Preliminary study on the genetic diversity and differentiation of three Chinese *Burguiera gymnorrhiza* populations

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Abstract: The genetic diversity and differentiation of three Chinese *Bruguiera gymnorrhiza* populations was examined in this study: Expected heterozygosity at species and population levels was 0.293 and 0.268, observed heterozygosity was 0.2745 and 0.2705, respectively. The genetic diversity between populations was 0.0830, which showed that among total heterozygosity, 8.3% came from inter-population. Gene flow was smooth, which was 3.34. [Nature and Science, 2004,2(2):67-72]

Key words: *Bruguiera gymnorrhiza;* genetic diversity; genetic differentiation

1 Introduction

The studies on genetic diversity of plants have much been developed in and abroad of recent years and most of them are studying the genetic structure (Spieth, 1974; Hoey, 1931; Hokanson, 1993) using allozyme as genetic markers. Such works can provide certain evidences for breeding, introducing a fine variety and protecting species resources. Mangroves are woody plant communities growing in tropical and subtropical areas along seashore and widely distributed in Guangdong, Guangxi, Hainan, Fujian and Taiwan provinces, China. It has distinguished characteristics such as vivipary, salinity-resistance and similar habitats. All of which made it far different from terrestrial plant communities. Although the energy ecology, physiology ecology and pollution ecology of mangroves have much been studied (Lin, 1990,1993,1997), little was known on its genetic structure. This article was aimed at this study, using *B. gymnorrhiza* as samples.

2 Materials and Methods

2.1 Sampling sites

Three sampling sites among mangrove distributing areas were selected, they are National Nature Mangrove Reserve of Dongzhai Harbor, Hainan Province (19°54'N，110°20'E), National Nature Mangrove and Bird Reserve at Futian and Neilingding, Shenzhen, Guangdong Province (22°32'N, 114°05'E) and Nature Mangrove Reserve of Longhai, Fujian Province (24°24'N, 117°55'E). *B gymnorrhiza* are natural and single forest in Dongzhai Harbor, Hainan Province, accompanying a few *Bruguiera sexangula* and *Bruguiera sexangula* var. *rhynchopetala*. *B. gymnorrhiza* in Shenzhen are scatterly distributed in *Kandelia candel* + *Aegiceras corniculatum* + *Avicennia marina* communities. *B. gymnorrhiza* population in Fugong is artificial forest, its transplanting time is more than ten years.

2.2 Sample collecting and treating

Fresh leaves of *B. gymnorrhiza* were collected in each site at random. The distance between each tree was more than five meters. Keeping these leaves fresh as well as taking them to laboratory as fast as possible. Mature hypocotyls of *B. gymnorrhiza* also could be collected, while the method was the same with leaves. Mature hypocotyls could be planted in fresh water washed sand in pots, when leaves grow up, using these leaves for analysis. As mangroves are full of tannin (Lin, 1984), the enzyme extracted solution was slightly modified according to Tris-HCl extracted solution (Wang, 1996). Leaves were grounded on ice-bath and then prepared for using.

2.3 Electrophoresis and analysis of zymogram

The enzymes were detected using polyacrylamide gel electrophoresis (PAGE) and horizontal sliceable starch gel electrophoresis (SGE). 15 loci encoded by 7

enzyme systems were studied. The concentrations of concentration gel and segregation gel were 2.5% and 7.0%, respectively, pH were 6.7 and 8.9, respectively in PAGE. According to separating degree and clarity of enzyme, hydrolysis potato starch (Sigma S-5691) and mixed starch were separately used. The concentration of starch was 12% and the corresponding buffers were Tris-Boric acid-EDTANa_4 (pH 8.6) (#10) and Tris-Boric acid-EDTANa_2 (pH 8.0) (TVB). The enzyme systems, E.C. No., numbers of loci and buffer systems in this study were show in Table 1. Histochemistry staining methods see Wang (1996), Vallejos (1983).

Table 1 The enzyme systems, E.C. No., buffer systems and numbers of loci in this study

Enzyme systems (Abbreviation in parentheses)	E.C. No.	Types of gels (Buffers in parentheses)	Numbers of loci
Malate dehydrogenase (MDH)	E.C.1.1.37	SGE(#10)	2
Malic enzyme (ME)	E.C.1.1.40	SGE(#10)	1
Esterase (EST)	E.C.3.3.3.-	SGE(TVB)	2
Peroxidase (POD)	E.C.1.11.1.7	PGE	2
Aspartate aminotransferase (AAT)	E.C.2.6.1.1	SGE(#10)	2
Alkaline phosphatase (ALP)	E.C.3.1.3.1	SGE(TVB)	3
Superoxide dismutase (SOD)	E.C.1.15.1.1	SGE(#10)	3

2.4 Calculating methods

2.4.1 Genetic Diversity Calculating

Percentage of polymorphic loci (*P*): $P=(k/n)\times 100\%$, *k*- numbers of polymorphic loci; *n*- total numbers of loci.

The criteria of polymorphic loci were according to 0.99 criteria (Nei, 1975), where the frequencies of the most common alleles was lower than or equal to 0.99.

Heterozygosity (*H*): percentage of heterozygous loci.

The expected heterozygosity (*He*):

$$He=\sum_{i=1}^{n}(1-\sum_{j=1}^{mi}q_{ij}^{2})/n$$

The observed heterozygosity (*Ho*):

$$Ho=\sum_{i=1}^{n}(1-\sum_{j=1}^{mi}p_{ij})/\mathrm{n}$$

q_{ij}– frequencies of j allele at i locus

p_{ij} – genotype frequencies of homozygotes of j alleles at i locus

Mean number of efficient alleles per locus (*Ae*):

$$Ae=\sum_{i=1}^{n}(1/\sum_{j=1}^{m}q_{ij}^{2})/\mathrm{n}$$

q_{ij}– frequencies of j alleles at I locus

Fixation index (*F*): $F=1-Ho/He$

2.4.2 Genetic differentiation calculating

Coefficient of gene differentiation: $G_{ST}=D_{ST}/H_T$

D_{ST} – gene diversity between populations; H_T – total genetic diversity

Genetic distance was detected using standard genetic distance (Nei, 1987):

$$I=\sum_{k}\sum_{i}XiYi/\sqrt{\sum_{k}\sum_{i}Xi^{2}\cdot\sum_{k}\sum_{i}Yi^{2}}$$

$D=-\ln I$

Gene flow was detected using formula:

$Nm=(1-F_{ST})/4F_{ST}$

3 Materials and Methods

3.1 Genetic diversity of *B. gymnorrhiza* populations

Fifteen enzyme loci encoded by 7 enzyme systems were detected in this article. 12 of that could be analyzed were shown in Table 2 and the allele frequencies see Table 2, too. Table 3 shows each indices of genetic diversity. Mean numbers of alleles per locus were 2.000 at population level, which was lower than that at species level (2.167). The value of A was equal at Hainan and Fugong populations. The mean number of efficient alleles per locus was 1.537 (at population level) and 1.575 (at species level), respectively, showing little difference. However, there was much difference at each

population, the range was from 1.487 to 1.576. The observed heterozygosity were also shown little difference at both population level and species level, which was 0.2705 and 0.2745, respectively, while the expected heterozygosity was 0.268 (at population level) and 0.293 (at species level), showing larger difference. The observed heterozygosity (0.2745) was lower than the expected heterozygosity (0.293) at species level, which showed the deficient of heterozygotes.

The trend was also been found from F value. At a total, each means of genetic diversity index was lower at population level than that at species level. We can see this at other species (Chen, 1997; Ge, 1997).

3.2 The genetic cifferentiation of *B. gymnorrhiza* populations

The differentiation degree between *B. gymnorrhiza* populations was lower. H_T was 0.2926, H_S was 0.2683, D_{ST} was 0.0243 and G_{ST} was 0.0830, which showed that among total heterozygosity there was only 8.3% coming from inter-population.

Table 2 Allelic frequencies of *B. gymnorrhiza* populations

Locus	Allele	Dongzhai Harbor of Hainan	Futian of Shenzhen	Fugong of Fujian	Species level
Mdh-1	A	1.000	0.953	1.000	0.984
	B	0.000	0.047	0.000	0.016
Mdh-2	A	0.220	0.488	0.044	0.248
	B	0.683	0.512	0.956	0.721
	C	0.098	0.000	0.000	0.031
Me-1	A	0.407	0.244	0.596	0.391
	B	0.593	0.756	0.404	0.609
Est-2	A	1.000	0.860	0.800	0.892
	B	0.000	0.140	0.200	0.108
Pod-1	A	0.964	1.000	0.973	0.977
	B	0.036	0.000	0.027	0.023
Pod-2	A	0.915	0.679	0.462	0.700
	B	0.053	0.231	0.205	0.156
	C	0.032	0.077	0.244	0.112
	D	0.000	0.013	0.089	0.032
Aat-1	A	0.529	0.593	0.182	0.480
	B	0.471	0.140	0.455	0.323
	C	0.000	0.267	0.364	0.197
Alp-2	A	1.000	1.000	1.000	1.000
Alp-3	A	0.491	0.389	0.250	0.403
	B	0.500	0.597	0.750	0.589
	C	0.009	0.014	0.000	0.008
Sod-1	A	0.471	0.477	0.458	0.471
	B	0.500	0.477	0.542	0.500
	C	0.029	0.047	0.000	0.029
Sod-2	A	0.740	0.907	1.000	0.866
	B	0.260	0.093	0.000	0.134
Sod-3	A	1.000	1.000	1.000	1.000

Table 3 Genetic diversity index of *B. gymnorrhiza* populations

	P	*A*	*Ae*	*He*	*Ho*	*F*
Dongzhai Harbor of Hainan	66.7%	1.917	1.489	0.259	0.2472	0.1655
Futian of Shenzhen	75%	2.167	1.545	0.287	0.2803	0.1308
Fugong of Fujian	66.7%	1.917	1.576	0.260	0.2840	0.0056
Mean	69.5%	2.000	1.537	0.268	0.2705	0.1006
Species level	83.3%	2.167	1.575	0.293	0.2745	0.2002

Table 4 Genetic differentiation in and between *B. gymnorrhiza* populations

Locus	H_T	H_S	D_{ST}	G_{ST}
Mdh-1	0.0315	0.0299	0.0016	0.0517
Mdh-2	0.4218	0.3531	0.0687	0.1629
Me-1	0.4859	0.4444	0.0415	0.0854
Est-2	0.2005	0.1869	0.0135	0.0675
Pod-1	0.0411	0.0407	0.0005	0.0114
Pod-2	0.4891	0.4385	0.0506	0.1035
Aat-1	0.6407	0.5610	0.0796	0.1243
Alp-2	0.0000	0.0000	0.0000	0.0000
Alp-3	0.4784	0.4587	0.0197	0.0412
Sod-1	0.5234	0.5222	0.0012	0.0023
Sod-2	0.2082	0.1845	0.0237	0.1136
Sod-3	0.0000	0.0000	0.0000	0.0000
Mean	0.2934	0.2683	0.0250	0.0636
Total loci	0.2926	0.2683	0.0243	0.0830

Table 5 Comparison between *B. gymnorrhiza* and mean of other species

	P	*He*	*A*	G_{ST}				
				Selfing	Outcrossing-wind	Outcrossing-Insect	Annual	Long-lived perennial
Other species	50%	14.9%	1.97	51%	9.9%	21%	3%	8%
B. gymnorrhiza	69.5%	26.8%	2.000			8.3%		

Since the genetic differentiation between populations was low, the genetic distance between these three *B. gymnorrhiza* populations would not be very large. The genetic distance between Hainan and Shenzhen was the smallest (0.038), and that between Shenzhen and Fugong was the biggest (0.0799).

Mean of genetic identity was 0.942. Gene flow was 3.43 calculated through F_{ST}.

4 Discussion

The level of genetic diversity in three *B. gymnorrhiza* populations was high, but the genetic differentiation between them (G_{ST} = 0.0830) was lower. This had something to do with the biological characteristics of this species.

B. gymnorrhiza was tropical plant, longevity, perennial and insect-pollinated. According to the statistic of Hamrick (1989) (Table 5), the biological characteristics of *B. gymnorrhiza* were according with those average value. But we found that the G_{ST} of *B. gymnorrhiza* was similar with that of wind-pollinated, outcrossing plants, while *B. gymnorrhiza* was insect-pollinated, and outcrossing plants. The reason is that the statistics coming from Hamrick was the results of terrestrial plants including 165 genus and 653 taxa, while mangroves grow in areas influenced by tide and its habitats are far different from terrestrial plants. At the same time, the gene flow between mangroves is quite smooth because of the movement of hytocytols. Wright (1931) suggested that when Nm was more than 1, gene flow could efficient prevent the genetic differentiation. The level of genetic diversity of plants was related to mating system, ecological and biological characteristics.

B. gymnorrhiza is one of widespread species of mangroves. Hamrick suggested that widespread species could keep genetic diversity within populations while the genetic differentiation rates between populations were low. The studies on *Kandelia candel* (Huang, 1996) and *Rhizophora stylosa* (Goodall, 1994) also showed the lower genetic differentiation between mangroves (*Kandelia candel* F_{ST} = 0.043, *Rhizophora stylosa* F_{ST} = 0.023, *Bruguiera gymnorrhiza* F_{ST} = 0.0679).

Fugong *B. gymnorrhiza* population was artificial forest; the hypocotyls were from Hainan and now are doing well after ten years. Its genetic diversity was close to Hainan *B. gymnorrhiza* population (0.259, 0.260, respectively) as well as other indices of genetic diversity were not far from difference. This phenomenon showed that the Fugong population was not suffered founder effect and bottleneck effect and keep the most genetic diversity in the population. The north border of *B. gymnorrhiza* was at the south of Zhangpu of Fujian Province in China; Fugong is at the north of Zhangpu. The genetic characteristics of Fugong *B. gymnorrhiza* were changed after fighting against cold and adapting to local habitats. For example, the *Alp*-1A allele was lost in Fugong *B. gymnorrhiza*, but the frequencies of the same alleles were 1.000 in Hainan and Shenzhen populations. This might be related to latitude, temperature and genetic drift.

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