

論文の内容の要旨

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氏 名 耿 其芳(Geng Qifang)

指導教員名 練 春蘭

論文題目 Genetic diversity and reproductive characteristics of dominant mangrove species in the  
coastline of South China

中国南沿岸域のマングローブ林における主要樹種の遺伝的多様性と繁殖実態の解明

Mangrove forests form conspicuous wetland ecosystems fringing extensive areas of coastlines of tropical and subtropical regions. Approximately 80 species from twenty plant families have been recognized, primarily consisting of trees and shrubs that normally grow above mean sea level in the intertidal zone of marine coastal environments or estuarine margins. Mangrove forests are extremely productive ecosystems that provide numerous good and services both to the marine environment and people including fisheries, timber and plant products, coastal protection, and tourism. Despite their ecological and economic importance, mangroves are being destroyed worldwide at an alarming rate due to a variety of destructive human practices, such as farmland conversion, embankment for aquaculture ponds, overharvesting for firewood and charcoal production, and recently rapid urban developments. The exploitation of mangroves has resulted in the loss of genetic diversity in mangrove ecosystems, as well as the loss of valuable forest resources.

In China, twenty-six (including one variety) mangrove species representing 15 genera and 12 families were recorded. Mangrove forests, occupying approximately 17,885 ha, naturally occur along the southeast coastline of four provinces: Hainan, Guangdong, Guangxi, and Fujian in addition to Hong Kong SAR, Macau SAR and Taiwan, intermittently extending from 18°N to 27°N. Nearly two-thirds of mangroves have been lost during the past 40 years. More than 80% of mangroves are secondary forests with 1-m-high trees and most populations are fragmented and scattered along the southeast coastline of China. Nowadays, the high priority of governments in China has been given to the conservation and sustainable management of mangrove forests. A total of 25 mangrove nature reserves including seven at the national level and six at the provincial level have been designated since 1980s, of which three belong to Ramsar international important wetlands. Surveys of genetic diversity of natural populations and mating system of mangrove species are necessary for effective mangrove forest management.

Seven mangrove species, *Acanthus ilicifolius* (Acanthaceae), *Aegiceras corniculatum* (Myrsinaceae), *Avicennia marina* (Avicenniaceae), *Bruguiera gymnorrhiza* (Rhizophoraceae), *Kandelia candel*

(Rhizophoraceae), *Lumnitzera racemosa* (Combretaceae), and *Rhizophora stylosa* (Rhizophoraceae) are dominantly distributed in the coastline of South China. In the present study, the genetic diversity and population genetic structure of these seven mangrove species in South China were estimated by polymorphic nuclear SSR and cpSSR markers. Moreover, the mating system, pollen and propagule dispersals and spatial genetic structure of *K. candel* in a natural population were also analyzed to reveal its reproduction characteristics.

### **1. Isolation and characterization of nuclear and chloroplast microsatellite markers in four mangrove species, *Acanthus ilicifolius*, *Aegiceras corniculatum*, *Avicennia marina* and *Lumnitzera racemosa***

I employed two techniques to isolate nuclear microsatellite regions. One is dual-suppression polymerase chain reaction (PCR) technique and the other was an improved technique for isolating codominant compound microsatellite markers. For *A. ilicifolius* and *A. corniculatum*, both two techniques were used and for *A. marina* and *L. racemosa*, only the improved technique was used. In *A. ilicifolius*, eight polymorphic nuclear SSR markers were developed. These markers showed polymorphism of two to eight alleles per locus, the observed and expected heterozygosities of these markers ranged from 0.200 to 0.875 and from 0.227 to 0.798, respectively. In *A. corniculatum*, 18 polymorphic nuclear SSR loci were isolated. These loci provided nuclear SSR markers with polymorphism of two to eight alleles per locus. The observed and expected heterozygosities ranged from 0.050 to 0.550 and from 0.097 to 0.736, respectively. From *A. marina*, 10 polymorphic nuclear SSR loci were isolated. These isolated loci provided compound SSR markers with polymorphism of two to six alleles per locus. The observed and expected heterozygosities ranged from 0.025 to 0.625 and from 0.096 to 0.767, respectively. From *L. racemosa*, nine polymorphic SSR loci were isolated. The number of alleles per locus ranged from two to nine. The observed and expected heterozygosities ranged from 0.025 to 0.350 and from 0.073 to 0.792, respectively.

Chloroplast microsatellite (cpSSR) markers were developed for the four mangrove species by two methods. First, the cpSSR regions were isolated by sequencing the non-coding regions of chloroplast DNA (cpDNA). Second, the six universal cpSSR loci (ccmp2, ccmp3, ccmp5, ccmp6, ccmp7 and ccmp10) were also used to screen their polymorphism in four species. One, three, seven and six polymorphic cpSSR loci in *A. ilicifolius*, *A. corniculatum*, *A. marina*, and *L. racemosa*, respectively were developed. Characterization of 229, 509, 369, and 216 individuals of *A. ilicifolius*, *A. corniculatum*, *A. marina*, and *L. racemosa*, collected from different natural mangrove populations (*A. ilicifolius*, 6; *A. corniculatum*, 14; *A. marina*, 10; *L. racemosa*, 6) in South China showed that these loci provide cpSSR markers with polymorphisms ranging from two to four alleles per locus and gene diversity between 0.005 and 0.675. Combining these cpSSR loci, three, five, 11 and four cpSSR haplotypes were detected in populations of *A. ilicifolius*, *A. corniculatum*, *A. marina*, and *L. racemosa* in the southern coastline of China, respectively.

### **2. Genetic diversity and differentiation of seven dominant mangrove species in southern Chinese populations revealed by nuclear microsatellite markers**

In this study, 6, 10, 11, 8, 10, 9 and 10 polymorphic SSR markers of *A. ilicifolius*, *A. corniculatum*, *A. marina*, *B. gymnorhiza*, *K. candel*, *L. racemosa* and *R. stylosa*, respectively, were employed to estimate wide range of genetic diversity, gene flow and population genetic structure. 6, 14, 10, 9, 10, 6, and 15 populations of *A. ilicifolius*, *A. corniculatum*, *A. marina*, *B. gymnorhiza*, *K. candel*, *L. racemosa* and *R. stylosa* in the coastlines of the South China were investigated in present study. For each species in the seven mangroves, number of alleles and allelic richness per locus per population ranged from 1.8 to 7.2 and 1.6 to 6.3, respectively. The observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity and inbreeding coefficient ( $F_{IS}$ ) had a mean value ranging from 0.088 to 0.544,

0.160 to 0.587, and 0.104 to 0.637, respectively. The genetic diversity was lowest in the *R. stylosa* but highest in the *K. candel* populations. These results indicate there are significant differences of genetic diversities among those seven mangrove species and most of the mangrove populations in coastlines of the South China may be reproduced by high inbreeding.

Significant genetic differentiations between the natural populations of all of these seven species were identified from the analysis. Overall genetic differentiation ( $F_{ST}$ ) for populations of each species ranged from 0.165 to 0.629, of which the highest  $F_{ST}$  was detected in *A. ilicifolius* and the lowest in *B. gymnorrhiza*. The hierarchical AMOVA showed that most of genetic variations were found among populations in *A. ilicifolius* (62.9%), *L. racemosa* (50.7%) and *R. stylosa* (58.0%) but the other four species within populations (*A. corniculatum*, 72.9%; *A. marina*, 75.1%; *B. gymnorrhiza*, 83.5%; *K. candel*, 79.9%). The population genetic structure also showed significant genetic differentiation between populations of each seven mangrove species. These results indicated that limited gene flow via pollen and propagate dispersal between populations may occur between studied populations of seven mangrove species in South China. Furthermore, in six mangrove species except *A. corniculatum* ( $P < 0.001$ ; Mantel test), no significant relationship between geographical and genetic distance ( $F_{ST}/(1 - F_{ST})$ ) was observed ( $P = 0.10$  to  $0.65$ ; Mantel test). The results indicate that there might be larger gene flow in *A. corniculatum* than in the other six species.

### **3. Diversity and genetic structure in populations of seven dominant mangrove species in southern China as revealed by cpSSR analysis**

Total seven, five, 13, 11, eight, 16, 8 cpSSR markers were used in the populations of *A. ilicifolius*, *A. corniculatum*, *A. marina*, *B. gymnorrhiza*, *K. candel*, *L. racemosa*, and *R. stylosa*, respectively. The cpSSR analysis showed that genetic diversity was also low within natural populations of seven dominant mangrove species in South China. Number of polymorphic loci per population ranged 0.3 to 2.1. The number of haplotypes per population ranged from 1.2 to 3.2. Gene diversity per population ranged from 0.050 to 0.263. Three, five, 11, five, ten, four and four chloroplast haplotypes were identified from all samples of *A. ilicifolius*, *A. corniculatum*, *A. marina*, *B. gymnorrhiza*, *K. candel*, *L. racemosa*, and *R. stylosa*, respectively. Among these chloroplast haplotypes, two, one, three, one, one, two, one haplotypes were dominantly distributed in most of the populations of *A. ilicifolius*, *A. corniculatum*, *A. marina*, *B. gymnorrhiza*, *K. candel*, *L. racemosa*, and *R. stylosa*, respectively. The cpSSR analysis indicated that pioneer haplotypes of seven species were created by the long-distance dispersal of seedlings/seeds originating from few mother haplotypes.

The overall  $G_{ST}$  values assessed by cpSSR DNA ranged from 0.173 to 0.923, indicating high genetic differentiation between populations of these seven mangrove species in South China. The low migration number ( $N_m$ ) values ranged from 0.042 to 2.391 in seven species. These results indicated limited propagate dispersal between populations may occur between studied populations of seven species.

Significant correlation between geographical distance and genetic distance ( $G_{ST}$ ) was observed in *A. marina* and *K. candel* ( $P < 0.01$ ; Mantel test), no significant relationship was found in other five species ( $P = 0.11$  to  $0.47$ ; Mantel test). Since chloroplast DNA of the mangrove species may be maternally inherited, the result indicated that some gene flow via propagule dispersal may be present in *A. marina* and *K. candel*, but not in the other five species.

### **4. Mating system, pollen and propagule dispersal, and spatial genetic structure in a high-density population of the mangrove tree *Kandelia candel***

We surveyed the mating system, pollen and propagule dispersal, and spatial genetic structure in a

0.55-ha high density natural population of *K. candel* in South China using nine and three polymorphic nuclear and cpSSR markers, respectively. In the study stand, all adult trees (n=2062) of *K. candel* were mapped and genotyped. 177 seedlings were arbitrarily selected from four plots to analyze the parentage of seedlings. Total 378 propagules were also arbitrarily chosen from 11 mother trees within the stand for paternity analysis.

High genetic diversity ( $H_E = 0.738$ ) were estimated by nuclear SSR markers in adults. Combining three cpSSR loci, 12 different chloroplast haplotypes were detected in the adults and six different chloroplast haplotypes were detected in the seedlings, of which one was specific to seedlings.

The mean value of Multilocus outcrossing rates ( $t_m$ ) was 0.905, which was significantly  $t_m$  less than 1, suggesting that selfing occurred in this stand. The mean value of biparental inbreeding rates ( $t_m - t_s$ ) was 0.058, which departed significantly from 0, indicating amount of biparental inbreeding. The estimated correlation of paternity ( $r_p$ ) was -0.021, which indicated that there must be a large number of individuals contributing to a well-mixed pollen pool in our study stand of *K. candel*.

For paternity analysis, the distance of pollen movement ranged from 0 to 74.0 m, with an average of  $15.3 \pm 15.0$  m (SD), with 97.3% of the assigned pollen traveled less than 50 m. Modeling for pollen dispersal by the two-component normal model, a short-distance ( $\sigma_1 = 4.5$ ) and long-distance dispersal ( $\sigma_2 = 35.1$ ) occupied a distribution frequency of 23.7% and 76.3%, respectively. The average pollen dispersal distance was 34.8 m. The distance of propagule dispersal ranged from 0.03 to 59.6 m, with a mean of  $9.4 \pm 13.8$  m (SD), with 82.1% of the assigned propagules traveled less than 20 m. Calculation by the two-component normal model revealed that 39.4% and the remaining 60.6% of the total pollen dispersal were of the short-distance type with  $\sigma_1 = 1.1$  and long-distance type with  $\sigma_2 = 24.0$ , respectively. The average propagule dispersal distance was 18.8 m. Paternity and parentage analysis and modeling estimations revealed the presence of an extremely short-distance component of pollen and propagule dispersal.

Adults of *K. candel* in the study stand showed significant spatial genetic structure at distances up to 32 m. A clumped distribution of cpSSR haplotypes was seen in *K. candel* adults. These results indicate that the *K. candel* population was initiated by limited propagule founders from outside by long-distance dispersal followed by limited propagule dispersal from the founders, resulting in a half-sib family structure.

## Conclusion

From the analysis of both nuclear and chloroplast microsatellite markers, it can be concluded that genetic diversity of seven dominant mangrove species in South China is very low except *K. candel* and the populations of these seven species may have been established mainly from few founders. Historical factors, such as repeated bottleneck effects, may also have played a role. The high level of genetic differentiation was observed in present populations of seven species, indicating the limited pollen and propagule dispersal distance. After initial establishment, there might be limited distance of propagule disperse from the founders. The study of pollen and propagule dispersals of *K. candel* approved the above hypothesis.

Moreover, the patterns of genetic diversity and population genetic structures of seven species are unique to each others. The differences reflect migratory potentials, extinction rates, adaptation, population sizes and other factors of respective species. Additionally, the present studied seven mangrove species have different habitats in the mangrove forests in South China.

This study addresses the urgent need for conservation and proper management of mangroves in China. Comparative studies of selected mangrove species were conducted in order to fill some of the large gaps in our knowledge of mangrove genetic resources, with emphases on their conservation genetics and reproductive biology.