論文題目：Microsatellite analysis of genetic structure of three dominant mangrove tree species in Iriomote Island, Japan

（西表島における優占マングローブ樹種三種のマイクロサテライトマーカーによる遺伝構造解析）

Introduction

Mangroves form the most unique and conspicuous wetland ecosystem worldwide with an approximate area of 240, 000 km^2 of sheltered coastlines. It servers as a protective barriers along the coast and also provides safe sties for many organism in costal-mangrove ecosystem. But for a last couple of decades, heavy exploitation of mangroves for excessive wood gathering, fishpond operations, mining, and development of coastal areas make a great threat to the world's mangroves. Conservation and ecological management of mangroves, therefore, now become the most important issues in the coastal areas of many countries.

Bruguiera gymnorrhiza (L.), *Kandelia candel* (L.) Druce and *Rhizophora stylosa* (Griff.) are three ecologically and somewhat economically important mangrove tree species of family Rizophoraceae. In Japan, these three species are mainly found in mangrove forest of southern part of Japan and mostly on the Ryukyu archipelago. Iriomote is one of the ecologically diverse Islands in the Ryukyu Islands where *B. gymnorrhiza*, *R. stylosa* and *K. candel* communities are found to be the most extensive and dominant over other mangrove species. These three species are ecologically important as they preserve shoreline from erosion and other natural calamities, and also enrich coastal-mangrove bio-resources. Economically they also provide some significant contribution to the mangrove-adjacent communities. Therefore, conservation and ecological management of these three mangrove trees are important for protecting coastal-marine ecosystem and uplifting socio-economical condition to the mangrove adjacent people.

Precise information of genetic diversity, geneflow, maternal lineages, and genetic structure and reproduction characteristics these species are particularly important in finding appropriate strategies and providing guidelines for the conservation and ecological management of these species in coastal-mangrove ecosystem. This information is needed for in-situ conservation, producing genetically improved planting stocks for afforestation, reforestation or enrichment planting and capturing maximum desirable genetic diversity in breeding program.

The use of molecular data in plant studies has increased dramatically during the last decade, as molecular methods are important tools for identifying appropriate population sources for reforestation or afforestation and conservation programs. In recent times microsatellites from nuclear DNA are now widely being used in detecting population genetic structure, testing percentage and relatedness, and assessing genetic diversity and recent population history. Recently, a cpDNA microsatellite region was also found to be highly polymorphic. Therefore, chloroplast microsatellite (cpSSR) polymorphism analysis can be a useful tool to reveal maternal lineages and to infer seed dispersal manners as cpDNA is maternally inherited in most of the angiosperms. In this study genetic diversity, geneflow, maternal lineages, genetic structure and reproduction characteristics of these three tree specie in their natural environment have been investigated using polymorphic nuclear and chloroplast microsatellite markers.

1. Development and characterization of nuclear microsatellite markers in Bruguiera gymnorrhiza, Kandelia candel and Rhizophora stylsoa

Ten polymorphic microsatellite loci were isolated from *B. gymnorrhiza* using a dual-suppression polymerase chain reaction (PCR) technique. These loci provided microsatellite markers with polymorphism of two to five alleles per locus within 216 individuals from nine natural populations of *B. gymnorrhiza* on Iriomote Island, Japan. In *K. candel*, eight codominant compound microsatellite markers *were* isolated. The isolated loci provided compound SSR markers with polymorphism of three to 11 alleles per locus within 24 individuals in natural mangrove forests located in four provinces in the southern part of China. In total, ten polymorphic microsatellite loci were isolated from *R. stylosa* using a dual-suppression PCR technique. These loci provided microsatellite markers with polymorphism of two to six alleles in each locus within 375 individuals from nineteen *R. stylosa* populations on three of the Sakishima Islands: Iriomote, Ishigaki and Miyako Island.

2. Population genetic structure of Bruguiera gymnorrhiza, Kandelia candel and Rhizophora stylosa in Iriomote Island, Japan revealed by nuclear microsatellite markers

Ten polymorphic microsatellies markers in *B. gymnorrhiza* and *R. stylsoa*, respectively and nine microsatellites markers in *K. candel*, were employed in this study to assess the wide range of genetic diversity, geneflow and population genetic structure these tree populations. Nine populations from each of *B. gymnorrhiza* and *R. stylosa* and seven populations in *K. candel* were investigated the present study. In nuclear SSR analysis, The low average number of alleles (mean, 2.6, 1.7, 2.3) and allelic richness (mean, 2.6, 1.7, 2.3) per locus per population and low heterozygosity (mean, 0.408, 0.252, 0.266 were detected in *Bruguiera gymnorrhiza*, *Kandelia candel* and *Rhizophora stylosa* respectively, indicating low levels of genetic diversity of the three species within natural populations in Iriomote Island. Inbreeding coefficient (F_{IS}) per locus within the natural populations of *B. gymnorrzha*, *K. candel* and *R. stylosa* was 0.233 (mean), 0.506 (mean) and 0.576 (mean), respectively indicate high inbreeding within the populations. Local adaptation after initial establishment and peripheral distribution of there plant species in Irimote Island by the influencing factors in lowering genetic diversity and inducing high inbreeding in Iriomote Island.

Significant genetic differentiations within the natural populations of all of these three species were identified from the analysis of molecular variances and over all F_{ST}. Low N_m value (2.235, 0.7165 and 1.238) in *B. gymnorrhiza*, *K. candel* and *R. stylosa*, respectively indicate low gene flow i.e., limited pollen or propagule dispersal within

populations which may cause high genetic differentiations. Pairwise genetic differentiations was not significantly correlated with geographic distances in *B. gymnorrhiza* and *K. candel*. But in *R. stylsoa* genetic differentiation was significantly correlated with geographic distances. Species characteristics, vegetation composition and origin of the species can influence the process of isolation by distances. Several ecological factors and special morphological characteristics of mangrove tree can influence gene flow and genetic differentiation within and between populations.

3. Development of chloroplast microsatellite (cpSSR) markers for maternal lineages analysis in Bruguiera gymnorrhiza, Kandelia candel and Rhizophora stylosa.

Chloroplast microsatellites markers have been developed from *B. gymnorrhiza*, *K. candel* and *R. stylosa*. Chloroplast DNA of *B. gymnorrhiza*, *K. candel* and *R. stylosa* were amplified by PCR using eight, six and four universal chloroplast primer pairs, respectively. The Universal primers amplified PCR products for all of the eight, six and four non-coding regions in *B. gymnorrhiza, K.candel and R.stylosa,* respectively. Eleven cpSSR markers in both *B. gymnorrhiza* and K. *candel* and eight markers in *R. stylosa* were isolated from their respective noncoding regions, which contained cpSSR loci. Among all of their respective cpSSR loci, seven, two and three loci showed polymorphism in *B. gymnorrhiza*, *K. candel* and *R. stylosa*, respectively, during screening throughout 216, 156 and 253 individuals, from different natural populations on Iriomote Island, Japan. The number of mononucleotide alleles per polymorphic cpSSR loci ranged from two to four in *B. gymnorrhiza*, and two in every locus of *K. candel* and *R. stylosa*.

4. Genetic structure and maternal lineages of Bruguiera gymnorrhiza, Kandelia candel and Rhizophora stylosa in Iriomote Island, Japan revealed by chloroplast microsatellite markers

Genetic diversity was also found low within natural populations of three dominant mangrove species in Iriomote Island, Japan. The number of alleles at each locus ranged from 2 to 4 in *B. gymnorrhiza*. In *K. candel* and *R. stylosa*, only 2 alleles at each locus were identified throughout the entire populations. Combination of alleles at different polymorphic cpSSR loci which are maternally inherited constituted very limited number of haplotypes i.e., five, three and two in *B. gymnorrhiza*, *K. candel* and *R. stylosa*, respectively. Though five haplotypes were detected in *B. gymnorrhiza* but two major types are commonly distributed throughout the entire populations. The same pattern is also found in *K. candel*. Among three haplotypes of *K. candel*, two types are mainly distributed throughout the entire Iriomote Island. These cpSSR results indicate only a few founders of three species in the entire Island. Therefore, a few founders might have been established at each watershed of Iriomote Island from the surrounding area after the last seismic uplift occurred ca. 1000 years. Local adaptation might have been happened there due to extreme environmental condition after their initial establishment and which may be the factor of inducing low genetic diversity within populations of three dominant mangrove species in the entire Iriomote Island.

The overall G_{ST} values assessed by cpSSR DNA were estimated as 0.310 in *B. gymnorrhiza,* 0.446 in *K. candel* and 0.257 in *R. stylosa*, respectively, indicating high genetic differentiation within populations. Significant correlations between cpDNA genetic distances and geographic distances were observed in *R. stylosa*. But IBD was not found significant for *B. gymnorrhiza* and *K. candel*. Same source of pioneer origin and limited geneflow between the neighboring populations may not process the IBD for these two species. There is a significant correlation between the genetic distances of

nuclear DNA and chloroplast DNA in *R. stylosa.* But significant correlation was not observed between genetic distances of nuclear and chloroplast DNA of *B. gymnorrhiza* and *K. candel.* From this evidence it can be inferred that pollen dispersal and propagule dispersal are clearly corresponded in *R. stylosa* but it they were not consistent in *B. gymnorrhiza* and *K. candel.*

5. Small scale genetic structure and reproduction characteristics in B. gymnorrhiza, K. candel and R. stylosa in Iriomote Island, Japan

Small scale genetic structure and reproduction dynamics of three dominant mangrove species: *B. gymnrrhiza, K. candel* and *R. stylosa* were investigated in a selected natural mangrove stand (300 m X 125 m) on the bank of the urauchi river (right side), Iriomote Island (24°24´41.1´´N, 12°34´638.8´´E), Japan. 147 adult trees and 44 seedlings in *B. gymnorrhiza*, 242 adult trees and 190 seedlings in *K. candel* and 192 adult trees and 188 seedlings in *R. stylosa,* were investigated for parentage analysis of seedlings. Besides, three mother trees for each of the three study species were selected within this stand and 48 propaugles from each mother trees from were analyzed to know about the movement of pollen in their Mating system. Genetic diversity within the adult trees and seedlings for all of the species in this natural stand were detected very low and showed the evidences of high inbreeding from the mean F_{IS} vales which were 0.2162 and 0.321, 0.261 and 0.333, and 0.621 and 0.633, respectively within the adult trees and seedlings of *B. gymnorrhiza, K. candel* and *R. stylosa.* Due to low polymorphism in the nuclear SSR and cpSSR analysis, no unambiguous parents were identified for any of the seedlings. Insufficiency of the polymorphic microsatellite markers or very low genetic diversity in the selected stand might be the limitation in parentage analysis of seedlings. However, parent child relationship for all of the three species was compared from the allelic distributions of each nuclear SSR loci and haplotype distributions of cpSSR loci. Both of these distributions assumed narrow range of pollen and propagules dispersal within the selected stand. Unambiguously detected pollen fathers of the propagules investigated from some selected mother trees were generally observed close to their respective mother trees. Generally high rate of selfing was observed in *K. candel* and *R. stylosa* from the analysis of propaugles in some selected mother trees. In case of *B. gymnorrhiza* selfing rate is lower than the rate observed in other two species. Pollen movement, efficiency of pollen vectors and species composition may largely influence the mating system of these three dominant species in their natural environment

Conclusion

From the analysis of both nuclear and chloroplast microstellite markers it can be concluded that genetic diversity of three dominant mangrove species in Iriomote Island is very low and these three species in might have been established in Iriomote Island mainly from two different mother types after the last seismic uplift occurred ca. 1000 years. After initial establishment they might have been dispersed rather narrow range, which may the most probable cause of high inbreeding and significant genetic differentiation within natural populations of all of the three dominant mangrove species in Iriomote Island, Japan. Reproduction characteristics and mating system of these three species could be identified clearly because of low polymorphism diversity. Insufficiency of polymorphic microsatellite markers or very low levels of genetic diversity in the mangroves of Iriomote Island are the limitation for parentage analysis for these three species.