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Local Adaptation in Natural Populations of *Toona ciliata* var. *pubescens* Is Driven by Precipitation and Temperature: Evidence from Microsatellite Markers

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Abstract: Environmental factors are strong drivers of local adaptation in forest tree species. *Toona ciliata* var. *pubescens*, an endangered tree species endemic to China, is widely distributed across Eastern and Southwestern China. In this study, we used 8 genomic microsatellite markers and 17 EST-SSR markers across nine populations from the Yunnan–Kweichow Plateau and Eastern China, to explore the adaptive variation and genetic structure of *T. ciliata* var. *pubescens*. Patterns of population structure were apparent using a Bayesian clustering program, STRUCTURE, which identified four distinct clusters. We identified four outlier loci that were potentially under selection using the Dirichlet-multinomial and hierarchic simulation models. Through the Mantel test, it was found that geographic and climatic factors have jointly affected the genetic structure of *T. ciliata* var. *pubescens* in the study area. Based on redundancy analysis (RDA), it was shown that the correlation between climatic variables associated with variation is stronger than that of geographic variables. It is worth mentioning that the eight alleles from outlier loci have potentially adaptive and are associated with either precipitation or temperature variables. All analyses revealed high genetic diversity and significant genetic differentiation in the populations of *T. ciliata* var. *pubescens*. This indicated that the climatic variables including precipitation and temperature are drivers of local adaptation in the populations of *T. ciliata* var. *pubescens*.

Keywords: *Toona*; Meliaceae; *T. ciliata* var. *pubescens*; adaptive variation; genetic conservation

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1. Introduction

Toona ciliata var. *pubescens*, known as "Chinese mahogany" in China, is deciduous, broad-leaved, fast-growing, entomophilous tree species of the *Toona* genus in the Meliaceae family. *T. ciliata* var. *pubescens* has red heartwood and an attractive growth grain, making it a valuable timber species in China [1–3]. Stands have been reduced due to continuously changing environmental, logging and slow regeneration [4]; in this case, *T. ciliata* var. *pubescens* is listed as a second-class endangered tree species in China [5–8]. Extant populations of *T. ciliata* var. *pubescens* are now mainly distributed along a 2000 km belt spanning the subtropical monsoon region, from the southwestern Yunnan–Kweichow Plateau to Eastern China. Due to the broad distribution of this species, its populations provide ideal material for the study of adaptive genetic variation.

Populations with a large geographical distribution tend to exhibit adaption to local environmental conditions [9]. Local adaptation occurs as populations evolve into different ecotypes in response to their local environmental conditions [10]. Local adaptation is a key driver of forest tree species' evolution and diversification. There are many examples of adaptive divergence in forest tree populations across a variety of spatial scales. Genetic differentiation in forest tree species is driven by environmental heterogeneity and the balance between selection and gene flow [11]. High levels of genetic variation among populations of forest tree species are common [12]. This variation often occurs along

climatic gradients of temperature and precipitation [13], indicating strong local adaptation to climate [14]. Local adaptation is also thought to be an important response to global climate changes [15]. To predict tree species' responses to a changing environment, it is first necessary to understand the adaptive genetic variation in natural populations. This information will assist forest resource managers in their efforts to mitigate the impacts of global climate change [16].

Adaptive genetic variation is defined as the variation found between the genomes in a species resulting from natural selection [17–19]. This variation is likely to emerge when selective forces are heterogeneous across a species' range. Genome-scan methods have been developed to study adaptive genetic variation by screening genetic markers and identifying those that are likely to be linked to the loci under selection [20–22]. By investigating the spatial distribution of alleles at different spatial scales (e.g., individual, population, metapopulation), these methods can determine the relationships between environmental variables and adaptive genetic variation.

At present, there are few reports and studies on the relationship between climate and environmental factors of *T. ciliata* var. *pubescens* and its adaptive genetic variation. The purpose of this work was to investigate the genetic structure and diversity of populations in *T. ciliata* var. *pubescens*, to examine the importance of geographical and climatic factors in constructing the genetic variation model of *T. ciliata* var. *pubescens*, and to identify the most important variables related to its genetic variation; this also provides a theoretical basis for its genetic conservation.

2. Materials and Methods

2.1. Sampled Populations

Nine natural populations of *T. ciliata* var. *pubescens* were chosen from two main regions, the Yunnan–Kweichow Plateau (YKP) and Eastern China (EC), including the cities of Binchuan, Yuanmou, Wuding, and Shizong in Yunnan Province; Ceheng in Guizhou Province; Xianju and Suichang in Zhejiang Province; Jingxian in Anhui Province; and Yifeng in Jiangxi Province. Both the YKP and EC belong to the subtropical monsoon climate. The YKP is high in the north and low in the south, descending in steps from north to south, with an altitude of 400–3500 m. Therefore, it has a rich and diverse natural environment, with significant climate differences. However, the EC has moderate annual temperatures, abundant rainfall, humid air, and four distinct seasons. Details of the environmental conditions characterizing each population are presented in Table 1 and Figure 1. Fresh leaves were collected from individual canopy trees for a total of 384 individual samples, of which 227 were from the YKP and 157 were from the EC. The leaves were dried rapidly by a ratio of silica gel to leaf biomass of 10:1. GPS (Magellan, eXporist 600, USA) was used to record the position of each individual sample.

Table 1. Geographic location and individual samples for nine natural populations of *T. ciliata* var. *pubescens*.

Population	No.	Province	Region	Longitude	Latitude	Altitude/m	Individual Samples
Yifeng	YF	Jiangxi	EC	114°29′ E	28°30′ N	375	65
Jingxian	JX	Anhui	EC	118°35′ E	30°31′ N	450	48
Xianju	XJ	Zhejiang	EC	120°32′ E	28°48′ N	620	24
Suichang	SC	Zhejiang	EC	119°12′ E	28°30′ N	510	20
Binchuan	BC	Yunnan	YKP	100°16′ E	25°02′ N	1520	60
Yuanmou	YM	Yunnan	YKP	101°49′ E	25°17′ N	1230	30
Wuding	WD	Yunnan	YKP	102°08′ E	25°47′ N	1702	29
Shizong	SZ	Yunnan	YKP	103°42′ E	24°21′ N	912	84
Ceheng	CH	Guizhou	YKP	105°40′ E	24°36′ N	710	24

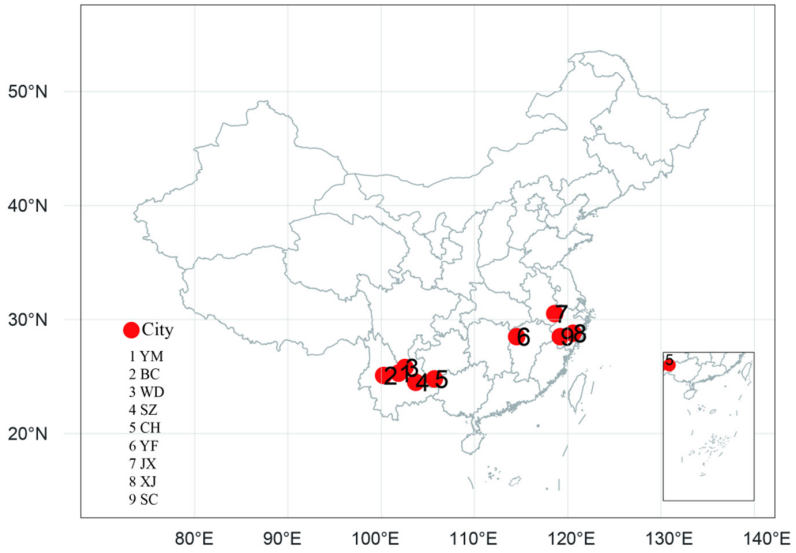

Figure 1. Distribution of sampled natural populations of *T. ciliata* var. *pubescens*. The red dot represents the location of the sampling city, and the numbers 1–9 represent the name of the city.

2.2. Molecular Analysis

Total genomic DNA was isolated from each sample with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Twenty-five microsatellite loci were analyzed (Table 2), including eight genomic microsatellite markers (Tc01~Tc08) [1] which were previously validated by our research group, and seventeen EST-SSR markers (Tc-A2~Tc-C81) which were obtained through transcriptome sequencing information on the roots, stems, and leaves of *T. ciliata* var. *pubescens*. PCR amplification was performed using the MYCYLE PCR Apparatus (BIORED, Hercules, CA, USA). A 10 µL reaction mix was used, comprising 1 µL genomic DNA, 0.2 µM for each forward primers and reverse primers, and 5 µL 2 × Master Mix (TSINGKE, Hangzhou, China). The reaction program of PCR was to undergo pre-denaturation at 94 °C for 4 min, undergo 35 denaturation-annealing cycles (1 min at 94 °C denaturation, 0.5 min at 52–55 °C annealing, and 1 min at 72 °C extension), and finally extend at 72 °C for 10 min. All PCR products were separated on the Qsep100 Genetic Analyzer (Bioptic, Xinbei Taiwan). The sizes of fragments were determined based on an internal lane standard (GeneScan 600 LIZ, Applied Biosystems, Foster City, CA, USA). The banding patterns were manually checked in the Q-Analyzer 2.0 software (Bioptic, Xinbei Taiwan).

Table 2. Characteristics of 25 microsatellite loci developed in *T. ciliata* var. *pubescens*.

Locus	Primer Sequences (5′–3′)	Repeat Motif	Allele Size (bp)	Ta (°C)	GenBank Accession No.
Tc01	F:GACTCGTGACACTTAGCCTGTA R:CTGGCGTAATCATGGTCATAC	(TTTCTC)7	231	55	DQ453903
Tc02	F:TAGGAAAGGCAAGGTGGG R:GGGTGGTCGATGAGGGTT	(AG)14	20	55	DQ453904
Tc03	F:AGTAATAGCCTGTAGAGCAG R:AGAGTGGGGTGGTCGATGAG	(AG)13	242	55	DQ453905
Tc04	F:GAAACCAGCAGGCAGAGC R:GAAGAAGGGTGAGCGAGA	(AG)10	230	55	DQ453906
Tc05	F:GATTACGCCAGGCAAACG R:TTGAATATGGGAGAAGGT	(CT)6	290	55	DQ453907
Tc06	F:ATGGATGAGTGTGCGATAGG R:TGTGATGTAGGAGTCTGAAC	(TC)7	280	55	DQ453912
Tc07	F:TGTCTCAGTTTATGCTGGCGT R:CTGCCCAATCAACAAGAG	(TC)8	260	55	DQ453914

Table 2. *Cont.*

Locus	Primer Sequences (5′–3′)	Repeat Motif	Allele Size (bp)	Ta (°C)	GenBank Accession No.
Tc08	F:TCAATGCAATTTAGGAGGAA R:TGCTTGTTGAACCCTGTG	(GA)8	291	52	DQ778303
Tc-A2	F:TGGAAAGATTTCATGGGCTC R:GGTGTGAATTGTCGGCTGTA	(TTC)5	199	60	MH593319
Tc-A3	F:AATGTGGAATGGAATGGAGC R:CGTCTCTTTCCCAACCTCAC	(TA)6	272	60	MH593320
Tc-A7	F:GGGTCCATTTCTCAGTGGTC R:TTCAACTCATCCCGTTCACA	(TGGGG)5	166	59	MH593321
Tc-A9	F:TCGGGTGGTAAGGCTAAAGA R:TTTTGCATTGCGTAGCATTC	(AGC)5	234	60	MH593322
Tc-A12	F:GAGATCGGTCCCTCTTCTCC R:TAGCGGAGGGGATAGGAAGT	(CTT)5	183	60	MH593323
Tc-B4	F:CCTGGGAAAGTTGTCAGCTC R:CAAGCTGGGTTTCTTCTTGG	(GCA)5	149	60	MH593324
Tc-B11	F:AGATCAAATCCGGGGAGATT R:CAGCAAAGCCAACTCATCAA	(TA)6	133	60	MH593325
Tc-B25	F:CAGTGCGATCATCACCCTTA R:GGTTCCGGGATTGTAGGACT	(TA)6	273	60	MH593326
Tc-B26	F:GGAGTTGCCATGGATGAAGT R:CCAGGATCAGCAACCTCAAT	(GCT)6	207	60	MH593327
Tc-B27	F:GGCAGAGAAGAGCGGTTTTA R:CGGATCTTTCGCAACGTAGT	(AG)8	191	60	MH593328
Tc-C11	F:CAAGCGAAGAGAGAGAAAGAGG R:ACCAAAGCTTTAGGCAGCAA	(AGA)5	195	60	MH593329
Tc-C26	F:AACAGAAATTCGCCAACCAG R:AATTCACACCAGCCACTTCC	(AAC)7	250	60	MH593330
Tc-C49	F:TAAACCTCCCAAGGTAGCGA R:GCACGACAGCACTTCATGTT	(TGC)6	267	60	MH593331
Tc-C50	F:TGGACACCAAGGGATCTAGC R:TTTGGGTCCATGATCCATCT	(AGC)5	235	60	MH593332
Tc-C66	F:GCACAGGTCATGAAAGAGCA R:CACCAGCAAAACGAAGAACA	(TA)6	250	60	MH593333
Tc-C77	F:CGGAAAAACCTCAATTGTTTTG R:TGCAATAACAGCACCAGCTC	(AT)8	229	60	MH593334
Tc-C81	F:AACGGTCAGAATCTGGATGG R:GCACCACCACCCCTAGAGTA	(GGT)6	269	60	MH593335

2.3. Climatic Data

Bio-climatic data for the 9 populations were extracted from the WorldClim 30-arcsec (ca.1 km) data set (http://www.worldclim.org/) [23]. In total, 22 environmental factors were considered. Three are geographical factors, namely, latitude of the population(Lat), longitude of the population(Lon), and elevation of the population (Ele). The remaining nineteen are bio-climatic factors (Bio1 to Bio19). The description of each bio-climatic factor is shown in Table 3.

2.4. General Statistical Analyses

The mean observed number of alleles (*Na*), effective number of alleles (*Ne*), observed heterozygosity (H_O), expected heterozygosity (H_E), and polymorphism information content (*PIC*) [24] were calculated for each population using POPGENE 32 (Version 1.32, Edmonton, AB, Canada) [25]. The analysis of molecular variance (AMOVA) for the genetic variation between regions and populations was conducted with ARLEQUIN 3.5 [26]. Significance testing was provided by 1000 permutations. The STRUCTURE 2.3.4 software (CA, USA) [27] was used to compute the most likely number of population clusters (K). The number of possible clusters (K) varied from 2 to 8 with ten replications each, and the most suitable value was selected through comparison. GenAlex6.502 was used for principal coordinate analysis (PCoA) to draw a scatter plot consisting of principal coordinates 1 and 2.

Table 3. Bio1–Bio19 for analyzing relative contributions.

Factors	Name	Description
Climate variables	Bio1	mean annual air temperature
	Bio2	mean diurnal air temperature range
	Bio3	isothermality
	Bio4	temperature seasonality
	Bio5	mean daily maximum air temperature of the warmest month
	Bio6	mean daily minimum air temperature of the coldest month
	Bio7	annual range of air temperature
	Bio8	mean daily mean air temperatures of the wettest quarter
	Bio9	mean daily mean air temperatures of the driest quarter
	Bio10	mean daily mean air temperatures of the warmest quarter
	Bio11	mean daily mean air temperatures of the coldest quarter
	Bio12	annual precipitation amount
	Bio13	precipitation amount of the wettest month
	Bio14	precipitation amount of the driest month
	Bio15	precipitation seasonality
	Bio16	mean monthly precipitation amount of the wettest quarter
	Bio17	mean monthly precipitation amount of the driest quarter
	Bio18	mean monthly precipitation amount of the warmest quarter
	Bio19	mean monthly precipitation amount of the coldest quarter

2.5. Identification of Outlier Loci

The detection of outlier loci was performed by means of two different software packages, ARLEQUIN 3.5 [26] and BayeScan [28]. Both methods use locus-specific genetic differentiation (FST) outliers to detect candidate markers under selection [29]. The ARLEQUIN software was used to perform analysis with a hierarchic simulation model. The hierarchic model is supposed to be the most suitable one for populations sharing recent common ancestry, reducing the number of false-positive outlier loci [30]. BayeScan is a Bayesian-based method that depends on a highly differentiated locus [28], relative to background differentiation levels, and selects sites with a false discovery rate (FDR) of less than 0.05 in the results. Both the ARLEQUIN and BayeScan analyses use default settings.

2.6. Genetic Variation Related with Climatic and Geographic Distance

To analyze the correlation between climatic, geographic, and genetic distances, a Mantel test was performed using the vegan package in R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria) [31]. Levels of significance was tested with 10,000 permutations. Pairwise genetic and geographic distance matrices were calculated using GenAlex and a standardized Euclidian distance matrix was produced for bioclimatic variables using SPSS v.22 (SPSS Inc., Chicago, IL, USA; http://www.spss.com). We performed redundancy analysis (RDA) by Canoco4.5 to examine the relative contribution of geographic and climatic data, and combination of the variables in driving genetic structure [31]. To avoid an overestimation of the contribution of environmental variables to the population structure, strongly correlated climatic and geographical factors were excluded from the original 22 variables by Pearson's correlation analysis. The eight remaining variables (Ele, Bio1, Bio2, Bio5, Bio8, Bio9, Bio16, and Bio18) were selected for RDA and environmental association analyses.

2.7. Associations between Microsatellite Allele Frequency and Climatic and Geographical Variables

Pearson's correlation analysis was used to investigate the associations between allele frequency of outlier loci and different climatic and geographical variables using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Markers with a frequency of less than 0.05 were removed from the analysis.

3. Results and Analysis

3.1. Genetic Variation and Population Structure

The statistical analysis results are presented in Table 4. The *Na* ranged from 2.64 in population XJ to 4.04 in population JX with a global mean of 3.46. The *Ne* ranged from 1.93 in population XJ to 2.82 in population CH, with an average of 2.29. It can be seen that there is a significant difference between the values of *Na* and *Ne*. The H_O was 0.32–0.74, and the mean value was 0.57. The H_E ranged from 0.43 to 0.61 with an average of 0.52. The mean of H_O was slightly higher than that of H_E.

Table 4. Summary of genetic variation in *T. ciliata* var. *pubescens* populations.

Population	*Na*	*Ne*	H_O	H_E	*PIC*
YF	3.40	2.08	0.58	0.49	0.42
JX	4.04	2.41	0.49	0.54	0.48
XJ	2.64	1.93	0.63	0.46	0.38
SC	2.76	2.12	0.48	0.51	0.42
YM	3.92	2.56	0.66	0.61	0.53
BC	3.32	2.32	0.74	0.50	0.42
WD	3.52	2.33	0.67	0.53	0.46
SZ	3.72	2.02	0.59	0.43	0.36
CH	3.83	2.82	0.32	0.60	0.54
Mean	3.46	2.29	0.57	0.52	0.45

Na: mean observed number of alleles; *Ne*: effective number of alleles; H_O: observed heterozygosity; H_E: expected heterozygosity; *PIC:* polymorphic information content.

When analyzing the genetic structure of the populations, the rate of change in the Napierian logarithm probability relative to the standard deviation (ΔK) showed a distinct ΔK peak at K = 4 (Figure 2A). Therefore, it indicated that populations of *T. ciliata* var. *pubescens* were structured into four distinct clusters. The clusters were closely related to geography (Figure 2B). As shown in the figure, the distribution was reasonable when K = 4, and the four groups could be divided into two regions (YKP and EC). Principal component analysis (PCoA) was conducted on all samples to further explore the genetic relationship between populations of *T. ciliata* var. *pubescens*. The results showed that the first three principal coordinates of the PCoA of 384 individuals explained 34.29%, 22.59%, and 12.61% of the genetic variation, respectively, and accounted for 69.49% of the total variation (Figure 3).

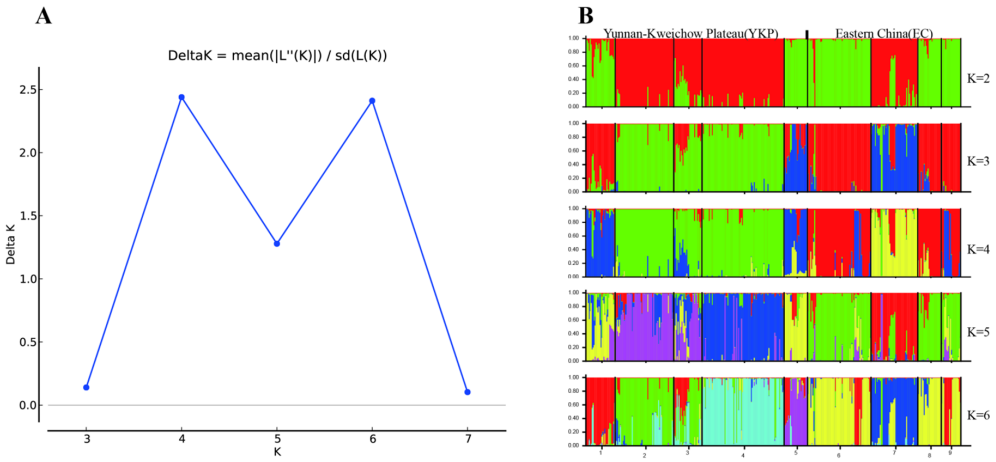

Figure 2. Analysis results of the genetic structure of the nine populations of *T. ciliata* var. *pubescens*. (**A**) The relationship between Delta K and K values. (**B**) The genetic structure of the *T. ciliata* var. *pubescens* population with different K values (K = 4–6).

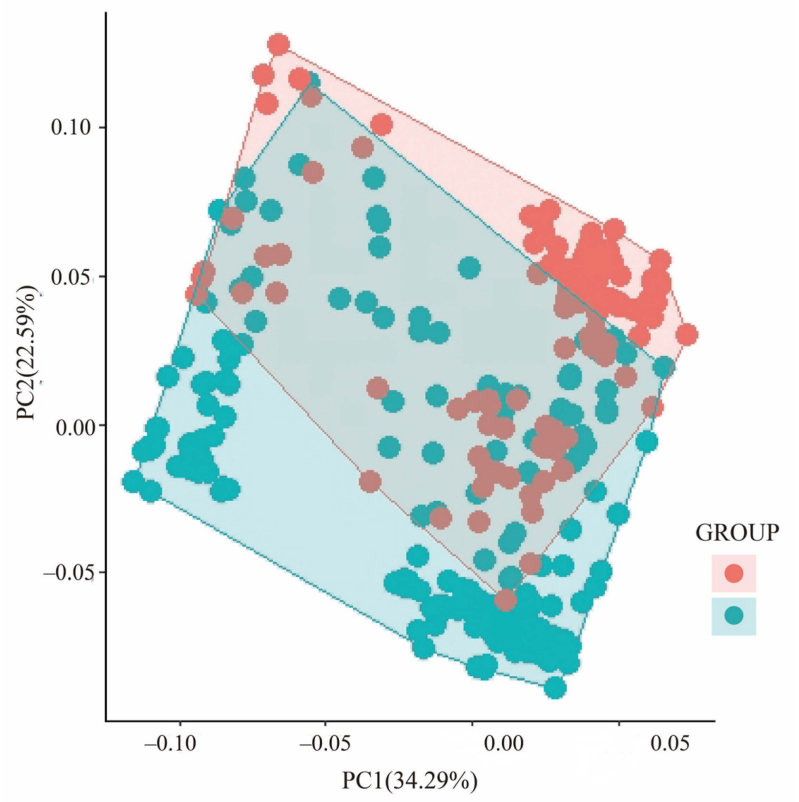

Figure 3. The principal coordinate analysis (PCoA) of the 384 *T. ciliata* var. *pubescens* individuals.

3.2. Genetic Differentiation and the Identification of Outlier Loci

The AMOVA showed highly significant genetic differentiation across levels of aggregation: among regions, among populations, and within populations (Table 5). This indicated that environmental factors partially affected the genetic variation. Genetic variation within populations has the highest variance component (59.1%); the remaining variations occur among regions and populations. The percentage of estimated genetic variation among regions is 10.5%, and the value among populations is 30.4% (Table 5).

Table 5. Analysis of molecular variance (AMOVA) for the genetic variation.

Source of Variation	d.f.	Sum of Squares	Percentage of Variation	*p*-Value
Among regions	1	784.051	10.5	0.001 **
Among populations within regions	8	1946.535	30.4	0.001 **
Within populations	375	4856.182	59.1	0.001 **
Total	384	7586.768		

** p-value $\leq$ 0.01.

Nine outlier loci (FDR = 0.05) were detected by BayeScan, including loci Tc01, Tc02, Tc05, Tc07, Tc-A7, Tc-B11, Tc-B25, Tc-C11, and Tc-C50. The hierarchical approach performed in ARLEQUIN reported six outlier loci with a significant *p*-value, including Tc05, Tc07, Tc-A7, Tc-B11, Tc-C49, and Tc-C66. Four outlier loci (Tc05, Tc07, Tc-A7, and Tc-B11) were the same in both analysis methods (Figure 4).

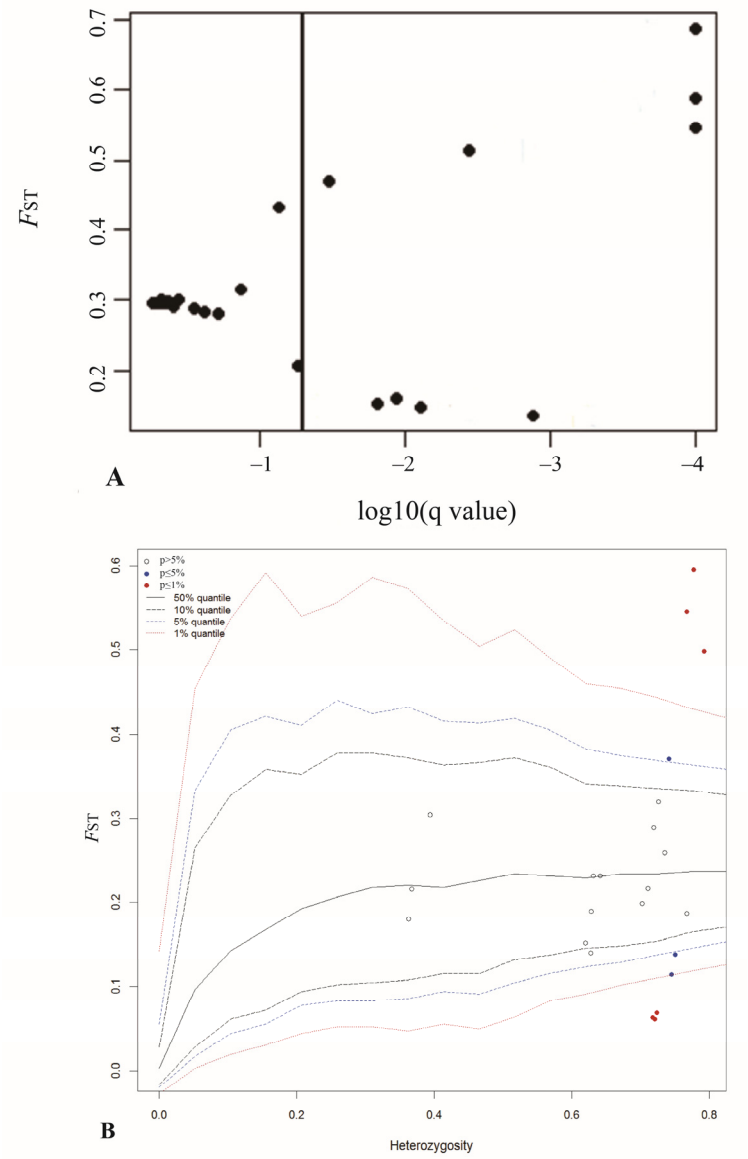

Figure 4. Results of the BayeScan search for putative outlier loci affected by selection. (**A**) This plot presents FST against log10(*q*-value), which is the FDR analog of the *p*-value. The line represents threshold FDR = 0.05 and the red dots are the outlier loci that are potentially affected by selection. (**B**) Outlier loci were detected under the hierarchical island model (HIM) with ARLEQUIN 3.5. Loci significant at 5% (blue dots) and 1% (red dots) are indicated.

3.3. Genetic Variation Explained by Climatic and Geographical Distance

The Mantel test revealed a significant correlation between genetic distance and geographical distance ($r = 0.3815$, $p = 0.002$), and a significant correlation between genetic distance and climatic distance ($r = 0.5624$, $p = 0.003$). The results of RDA explained that the variation related with climatic variables played a more important role than that linked to elevation (29.4% and 4.8%, respectively). The percentages of the total eigenvalues of axes 1 and 2 were 31.43% and 21.49%, respectively (Figure 5).

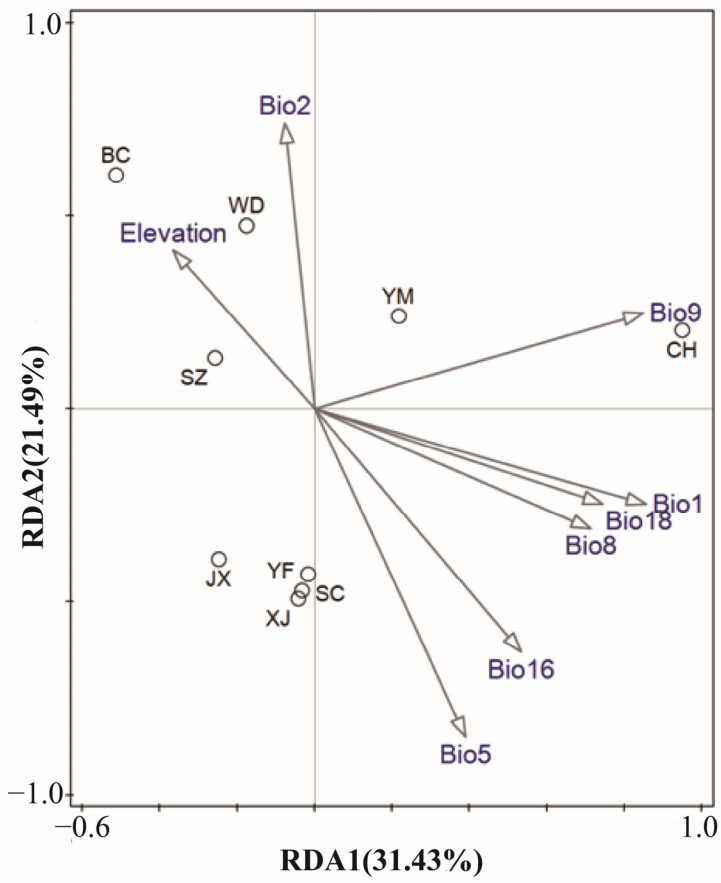

Figure 5. RDA results of relative contributions of climate and altitude.

3.4. Associations between Microsatellite Allele Frequency and Climatic and Geographical Variables

The correlations between allele frequencies and the eight selected environmental variables are presented in Table 6. Six alleles (Tc05-1, Tc05-7, Tc07-2, Tc-A7-1, Tc-B11-2, and Tc-B11-4) were associated with temperature variables, namely, mean annual air temperature (Bio1), mean diurnal air temperature range (Bio2), mean daily maximum air temperature of the warmest month (Bio5), mean daily mean air temperatures of the wettest quarter (Bio8), and mean daily mean air temperatures of the driest quarter (Bio9). Four alleles (Tc05-7, Tc07-2, Tc07-5 and Tc-B11-5) were associated with precipitation variables, namely, mean monthly precipitation amount of the wettest quarter (Bio16) and mean monthly precipitation amount of the warmest quarter (Bio18).

Table 6. Pearson's correlation between allele frequency at outlier loci and geographic and climatic factors.

Allele	Elevation	Bio1	Bio2	Bio5	Bio8	Bio9	Bio16	Bio18
Tc05-1	−0.151	0.227	−0.384	0.693 *	−0.066	−0.085	0.410	−0.039
Tc05-2	−0.024	0.058	−0.180	0.420	−0.063	−0.126	0.268	−0.179
Tc05-4	0.264	−0.280	0.555	−0.478	−0.307	−0.011	−0.511	−0.423
Tc05-5	−0.012	−0.442	0.144	−0.436	−0.163	−0.366	−0.410	−0.202
Tc05-6	0.626	−0.550	0.183	−0.559	−0.522	−0.326	−0.434	−0.375
Tc05-7	−0.296	0.810 **	−0.129	0.269	0.720 *	0.841 **	0.501	0.841 **
Tc07-1	0.212	−0.251	0.575	−0.487	−0.295	0.018	−0.444	−0.328
Tc07-2	0.234	−0.453	0.731 *	−0.787 *	−0.457	−0.065	−0.669 *	−0.436

Table 6. *Cont.*

Allele	Elevation	Bio1	Bio2	Bio5	Bio8	Bio9	Bio16	Bio18
Tc07-3	−0.004	0.266	0.017	0.349	0.170	0.169	0.363	−0.077
Tc07-4	−0.398	0.322	−0.472	0.661	0.255	0.032	0.467	0.217
Tc07-5	−0.246	0.432	−0.587	0.447	0.520	0.159	0.480	0.675 *
Tc07-6	−0.227	−0.113	0.117	−0.184	0.021	−0.098	−0.076	0.089
Tc07-7	0.540	−0.561	0.369	−0.654	−0.523	−0.269	−0.612	−0.510
Tc-A7-1	−0.176	0.211	−0.454	0.728 *	−0.043	−0.141	0.430	−0.023
Tc-A7-2	−0.082	0.161	−0.251	0.496	0.052	−0.052	0.361	−0.059
Tc-A7-4	0.232	−0.185	0.546	−0.451	−0.224	0.091	−0.457	−0.327
Tc-A7-5	0.008	−0.442	0.207	−0.475	−0.193	−0.333	−0.439	−0.227
Tc-A7-6	0.626	−0.550	0.183	−0.559	−0.522	−0.326	−0.434	−0.375
Tc-B11-1	0.231	−0.253	0.541	−0.463	−0.291	0.003	−0.436	−0.336
Tc-B11-2	0.214	−0.311	0.809 **	−0.808 **	−0.356	0.136	−0.635	−0.315
Tc-B11-3	0.077	−0.258	−0.060	0.221	−0.247	−0.431	0.043	−0.501
Tc-B11-4	−0.437	0.505	−0.454	0.715 *	0.338	0.250	0.559	0.360
Tc-B11-5	−0.225	0.416	−0.570	0.398	0.570	0.156	0.488	0.688 *
Tc-B11-7	0.536	−0.558	0.371	−0.653	−0.521	−0.266	−0.612	−0.511

* p-value $\leq$ 0.05, ** p-value $\leq$ 0.01.

4. Discussion

4.1. Population Structure and Genetic Differentiation

Quantifying local adaptation is fundamentally important for population conservation, evolution, and global-change biology [32,33]. Population analyses of adaptive genetic variation have become more sophisticated since FST was first used to investigate local adaptation at the molecular level in 1973 [34–36]. In this study, we used landscape genomics modeling to infer local adaptation to climatic and geographical factors in natural populations of *T. ciliata* var. *pubescens*. We found high levels of intra-population genetic variation, consistent with other studies of broad-leaved trees [37–40]. Our STRUCTURE analysis showed that the nine populations of *T. ciliata* var. *pubescens* were most parsimoniously grouped into four distinct genetic clusters (cluster1–cluster4).

These clusters likely relate to ecotypes from different geographical regions: cluster1 and cluster2 from the Yunnan–Kweichow Plateau (YKP); cluster3 and cluster4 from Eastern China (EC). However, when two clusters were specified (K = 2) in the STRUCTURE analysis (Figure 2B), the populations did not segregate by region (e.g., BC, WD, SZ, and JX were in one cluster). One possible reason for this could be historical gene flow [41–43] between populations of *T. ciliata* var. *pubescens*. Estimating gene flow is a vital element in local adaptation studies, since it can have a strong (often antagonistic) effect on local genetic structure [44,45]. However, due to climate change and habitat fragmentation, gene flow is likely restricted among populations of *T. ciliata* var. *pubescens* [18]. We speculate that due to the large distance and limited gene exchange, the genetic variation of the population will become smaller and smaller.

Two hypotheses could explain the genetic differentiation among populations observed in this study. First, the distance between the two regions of the Yunnan–Kweichow Plateau and Eastern China (>1000 km) may have inhibited gene flow. Second, significant environmental differences between the two regions may have driven divergent selection and local adaptation in the two regions. Populations in the two regions may represent different ecotypes. Ecotype differentiation can be evaluated by multivariate environmental and genetic distance analyses [46,47]. RDA analysis can combine multiple environmental factors simultaneously, and its results are intuitive, clear, and contain a large amount of information. In this study, the Mantel test and RDA showed that geographical (elevation) and climatic factors have jointly affected the genetic differentiation of *T. ciliata* var. *pubescens*.

4.2. Outlier Loci Detection and Associations with Environmental Variables

Four out of twenty-five loci from this study were identified as outlier loci by BayeScan and ARLEQUIN. This result is consistent with previous landscape genomics studies, such

as the 2 outlier loci out of 15 loci in *Quercus rubra* L. and *Quercus ellipsoidalis* identified by E. J. Hill [48], and the 19 outlier loci out of 144 loci in *Picea abies* identified by L. Karst [49].

We expected that the outlier loci would be associated with the environmental variables. Pearson's correlation between allele frequency of outlier loci and geographical and climatic factors showed that climatic variables, such as temperature and precipitation variables (Bio1, Bio2, Bio5, Bio8, Bio9, Bio16, and Bio18), were the main environmental variables affecting the adaptation of *T. ciliata* var. *pubescens*. Temperature and precipitation strongly affect the survival of plants, often via complex pathways [50]. Low temperature has adverse effects on plants from small to cellular levels, as well as on tissues and organs, thereby inhibiting their growth, development, and even survival. Precipitation limitations, especially insufficient precipitation during the growing season, can have limitations similar to low temperature on plant growth, development, and reproduction. These environmental factors are likely to act as major drivers of selection in plants [51]. We found the temperature and precipitation variables to be correlated with several of the allele frequencies at the outlier loci in *T. ciliata* var. *pubescens* that we studied. Previous studies have shown the prominent role of temperature and precipitation in driving adaptation of plants [52–55]. The correlations between climate and signals of adaptation in *T. ciliata* var. *pubescens* studied here indicate that there should be ample standing genetic variation available in populations of *T. ciliata* var. *pubescens* for adaptation to occur in the course of climate change.

5. Conclusions

As a precious tree species in China, *T. ciliata* var. *pubescens* is widely distributed, and studying its genetic variation is of great significance. Our study utilized 25 microsatellite markers to conduct genetic variation analysis on 384 samples from the YKP and EC, that revealed a high genetic diversity and significant genetic differentiation among populations of *T. ciliata* var. *pubescens*. Significant correlations between genetic distance and climatic and geographical distance were identified, which suggested that climatic and geographical variables were responsible for the adaptive variation among populations. The RDA results indicate that climatic variables had a stronger association with genetic variation than elevation. Eight alleles from four outlier loci are potentially associated with adaptation to two climatic factors: precipitation and temperature. We thus predict that the genetic diversity of *T. ciliata* var. *pubescens* will decline due to future climate change, which may exacerbate the endangered form of *T. ciliata* var. *pubescens*. Therefore, it is necessary to strengthen the conservation of different geographic populations of *T. ciliata* var. *pubescens*, and more research is needed to assess the capabilities of local adaptation to new environmental conditions in populations of *T. ciliata* var. *pubescens*. This study analyzed the results of local adaptation of *T. ciliata* var. *pubescens* from the perspectives of climate factors and altitude. As for the impact of other factors such as terrain, soil, and human interference on its distribution, further collection and accumulation of relevant data are needed for analysis to reveal its causes more comprehensively. In summary, in order to implement effective management and ecological conservation, more research is needed to evaluate the local adaptability of *T. ciliata* var. *pubescens* populations to new environments.

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