Mating System and Outcrossing Rates of Four *Bruguiera Gymnorrhiza* Populations of Mangrove, China

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Abstract: Using polymorphic loci of allozyme as genetic marker and horizontal sliceable gel electrophoresis as method to examine the genotypes of 4 *Bruguiera gymnorrhiza* populations in Fugong of Fujian (24°24′), Futian of Guangdong (22°32′), Shankou of Guangxi (21°28′) and Dongzhai Harbor of Hainan (19°51′), China. The mating system was also decided by Multi Locus Testing. The polymorphic loci selected for analyzing were different in these four areas: *Mdh*-1, *Mdh*-2, and *Me*-1 in Fugong, Guangxi and Hainan as well as *Mdh*-1, *Mdh*-2, *Aat*-1, *Aat*-2 in Shenzhen. The results showed that the difference between outcrossing rates of polymorphic loci were quite obvious, that of Fugong was the biggest, which was 0.845 and that of Hainan was the smallest, which was 0.267. The different value between outcrossing rates of polymorphic loci and the mean of outcrossing rates of monomorphic loci in these four areas were vary, which showed that the mating system of Fugong, Guangxi and Hainan were slightly biaparental inbreeding and that of Guangxi was at random. *Bruguiera gymnorrhiza* was mixed-mating species and its mating system was mainly outcrossing. This was influenced by factors such as plant population density and structure, the adaptability of pollinator and whether it's self-incompatibility or not. [Nature and Science 2003;1(1):42-48].

Key words: mangrove; mating system; *Bruguiera gymnorrhiza*

1. Introduction

Mating system is determinant for genetic differentiation of inter-population and intro-populations (Brown, 1989; Brown, 1989; Hamrick, 1989). As a bridge connecting two generations, mating system also determines the genotype distribution and population dynamic of offspring populations (Hamrick, 1982) . Many researchers have emphasized the functions of mating system on gene flow, genetic structure of populations and potential evolution of plant population (Brown, 1979; Hamrick, 1979; Loveless, 1984). Recently, the studies involved plant mating systems have been thought a lot of. For example, in the article of Barrett and Eckert (1990), 129 plants' mating systems have been concerned. Polymorphic loci are useful for estimating plant mating systems (Brown, 1978; Hamrick, 1989), these have been affirmed in recent studies (Moran, 1989; Sampson, 1989; Warwick, 1989; Morgan, 1990; Watkins, 1990; Sun, 1998), and have provided rapid and efficient methods for studying genetic diversity of plant populations. There are many models used for estimating mating system, and the classical one is Mixed Mating Model (Fyffe, 1951). On the basis of above, Shaw et al. (1982) and Ritland (1981) published Multi Locus Testing (MLT) Model, which is the most popular procedure used in estimating outcrossing rate. In this study, we also use this MLT.

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. They have distinct characteristics such as vivipary, salinity-resistance and similar living habitats. All of these made them far different from terrestrial plant communities. Although the energy ecology, physiology ecology and pollution ecology of mangroves have much been studied since 1980s, the studies on mating system are not much concerned.

Several examples of mating system of mangroves were used, such as characteristic that the hypocotyls still stay in the mother trees after they maturated, so it is easy to observe the offspring separation rate. For example, the outcrossing rates of *Rhizophora mangle* (Lowenfeld, 1992; Klekowski, 1994) and *Kandelia candel* (Chen, 1996) were estimated by observation of chlorophyll-deficient heterozygotes of offspring. Recently, allozymic polymorphic loci were used as genetic markers to estimate mating system of *Kandelia candel* by Sun et al. (1998). In this study, *Bruguiera gymnnorrhiza*, which is widespread species in mangrove, was used as materials to study mating system through MLT procedure. Upon this study, genetic diversity and genetic differentiation will be much better understood.

2. Materials and Methods

2.1 Sampling Sites

Bruguiera gymnorrhiza is widespread thermophile (Lin, 1984), pollinated by birds or butterflies, hermaphrodite, which is naturally distributed along seashore in Guangdong, Guangxi, Hainan, Fujian and Taiwan

Provinces, China. Five sampling sites were selected in these areas according to limited conditions. Climate, chemical and physical characteristic of soils in sampling sites were shown in Table 1.

Table 1. Climate and Chemistry-Physic Characteristic of Soils in Research Sites of *Bruguiera gymnorrhiza* Populations

Items	Fugong	Shenzhen	Guangxi	Hainan
Latitude	24°24′	22°32′	21°28′	19°51′
Longitude	117°55′	114°05′	109°43′	110°24′
annual mean temperature℃	21.0	22.0	22.6	23.8
annual temp. difference℃	16.7	14.0	15.2	11.3
rainfall mm	1365.1	1926.7	1796.8	1697.8
Organism%	2.67	3.62	4.94	7.38
total N%	0.54	0.53	0.50	0.55
total P（P_2O_5%）	0.018	0.021	0.009	0.011
total salinity%	0.821	0.844	1.590	5.921
content of Cl^-%	13.280	4.195	6.938	7.266
PH	6.92	6.55	3.16	3.01

Fugong site is located in Longhai Mangrove Protect Area, Guoxia Village, Fugong Town, Longhai, Fujian Province. This protected area is in the entrance of Jiulong River, where belongs to subtropical climate. Several species of mangroves are natural growing, which includes *Aegiceras corniculatum*, *Avicennia marina*, *Acanthus ilicifolius* and *Acanthus xiamenensis*.

Shenzhen site is located in Futian National Mangrove Protect Area, northeast to Shenzhen Bay, Guangdong Province, where is 11 km long and 10 to 200 m wide. This area is close to subtropical sea, belongs to subtropical monsoon climate. The main species are *Kandelia candel*, *Aegiceras corniculatum* and *Avicennia marina*, and secondly are *Bruguiera gymnorrhiza*, *Acanthus ilicifolius* and *Excoecaria agallocha*, sum up to five families, six genera and six species. Semi-mangrove species such as *Acrostichum aureurm*, *Thespesia populnea* and *Pluchea indica* also exist.

Guangxi site is located in Shankou National Mangrove Ecological Nature Protect Area, Yingluo bay, Hepu county, southeast of Guangxi district, where is located in the marginal terrain between north torrid zone and south sub-torrid zone, belongs to north monsoon torrid zone. Mangrove in Yingluo bay lies in high tide beach, which is 86.67 hm^2 large. *Kandelia candel* and *Aegiceras corniculatum* live at the edge of the zone, accompanying with scattered *Avicennia marina*. *Bruguiera gymnorrhiza* is a small community near to seashore.

Hainan site is located in Dongzhai Harbor National Mangrove Protect Area, Qiongshan, Hainan. Mangrove species are quite lot more here and sum up to 12 families, 14 genera and 18 species. Furthermore, another 8 species were introduced. *Bruguiera gymnorrhiza* is natural and pure forest, accompanying by *Bruguiera sexangula*, *Ceriops tagal* and *Kandelia candel*.

2.2 Sample Collecting and Treating

2.2.1 Sample Collecting

Five to eight trees, used as mother family arrays, were selected at random in each site when hypocotyls were mature. Five to eight hypocotyls were sampled on each tree as progeny arrays. Took these hypocotyls back to lab and planted them in fresh water washed sand. Hypocotyls in the same mother family arrays should be planted in the same pot. When leaves grew up, sampled fresh leaves according to progeny arrays.

2.2.2 Sample Treating

Fresh young leaves were taken into analysis. The leaves were cut into small pieces, and put into mortars. Suitable Tris-HCl extracted buffer were added, and the leaves were ground. All of these above processes would be on the ice bath. When the leaves were ground into even pasty, 4×7 mm wicks were used to absorb even pasty liquid (i.e. crude enzyme extracted solution). Loading sample and running with horizontal sliceable gel electrophoresis. Those enzymes must be analyzed as quickly as possible. As mangroves are full of tannin (Lin, 1984), the enzyme-extracted buffer was slightly modified according to Tris-HCl extracted solution (Wang, 1996; Chen, 1997).

2.3 Electrophoresis and Analysis of Zymogram

Horizontal sliceable starch gel electrophoresis (SGE) was used to detect the genotypes of progeny and parent. Hydrolyzed potato starch made by Sigma (s-5691) was used and its concentration was 12.75%, the corresponding gel buffer system was Tris-Boric Acid-EDTANa_4 （#10）（pH8.6）(Wang, 1996).

The enzyme systems, E.C. No., numbers of loci and buffer systems used in this study were show in Table 2. Locus and their numbers were different in each site (Table 3).

Table 2. The Enzyme Systems, E.C.No., Numbers of Loci and Buffer Systems of *Bruguiera gymnorrhiza* Populations

Enzyme system (Abbreviation in parentheses)	E.C.No.	Type of gel (buffer in parentheses)	numbers of loci
Malate dehydrogenase (MDH)	E.C.1.1.37	SGE（#10）	3
Malic enzyme (ME)	E.C.1.1.40	SGE（#10）	1
Aspartate (AAT)	E.C.2.6.1.1	SGE（#10）	2

Table 3. Loci of *Bruguiera gymnorrhiza* Populations Used in Mating System

Locus	Fugong	Shenzhen	Guangxi	Hainan
Mdh-1	+	+	+	+
Mdh-2	+	+	+	+
Me-1	+		+	+
Aat-1		+		
Aat-2		+		

2.4 Estimating Methods of Outcrossing Rates

Mating system of *Bruguiera gymnorrhiza* populations were estimated by MLT-1（Ritland, 1990）. This procedure also can estimate maternal plant genotype and pollen pool allelic frequencies at the same time. The included estimation parameters were shown as follows:

tm—multiloci outcrossing rate

ts—mean of single locus outcrossing rate

tsa—outcrossing rate of each locus using MLT

tm-ts—difference between tm and ts. If tm-ts is bigger than zero, which indicated that the parents are inbreeding. The estimation of tm, ts, tsa is through the comparison between maternal genotype and progeny genotype. Computer was used to do all of these estimations. Estimating the multiloci will have much priority because all of the results are on the base of all loci, which avoid some problems in using single locus (Green, 1980; Shaw, 1982), such as affected by selection or random mating（Ritland, 1981）.

3. Results

3.1 Allelic Frequencies of Hypocotyls, Maternal Plants and Pollen Pool in Each *Bruguiera gymnorrhiza* Population

Allelic frequencies of hypocotyls, maternal plants and pollen pool of four populations were shown in Table 4. The results showed that there were certain difference in these three items at different loci and different populations. In Shenzhen population, allelic frequencies difference between pollen pool and hypocotyls were small, but that between pollen pool and maternal plants were quite large. Same phenomenon also existed in other three populations. This might be due to the different pollen amounts produced by different mature individual. In addition, tree ages might also affect pollen amounts. There were some reports on this problem in conifer (Muller, 1984). Both limited spreading of pollen and selecting function of gamete can result in allelic frequencies difference of pollen pool and maternal plants (Apsit, 1989). Except for some alleles in certain loci, such as *Aat*-1C in Shenzhen population, *Mdh*-1A in Guangxi population, *Me*-1B in Hainan population and *Mdh*-1A in Fugong population, there were slight difference between pollen pool and maternal plant, which showed that the pollen contribution of maternal plant to this allele was equal.

3.2 Outcrossing Rates of Each *Bruguiera gymnorrhiza* Population

The outcrossing rates of each population estimated by MLT and those of single locus were shown in Table 5-8. The results showed that tm in each population differed quite big. tm in Fugong population was the biggest, which was 0.845 and that in Hainan population was the smallest, which was 0.267. tm in Guangxi population was similar with that in Fugong population, which was 0.820, while tm in Shenzhen population was 0.561. The tm of *Kandelia candel* population was 0.697 (Sun, 1998), that is in the same family with *Bruguiera gymnorrhiza*. tm in Guangxi and Fugong were little higher while that in Shenzhen and Hainan was little lower.

Except for Guangxi population, the tm-ts of other three populations were positive which showed that these three populations were slightly inbreeding while Guangxi population was random mating. Reasons for these might be focus on different population structures.

Outcrossing rates of each locus were also estimated by MLT using single locus. The results showed that the mean of single locus outcrossing rates differed not too much in Guangxi (0.817), Shenzhen (0.816) and Fugong (0.812). But that in Hainan was much lower (only 0.502). There was some difference on the estimated value at each locus of each population. The significance of locus outcrossing rates of each population by χ^2 tests showed that the difference in

Table 4. Allelic Frequencies of Seeds, Maternal Plant and Pollen Pool of *Bruguiera gymnorrhiza* Populations （standard errors in parentheses）

Locus	Allele	Fugong			Shenzhen			Guangxi			Hainan		
		Maternal	pollen pool	*hypo-cotyles	Maternal	pollen pool	Hypo-cotyles	Maternal	Pollen pool	Hypo-cotyles	Maternal	pollen pool	Hypo-cotyles
Mdh-1	A	0.917	0.984	0.971	0.750	0.930	0.934	0.900	0.989	0.974	0.583	0.351	0.489
		(0.079)	(0.013)	(0.020)	(0.125)	(0.025)	(0.028)	(0.095)	(0.009)	(0.018)	(0.142)	(0.135)	(0.052)
	B	0.083	0.016	0.029	0.250	0.070	0.066	0.100	0.011	0.026	0.417	0.649	0.511
		(0.079)	(0.013)	(0.020)	(0.125)	(0.025)	(0.028)	(0.095)	(0.009)	(0.018)	(0.142)	(0.135)	(0.052)
Mdh-2	A	0.833	0.903	0.914	0.750	0.955	0.934	0.800	0.971	0.950	0.833	0.979	0.957
		(0.108)	(0.041)	(0.034)	(0.354)	(0.021)	(0.028)	(0.126)	(0.009)	(0.024)	(0.108)	(0.007)	(0.021)
	B	0.167	0.097	0.086	0.250	0.045	0.066	0.200	0.029	0.050	0.167	0.021	0.043
		(0.108)	(0.041)	(0.034)	(0.354)	(0.021)	(0.028)	(0.126)	(0.009)	(0.024)	(0.108)	(0.007)	(0.021)
Me-1	A	0.583	0.487	0.529				0.200	0.064	0.064	0.333	0.586	0.404
		(0.142)	(0.085)	(0.060)				(0.126)	(0.030)	(0.028)	(0.136)	(0.138)	(0.051)
	B	0.417	0.513	0.471				0.300	0.581	0.462	0.417	0.404	0.574
		(0.142)	(0.085)	(0.060)				(0.145)	(0.107)	(0.056)	(0.142)	(0.138)	(0.051)
	C	0.000	0.000	0.000				0.500	0.355	0.474	0.083	0.010	0.021
		(0.000)	(0.000)	(0.000)				(0.158)	(0.104)	(0.057)	(0.080)	(0.004)	(0.015)
Aat-1	A				0.333	0.233	0.263						
					(0.136)	(0.102)	(0.051)						
	B				0.500	0.639	0.579						
					(0.144)	(0.113)	(0.057)						
	C				0.167	0.128	0.158						
					(0.108)	(0.049)	(0.042)						
Aat-2	A				0.417	0.380	0.382						
					(0.142)	(0.136)	(0.056)						
	B				0.583	0.620	0.618						
					(0.142)	(0.136)	(0.056)						

* The reproductive organ of *Bruguiera gymnorrhiza* is hypocotyl but not seed

Table 5. Outcrossing Rates of Fugong *Bruguiera Gymnorrhiza* Population (standard errors in parentheses)

Locus	Tsa	Tm	Ts	tm-ts
Mdh-1	0.643（0.454）	0.845（0.276）	0.740（0.162）	0.104（0.138）
Mdh-2	0.476（0.193）			
Me-1	1.318（0.141）			
Mean	0.812			
$\chi^2=\Sigma Ii(ti-t)^2=2.468$ df=2 p>0.05				

Table 6. Outcrossing Rates of Shenzhen *Bruguiera gymnorrhiza* Population （tandard errors in parentheses）

Locus	Tsa	Tm	Ts	Tm-ts
Mdh-1	0.511（0.182）	0.561（0.154）	0.530（0.131）	0.032（0.063）
Mdh-2	0.751（0.096）			
Aat-1	0.001（0.086）			
Aat-2	1.999（0.007）			
mean	0.816			
$\chi^2=\Sigma$ ii（ti-t）^{2}=208.21 df=3 p<0.001				

Table 7. Outcrossing Rates of Guangxi *Bruguiera gymnorrhiza* Population （standard errors in parentheses）

Locus	Tsa	Tm	Ts	Tm-ts
Mdh-1	0.744（0.088）	0.820（0.100）	0.835（0.109）	-0.015（0.025）
Mdh-2	0.774（0.088）			
Me-1	0.932（0.187）			
Mean	0.817			
$\chi^2=\Sigma$ Ii（ti-t）^{2}=0.155 df=2 p>0.05				

Table 8. Outcrossing Rates of Hainan *Bruguiera gymnorrhiza* Population （standard errors in parentheses）

Locus	Tsa	Tm	Ts	tm-ts
Mdh-1	0.001（0.000）	0.267（0.010）	0.242（0.100）	0.025（0.025）
Mdh-2	0.764（0.230）			
e-1	0.741（0.472）			
Mean	0.502			
$\chi^2=\Sigma$ Ii（ti-t）^{2}=0.900 df=2 p<0.05				

Guangxi and Fugong was not too much while that in Hainan and Shenzhen was quite large. The lower outcrossing rates in Hainan and Shenzhen might be due to the lopsided distribution of outcrossing rates between each locus in populations. At the same time, ts-ta also differed quite large in different populations. The estimated outcrossing rate of single locus was easily affected by irregular distribution of individual in natural population. Chen (1997) has obtained the similar conclusion when studying *Cyclobalano glauca* populations.

4. Discussion

The outcrossing rates of different *Bruguiera gymnorrhiza* populations differed quite large. The outcrossing rates of Fugong population was the biggest, which was 0.845 and that of Hainan population was the smallest, which was 0.267. The discrepancy was 0.578. Hainan, Shenzhen and Fugong populations were slightly inbreeding and Guangxi population was random mating from the ts-tm. As a result, it can be inferred that *Bruguiera gymnorrhiza* was mix-mating system, mainly outcrossing. Kondo et al. （1987） also found that *Bruguiera gymnorrhiza* and *Rhizophora mucronata* might be outcrossing and inbreeding, which had the same results with Tomlinson （1986）.

Species in *Bruguiera* were pollinated by birds and butterflies through the study on anadem biology by Tomlinson et al. （1979） and Tomlinson （1986）. Moreover, the mating systems of entomoplily are generally outcrossing (Barrett, 1990). So the mating system of *Bruguiera gymnorrhiza* gives priority to outcrossing. The similar conclusion was obtained when studying another mangrove—*Kandelia candel* (Sun, 1998; Chen, 1996). Chen et al.（1996） found that the outcrossing rates of *Kandelia candel* in Fugong and Zuta was 0.197 and 0.177, respectively, using chlorophyll-deficient mutants. Sun et al.（1998）found the tm was 0.679 and 0.797 in two *Kandelia candel* populations, Hongkong. As a result, *Kandelia candel* also gives priority to outcrossing due to its characteristic of entomoplily.

At the same time outcrossing rates between multiloci differed quite large. It was a common phenomenon in mix-mating species that there were difference in outcrossing rates between populations（Schoen, 1982）. Though Lande and Schemske （1985） had pointed out that significant outcrossing and inbreeding might exist in equilibrium populations, environment factors and statistic error might disrupt such equilibrium and brought distinct outcrossing rates. Some genetic factors also have functions to certain extend (Brown, 1989), such as pollination types, pollinator adaptability, anadem structure, population size and density, inbreeding incompatibility and genetic controlling, etc.

Similarly, outcrossing rates differences between *Bruguiera gymnorrhiza* populations were also affected by plant density, population structure, pollinator adaptability and activity as well as whether they were inbreeding incompatibility or not.

First, the larger the population density, the higher outcrossing level between relatives, owing to the large connection between plants. *Bruguiera gymnorrhiza* is inbreeding compatibility （Tomlinson, 1986）. The opportunity of outcrossing and inbreeding for inbreeding compatibility species is much more when population density is lower. Thereby the outcrossing rates decreases. In Shenzhen site, individual of *Bruguiera gymnorrhiza* population scattered distributed while the density was low. This caused the individuals to accept small amount pollen offered by off-relativities. Chen （1995） also found that outcrossing rates was positive correlation with plant density when he studied *Cyclobalano glauca* population, a kind of inbreeding compatibility species. However, the relationship between outcrossing rates and plant density was relatively complex according to entomoplily, sometimes it was positive and sometimes it was negative. For example, outcrossing rates and plant density was negative correlated in *Phloxdrum moradii*, which was inbreeding incompatibility. Watkin et al. （1990） argued that the plant density was lower and the pollinator activity distance was longer, so they could contact off-relativities, and outcrossing happened.

Population structure is the most important factor on mating system when estimating the outcrossing rates or inbreeding rates. Using single locus to estimate outcrossing rates is not quite right because this is including real outcrossing and inbreeding. However, theoretically, multiloci estimation was slightly affected by consanguinity mating, and could commendably estimate inbreeding. In this study, Shenzhen, Hainan and Fugong population were slightly parental inbreeding according to tm-ts.

Pollinator adaptability and active ability also have effect on mating system, particularly entomoplily. An example is *Banksia cuneata*. Schemske and Lande （1985） showed that pollinator activity might cause outcrossing rates varied in some kinds of entomoplily. *Bruguiera gymnorrhiza* is entomoplily, big flower, pollinated by birds (Tomlinson, 1986). Thus the lower outcrossing rates in Hainan population might be affected by bird's activity to some extent, which include looking for foods, suckling and changing in bird's population structure.

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