At the same time, the maximum allele richness was also the DL subpopulation, and the minimum allele richness was also the DC and HQ subpopulations. According to the number of private alleles in each subpopulation, KW and QL subpopulations had one private allele at locus TJ55 and TJ79 respectively. The DL subpopulation had a private allele at loci TJ55, TJ28 and TJ10 respectively. The number of private alleles in XY subpopulation is the highest, with 3 alleles at locus TJ21 and 2 alleles at locus TJ45.

Table II. Genetic diversity indices of 7 *T. jackii* subpopulations

Subpopulation	Observed heterozygosity (H_O)	Expected heterozygosity (H_E)	Average number of alleles (A)	Average allele richness (A_R)	Private alleles
KW	0.5750	0.4939	3.4	3.4	1
WY	0.5375	0.5143	3.6	3.6	0
DC	0.5188	0.5183	3.1	3.1	0
QL	0.4938	0.4779	3.3	3.3	1
DL	0.6027	0.5471	4.1	3.8	3
XY	0.4688	0.4453	3.6	3.5	5
HQ	0.6023	0.5451	3.1	3.1	0

In terms of the inbreeding coefficient (Table III), generally speaking, the inbreeding coefficients of three subpopulations were less than 0 and those of four subpopulations were greater than 0, while the F_{IS} values were basically close to 0. For locus TJ75, its inbreeding coefficient in all subpopulations was negative, indicating that there might be a problem of excessive heterozygosity at this locus. In addition, F_{IS} values of subpopulations DC and HQ were larger in general, indicating that there was obvious lack of heterozygosity in these two subpopulations.

Table III. Inbreeding coefficient FIS of 8 loci in *T. jackii* subpopulation

Locus	KW	WY	DC	QL	DL	XY	HQ
TJ10	-0.333	0.144	0.200*	0.118	-0.022	-0.283	0.099
TJ21	0.434*	-0.313	-0.012	-0.088	-0.164	-0.167	-0.109
TJ28	-0.056	-0.013	NA	-0.056	-0.127	0.000	-0.135
TJ45	0.198*	-0.139	0.205*	0.157	0.517*	0.226*	0.404*
TJ55	-0.027	-0.118	-0.073	-0.048	-0.033	0.000	-0.050
TJ62	-0.111	0.330*	0.296*	-0.048	0.067	0.426*	0.816*
TJ75	-1.000	-0.520	-0.600	-0.407	-0.473	-0.375	-0.720
TJ79	0.340*	0.247*	0.485*	-0.056	0.345*	0.302*	0.417*
Total	-0.060	-0.030	0.119*	-0.034	0.049	0.035	0.171*

Note: NA indicates that the data can not be calculated; *: $P<0.05$.

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3.2 Subpopulation Genetic Structure

Cluster analysis of the subpopulation of *T. jackii* was carried out by STRUCTURE software. Since there were seven subpopulations of *T. jackii* in total, K=7 was set for analysis, and the relationship among lnP(*D*), ΔK value and K value was calculated as shown in Figure 2, showing that the optimal group number of the subpopulation of *T. jackii* according to heredity was 2, but the corresponding ΔK value was less than 30. This is insufficient to explain the division into 2 categories. The results of clustering division of seven subpopulations with K=2 are shown in Figure 3. According to the proportion of each subpopulation in the two groups, the clustering results are shown on the geographical topographic map (Fig 4). It can be seen that although there is a certain geographical pattern in the seven subpopulations of *T. jackii*, the degree of mixing among the subpopulations is high, and the seven subpopulations should be treated as a collective population as a whole.

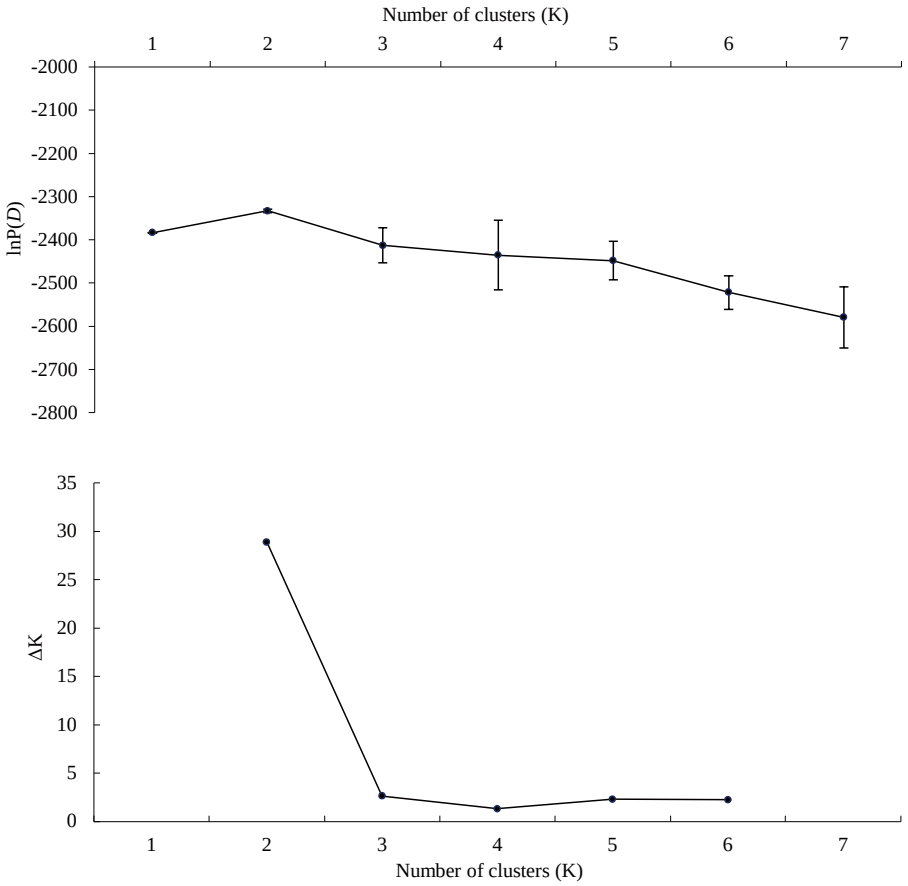

Fig 2: Relationship among lnP(D), ΔK value and K value for STRUCTURE analysis

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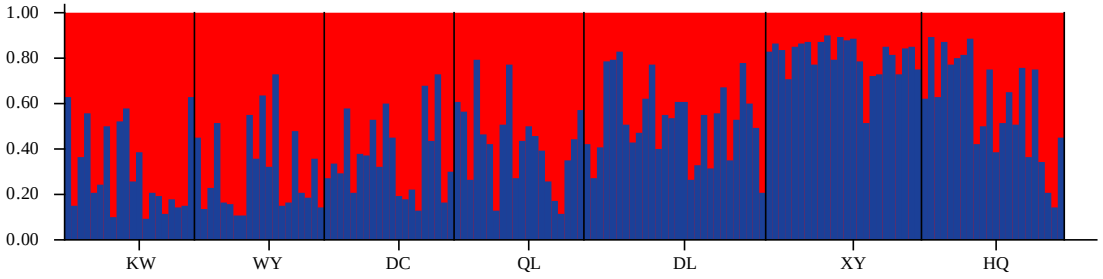

Fig 3: STRUCTURE cluster diagram of seven subpopulations of *T. jackii* (K=2)

Note: Red and blue represent two groups. Each vertical bar represents an individual. The length of red and blue in the vertical bar indicates the proportion of the individual to another group.

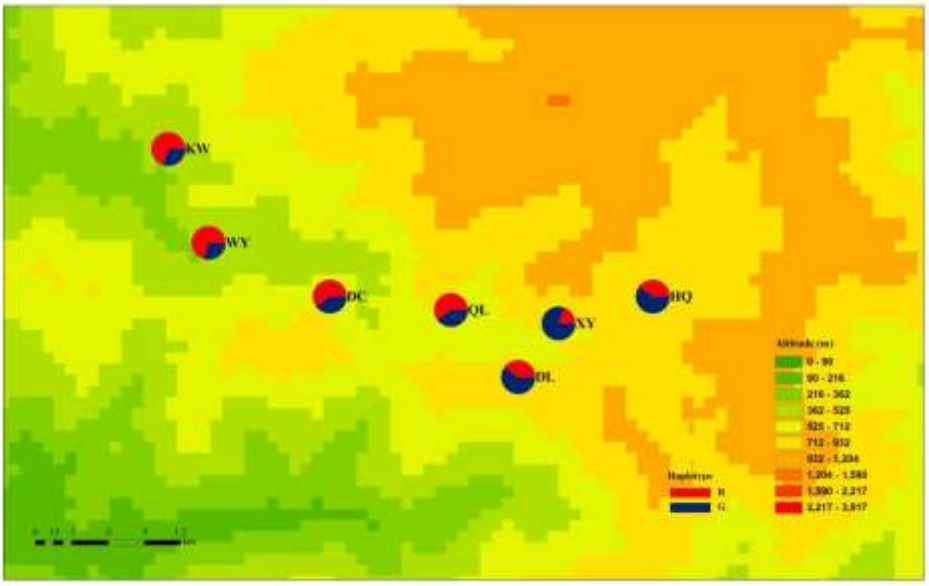

Fig 4: STRUCTURE distribution map of 7 *T. jackii* subpopulations (K=2)

3.3 Mantel Inspection

Genetic differentiation coefficients F_{ST} and R_{ST} were used to measure the degree of genetic differentiation among seven subpopulations of *T. jackii*. The F_{ST} and R_{ST} values between every two of the seven subpopulations are shown in Table IV. The F_{ST} ranges from -0.003 to 0.167. Among them, the WY and QL subpopulations have the smallest genetic differentiation, and the XY and KW subpopulations have the largest genetic differentiation, and the overall F_{ST} is 0.064. R_{ST} ranges from -0.014 to 0.255, with the largest in subpopulations XY and WY and the smallest in subpopulations WY and QL. The overall R_{ST} is 0.089. In addition, the F_{ST} and R_{ST} values between XY subpopulation

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and other subpopulations are much larger than those between other subpopulations.

The genetic similarity and genetic distance among the seven subpopulations of *T. jackii* are shown in Table V, from which we can see that the genetic similarity among the seven subpopulations ranges from 0.8180 to 0.9804, with an average of 0.9155; the genetic distance among subpopulations ranges from 0.0198 to 0.2009, with an average of 0.0895. Among them, WY and QL subpopulations have the highest genetic similarity and the smallest genetic distance. The genetic similarity with XY and WY subpopulation is the lowest and the genetic distance is the largest. Thus, it can be seen that those with the longest geographical distance among subpopulations do not show the largest genetic distance, and those with the shortest geographical distance do not show the smallest genetic distance.

To further test the relationship between genetic distance and geographical distance among the subpopulations of *T. jackii*, the correlation between genetic distance and geographical distance between every two subpopulations was tested by Mantel test with F_{ST}, $F_{ST}/(1-F_{ST})$, standardized genetic differentiation coefficient F'_{ST} and R_{ST} as genetic distance (Fig 5). The results show that there is no significant correlation between genetic distance and geographical distance among seven subpopulations ($p > 0.05$)

Table IV. Matrix of FST and RST between 7 *T. jackii* subpopulations

	KW	WY	DC	QL	DL	XY	HQ
KW	-	0.063	0.113	0.056	0.069	0.242	0.101
WY	0.051	-	0.091	-0.014	0.071	0.255	0.067
DC	0.032	0.021	-	0.074	0.012	0.153	0.025
QL	0.072	-0.003	0.046	-	0.041	0.203	0.036
DL	0.052	0.024	0.040	0.017	-	0.129	0.023
XY	0.167	0.159	0.135	0.119	0.088	-	0.060
HQ	0.089	0.060	0.044	0.049	0.026	0.027	-

Note: R_{ST} (above diagonal) and F_{ST} (below diagonal).

Table V. Genetic identity and genetic distance among subpopulations of *T. jackii*

	KW	WY	DC	QL	DL	XY	HQ
KW	-	0.9288	0.9468	0.9110	0.9275	0.8202	0.8848
WY	0.0739	-	0.9534	0.9804	0.9523	0.8180	0.9063
DC	0.0547	0.0477	-	0.9304	0.9334	0.8455	0.9263
QL	0.0932	0.0198	0.0722	-	0.9624	0.8704	0.9265
DL	0.0753	0.0489	0.0689	0.0383	-	0.8964	0.9480
XY	0.1983	0.2009	0.1678	0.1388	0.1094	-	0.9564
HQ	0.1224	0.0984	0.0765	0.0763	0.0534	0.0446	-

Note: Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

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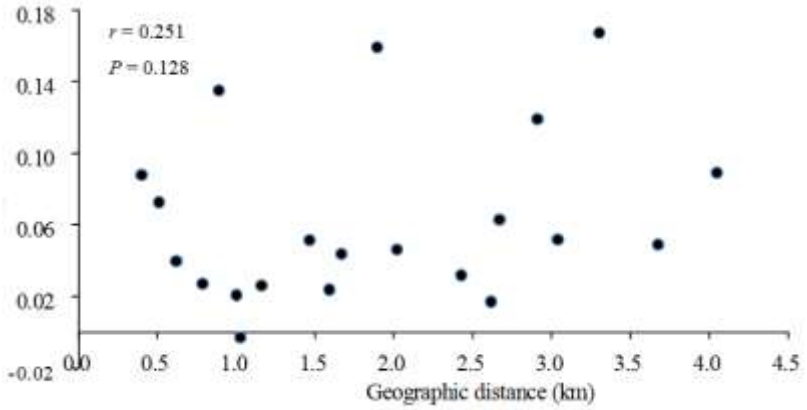
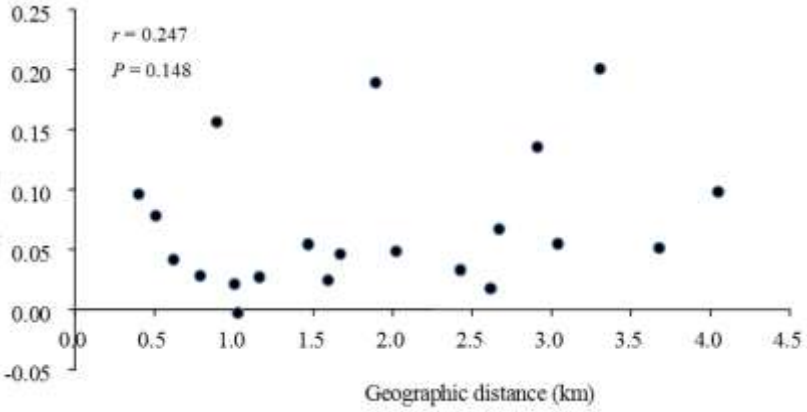
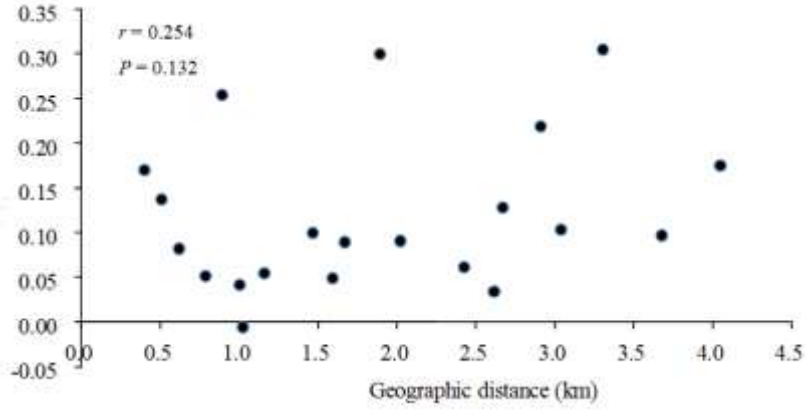


A. Mantel test of relationship between FST and geographic distance among subpopulations of *T. jackii*



B. Mantel test of relationship between FST/(1-FST) and geographic distance among subpopulations of *T. jackii*



C. Mantel test of relationship between FST and geographic distance among subpopulations of *T. jackii*

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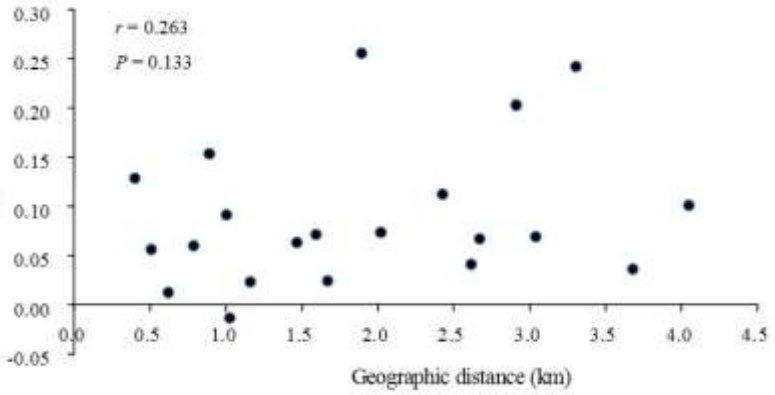

D. Mantel test of relationship between RST and geographic distance among subpopulations of *T. jackii*

Fig 5: Mantel test of relationship between genetic distance and geographic distance among subpopulations of *T. jackii*

Seven subpopulations of *T. jackii* are distributed on both sides of the creek, and the creek runs through them from the highest altitude to the lowest altitude. Considering that HQ subpopulation has the highest altitude, its seeds may spread to other subpopulations along with the water flow. Therefore, taking HQ subpopulation as the starting point and F_{ST} as the genetic distance, regression analysis was made on the genetic distance and geographical distance between HQ subpopulation and other subpopulations. The results are shown in Fig 6, from which it can be seen that there is a significant correlation between the genetic distance and geographical distance between HQ subpopulation and other subpopulations. This indicates that *Torreya jackii* seeds can move from upstream to downstream along with the water flow direction. Meanwhile, HQ subpopulation may also be the source population of other subpopulations.

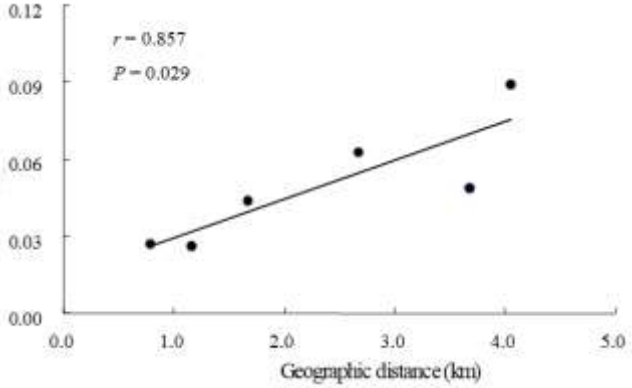

Fig 6: Regression analysis of genetic relationship between subpopulation HQ and other subpopulations

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The results of molecular variance analysis (AMOVA) showed that (Table VI) the genetic variation within the subpopulation of *T. jackii* accounts for 94% of the total variation, and the genetic variation among subpopulations accounts for 6%. This indicates that the genetic variation of the subpopulation of *T. jackii* is mainly distributed within the subpopulation.

Table VI. Analysis of molecular variance (AMOVA) of *T. jackii* subpopulations

Source of variation	*df*	SSD	MSD	Variance component	Total variance	*P*-value
Among subpopulations	6	47.432	7.905	0.135	6%	<0.001
Within subpopulation	301	590.036	1.960	1.960	94%	<0.001

Note: *P* value is calculated using 9999 random repetitions.

3.4 Bottleneck Effect Detection

According to the analysis results of BOTTLENECK (Table VII), the detection results of KW, WY, DC, QL, DL, XY subpopulations under IAM model, SMM model and TPM model have not reached significant level, while the detection results of allele frequency distribution are all in L-shaped state, which indicates that these six subpopulations have not experienced bottlenecks recently. However, the Sign test results of HQ subpopulation under IAM model and TPM model all reach significant level, and the test results of Wilcoxon test all reach extremely significant level, indicating that HQ subpopulation has experienced a bottleneck in the near future.

Table VII. Results from the BOTTLENECK test for subpopulations of *T. jackii*

subpopulation	IAM		SMM		TPM		Allele frequency distribution test
	Sign test	Wilcoxon test	Sign test	Wilcoxon test	Sign test	Wilcoxon test	
KW	0.1683	0.1914	0.4752	0.7695	0.4419	0.2734	L-shaped
WY	0.1934	0.1914	0.4360	0.7695	0.4725	0.3711	L-shaped
DC	0.0860	0.1484	0.1366	0.1484	0.0980	0.1484	L-shaped
QL	0.4040	0.1914	0.5239	0.5273	0.4378	0.1914	L-shaped
DL	0.4845	0.3203	0.4063	0.7695	0.5075	0.4727	L-shaped
XY	0.4385	0.5781	0.2293	0.9727	0.4387	0.6797	L-shaped
HQ	0.0384*	0.0059**	0.2368	0.0977	0.0457*	0.0059**	L-shaped

Note: *: *P*<0.05，**: *P*<0.01.

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IV. DISCUSSION

4.1 Subpopulation Genetic Structure

The average observed heterozygosity and expected heterozygosity were 0.5427 and 0.5060 in seven subpopulations of *T. jackii* in Baiyunyuan Forest Park, Tonglu County, Zhejiang Province, indicating that the overall genetic diversity of *T. jackii* in this area was at a high level. In addition, the average number of alleles, allele richness, observed heterozygosity and expected heterozygosity among the seven subpopulations had little difference, indicating that the distribution pattern of patches in the forest had no significant influence on the genetic diversity of the subpopulation of *T. jackii*. *T. jackii* Tonglu population has more plants than other populations in the whole distribution area, and *T. jackii* plants are relatively widely distributed in this area. At the same time, the terrain in this area is complex, with diverse habitats. The high spatial heterogeneity makes it possible for different genotypes of *T. jackii* to grow, so the total genetic diversity is at a high level. In addition, the reason may also be that the remaining subpopulations still maintain a certain intensity of gene flow, alleviating the isolation effect caused by being in different patches[37-40]. It is generally believed that wind-pollinated plants have strong gene diffusion ability[41-42]. *T. jackii* relies on wind-pollinated plants, so it theoretically has the ability of long-distance gene diffusion. In addition, the geographical distance among seven *T. jackii* subpopulations is less than 5 km, so there should be no great obstacle to gene communication among subpopulations through pollen. Therefore, this dioecious feature of wind-borne pollination can, to a certain extent, ensure that *T. jackii* maintains a relatively stable genetic diversity in different patches, thus maintaining the long-term viability of the population.

Mountainous areas of Zhejiang Province and Fujian Province are the key areas of terrestrial biodiversity in China[43], and they are also the areas where flora in East China is concentrated. Among it, subtropical endemic species account for a large proportion, but many species are endangered, and most of these species are in patch distribution pattern in the forest, which is very similar to the survival state of *T. jackii*. *T. jackii* is an endemic endangered tree species in mountainous areas of Zhejiang Province and Fujian Province. Seen from the results of this study, woody plants with similar breeding system characteristics as *T. jackii* may still have optimistic long-term survival prospects in this state.

On the other hand, the short duration of isolation among the subpopulations of *T. jackii* may also be the reason why its genetic diversity has not changed significantly. *T. jackii* is a perennial woody plant with a long generation period, but the number of generations experienced by *T. jackii* plants in seven subpopulations may be relatively limited, so the genetic effects caused by mutual isolation may not be revealed yet, which is similar to Muir et al. ' s research results on *Quercus petraea*[44].

In addition, the subpopulations growing in the cliff habitat have unique genetic characteristics. Although they do not have high genetic diversity, they have abundant private alleles, which preserve

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a rich gene pool for *T. jackii*, and can also provide a "corridor" or "stepping stone" for gene exchange for the subpopulation growing on both sides of the cliff. Therefore, they have important conservation value.

4.2 *T. jackii* Protection Strategy

The population of Baiyunyuan Forest Park in Tonglu of *T. jackii* has been divided into seven subpopulations isolated from each other in spatial distribution, but the research results in this paper show that its overall genetic diversity is still at a high level, which indicates that this patch-like spatial pattern has no significant impact on the genetic diversity of its population. Combined with field observation, it is found that there are still a certain number of seedlings and young trees in the population of *T. jackii* Tonglu Baiyunyuan Forest Park, and the population can still be naturally renewed in a short period of time, so the local protection strategy should be emphasized for this population. However, in the process of in-situ protection, some shrubs or small trees around *T. jackii* should be properly thinned, especially those vines entangled on *T. jackii* plants, so as to reduce the influence on the pollen and seed diffusion of *T. jackii*. In addition, we can artificially spread seeds to increase the distance of gene diffusion and promote gene exchange between patches, thus reducing the risk of inbreeding. At the same time, it is necessary to strengthen the management of seedlings and prohibit local villagers from picking *T. jackii* seeds at will.

Seen from each subpopulation, DL subpopulation has the largest number of plants, relatively high genetic diversity and abundant private alleles, and thus should be given priority protection, and the core protection plot of *T. jackii* can be considered in this area. In addition, there are abundant private alleles in XY subpopulation. However, due to the tourism development in recent years, tourism plank roads have been built on the cliffs one after another, which poses a serious threat to *T. jackii* plants growing on the cliffs. In the field observation, it was found that most of the *T. jackii* plants growing on both sides of the plank road had been cut down, while the *T. jackii* seeds near the plank road were easy to be picked. Therefore, this area should also be the object of key protection. Relevant management departments should strengthen publicity and set up warning signs on both sides of the plank road to prevent the *T. jackii* plants on the cliff from continuous damage.

In addition, because of the high overall genetic diversity of *T. jackii* Tonglu population, the sampling of this population can be given priority in ex-situ conservation. In the process of germplasm collection in this population, while fully sampling the population, attention should also be paid to the sampling interval to increase the representativeness of the sampled individuals and avoid repeated sampling of genetically similar individuals. At the same time, priority can be given to collecting *T. jackii* plants on cliffs for germplasm preservation.

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