Forest Ecology and Management 256 (2008) 1143–1150

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Roles of seed and pollen dispersal in natural regeneration of *Castanopsis fargesii* (Fagaceae): Implications for forest management

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ARTICLE INFO

Article history:
Received 27 December 2007
Received in revised form 9 June 2008
Accepted 10 June 2008

Keywords:
Castanopsis fargesii
Seed and pollen dispersal
Successional stages
Natural regeneration
Second-growth forests

ABSTRACT

Natural regeneration is an important process to reverse the loss of forests. Understanding the process of natural regeneration is crucial for achieving sustainable forest management. In this study, we examined the effects of seed and pollen dispersal in naturally regenerated populations of *Castanopsis fargesii*. Genetic variation in six populations along two successional series (three successional stages in each series: early, pre-climax, and climax) was assayed using RAPD (random amplified polymorphic DNA) markers. High genetic variability was observed as measured with Shannon's information index. A majority of genetic variation was distributed within populations ($\Phi_{st} = 0.1271$) and significant isolation by distance existed among populations. A contrasting pattern of genetic variation along these two series was observed, representing different scenarios of natural regeneration processes. The ratio of the number of migrants between the mature populations to the number of migrants from the mature to immature populations was estimated as 1.146 ± 0.099 to 1.981 ± 0.164, implying that comparable seed and pollen dispersal might exist at a fine spatial scale ($\sim$2 km^2). The results suggest the critical role of seed dispersal in shaping genetic composition and diversity in the second-growth forests. Barriers to seed dispersal from a variety of genetic sources could result in low genetic diversity in naturally regenerated populations. Management that facilitates the connectivity of newly regenerated stands with mature forests could be effective for natural regeneration given the predominant role of short-distance dispersal of seeds in this species.

1. Introduction

Forest loss due to the impacts of human and natural disturbances has become a central issue in the sustainable management of forests and other natural resources. The success of ecological restoration is related to the process of natural regeneration of different species. Natural regeneration refers to the renewal process of harvested or disturbed forest stands from natural seed fall or from stump or root sprouting. In spite of the slow process of natural regeneration, its importance has been appreciated in restoring biodiversity, which is difficult to achieve through artificial regeneration (Kozlowski, 2002; Slocum et al., 2004). In flowering plants, individual recruitment is mediated through seed dispersal. In the regeneration phase, seed dispersal from the adjacent stands often plays a critical role for species incapable of vegetable propagation (Gorchov et al., 1993; Duncan and Chapman, 1999; Standish et al., 2007). Pollen dispersal is also important for facilitating natural regeneration by maintaining genetic diversity.

The genetic consequences of natural regeneration have rarely included the use of molecular markers. Early studies have concentrated on genetic evaluations related to the perspective of conventional silviculture and forest management. A few empirical studies demonstrated that the genetic composition of naturally regenerated populations was affected by adjacent (Sezen et al., 2005) or distant mature populations (Cespedes et al., 2003; Goto et al., 2004). These findings support the idea that seed and pollen dispersal from the mature forests are critical in shaping the genetic consequences of secondary forests, but fail to evaluate the relative roles of seed and pollen dispersal. Our current knowledge of the relative roles of seed and pollen dispersal mainly comes from assays using nuclear and organelle molecular markers (Ennos, 1994; Petit et al., 2005). However, organelle genomes (chloroplast

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doi:10.1016/j.foreco.2008.06.014

or mitochondrial DNA) are conservative and frequently little variation is found in plant populations (e.g., Lu et al., 2006), especially at smaller spatial scales where a population usually contains one or a few haplotypes. Therefore, it may be difficult to use organelle markers to assess the relative roles of seed and pollen dispersal in natural regeneration at these spatial scales.

Use of the populations in different regeneration phases is a promising approach for evaluating the impacts of seed and pollen dispersal, alternative to the one using both nuclear and organelle (maternally or paternally inherited) genomic markers. Gene migration can be mediated through both seed and pollen dispersal between reproductively mature stands, but only through seed dispersal from the reproductively mature to immature stands. This feature can be used to infer the relative magnitudes of gene flow to the newly regenerated stands, compared with the extent of gene flow between the reproductively mature stands. The results may also help in understanding how genetic diversity changes along the process of natural regeneration.

The purpose of this study is to investigate a new method to infer pollen and seed dispersal at small spatial scales, for example, a few square kilometers, and to apply it to assess the differences of seed and pollen dispersal in naturally regenerated populations of *Castanopsis fargesii* Franch. This species is an important long-lived monoecious species and establishes a canopy of evergreen broadleaved forests in the subtropical region in China and Southeast Asia. Adult trees can attain 30 m in height and 0.8 m in diameter at breast height (DBH). The species is wind-pollinated and flowers in May (Chen et al., 2003). Seeds are predominantly dispersed by natural gravity and by rodents, such as squirrels and mice. An early molecular marker study indicated that a small proportion of variation was distributed among populations in the southern range of this species (Zhu et al., 2002), suggesting the presence of an extensive gene flow between the mature populations. A separate study with different species in the same family (*Fagus crenata*) revealed that a dominant contribution to gene flow came from pollen other than from seeds from mature populations (Tomaru et al., 1998). However, effects of pollen and seeds dispersal in natural regeneration at the fine spatial scale are not known, especially the extent of seed dispersal to the newly generated populations because seed dispersal is often underestimated (Bacles et al., 2006). Implications needs to be explored for sustainable forest management based on inferences from seeds and pollen dispersal and the impacts on naturally regenerated populations of this species. To gain a preliminary insight into the relative roles of seed and pollen dispersal in naturally regenerated populations, RAPD (random amplified polymorphic DNA) markers were selected because no prior knowledge about primer sequences was required (Zhu et al., 2002). Also, RAPDs may have no essential differences from other dominant markers when the experiments are well controlled, such as IRAP and AFLP markers (Schlotterer, 2004).

2. Materials and methods

2.1. A method to infer seed and pollen dispersal

The number of migrants between a pair of populations for diploid nuclear markers can be estimated according to Wright's (1969) formula $Nm = (1/F_{st} - 1)/4$ although some assumptions underlying this model may be violated (Whitlock and McCaulery, 1999). This method is an indirect way of estimating past gene flow based on the spatial distribution of gene frequencies and may be more effective than direct monitoring methods, especially for species with frequently demographic changes (Slatkin, 1987). Here, F_{st} can be replaced with other measurements of population genetic differentiation, such as G_{st} for the multiple allele case and Φ_{st} estimated with dominant markers (Grashof-Bokdam et al., 1998). Both seed and pollen dispersal can contribute to gene flow between populations at the pre-climax and climax stages. The number of migrants per generation is expressed as $N_{(M)}(m_{S(M)} + m_{P(M)}/2)$, where $N_{(M)}$ is the effective size of the mature populations and $m_{S(M)}$ and $m_{P(M)}$ are the migration rates of seeds and pollen between the mature populations, respectively. Half the migration rate of pollen ($m_{P(M)}/2$) is because pollen dispersal is haploid dispersal in comparison with diploid seed dispersal (Hu and Ennos, 1999). From the genetic differentiation between mature populations, denoted by $\Phi_{st(M)}$, the number of migrants is $N_{(M)}(m_{S(M)} + m_{P(M)}/2) = (1/\Phi_{st(M)} - 1)/4$.

Migration between the immature and mature populations is unidirectional (from the mature to immature population by seed dispersal), analogous to the situation of island-mainland structure where an island population receives migrants from the mainland or to the source–sink model of metapopulation structure. The difference between the island and mainland populations in terms of Wright's F_{st} can be defined as $F_{st} = 1 - H_I/H_C$ (Hudson, 1998), where H_I and H_C are the heterozygosities in the island and mainland populations, respectively. Hudson (1998) showed that F_{st} has the expression similar to Wright's results and is applicable to the case of unidirectional migration (from the mainland to island). Application of Hudson's (1998) results to the present study gives $F_{st} = (1 + 4N_{(I)}m_{S(I)})^{-1}$, where $N_{(I)}$ is the immature population size (not the effective population size) and $m_{S(I)}$ is the immigrant rate of seeds to the immature population. Note that there is no need to assume the equilibrium between the effects of migration and genetic drift in using the above equation (Hudson, 1998). Here we directly estimated population genetic differentiation between the immature and mature populations with AMOVA instead of estimating H_I and H_C under Hardy–Weinberg equilibrium (HWE) assumption. Let $\Phi_{st(I)}$ be the measure for the genetic differentiation between the immature and mature populations and then $N_{(I)}m_{S(I)} = (1/\Phi_{st(I)} - 1)/4$. An alternative method introduced by Yeh and Hu (2005) requires the information of effective mainland population size and is not employed here.

The ratio of the number of migrants between the mature populations to the number of migrants from mature to immature populations, denoted by r, can be estimated by:

$$r = \frac{N_{(M)}(m_{S(M)} + m_{P(M)}/2)}{N_{(I)}m_{S(I)}} = \frac{1/\Phi_{st(M)} - 1}{1/\Phi_{st(I)} - 1}. \quad (1)$$

When the number of migrating seeds between the mature populations is comparable with the number of migrants from the mature to immature populations, i.e., $N_{(M)}m_{S(M)} \approx N_{(I)}m_{S(I)}$, the ratio of pollen to seed dispersal in each series, i.e., $N_{(M)}m_{P(M)}/N_{(I)}m_{S(I)}$ (or $N_{(M)}m_{P(M)}/N_{(M)}m_{S(M)}$) $= 2(r - 1)$. When pollen dispersal is predominant in migration between the mature populations ($N_{(M)}m_{S(M)} \approx 0$), $(N_{(M)}m_{P(M)}/N_{(I)}m_{S(I)}) \leq 2r$.

The variance for the ratio r can be expressed as:

$$V(r) = V\left(\frac{\Phi_{st(I)}(1 - \Phi_{st(M)})}{\Phi_{st(M)}(1 - \Phi_{st(I)})}\right). \quad (2)$$

Suppose that $\Phi_{st(M)}$ and $\Phi_{st(I)}$ are independent, which is biologically reasonable. The variance for a product of two independent variables ($\Phi_{st(I)}(1 - \Phi_{st(M)})$) is given by

$$V(\Phi_{st(I)}(1 - \Phi_{st(M)})) = \hat{\Phi}^2_{st(I)}V(\Phi_{st(M)}) + (1 - \hat{\Phi}_{st(M)})^2 V(\Phi_{st(I)}) + V(\Phi_{st(I)})V(\Phi_{st(M)}) \quad (3)$$

Replacing $\Phi_{st(I)}$ and $1 - \Phi_{st(M)}$ in Eq. (3) with $\Phi_{st(M)}$ and $1 - \Phi_{st(I)}$, respectively, gives the expression for $V(\Phi_{st(M)}(1 - \Phi_{st(I)}))$. Simi-

larly, replacing $1-\Phi_{st(M)}$ in Eq. (3) with $\Phi_{st(M)}$ gives the expression for $V(\Phi_{st(M)}\Phi_{st(I)})$. Therefore, according to Kendall and Stuart's (1969, pp. 132–133) formula, we obtained

$$V(r)=\left(\frac{A}{B}\right)^2\left(\frac{V(A)}{A^2}+\frac{V(B)}{B^2}-\frac{2\,\mathrm{cov}(A,B)}{AB}\right), \quad (4)$$

where $A=\hat{\Phi}_{st(I)}(1-\hat{\Phi}_{st(M)})$, $B=\hat{\Phi}_{st(M)}(1-\hat{\Phi}_{st(I)})$ and $\mathrm{cov}(A,B)=-\hat{\Phi}_{st(I)}V(\hat{\Phi}_{st(M)})-\hat{\Phi}_{st(M)}V(\hat{\Phi}_{st(I)})+V(\hat{\Phi}_{st(I)}\hat{\Phi}_{st(M)})$. Eq. (4) can give an appropriate estimate of the standard deviation for the migration ratio r, especially when the sample sizes are not too small.

2.2. Study sites and sample collections

The north boundary of the distribution of *C. fargesii* in East China is located in Tiantong, Ningbo City, China. This species is one of the dominant species within Tiantong National Forest Park, but is absent outside the park (less than a few square kilometers in the whole park). The plant community around this park mainly consisted of rice paddy, *Myrica rubra*, orange plantations, and other secondary forests dominated by *Cyclobalanopsis glauca*, *Quercus fabri*, and bamboo species (Song and Wang, 1995). In this study, we selected populations of *C. fargesii* in two successional series that were physically isolated by the central hills and Tiantong Temple buildings in Tiantong National Forest Park (Fig. 1). In each series, three age-classes of stands were selected: sapling stands (<10 years, on average), adult stands (around 40 years old, on average), and old stands (more than 80 years old, on average). One series (hereafter called Series I; Table 1) was located on the west side of the park, across the southwest boundary. Three stands in different successional stages were selected along the direction from the southwest to northeast sides (Fig. 1). The stand or population (coded by "A"), outside the south boundary of the park, consisted of immature individuals (early successional stage), with the diameter at breast height (DBH) being less than 2.5 cm. The second stand (coded by "B") grew with the dominant species *Schima superba* in the pre-climax stage. A majority of *C. fargesii* individuals were reproductively mature, with the DBHs from 16 to 30 cm. The stand at the north end

Table 1
The successional stages and the diameter at breast height (DBH) of the largest individuals for the six selected stands of *Castanopsis fargesii* Franch

Series	Populations	DBH (cm)	Successional stages
I	A	~2.5	Early stage (immature stand)
	B	~30	Pre-climax stage (mature stand)
	C	~60	Climax stage (mature stand)
II	D	~4.0	Early stage (immature stand)
	E	~35	Pre-climax stage (mature stand)
	F	~65	Climax stage (mature stand)

These six stands were distributed between two successional series (from reproductively immature to mature stages).

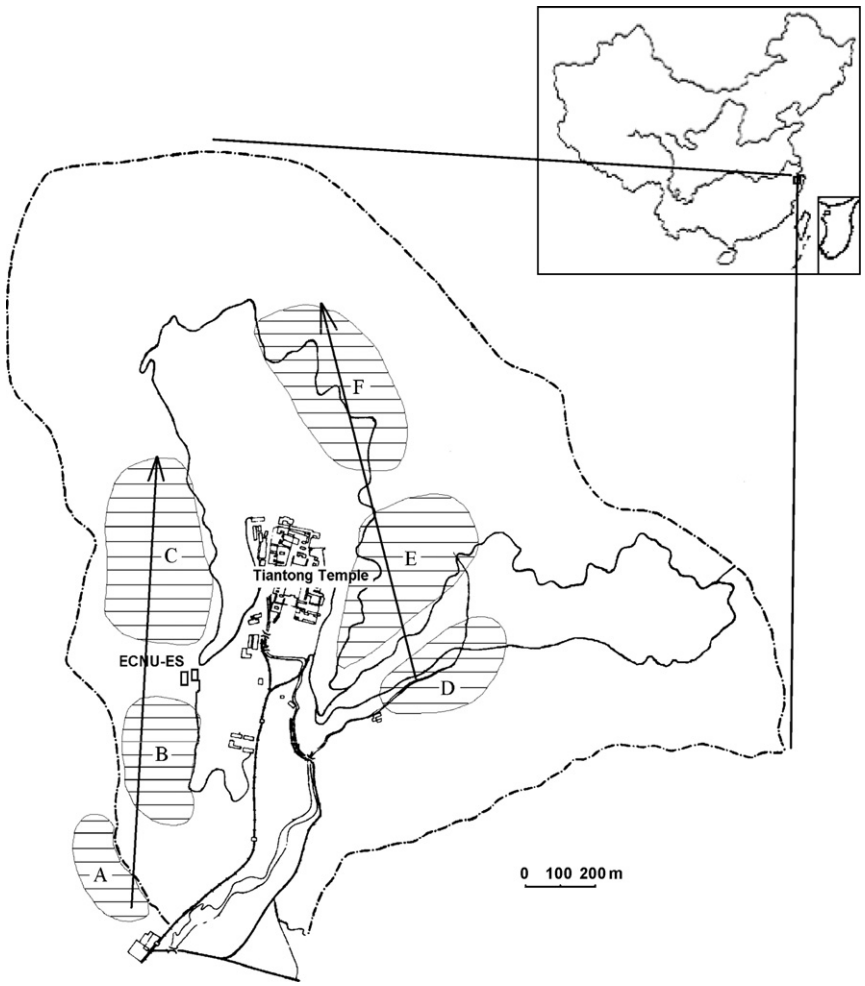

Fig. 1. Sampling sites of 6 populations of *Castanopsis fargesii* at different successional phases. Arrows indicate successional direction from the early stage to the pre-climax and climax stage. Series I (populations A, B, and C) and Series II (populations D, E, and F) was physically isolated by hills, temple and other buildings and roads.

(coded by "C") was the old-growth forest at the climax stage, where *C. fargesii* was the dominant species in its resident plant community (Song and Wang, 1995), with the largest DBH being about 60 cm.

The second series (hereafter we call it Series II; Table 1) was distributed in the east side of the park. Three stands in different successional stages were also selected along the direction from the southeast to northeast (Fig. 1). The sapling stand (coded by "D") on the southeast side grew in the forest mixed with species *Cunninghamia lanceolata*, and the individual DBHs were less than 4 cm. The reproductively mature stand (coded by "E") close to Tiantong Temple grew with the dominant species *Lithocarpus glaber* in the pre-climax stage, and the individual DBHs ranged from 20 to 35 cm. The northeast stand (coded by "F") was an old-growth forest in the climax stage and the largest DBH was about 60 cm. *C. fargesii* was the dominant species in the climax stage (Fig. 1).

Samples were collected at random for all stands in the pre-climax and climax stages separated by a minimum distance of at least 20 m. Samples for the sapling stands included as many samples as possible (about 30 individuals) since a few individuals were in this stage. Five to ten healthy leaves were harvested from each sampled individual and were taken to laboratory for DNA extraction within 1 day.

2.3. DNA isolation and assays

DNA was isolated with a modified Doyle and Doyle's (1987) CTAB procedure (Fan et al., 2004). Fresh leaves were ground to powder with a cold grinder, suspended with 750 μl homogenate buffer (100 mmol/l Tris–HCl (pH 8.0), 20 mmol/l EDTA (pH 8.0), 1.4 mmol/l NaCl, 0.3% β-mercaptoethanol, v/v, and 2% PVP, w/v), and then centrifugated at 12,000 rpm for 20 min at 4 °C. After centrifugation, the sediments were suspended with a 750 μl 60 °C extraction buffer (100 mmol/l Tris–HCl (pH 8.0), 1.4 mol/l NaCl, 20 mmol/l EDTA (pH 8.0), 2% CTAB, w/v) and incubated for 90 min. The mixture was treated twice by shaking with 0.5 ml of chloroform/isoamyl alcohol, followed by centrifugation to separate phases and the removal of aqueous layer. Then 2 μl 10 μg/ml RNaseA was added and incubated at 37 °C for 30 min and the mixture was treated with 0.75 ml of chloroform/isoamyl alcohol, followed by centrifugation at 14,000 rpm under 4 °C for 10 min. We added 2 volumes of 100% ethanol to the aqueous layer, followed by the 30 min incubation at −20 °C and centrifugation at 12,000 rpm. The sediment was washed and precipitated with cold 70% ethanol and finally the DNA was dissolved in 1.5 ml TE buffer and stored at 4 °C. Reactions were carried out using a PTC-220 DNA engine DAYD™. Negative controls were included in each thermocycler run to check for contamination.

A set of random 10-mer RAPD primers were purchased from Sagon Inc., Shanghai. After screening more than 100 arbitrary primers, 11 primers that consistently amplified clear banding patterns were selected for further studies (Table 2). RAPD assays were conducted using the conditions described in a previous study (Fan et al., 2004). Samples were amplified twice to ensure banding reproducibility and stability. 5 μl amplification products were separated on 1.6% agarose gel in 0.5× TBE buffer and visualized by staining with ethidium bromide and photographed over UV light with Bio-Rad Gel Doc 2000™.

2.4. Data analysis

Each polymorphic PCR banding site was treated as a single locus and scored for presence and absence. Population genetic diversity can be estimated from the frequencies of dominant markers under the assumption of HWE (Lynch and Milligan, 1994). This assumption, however, is invalid in this study since the immature populations did not undergo the process of gamete association to produce progeny and their genetic diversity solely resulted from the founder effects. Population genetic diversity at each locus in each population was calculated using Shannon's diversity index, i.e., $-\sum_{j=1}^{2} p_{ij} \ln p_{ij}$ for the diversity at the jth locus in the ith population, where p_{ij} is the frequency of the jth band (presence or absence) in the ith population.

Analysis of molecular variance (AMOVA; Arlequin ver. 3.0; Excoffier and Schneider, 2005) was conducted to estimate the genetic variation among series and within and among populations in series. Measure of population genetic differentiation Φ_{st}, analogous to Wright's F_{st}, was used, removing the HWE assumption (Grashof-Bokdam et al., 1998), and the standard deviation of Φ_{st} was estimated using the jackknifing method (Weir, 1996). Significance tests of the estimated Φ_{st} were conducted by 1000 permutations with Arlequin ver. 3.0 to calculate P-values. Genetic distances between populations were calculated according to the formula similar to Nei's (1972). UPGMA method was used to draw the tree of genetic relationships among all populations according to the genetic distances matrix with PHYLIP ver. 3.67 (Felsenstein, 1989). One hundred trees were generated through the bootstrapping method and a final consensus tree was constructed using PHYLIP. The relationship between the population genetic differentiation (or the population genetic distance) and the geographical distance was examined using regression analysis. Pairwise population geographical distances were calculated on the basis of the center points in each population since the coordinates for individuals were not recorded at a fine spatial scale.

Table 2
Eleven oligonucleotide primer sequences and the number of stable RAPD bands observed in *Castanopsis fargesii* Franch

Primers	Sequence 5′–3′	Number of bands
A07	GAA ACG GGT G	6
AG01	CTA CGG CTT C	8
AM14	TGG TTG CGG A	7
S1221	CAC ACC GTG T	7
S1361	TCG GAT CCG T	5
S2068	CAT ACG GGC T	5
S2116	AGG GTC CGT G	5
S2140	TGG TAC CTG G	5
S2144	ACC TGC CAA C	5
S307	GAG CGA GGC T	6
S90	AGG GCC GTC T	4

A total of 63 useful markers were obtained in this study.

3. Results

3.1. Genetic variation among successional stages

A total of 63 polymorphic loci generated by 11 primers were observed (Table 2). The number of polymorphic loci varied with primers from 4 to 8 per primer. Based on the multilocus genotypes, each of 168 individuals from 6 populations was unique. No population-specific alleles were observed.

In all 6 populations, the observed numbers of alleles per locus varied from 1.964 to 1.982 (Table 3). A contrasting pattern between the two successional series was observed with Shannon's diversity index. In Series I, the genetic diversity was larger in the sapling (0.514 ± 0.207) and the pre-climax (0.483 ± 0.221) populations than in the climax population (0.453 ± 0.231). In Series II, the genetic diversity gradually decreased from the climax population (0.504 ± 0.207) to the pre-climax population (0.487 ± 0.222) and to the sapling population (0.484 ± 0.222). Differences between Series I and Series II and between populations within series were significant

Table 3
Sample sizes and estimates of genetic diversity in terms of Shannon's diversity index for 6 natural populations of *Castanopsis fargesii* at different successional stages

Populations	A	B	C	D	E	F
Sample sizes	23	29	29	29	29	29
Number of alleles per locus	1.970	1.976	1.964	1.964	1.964	1.982
Shannon's diversity indices						
Mean	0.514	0.483	0.460	0.484	0.487	0.504
S.E.	0.026	0.028	0.029	0.028	0.028	0.026
S.D.	0.207	0.221	0.231	0.222	0.222	0.207

Standard errors (S.E.) and standard deviations (S.D.) were estimated among 63 polymorphic RAPD markers.

Table 4
Analysis of molecular variance (AMOVA) assessed with 63 RAPD markers for 6 populations of *Castanopsis fargesii* at different successional stages in Tiantong National Forest Park, Ningbo city

Variation resource	d.f.	Sum of squares	Mean squares	Variance components	Percentage of total variance	*P*-value
Between series	1	143.068	143.068	1.319	10.29	<0.001
Among populations	4	128.938	32.234	0.770	6.01	<0.001
Within populations	162	1737.631	10.726	10.726	83.70	<0.001

P-values were calculated from 1000 permutations with Arlequin (ver. 3.0; Excoffier and Schneider, 2005).

(*P*-value < 0.0001; Table 4). The genetic diversity in the whole pooled population was 0.556 ± 0.169.

3.2. Genetic structure

When all six populations were jointly analyzed, the multilocus estimate Φ_{st} was 0.127 (*P*-value < 0.001), indicating that a majority of genetic variation was distributed within populations. Individual estimates of Φ_{st} at different loci varied, mainly ranging from 0.01 to 0.4 (Fig. 2). Two-level hierarchy AMOVA indicated that there were significant differences between Series I and Series II and among populations at different successional stages (Table 4). Most genetic variation occurred within populations (83.70%). The genetic variation between Series I and Series II accounted for 10.29%, much greater than the genetic variation among populations within series (6.01%).

Estimates of multilocus Φ_{st} for pairwise populations are summarized in Table 5. Each estimate was significantly different from zero (*P*-value < 0.001). In each of these two series, population genetic differentiation between sapling and pre-climax populations or between sapling and climax populations was greater than that between pre-climax and climax populations. The genetic differentiation between the population in Series I and the population in Series II was much greater than that between the populations within each series, consistent with the results of two-level hierarchy AMOVA (Table 4).

The average genetic distances between Series I and Series II (*D* = 0.105 ~0.149) were greater than those between the populations within each series (0.049–0.095 in Series I, 0.032–0.046 in Series II). The consensus tree indicated that Series I and Series II were separated with a probability of 100% (Fig. 3). In each of these two series, the populations at the pre-climax and climax stages were first clustered and then jointed with the sapling population. Population genetic differentiation (Φ_{st}) was significantly correlated with the geographical distance (log transformed) ($\Phi_{st}/(1-\Phi_{st})$ = 0.0131 + 0.1023 ln(geographical distance), R^2 = 0.3528, *P*-value = 0.0195; Fig. 4). This was the same case for the test of the correlation between the genetic and geographical distances

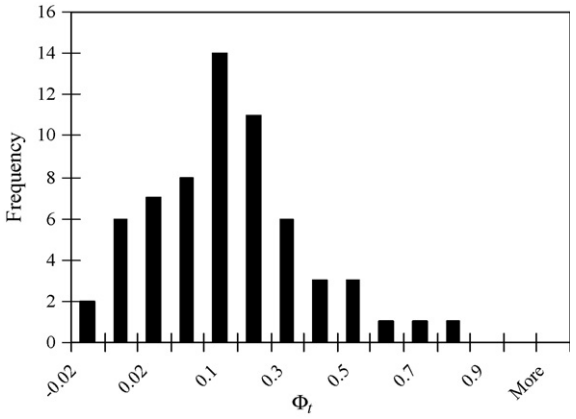

Fig. 2. The frequency distribution of population differentiation in terms of Φ_{st} for 63 RAPD markers, showing that Φ_{st}'s for most loci distributed within the range from 0.01 to 0.4. Results were calculated using all six populations and 63 RAPD markers with AMOVA (Arlequin ver. 3.0; Excoffier and Schneider, 2005).

Table 5
Average pairwise Φ_{st} estimates (above diagonal) and their standard deviations in parentheses obtained through AMOVA with the sampling data sets generated by the Jackknifing method

Populations	A	B	C	D	E	F
A	–	0.116 (0.003)	0.125 (0.005)	0.183 (0.004)	0.163 (0.004)	0.162 (0.005)
B	1.908 (0.071)	–	0.062 (0.002)	0.143 (0.004)	0.144 (0.004)	0.137 (0.004)
C	1.756 (0.079)	3.796 (0.163)	–	0.193 (0.005)	0.170 (0.005)	0.182 (0.005)
D	n.a.	1.495 (0.047)	1.044 (0.038)	–	0.030 (0.002)	0.051 (0.002)
E	1.281 (0.041)	1.490 (0.058)	1.225 (0.053)	8.133 (0.498)	–	0.026 (0.002)
F	1.295 (0.044)	1.578 (0.059)	1.126 (0.042)	4.698 (0.235)	9.347 (0.842)	–

All pairwise Φ_{st}'s were sinificantly different from zero (*P*-value < 0.001). Estimates of the number of migrants (below diagonal) were calculated from $(1/\Phi_{st} - 1)/4$ and their standard deviations in parentheses obtained through the Jackknifing method. n.a.: Not applicable.

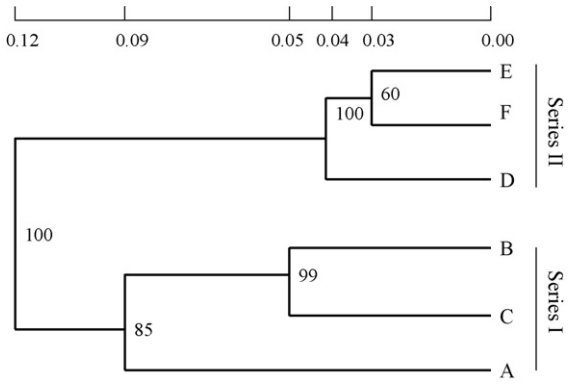

Fig. 3. Dendrogram shows the genetic relationships among 6 populations of *Castanopsis fargesii* at different successional stages. The consensus tree was constructed on the basis of 100 bootstrapping replicates obtained with PHYLIP ver. 3.67 (Felsenstein, 1989). UPGMA method was used according to population genetic distance matrix defined in the main context. The numbers shown on the tree indicated the times that the internal branch occurred out of 100 replicates.

($D = 0.0333 + 0.0162$(geographical distance), $R^2 = 0.3919$, P-value = 0.0125), indicating the presence of significant effects of isolation by distance.

3.3. Migrant exchange

Estimates of the number of migrants for pairwise populations are summarized in Table 5. The average numbers of migrants among populations within each series (average number of migrants = 1.756–3.796 for Series I and 4.698–9.347 for Series II) were greater than those between the populations in Series I and the populations in Series II (average number of migrants = 1.044–1.578). Dispersal mainly occurred among populations within each successional series rather than among populations between the two successional series. Also, migration from the pre-climax to the sapling populations was more extensive than that from the climax to the sapling populations because of the effects of isolation by distance (Table 5).

Since there was a significant difference between Series I and Series II (Table 4), the relative numbers of migrants between the mature populations to those from the mature to the immature populations, r, were assessed separately in each series. In Series I, Φ_{st} between populations B and C was used as $\Phi_{st(M)}$ (Table 5), and the estimates of r were 1.825 ± 0.090 to 2.161 ± 0.131 (Table 6). In Series II, Φ_{st} between populations E and F was used as $\Phi_{st(M)}$ (Table 5),

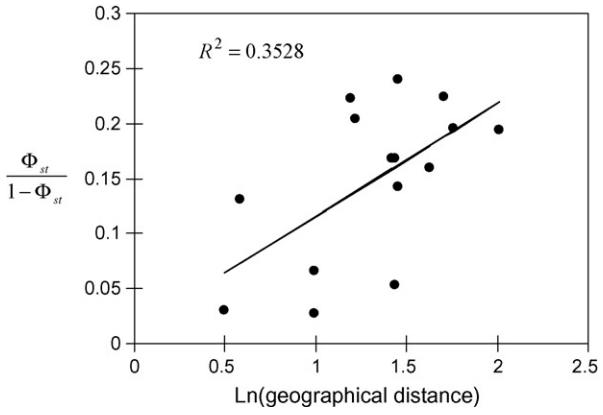

Fig. 4. Test of the effects of isolation by distance (IBD), showing the presence of a significant IBD effect on population differentiation ($\Phi_{st}/(1-\Phi_{st}) = 0.0131 + 0.1023$ ln(geographical distance), P-value = 0.0195).

Table 6
Estimates of the ratio of the relative number of migrants between the mature populations to that from the mature to immature populations

Series	Immature–mature populations	$r \pm$ S.D.
I. $\Phi_{st(M)} = 0.062 \pm 0.002$	A–B	1.989 ± 0.100
	A–C	2.161 ± 0.131
	A–B + C*	1.825 ± 0.090
II. $\Phi_{st(M)} = 0.026 \pm 0.002$	D–E	1.146 ± 0.099
	D–F	1.981 ± 0.164
	D–E + F	1.280 ± 0.105

Standard deviations (S.D.) were estimated according to Eq. (4).
* B + C: populations B and C were pooled as one population for analysis and E + F: populations E and F were pooled as one population for analysis.

and the estimates of r were 1.146 ± 0.099 to 1.981 ± 0.164 (Table 6). These estimates indicate that the number of migrants between the mature populations was not substantially greater than the number of migrants from the mature to immature populations.

4. Discussion

In this study, we investigated a new method to assess the difference in seed and pollen dispersal in naturally regenerated populations along different successional stages. This method only uses nuclear molecular markers, such as microsatellites and RAPDs markers, in contrast to previous methods that use both biparentally and paternally or maternally inherited markers (Ennos, 1994; Hu and Ennos, 1999; Petit et al., 2005). Our results also confirmed the prediction that genetic differentiation was greater between reproductively immature (populations A and D) and mature (populations B, C, E, and F) populations rather than between reproductively mature populations. A small proportion of genetic variation occurred among populations in *C. fargesii*, similar to the findings for many other tree species (Hamrick et al., 1992; Nybom and Bartish, 2000). The genetic diversity in the naturally regenerated populations of *C. fargesii* varied with successional series. Different spatial patterns of genetic diversity were evident for the populations changing from the immature to mature phases. The number of migrants from the mature to immature stands was relatively substantial in comparison with the number of migrants between the mature populations. These results highlighted the role of seed dispersal from the adjacent old-growth forests to the second-growth forests in shaping their genetic composition.

In comparison with the genetic diversity within populations in other species using RAPD markers (Nybom and Bartish, 2000), a relatively high genetic variability existed in the populations of *C. fargesii*. This result was consistent with the prediction from the perspective of life history, such as wind pollination and late-successional stage (Hamrick and Godt, 1989; Nybom and Bartish, 2000). Also, our Shannon diversity indices were slightly higher than another study (Zhu et al., 2002). This was probably attributable to the small human perturbations in the national forest parks.

Our results indicated that the extent of population genetic differentiation was related to the population history in different successional stages. As expected, more extensive exchanges of migrants occurred between the pre-climax (populations B or E) and climax populations (populations C or F) than the migrants from the reproductively mature (populations B, C, E or F) to immature populations (populations A or D). This is because the immature populations evolve like sinks and only receive individuals from the mature populations (short- or long-distance immigrants). Pollen dispersal as an additional vector of gene flow facilitates the reduction in genetic differentiation between the

mature populations. The effective pollen dispersal between the mature populations was essentially related to the reproductive systems of this species. Although information on the outcrossing rate in *C. fargesii* was not available, a mixed to predominantly outcrossing system was recorded in closely related species (Bacilieri et al., 1996; Chen and Song, 1997; Wang, 2003). A negative relationship between population differentiation and outcrossing rate (t) can be viewed from a theoretical expression, $F_{st} = (1 + (1 + t/2) \times 4Nm)^{-1}$, which was derived by replacing the effective population size N in $F_{st} = (1 + 4Nm)^{-1}$ with $N(1 + (1 - t/1 + t))^{-1}$ under certain assumptions (Caballero and Hill, 1992).

In relation to the pattern of genetic diversity along a successional gradient, our results implied that different scenarios of natural regeneration existed between Series I and Series II. The contrasting spatial pattern of genetic diversity was associated with the process that produced the secondary-growth stands. Establishment of the second-growth forests was involved in the migrants from the mature populations and hence founder events were important in shaping genetic composition (Sezen et al., 2005). The theoretical expectation for neutral genes is that the genetic variation is lower in newly established populations than their source populations since colonists can be viewed as a sample of source populations. This is similar to the relationship between genetic variations in central and marginal populations, as implied from many records of genetic diversity in island populations (Frankham, 1997). The lowest genetic variation in the sapling population (D) in Series II reflected such a biologically sampling process (Table 3). A similar pattern was found in *Primula elatior* (Jacquemyn et al., 2004) where the total gene diversity was slightly higher in the old (total heterozygosity $H_T = 0.2987$) than in the young populations ($H_T = 0.2828$). The possibility for the occurrence of diversified selection or genetic hitchhiking effects could be excluded since our RAPD markers in all mature populations were tested to be neutral with Ewens–Watterson test (Manly, 1985; Yeh et al., 1997) (results not shown here).

Series I represented the scenario distinct from the above expected pattern occurred in Series II, where genetic variability was greater in the early successional stage (populations A and B) than in the climax stage (population C). Related evidence in other species was also recorded (Frankham, 1997). One possible reason was the occurrence of immigration from Series II to Series I although a larger genetic differentiation existed between Series I and II than among populations in each of the two series (Table 5). This could be inferred from the observations of population genetic differentiation between Series I and Series II (Table 4). Population B had the smallest Φ_{st} with the populations in Series II ($\Phi_{st} = 0.1368–0.1438$), followed by population A ($\Phi_{st} = 0.1619–0.1817$) and population C ($\Phi_{st} = 0.1697–0.1934$). Strong evidence is that an allele at a locus generated by primer S2140 was observed in population B and all populations in Series II but not in populations A and C, implying that this allele came from Series II. This probably explains why population B had the largest genetic diversity in Series I.

Enhanced genetic diversity in the early successional stage has been reported in other plant species, such as in *Empetrum hermaphroditum* (Szmidt et al., 2002), *Swietenia macrophylla* (Cespedes et al., 2003), and *Betula maximowicziana* (Goto et al., 2004). Genetic diversity in population A was mainly affected by seed dispersal from population B. This can be inferred from the smallest Φ_{st} between populations A and B among all pairwise Φ_{st}'s between population A and all other populations (Table 5). Strong evidence is that an allele generated by primer S307 was observed in populations A and B and all populations in Series II, but not in population C, implying that this allele in population A might come from population B or from Series II. The spatial pattern of genetic diversity in Series I implied that the genetic diversity in the newly generated populations could be greater than in the mature populations (Frankham, 1997).

An insight into the effects of seed and pollen dispersal can be gained from the estimates of the ratio of the number of migrants between the mature populations to that from the mature to the immature populations. If the number of migrating seeds between the mature populations is comparable with the number of migrants from the mature to the immature populations, the ratio of pollen to seeds dispersal in each series is around or smaller than 2.0 on average (Table 6). If pollen dispersal is predominant in migration between the mature populations, the ratio of pollen to seeds dispersal in each series is around or less than 4 on average. In each case, the number of migrants between the mature populations was not substantially greater than the number of migrants from the mature to immature populations. This suggested that seed dispersal likely had larger effects in the early than in the late phases of natural regeneration of *C. fargesii* at a final spatial scale.

The above inference was consistent with the reproductive ecology of this species at the fine spatial scale. Seeds of *C. fargesii* are mainly dispersed by gravity and usually not far away from maternal trees. Rodents, such as Edward's long-tailed rat (*Leopoldamys edwardsi*), may serve as a secondary seed disperser (Xiao et al., 2003). Thus, seed dispersal exhibits a highly leptokurtic distribution with a long tail. Pollen dispersal in *C. fargesii* is mediated by wind and displays a relatively less leptokurtic distribution in space. The average distance of pollen dispersal is expected to be greater than the average distance of seed dispersal. The relative numbers of migrants by pollen dispersal versus those by seed dispersal are scale-dependent, with a smaller value or an approximate unity at a small or sufficient large scale and a large value at a moderate scale (McCauley, 1997). In this study at the fine spatial scale used in this study, our estimates reflected the scale-dependent expectation for the dispersal of seeds and pollen within a short distance (Cozzolino et al., 2003; Oddou-Muratorio et al., 2001; Squirrell et al., 2001; Trapnell and Hamrick, 2004).

Our results suggest a strategy for managing the second-growth forests from the perspective of genetic diversity. Although the secondary forests cover more area than the old-growth forests in many countries, their genetic variation is rarely stressed (Cespedes et al., 2003; Sezen et al., 2005). We frequently emphasize a large ratio of pollen/seed dispersal among mature populations at a moderate or large spatial scale in wind-pollinated species (Ennos, 1994; Petit et al., 2005). Effects of seed dispersal were underestimated at the fine spatial scale. Our results suggested the important effects of seed dispersal on the second-growth forests at the early stage of natural regeneration. One concern on the secondary forests is their evolutionary genetic potential and hence their sustainability (or extinction) in changing environments. This is ultimately related to the level of genetic diversity in the regenerated forests. The dependent property of the second-growth forests (especially the founding populations) on the old-growth forests (or the source pool) implies the critical importance of seed and pollen dispersal, especially seed dispersal (Gorchov et al., 1993; Duncan and Chapman, 1999; Standish et al., 2007). Barriers to seed dispersal from a variety of genetic sources may result in naturally regenerated populations possessing low genetic diversity that are susceptible to disturbance. Forest managers should, therefore, attempt to reduce such barriers.

One general consideration is that forest managers should take into account the properties of species-specific life history and the ecology of seed dispersal. For example, the vector of seed dispersal and seed longevity could affect seed recruitment to natural regeneration and these properties vary with species (Standish et al., 2007; references therein). Landscape fragmentation, the

frequent outcome of natural or human disturbances, could be a stronger physical barrier to seed recruitment for animal-dispersed species than for wind-dispersed species (Verheyen et al., 2003). However, construction of corridors may be an effective way to minimize the negative effects of fragmentation on seed dispersal in addition to maintaining plant species richness (Damschen et al., 2006). Wildlife corridors were also believed not only to help to help conserve biodiversity but also to facilitate seed dispersal and forest regeneration to abandoned lands (Beier and Noss, 1998). For *C. fargesii*, short-distant seed dispersal by natural gravity plays a dominant role in natural regeneration and the long-distance dispersal by rodents is negligible (Xiao et al., 2003). Thus, the approach that minimizes landscape fragmentation could be important in combination with other approaches that rely on natural processes or artificial plantations (Duncan and Chapman, 1999; Standish et al., 2007).

Acknowledgements

We appreciate two anonymous referees, associate editor, and Todd Fredericksen for constructive comments and suggestions that improved the early versions of this article, Fangliang He for kind support, and Michel E. Rahbeh for linguistic checks. This work was supported by National Natural Science Foundation of China (30470287) and the Program for New Century Excellent Talents in University (NCET-05-0431) of China to XYC.

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