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Chloroplast DNA diversity in *Castanopsis hystrix* populations in south China

J. Li [a,b], X.J. Ge [a,c], H.L. Cao [a], W.H. Ye [a,*]

[a] *South China Botanical Garden, The Chinese Academy of Sciences, Guangzhou 510650, PR China*
[b] *Graduate School of the Chinese Academy of Sciences, Beijing 100049, PR China*
[c] *School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, PR China*

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Abstract

Nineteen Chinese populations of *Castanopsis hystrix* were examined to quantify genetic diversity and genetic structure at chloroplast DNA. Microsatellites (SSR) were analyzed by PCR using conserved primers. The average within population gene diversity (H_S), the total gene diversity (H_T), and the differentiation for unordered alleles (G_{ST}) and for ordered alleles (N_{ST}) were measured. Fourteen different haplotypes were detected, two of them very common. The level of differentiation among populations (G_{ST} = 23.6%) indicates a highly efficient seed dispersal mechanism. In addition, the difference between G_{ST} and N_{ST} for the species is not significant, suggesting that the phylogeographic structure is weak or absent. The geographical pattern of *C. hystrix* haplotypes could be attributed to its migration from the numerous and scattered refugia, where the species confined during the last glacial period. These results provide an important insight into patterns of postglacial recolonization of this tree species.

Keywords: cpDNA; SSR; Genetic differentiation; *Castanopsis hystrix*

1. Introduction

The modern distribution of plant and animal taxa is determined not only by the current environment, but also by historic events such as the last glacial period (Hengeveld, 1989). Although the land in south China has never been covered by ice sheets, the tremendous temperature and climatic changes might have influenced species' distributions and evolution. The Holocene postglacial history of many trees is characterized by the northward expansion of southern refugial populations following the retreat of the ice sheets. The last glacial age might have been an important factor in determining the genetic structure and phylogeography of plant species in south China.

Fossil pollen deposits can be used to reconstruct long-term vegetation dynamics, such as changes in the distribution and abundance of plant species during the quaternary period. But for the species which produce only small amount of pollen grains, occurrences of their pollen grains in the fossil pollen record may be therefore too infrequent to allow detailed reconstruction of the species' past range and migration patterns. An alternative approach to the study of postglacial changes in plant distributions developed in recent years is the use of molecular markers (Ferris et al., 1999; Hewitt, 1999). Currently existing plant populations are expected to retain the genetic traces resulting from the migration routes they followed during the past, which could be revealed by studying the spatial distribution pattern of molecular markers. Chloroplast markers have been widely applied to studies of population history in trees (e.g. Walter and Epperson, 2001; Rendell and Ennos, 2003; Cheng et al., 2005). Firstly, because chloroplast genomes are haploid, their effective population size in monoecious outcrossing plants is half of diploid nuclear genomes. As a result, chloroplast-specific markers are considered good indicators of historical bottlenecks, founder effects and genetic drift. The use of chloroplast microsatellites has allowed the examination of these events at a finer level of detail than it was previously possible. Secondly, because chloroplasts evolve slowly and exhibit little variation at the intraspecific level (Clegg et al., 1994), cpDNA markers have been widely used for phylogenetic inference (Olmstead and Palmer, 1994) and to some extent, for within-species genetic studies (Soltis et al., 1992; Ennos et al., 1999). However, microsatellite markers are

* Corresponding author. Tel.: +86 20 37252996.
E-mail addresses: lijing@scib.ac.cn (J. Li), why@scib.ac.cn (W.H. Ye).

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also used to find fine-scale polymorphism because of their highly polymorphic nature (Jarne and Lagoda, 1996; Powell et al., 1996). Thirdly, chloroplast DNA is maternally inherited, i.e. through the seeds in most angiosperms (Dumolin et al., 1995; Rajora and Dancik, 1992; Mogensen, 1996). Therefore cpDNA, which generally reflects seed dispersal and maternal gene flow, is an effective tool for genetic variation studies and for identifying postglacial migration routes (McCauley, 1994). Finally, the geographically structured cpDNA variations permit the elucidation of evolutionary history and the study of intraspecific phylogeography (Soltis et al., 1997).

Castanopsis hystrix (Fagaceae) is an evergreen tree species, as one of fagaceous species originated in the south China and Indochina (Li, 1996). The present-day distribution of *C. hystrix* in China extends from Taiwan, along Fujian, Guangdong, Guangxi, Yunnan, to south Tibet, expanding southwards as far as Hainan island. *C. hystrix* is one of the most important and dominant species of the evergreen broad-leaved forests in subtropical China. The extensive use of *C. hystrix* wood for many purposes, such as construction and boat building, resulted in the fragmentation of the once continued and broad natural distribution and thus in population shrinkage and possible genetic erosion. For both conservation and forestry management, therefore it is essential to be able to assess *C. hystrix*'s genetic diversity. Although pollen data show that the *C. hystrix* forests were present in southern China through the quaternary period, some reduction occurred in glacial period (Zheng, 1991; Sun et al., 1999; Sun and Li, 1999; Zheng and Lei, 1999; Zheng et al., 2004) and the information of its genetic diversity and population history is very limited.

Main objective of the present study was to assess the cpDNA variation and diversity among and within *C. hystrix* natural populations, to investigate whether its genetic diversity follows a geographic pattern and discuss the forces and events that led to this pattern.

2. Materials and methods

2.1. Collection of samples

Nineteen populations of *C. hystrix* (380 individuals), comprising 20 individuals per population, were sampled in south China (Table 1 and Fig. 1). Sample collections covered most of the known populations for this species in mainland China. To avoid sampling clones or close relatives, all individuals chosen were separated by at least 20 m, except for the population Yanyang which is a very small population (the respective number 7), where the least distance between plants was 10 m based on the sample size and the availability. Approximately 15 leaves per individual were sampled. Leaf material collected in the field was dried immediately using silica gel and stored at room temperature until DNA extractions were completed.

2.2. DNA extraction

Genomic DNA was extracted following the cetyltrimethyl ammonium bromide procedure (Doyle, 1991). One to two grams of the dried leaves were submerged in liquid nitrogen, and then ground into powder, added to 750 μl of 2× CTAB (2% (w/v) hexadecylcetytrimethylammonium bromide) in a 1.5 ml centrifuge tube and incubated at 65 °C for 45–60 min. A volume of 750 μl of 24:1 chloroform:*iso*-amyl alcohol was added. The tubes were mixed evenly and centrifuged at 10,000 rpm for 10 min. The top layer was pipetted into a clean tube, and the above step was repeated. Finally the supernatant was transferred to a clean tube to which 600 μl of isopropanol and 150 μl of sodium chloride were added. It was kept at −20 °C for more than 30 min and centrifuged at 10,000 rpm for 10 min. The supernatant was discarded and the precipitate was washed with 500 μl of 75% ethanol two times, dried at room temperature and then dissolved in 100 μl of TE (Tris–EDTA buffer, pH 8).

Table 1
The studied populations of *Castanopsis hystrix*

Population no.	Population name	Province region	Latitude	Longitude	Altitude (m)
1	Jianfengling	Hainan	18°44′N	108°52′E	900
2	Nanjing	Fujian	24°31′N	117°21′E	300
3	Nanhuasi	Guangdong	24°33′N	113°41′E	120
4	Deqing	Guangdong	23°13′N	111°56′E	350
5	Jintong	Guangdong	22°24′N	110°44′E	350
6	Zijin	Guangdong	23°42′N	114°54′E	150
7	Yanyang	Guangdong	24°15′N	116°11′E	150
8	Huadu	Guangdong	23°24′N	113°11′E	200
9	Heyuan	Guangdong	23°21′N	114°32′E	120
10	Enping	Guangdong	22°15′N	112°06′E	150
11	Luofushan	Guangdong	23°16′N	114°03′E	100
12	Gongcunzhang	Guangdong	23°27′N	115°23′E	200
13	Longsheng	Guangxi	25°50′N	109°53′E	200
14	Jinxiu	Guangxi	24°08′N	110°11′E	300
15	Damingshan	Guangxi	23°10′N	108°16′E	450
16	Xishuangbanna	Yunnan	21°52′N	100°26′E	350
17	Simao	Yunnan	24°16′N	100°48′E	1900
18	Xichou	Yunnan	23°25′N	104°41′E	1600
19	Maguan	Yunnan	23°06′N	104°02′E	1700

2.3. Chloroplast microsatellites

Because the chloroplast genome is haploid and does not undergo recombination it can be viewed as a single locus and all sequence variation can be interpreted as giving rise to different haplotypes of the genome. The chloroplast genome may alternatively be viewed as a circular haploid chromosome wherein sequence variation generates different alleles within the individual (nonrecombining loci). In either case, for ease of presentation and discussion, we used the terms 'locus' to refer to a cpSSR site (defined by the termini of a PCR primer pair), and 'alleles' to refer to length variants at a cpSSR site.

Seven universal primers described in Weising and Gardner (1999) and Chung (2002) were used in this study (Table 2). Four of these primers are flanking angiosperm chloroplast microsatellites and the remaining three are flanking cucumber chloroplast microsatellites. PCR amplification was performed in a 20 μl reaction mixture consisting of 50 ng of template DNA, 10 mM of Tris–HCl (pH 9.0), 50 mM of KCl, 0.1%