報告番号	※　　第　　号

主　論　文　の　要　旨

論文題目　Genetic diversity, spatial genetic structure, and gene dispersal of *Parashorea malaanonan* (Dipterocarpaceae) in Mt. Makiling Forest Reserve, Philippines
（Mt. Makiling 森林保護区（フィリピン）における *Parashorea malaanonan*（フタバガキ科）の遺伝的多様性、空間遺伝構造、および遺伝子散布）

氏　　名　TINIO Crusty Estoque

論　文　内　容　の　要　旨

Parashorea malaanonan, known locally in the Philippines as ‘bagtikan,’ was categorized as critically endangered on the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species. Its genetic resources are believed to have been rapidly reduced due to international timber trade, massive (illegal) logging, shifting cultivation and land-use conversion. In order to provide how best to conserve genetic resources of this species, I studied the genetic diversity, spatial genetic structure, and gene dispersal at the fine scale (20-ha plot) and at the landscape (forest reserve) scale. The study was conducted in one of the remaining intact secondary climax forests, the Mt. Makiling Forest Reserve (MMFR), which is also an ASEAN heritage site.

The first study was on the development of 48 microsatellite markers (or simple sequence repeats) from genomic DNA of *P. malaanonan* using next-generation sequencing. Forward primers with universal primer tail sequences and reverse primers with a PIG-tail sequence for 48 microsatellites were designed. The polymerase chain reaction amplification and polymorphism tests yielded final 20 microsatellite markers with stable polymorphism. The genetic diversity parameters such as the number of alleles, observed heterozygosity and expected heterozygosity were high at each locus and the total exclusion probabilities for the 1st and 2nd parents over the 20 loci were also high (0.99932499 and 0.99999723, respectively). One locus departed significantly from Hardy-Weinberg equilibrium and no significant linkage disequilibrium was detected for any pair of the loci. Therefore, these newly developed microsatellite markers are useful for future work on population genetics, conservation genetics, and molecular ecology of the focal

species.

In the second study, at the fine scale, I investigated the current pattern of genetic diversity and determined the existence and intensity (indicated by Sp values) of spatial genetic structure (SGS), then inferred from SGS the neighborhood size and historical gene dispersal in adult, sapling, and seedling life stages. I used 16 microsatellite markers for the 32 adults, 161 saplings, 199 seedlings collected from a 20-ha plot, which was expanded from the 4-ha JIRCAS plot, within Molawin-Dampalit (one of the four subwatersheds of MMFR). Genetic diversity was high in all three stages, although there was a decreasing pattern of genetic diversity from adult to seedling stages. Significant SGS existed in the sapling and seedling stages, but not in the adult stage, which is due to very low population density. Sp range (0.009-0.025) falls within Sp values for plants with outcrossing breeding system and with wind and gravity dispersed seeds. Historical gene dispersal parameters converged only for the seedling stage and the estimated values (neighborhood size, N_b, = 20. 5 individuals and historical gene dispersal distance, σ, = 94.71 m) indicated short distance gene dispersal via seeds, which is common among lowland dipterocarps. Therefore, for *ex situ* conservation, an interval of collection of seedlings should be larger than about 100 m to avoid sampling of half-sibs.

In the third study, parentage of saplings and seedlings, mating system, and gene dispersal were investigated using parentage analysis and analyses with a mixed mating model and a neighborhood model with the same microsatellite markers and DNA materials as used in the second study. It was confirmed that *P. malaanonan* was highly outcrossed from the results of all analyses. The seed dispersal distance estimated using parentage and neighborhood-model analyses was short (less than 100 m), whereas the pollen flow distance estimated using neighborhood-model analysis was extensive (466.7 m), which may be resulted from the low population density (0.30 trees ha^{-1}). Furthermore, both parentage and neighborhood-model analyses revealed no effects of the sizes of a diameter at breast height on reproductive success of adult trees.

In the fourth study, at the landscape scale, I estimated the genetic diversity along altitudinal gradients (low, middle, upper) and at the forest reserve scale using the two subwatersheds of 904 ha in total, and sought for an evidence of recent population bottleneck. Also, I determined the existence of SGS and inferred the historical gene dispersal distance, genetic neighborhood area, and effective population size. Sixteen microsatellite markers were analyzed on 269 adult trees collected from 68 to 614 m above sea level. Results showed no significant differences in genetic diversity parameters across altitudinal gradients and no

evidence of recent population bottleneck, indicating quantitatively homogenous distribution of genetic diversity at the landscape scale. Furthermore, significant but very weak SGS existed and the neighborhood size (N_b = 185 individuals) and historical gene dispersal (σ = 704 m) were within the range of values, N_b =20–350 and σ = 150–1200, which is a typical characteristic of tropical trees at mixed tropical forests. Based on the effective population size of 185 and population density of adult trees, the minimum conservation area was estimated to be about 600 ha.

Based on the findings, I propose appropriate strategies for genetic conservation and management of *P. malaanonan*: (1) the high genetic diversity of the species should be continuously conserved *in situ* in the MMFR; (2) for *ex situ* conservation, an interval of collection of seedlings should be greater than about 100 m to avoid sampling of half-sibs; and (3) larger than the minimum conservation area of about 600 hectares should be conserved at the forest reserve level (MMFR).