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RESEARCH ARTICLE

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Genetic diversity of *Lithocarpus harlandii* populations in three forest communities with different succession stages

Abstract By using random amplified polymorphic DNA (RAPD) technique, this paper studied the genetic diversity and genetic differentiation of *Lithocarpus harlandii* populations in three forest communities (coniferous forest, coniferous and broad-leaved mixed forest, and evergreen broad-leaved forest) with different succession stages in Tiantai Mountain in Zhejiang Province. The results showed that a total of 173 repetitive loci were produced in 60 individuals of *L. harlandii* by 12 random primers, among which, 152 loci were polymorphic, and the total percentage of polymorphic loci was 87.86%. The average percentage of polymorphic loci of the populations was 65.32%, and their total genetic diversity estimated by Shannon information index was 0.4529, with an average of 0.3458, while that judged from Nei's index was 0.3004, with an average of 0.2320. The percentage of polymorphic loci, Shannon information index, and Nei's index of the populations were in the sequence of coniferous forest community > coniferous and broad-leaved mixed forest community > evergreen broad-leaved forest community. Analysis of molecular variance (AMOVA) showed that 72.85% of genetic variance was found within the populations, and 27.15% of genetic variance resided among the populations. The coefficient of gene differentiation was 0.2277, and the gene flow was 1.6949. The genetic structure of *L. harlandii* was influenced not only by the biological characteristics of this species, but also by the microenvironment of different communities. The mean of genetic identity among three populations of *L. harlandii* was 0.8662, and the mean of their genetic distance was 0.1442. The genetic similarity between coniferous and broad-leaved mixed forest community and evergreen broad-leaved forest community was the highest, while that between evergreen broad-leaved forest community and coniferous forest community was the lowest. The unweighted pair group method with arithmeticmean (UPGMA) cluster analysis based on Nei's genetic distance showed that coniferous and broad-leaved mixed forest community first gathered with evergreen broad-leaved forest community, and then with coniferous forest community.

Keywords *Lithocarpus harlandii*, RAPD, genetic diversity, genetic differentiation, community succession

1 Introduction

Plant community succession is a dynamic process where a plant community is replaced by another. In the subtropical mountainous region of eastern China, there universally exists a succession trend from *Pinus massoniana* forest to evergreen broad-leaved forest (Zhou et al., 1999). Since water and temperature conditions are good in Tiantai Mountain in Zhejiang Province, plant growth is flourishing, flora is complex (Jin, 1994), and the community types are varied. The integrity successional series from pioneer communities of coniferous forests to evergreen broad-leaved forest climax community exist in Tiantai Mountain, thus, it is an ideal area for community succession research. The community structure can be determined by the dominant populations and the main features of the community environment and studies on the dynamics of dominant populations in forest communities have great significance to clarify the succession law of forest communities (Shi et al., 2003). Community succession is also a process where the population genetic diversity changes gradually. The genetic diversities of different dominant populations in different successional communities are extremely varied, and not consistent

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with the form displayed by shape (Wang et al., 2004). *Lithocarpus harlandii* is one of the main constructive species in the subtropical evergreen broad-leaved forest of China (Jin, 1999). It widely distributes in the community with different succession stages in Tiantai Mountain. The photosynthetic ecophysiological characteristics of *L. harlandii* in the different habitats in Tiantai Mountain had been studied (Ke et al., 2004). However, there are no studies on the genetic diversity of *L. harlandii* populations in forest communities with different succession stages.

Molecular markers used in the study of genetic diversity mainly include allozymes, random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), inter-simple sequence repeat (ISSR) and amplified fragment length polymorphism (AFLP) and so on. Among which, the RAPD technology has been widely used in recent years to characterize the genetic diversity and genetic structure of woody plants because of it is simple, rapid, economical, low DNA dosage and good polymorphism (Palacios and Gonzalez, 1997; Xia et al., 2001; Li and Jin, 2005). Recent studies showed that RAPD could be used as reliable molecular markers just like AFLP (Kjølner et al., 2004). The aim of this study is to understand succession and the close relations between succession and population genetic behavior through the analysis of the genetic diversity of *L. harlandii* populations in the forest communities with different succession stages in Tiantai Mountain in Zhejiang Province using RAPD technology. It has the vital significance both in theory and in practice (Wang et al., 2004).

2 Materials and methods

2.1 Study area

Tiantai Mountain is located in central subtropical China (29°09′–29°28′N, 120°50′–121°24′E) and the vegetation is made up of evergreen broad-leaved forests (Jin, 1998). The coniferous forest is made up of *P. massoniana* with a simple community structure. *P. massoniana* is dominant in the community, the canopy density of the community is low (45%), understory light is sufficient, understory plants are dense and the positive species such as *Schima superba* and *L. harlandii* have emerged. The dominant specie of the coniferous and broad-leaved mixed forest community is *P. massoniana*, the codominant species are *S. superba* and *Castanopsis eyrei*. The community's light, soil, water and other conditions are changed due to the growth of *P. massoniana*; the habitat is improved and the suitable species increased. The canopy density of the community increased (74%), and some of the shade-tolerant plants emerged and settled down. The evergreen broad-leaved forest is made up of *C. eyrei* with complex community structure. The canopy density of the community is big (91%), and the understory species and individuals are few.

2.2 Methods

2.2.1 Sample collection and processing

In May 2002, according to the different stages of succession communities, three communities, i.e., coniferous forest, coniferous and broad-leaved mixed forest, and evergreen broad-leaved forest in Shiliang Senic in Tiantai Mountain were chosen for plant sample collection. The sampling spot for the coniferous forest was 800 m away from that of the coniferous and broad-leaved mixed forest, and that of the broad-leaved mixed forest was 600 m away from that of the evergreen broad-leaved forest, and that of the evergreen broad-leaved forest was 1300 m away from that of the coniferous forest. P_1, P_2, P_3, were designated as the population of *L. harlandii* in the coniferous forest, coniferous and broad-leaved mixed forest, and evergreen broad-leaved forest, respectively. Fresh tender leaves were collected randomly from 20 adult plants with distances of longer than 30 m in three populations. The leaves were kept at 4°C and were taken back to the laboratory. Leaves were washed and stored at −70°C until DNA extraction.

2.2.2 DNA extraction and quantification

DNA was extracted following the optimized sodium dodecyl sulfate (SDS) method (Li et al., 2002). DNA was then run on a 0.8% agarose gel and photographed on a GIS-2008 gel imaging and analysis system (Shanghai Tanon Science & Technology Co., Ltd.). The DNA concentration was determined and stored at −20°C for RAPD amplification.

2.2.3 PCR amplification and products identification

Random primers were purchased from Sangon Inc., Shanghai, China. The determined optimal reaction system was as follows: 1 × *Taq* polymerase buffer (10 mmol/L Tris-HCl, pH 9.0, 50 mmol/L KCl, 0.1% Triton X-100), 0.15 mmol/L each of dATP, dCTP, dGTP or dTTP, 0.75 U *Taq* DNA polymerase (Huamei Inc., Shanghai, China), 10 ng template DNA, 3.6 pmol primer (Sangon Inc., Shanghai, China), 1 μg/μL bovine serum albumin (BSA) in the total 10 μL reaction volume. The reference substance was run as well by replacing template DNA with double-distilled water. The determined optimal PCR cycle program included an initial 5 min denaturation at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 40°C and 1.5 min at 72°C, and 5 min final extension at 72°C. Amplification reaction was performed in a P × 2 Thermal Cycler (Thermo Hybaid, Inc.). PCR product was electrophoresed in 1.4% agarose gel (stained with 0.5 μg/mL ethidium bromide). The electrophoresis buffer was 0.5 × TBE. Images were photographed with GIS gel imaging and analysis system (Shanghai Tanon Science & Technology Co., Ltd.).

2.2.4 Data analysis

200 bp DNA ladder was the reference to the standard molecular weight marker. A data matrix of RAPD profiles was obtained based on the locations of the reaction products. The presence of the fragment was represented with "1" and the absence was represented with "0". The matrix was used as the input for POPGENE32 software. The percentage of polymorphic loci, Nei's gene diversity, and Shannon's information index, genetic differentiation coefficient (G_{st}), genetic identity and genetic distance between populations were estimated. Gene flow (N_m) was estimated according to the value of G_{st}. According to the genetic distance between populations, unweighted pair group method arithmetic average (UPGMA) cluster analysis was performed to demonstrate the relationships among populations. Simultaneously, AMOVA (analysis of molecular variance) 1.55 software was used to analyze the distribution of genetic variation within and among populations (Excoffier et al., 1992).

3 Results

3.1 Genetic diversity of *L. harlandii* populations

Twelve random primers (Table 1) with clarity and reproducibility of banding patterns were screened from 100 primers for RAPD analysis of a total of 60 individual DNA samples of three *L. harlandii* populations. A total of 173 repetitive loci were produced, among which 152 loci were polymorphic, and the total percentage of polymorphic loci was 87.86% (Table 2). RAPD amplification of *L. harlandii* population with primer S71 in the coniferous forest is given in Fig. 1. The percentage of polymorphic loci of three *L. harlandii* populations ranged between 57.23% and 75.72%, with an average of 65.32%. The percentage of polymorphic loci of P_1 population was the highest, while that of P_2 population was second and that of P_3 population was the lowest.

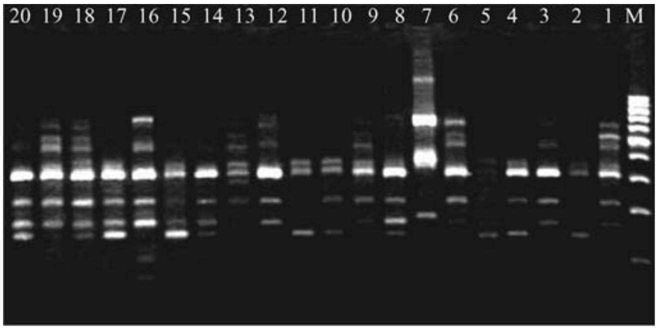

Fig. 1 RAPD amplification of *L. harlandii* population in coniferous forest with primer S71
M: 200 bp DNA ladder molecular weight marker; 1–20: individuals of *L. harlandii*

Estimated by Shannon information index, the total genetic diversity of three *L. harlandii* populations was 0.4529 (Table 2), while the genetic diversity of populations ranged between 0.3136 and 0.3848, with an average of 0.3458. That of P_1 population was the highest, while that of P_2 population was next and that of P_3 population was the lowest. Estimbaed by Nei's index, the total gene diversity was 0.3004, and those of populations ranged from 0.2119 to 0.2561, with an average of 0.2320. The genetic diversity estimated by Nei's index was lower than that estimated by the Shannon information index, but the rank order was consistent, i.e., that of P_1 population is the highest, that of

Table 1 Sequences of 12 random primers used in RAPD analysis

primers	sequences (5′-3′)	primers	sequences (5′-3′)	primers	sequences (5′-3′)
S329	CACCCCAGTC	S309	GGTCTGGTTG	S70	TGTCTGGGTG
S332	TCAACGGGAC	S112	ACGCGCATGT	S72	TGTCATCCCC
S306	ACGCCAGAGG	S62	GTGAGGCGTC	S73	AAGCCTCGTC
S303	TGGCGCAGTG	S69	CTCACCGTCC	S71	AAAGCTGCGG

Table 2 Genetic diversity for three populations of *L. harlandii*

	no. of samples	total of loci	no. of polymorphic loci	percentage of polymorphic loci/%	Shannon information index	Nei's index
P_1	20	173	131	75.72	0.3848	0.2561
P_2	20	173	109	63.01	0.3391	0.2279
P_3	20	173	99	57.23	0.3136	0.2119
Average	20	173	113	65.32	0.3458	0.2320
Total	60	173	152	87.86	0.4529	0.3004

P_2 population was second, and that of P_3 population was the lowest.

3.2 Genetic differentiation among *L. harlandii* populations

Analysis of molecular variance (AMOVA) showed that 72.85% of genetic variance was found within populations and 27.15% of genetic variance resided among populations (Table 4). The genetic variance within and among populations were extremely significant ($\phi\text{st} = 0.2715$, $P < 0.001$). The ratio of genetic diversity among populations estimated by Shannon's information index is shown in Table 3, among which, 76.35% of genetic variance was found within populations and 23.65% of genetic variance resided among populations. The coefficient of gene differentiation (G_{st}) was 0.2277 and the gene flow (N_m) estimated from G_{st} was 1.6949. All the estimates of genetic differentiation showed that genetic variance was found both within and among populations, but mainly resided within populations.

3.3 Genetic distance among three *L. harlandii* populations

According to the Nei's method (Nei, 1972), genetic similarity and genetic distance were calculated among three populations of *L. harlandii* (Table 5). The genetic similarity among three populations of *L. harlandii* ranged between 0.8347 and 0.9074, with an average of 0.8662. The genetic similarity between P_2 population and P_3 population was the highest, while that between P_1 population and P_3 population was the lowest. The genetic distance among populations ranged between 0.0971 and 0.1807, with an average of 0.1442. The unweighted pair group method with arithmetic mean (UPGMA) cluster analysis based on the genetic distances among three populations (Fig. 2) showed that the lowest genetic distance was between populations P_2 and P_3 and gathered together first, and then clustered with P_1 population.

4 Discussion

Genetic diversity is the result of the long-term evolution of species, and is a prerequisite for population survival and development. The parameters such as the percentage of polymorphic loci, Shannon information index and Nei's index are the major indicators to measure the population genetic diversity within the populations. The results showed that the average percentage of polymorphic loci of three *L. harlandii* populations was 65.32%, and the total percentage of polymorphic loci was 87.86%. The total genetic diversity estimated by Shannon information index was 0.4529, with an average of 0.3458, while that estimated from Nei's index was 0.3004, with an average of 0.2320. It indicated that the genetic diversity of *L. harlandii* was relatively high and it might be due to the biological characteristics of this species. Hamrick et al. (1981) pointed out that the plant with a high level of genetic variation is always the species that is long-lived, has wide geographic distribution, has wind-borne pollination, is hybrid and is highly solid. *L. harlandii* is widely distributed in the subtropical regions of China, and is one of the main constructive species which has all the above features. Therefore, it displays high genetic diversity.

In the subtropical regions, the coniferous forest is the early successional community, followed by coniferous and broad-leaved mixed forest community, and finally developing into a climax community of evergreen broad-leaved forest. *L. harlandii* is distributed in all different successional series communities with many seedling sources and is widely distributed. The results showed that the percentage of polymorphic loci, Shannon information index, and Nei's index of the three *L. harlandii* populations were the largest in the coniferous forest community, then decreased in coniferous and broad-leaved mixed forest community, and that in evergreen broad-leaved forest community was the least. Previous studies showed that genetic diversity of

Table 3 Partitioning of the genetic diversity within and among three populations of *Lithocarpus harlandii*

Shannon information index		Nei's index	
Genetic diversity within population, H_{pop}	0.3458	Gene diversity within population, H_s	0.2320
Total genetic diversity, H_{sp}	0.4529	Total gene diversity, H_t	0.3004
Ratio of genetic diversity within population, H_{pop}/H_{sp}	0.7635	Ratio of gene diversity within population, H_s/H_t	0.7723
Ratio of genetic diversity among populations, $(H_{sp}–H_{pop})/H_{sp}$	0.2365	The coefficient of gene differentiation, G_{st}	0.2277

Table 4 Analysis of molecular variance (AMOVA) within and among populations of *Lithocarpus harlandii*

source of variation	degree of freedom	sum of squared deviations	mean squared deviations	variance component	percentage of total variance (%)	*P*-value
Among populations	2	335.2333	167.617	7.3893	27.15	<0.001
Within populations	57	1130.3000	19.830	19.8298	72.85	<0.001

The *P*-value was estimated after 1000 random permutations.

Table 5 Genetic identity and genetic distance among three populations of *L. harlandii*

population	P_1	P_2	P_3
P_1	–	0.8566	0.8347
P_2	0.1548	–	0.9074
P_3	0.1807	0.0971	–

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

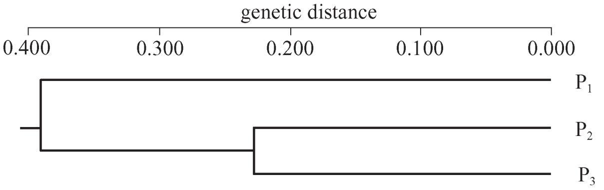

Fig. 2 Cluster figure produced by POPGENE32 soft for three populations of *Lithocarpus harlandii*

different dominant populations in different successional series communities extremely varied. The genetic diversity of *S. superba* was the largest in coniferous forest, decreased in coniferous and broad-leaved mixed forest, and was the least in evergreen broad-leaved forest. The genetic diversity of *Castanopsis chinensis* was the least in coniferous forest, increased in coniferous and broad-leaved mixed forest, and was the largest in evergreen broad-leaved forest. *Cryptocarya chinensis* does not distribute in coniferous forest. The genetic diversity of *C. chinensis* increased from coniferous and broad-leaved mixed forest to evergreen broad-leaved forest, but there was little difference between them (Wang et al., 2004). Compared with these dominant species, the genetic diversity of *L. harlandii* was similar to that of *S. superba*, but opposite to that of *C. chinensis*. This might due to the small canopy density and enough light in the early successional community. *L. harlandii* is a positive plant and plants with different genotypes could grow in that environment and result in high genetic diversity. With the succession carrying on, canopy density of the community is gradually increased, the renewal of *L. harlandii* is restricted and only those shade-tolerant plants can survive. Consequently, the genetic diversity is gradually decreased in coniferous and broad-leaved mixed forests and evergreen broad-leaved forests. The UPGMA cluster analysis based on Nei's genetic distance also showed that the genetic distance was smaller between coniferous and broad-leaved mixed forest communities and evergreen broad-leaved forest community and the genetic similarity was higher. The genetic distance was larger between the coniferous forest community and these two communities and the genetic similarity was lower. It indicated that the small environment tended to be more consistent in the development process of succession from coniferous and broad-leaved mixed forest to evergreen broad-leaved forest. The genetic diversity of the three *L. harlandii* populations might be the result of adapting to their own habitats from different communities.

There are many factors that can influence the genetic differentiation of plant populations, but the primary factors are the heterogeneity of the microhabitat and gene flow (Chen and Song, 1998). AMOVA showed that, in the total genetic diversity of *L. harlandii*, 72.85% of genetic variance was found within populations and 27.15% of genetic variance resided among populations. The results estimated by Shannon's information index also showed that, in the total genetic diversity, 76.35% of genetic variance was found within populations and 23.65% of genetic variance resided among populations. The coefficient of gene differentiation (G_{st}) was 0.2277. All the results showed that genetic variance was found both within and among populations, but mainly resided within populations. Previous studies showed that the impact of different community types on the genetic differentiation of different species was different. That of *Castanopsis fargesii* was 4.29% (Zhu et al., 2002), that of *S. superba* was 4.01% (Wang et al., 2000), that of *C. chinensis* was 24.64% (Wang et al., 2000) and that of *Gordonia acuminata* was 10.21% (Cao et al., 2005). Compared with the above, genetic differentiation is relatively high among *L. harlandii* populations in the three different successional series communities, and is similar to that of *C. chinensis*. Wright (1931) indicated that when $N_m > 1$, the species would become genetically homogeneous. Whereas, when $N_m < 1$, gene flow might be the main reason for population genetic differentiation. The gene flow (N_m) of *L. harlandii* was 1.6949 in this study, which indicated that gene flow was not the main factor affecting genetic differentiation of *L. harlandii* populations, and the major factor was the heterogeneity of the microhabitat. Different genotypes had various suitabilities in different habitats. This led to the same genotype of individuals gathering in the microhabitat, which comparatively adapts, thus resulting in genetic differentiation (Chen and Song, 1998). Adaptation to different ecological microhabitats could lead to different genetic structures of different populations (Su et al., 1997). Although the three *L. harlandii* populations distributed in a limited geographical scope, species composition is simple in the arbor layer of *P. massoniana* forest, the canopy density of the community is low, understory light is sufficient, and humidity inside the forest is low. While the community structure in evergreen broad-leaved forest is complex, both canopy density and humidity inside the forest are bigger. Therefore, ecological factors such as light, soil and moisture are all different in different successional series communities, which lead to different microhabitats in different communities. The genetic differentiation of *L. harlandii* populations in different microhabitats arise due to the selective pressure. Although no morphological differentiation of *L. harlandii* were found in different communities, the genetic differentiation of *L. harlandii* is relatively high on the level of community. This suggests that species can be influenced by different communities on the molecular level. The genetic differentiation among *L. harlandii* populations could be influenced not only by the biological characteristics of this species, but also by the microenvironment of different communities.

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