Size-class Differences in Genetic Structure of *Metasequoia glyptostroboides* Hu et Cheng (Taxodiaceae) Plantations in Shanghai

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Abstract

We report RAPD variation in four size-classes of *Metasequoia glyptostroboides* plantations in Shanghai. Seven primers produced 48 discernible DNA fragments with 46 being polymorphic. The mean percentage of polymorphic loci (P_{95}) was 84.9% (ranged from 81.3% to 87.5%). The mean number of alleles per locus (A) ranged from 1.57 to 1.65 with a mean of 1.60. The mean heterozygosity (H_e) was 0.3502 (0.3338-0.3663). These data were higher than the estimates of a collection of individuals from natural populations (Li *et al.,* 1999). Little differences were observed among size-classes and the G_{ST} was 0.097, indicating that *M. glyptostroboides* trees planted recent decades were highly similar in genetic composition.

Key words: Metasequoia glyptostroboides Hu et Cheng, dawn redwood, plantation, RAPD, size-class.

Introduction

Metasequoia glyptostroboides Hu et Cheng, a Tertiary relict species native in China, was known to the scientific world since 1941, the same year that the genus had been established by Shigeru Miki, a Japanese paleobotanist, based on the well-preserved fossils of cones and foliages (Miki, 1941). The discovery of the living fossil has aroused widespread interest; and subsequently, *M. glyptostroboides* has been widely propagated around the world. Today it exists in plantations and as solitary trees, growing in more than 60 countries.

Viewed from the distribution and quantity, *M. glyptostroboides* has been successfully recovered and seems no longer to be threatened, though it was still listed in 1999 as a species needing first-class conservation by the State Council of People's Republic of China. However, some other factors – for instance, genetic variation – may influence the viability of plant species. Genetic variation not only has instant effects on the adaptation of the population (Martins and Jain, 1979), but also it is thought to be indicative to overall species vitality and the potential for evolutionary response to environmental changes (Cruzan, 2001; Ellstrand and Elam, 1993). Species with low or no genetic variation are thought to be sensitive to environmental changes and easy to go extinction. For example, American chestnut (*Castanea dentata*), once a dominant species in the eastern deciduous forests, suffered severe damages after chestnut blight arriving in the North America around 1900 and is threatened now. An explanation of the American chestnut's plight is its narrow genetic diversity compared to congener species (Huang *et al.,* 1998).

Despite the worldwide concerns for *M. glyptostroboides,* there is little information on the genetic variation in its natural populations and plantations. Kuser *et al.* (1997) has compared the genetic variation in two *ex situ* seedlots collected in 1947 and 1990, respectively. Only three of 15 isozyme loci were polymorphic and genetic variation measured higher in the 1990 seedlot. This pattern of allozyme variation may not represent the level of genetic diversity at other marker loci covering the entire genome of the species. More polymorphic molecular markers should be employed to complement allozyme studies.

Of the various DNA markers recently developed for plant research, random amplified polymorphic DNA (RAPD) has become increasingly popular in studies of natural and artificial plant populations (Nybom and Bartish, 2000). Li *et al.* (1999) examined 18 natural-like and 9 grafting or planted individuals of *M. glyptostroboides* using RAPD markers, and found low RAPD-based genetic variation compared to other conifers. In this study, we address the following questions by analyzing the genetic composition of *M. glyptostroboides* plantations in Shanghai using RAPD markers: What is the extent of genetic variation in *M. glyptostroboides* plantations in Shanghai in comparison with the genetic variation of wild populations? Are there any size-class differences in the genetic structures of plantations and thereby any differences of individuals planted at different times?

Methods

Plant material

In April of 2002, a total of 88 foliar samples of *M. glyptostroboides* were collected from 4 plantations – namely, Zhongshan Park, Tongji University's campus, Shanghai Normal University's campus, and East China Normal University's campus in Shanghai. The plantations were reintroduced from Xiaohe Town of Lichuan County, Hubei Province. The diameters at breast-heights of sampling individuals were recorded and were divided into 4 size classes (*table 1*). The fresh leaves were stored at –70 °C till grinding and extracting the DNA, and usually were subject to experiments within one week.

DNA extraction and PCR condition

Total plant DNA for PCR was isolated from *Metasequoia* leaves, according to a protocol modified from Doyle & Doyle's method (Doyle and Doyle, 1987). A set of seven oligonucleotide primers (Sagon Inc., Shanghai), which could amplify repro-

Table 1. – Classification of size-classes of *Metasequoia* individuals and the sample size. Approximative planted time was calculated with the regression equation based on the data observed in a plantation of Hubei Province (*Fig. 2*).

Size-class	Diameter at breast-height (cm)	Approximative plantation time	Sample size
1	<10	After 1992	14
2	10~20	1983-1991	24
3	20~30	1974-1982	34
4	>30	Before 1973	16

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ducible products, were used to characterize the 88 individuals. The sequence of the seven primers (5'-3') is: S21=CAGGCC-CTTC, S123=CCTGATCACC, S1200=GTGAACGCTC, S2040= CCAGGTGTAG, S2029=AGGCCGGTCA, S2140=TGGTAC-CTGG, S2154= ACCGTGGGTG. The procedure of RAPD-DNA amplification followed that described previously in 25 µl reaction volume (SHEN, 2002). 5 µl amplification product were analyzed by electrophoresis on 1.6% agarose gel in 0.5 x TBE buffer. The gels were observed and photographed with Bio-RAD Gel Doc2000™.

Data analysis

The RAPD data were scored as band present or absent. Assuming at Hardy-Weinberg equilibrium, allele frequencies (p and q) for RAPD loci were estimated using the homozygous null genotype $\sqrt{q^2} = q$ and $p = 1\text{-}q$. P_{95}, P_{99}, and H_e at the population and at the species level were calculated using TFPGA (Tools For Population Genetic Analyses) v1.3 (MILLER, 1997). SHANNON's diversity (LEWONTIN, 1972) was calculated to provide a relative estimate of the degree of variation within each population by using the formula: $SI = -\Sigma p_i log_2 p_i$, where p_i is the frequency of presence or absence of each RAPD band. SI and the effective number of alleles per locus (A_e, the reciprocal of homozygosity) (HARTL and CLARK, 1989) were estimated using Popgene 1.31 (YEH *et al.,* 1999). Mean number of alleles per locus (A) and G_{ST} were also calculated. An UPGMA dendrogram was constructed based on the matrix of NEI's (1978) distance using TFPGA v1.3 (MILLER, 1997).

Results

Of the 48 RAPD fragments scored, 46 fragments (96%) were polymorphic using the 95% criterion. A ranged from 1.81 to 1.88 (*table 1*) with a mean of 1.85. The percentage of polymorphic loci (P_{95}) ranged from 81.3% to 87.5% with a mean of 84.9%, P_{99}s were the same (89.6%) for the four size-classes, the effective number of alleles per locus (A_e) ranged from 1.57 to 1.65 with a mean of 1.60, and the expected heterozygosity (H_e) ranged from 0.3338 to 0.3663 with a mean of 0.3502, the SHANNON information index ranged from 0.4830 to 0.5236 with a mean of 0.5009 (*table 2*).

NEI's (NEI, 1978) genetic identity and distance were estimated for all 6 pairs comparison among the size classes (*table 3*). The mean identity was 0.937, ranging from 0.8956 to 0.9600; the mean genetic distance was 0.066, ranging from 0.0408 to 0.1103. The G_{ST} was 0.097. These estimates indicate low genetic differentiation among size-classes. Genetic relationships among size classes were further examined by UPGMA clustering using NEI's genetic distance. The UPGMA dendrogram revealed two groups: one group contained the largest size-class (size class 4) and another group included the other three size-classes (size classes 1, 2, and 3).

Table 2. – Genetic diversity of *Metasequoia* collections of different size classes.

Size-class	P_{95}	P_{99}	A	Ae	He	Shannon index
1	85.4	89.6	1.85	1.58	0.3463	0.4939
2	81.3	89.6	1.81	1.57	0.3338	0.4830
3	87.5	89.6	1.88	1.65	0.3663	0.5236
4	85.4	89.6	1.85	1.60	0.3542	0.5029
Mean	84.9	89.6	1.85	1.60	0.3502	0.5009
Total	95.8	100	1.96	1.66	0.3878	0.5533

Table 3. – NEI's unbiased genetic distance (under diagonal) and identity (above diagonal) (NEI, 1978) among 4 size-classes of *Metasequoia* glypostroboides plantations in Shanghai.

Size-class	1	2	3	4
1	-	0.9581	0.9554	0.8968
2	0.0428	-	0.9600	0.8956
3	0.0456	0.0408	-	0.9534
4	0.1090	0.1103	0.0477	-

Discussion

Gymnospermous species usually has high within-population genetic diversity (HAMRICK and GODT, 1989; NYBOM and BARTISH, 2000). Within-size class RAPD genetic variation in *Metasequoia* plantations (H_S = 0.350) is higher than the mean within-population diversity of plant species (H_{pop} = 0.214), but slightly lower than that of gymnosperms (mean = 0.386, based

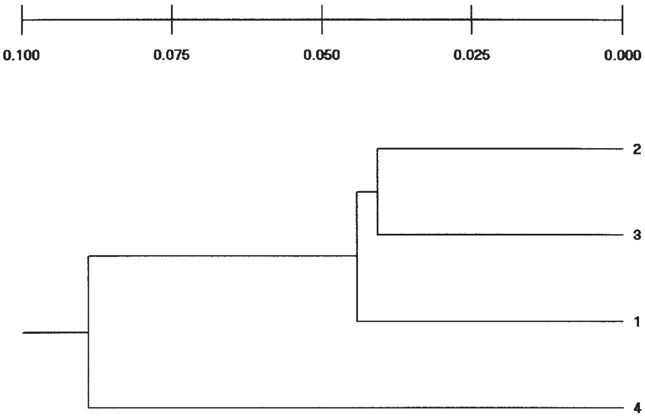

Figure 1. – UPGMA dendrogram of RAPD markers based on NEI's genetic distance between 4 size-classes of *Metasequoia glyptostroboides* plantations.

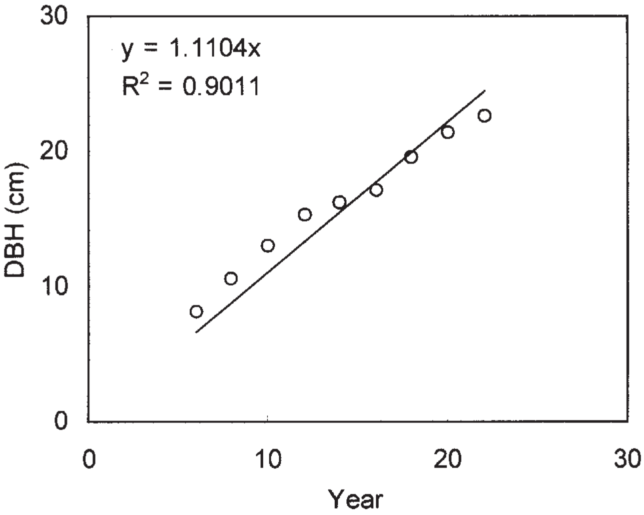

Figure 2. – Relationship between diameter at breast height (DBH, cm) and growth year. Data were cited from Forest in China (The Editorial Board of Forest in China, 1999).

on five species) (Nybom and Bartish, 2000). Lower RAPD-based genetic variation (P = 0.53) was also observed in wild *Metasequoia* populations using RAPD markers (Li *et al.,* 1999). However, compared to Li *et al.'s* (1999) result, plantations in Shanghai had higher genetic variation. It might be due to the small sample size in their study. In total, they analyzed 27 individuals, among which 8 were cuttings and one was planted.

Allozyme analysis indicated the same situation in the genetic variation of *M. glyptostroboides.* Frequency analysis of foliar allozymes showed that only 3 out of 15 loci ($P = 20.0\%$) were polymorphic in individuals germinated from the 1990 seedlot, while only 2 out of 15 ($P = 13.3\%$) were polymorphic in the 1947 seedlot (Kuser *et al.,* 1997). The mean number of alleles per locus was 1.40 and 1.70 in individuals of 1947 and 1990 seedlots, respectively. Expected heterozygosity was 0.083 in seeds from 1947 and 0.091 of the 1990 seedlots, respectively. These data are much lower than those of gymnosperms (Hamrick and Godt, 1989).

Genetic variation provides the potential to adapt the changing environment. Therefore, maintenance of genetic variation is an important objective of biodiversity conservation. Commercial management and breeding of plant species often leads to changes in the pattern of, and usually to the reduction in genetic diversity (Harlan, 1975). However, studies examining the impact of regeneration on genetic diversity have produced mixed results. Knowles found no difference in genetic diversity between fire-origin and artificially regenerated stands of jack pine and black spruce (Knowles, 1985). Lower, but not significantly, expected heterozygosity was observed in naturally generated stands than the planted or unharvested stands (Thomas *et al.,* 1999). In contrast, Gomory reported that planted stands of Norway spruce had significant less genetic diversity than unharvested or naturally regenerated stands (Gomory, 1992).

The difference between plantation and naturally regenerated or unharvested stands comes from two different sources. If seeds were collected from few maternal individuals, lower genetic diversity and/or biased genetic composition are usually observed (Chen, 1999). On the other hand, selection during nursery production may be less intense than that experienced at the early establishment phase in natural stands (Muona *et al.,* 1988), leading to higher levels of genetic diversity in artificially regenerated stands.

RAPD markers usually detected higher differentiation than allozymes did (Nybom and Bartish, 2000). However, little differences were found among size-classes of *Metasequoia* plantations in Shanghai. G_{ST} (0.097) was much lower than average of plant species analysed and gymnosperms (0.29 and 0.18, respectively) using RAPDs (Nybom and Bartish, 2000) and comparable with those using allozymes (Hamrick and Godt, 1989). The same population of ponderosa pine (Linhart *et al.,* 1981), or *Camellia japonica* (Ueno *et al.,* 2002) also expressed lower differentiation in size-class compared to in space. Similar results were observed in the genetic structure of seeds in successive years (Gregorius *et al.,* 1986) or in successive soil layers (Morris *et al.,* 2002). These indicated that differentiation in time is usually lower than in space (Linhart *et al.,* 1981). Demographic equilibrium in the old-growth forest might explain the little differences among size-classes (Ueno *et al.,* 2002). However, in the present study, the little differences reflected that *M. glyptostroboides* individuals planted in recent decades were similar in genetic composition. Almost all the seeds and seedlings planted all over the world were from Xiaohe of Lichuan, Hubei Province. The same maternal pool and random distribution of seeds and seedlings might contribute to the little differences in genetic composition among size-classes.

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