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Genetic diversity, spatial genetic structure, and gene dispersal of *Parashorea malaanonan* (Dipterocarpaceae) in Mt. Makiling Forest Reserve, Philippines

Parashorea malaanonan is an ecologically and economically important timber species of the family Dipterocarpaceae. Genetic resources of the species (and other dipterocarps) are believed to have been damaged by the long period of extensive timber harvesting and other anthropogenic activities in Philippine forests. Thus, it has been categorized as critically endangered in the IUCN Red List of Threatened Species. The episodic production of its seeds every ca. 5-7 years and their desiccation sensitivity pose challenges for the sustainable utilization and maintenance of the species' genetic diversity. The main strategy of the Philippine government for conserving the country's forest tree genetic resources is the establishment of a network of protected areas. Apart from this, there have been no serious sustainable programs for conserving genetic resources of timber trees (particularly dipterocarps) in the country. In order to facilitate efforts to formulate optimal strategies for conserving genetic resources of *P. malaanonan*, I studied the genetic diversity, spatial genetic structure (SGS), and gene dispersal in a population of the species at fine scale (in a 20-ha plot) and landscape (forest reserve) scale. The examined material was growing in the Mt. Makiling Forest Reserve (MMFR) on Luzon Island, one of the earliest national parks established in 1910. The area was logged in the mid-1940s, then left with little or no human intervention, and is now regarded as one of few remaining old-growth secondary forests in the country.

To acquire the genetic information required for analyses of genetic diversity and SGS at both fine and landscape scales, and parentage and gene dispersal via seeds and pollen in *P. malaanonan* populations, suitable markers were needed. Thus, my first study focused on development of 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. Polymerase chain reaction amplification and polymorphism tests yielded 20 microsatellite markers with stable polymorphism. 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 (0.999325 and 0.999997, respectively). One locus departed significantly from Hardy-Weinberg equilibrium and no significant linkage disequilibrium was detected for any pair of the loci. Therefore, these 20 newly developed microsatellite markers will be useful for future work on population genetics, conservation genetics, and molecular ecology of the focal species.

In a second study, I investigated the fine-scale level of genetic diversity and presence and intensity of SGS in adult, sapling, and seedling life stages of the species using the developed microsatellite markers, then inferred the neighborhood size and historical gene dispersal distance based on the SGS. For this, I analyzed 10 microsatellite markers in samples of 32 adults, 161 saplings, 199 seedlings in a 20-ha plot. The results showed that the current level of genetic diversity across the examined life stages in the *P. malaanonan* population is high and random mating has occurred within the plot. Values of the kinship (or coancestry) coefficient (F_{ij}) were positively significant up to 40 m for saplings and up to 50 m for seedlings. No significant SGS among adults was detected, primarily due to low population density resulting from demographic thinning, but strong SGS was detected among seedlings and saplings, primarily due to allele aggregation of maternal half-sibs, manifested by their observed clumping near parent trees in the field. The strong SGS among seedlings and, based on the SGS results, the estimated neighborhood size (20.15) and historical gene dispersal (94.71 m) may reflect short distance gene dispersal via seeds for *P. malaanonan*. Based

on the SGS findings, it is suggested that seedlings and saplings spaced more than about 50 m apart should be collected when sampling, to reduce risks of sampling closely genetically related specimens.

In a third study, the parentage of offspring, mating system, and gene dispersal in the population were investigated using parentage analysis of saplings and seedlings and neighborhood model analysis of data pertaining to seedlings, with the same plant materials and their microsatellite genotypes as used in the second study. The results showed that 24 of the 27 adult trees had contributed genes to at least four offspring, and there was strong variation in the production of offspring as seed parent trees among adult trees. This may have resulted in lower allelic richness in seedlings, and may have increased the intensity of SGS. *P. malaanonan* is highly outcrossing, and this was confirmed by low (close to zero) estimated rates of self-fertilization. This has important implications for genetic conservation because random mating usually occurs in outcrossing species, which promotes genetic diversity by increasing gene flow between populations. The seed dispersal distance estimated from parentage and neighborhood-model analyses was short (less than 100 m), confirming the restricted seed dispersal of *P. malaanonan.* However, the direct method, i.e., neighborhood model analysis confirmed that long-distance pollen dispersal, primarily due to the low population density, occurs in the *P. malaanonan* population.

In a fourth, landscape-scale study, I investigated levels of genetic diversity among adult trees of the species at three elevations (low, middle, upper) and at the forest reserve scale using the microsatellite markers and sought evidence of recent population bottlenecks. I also determined the existence and intensity of SGS in the population, and inferred the historical gene dispersal distance and genetic neighborhood size and area based on the SGS. In this study, 14 microsatellite markers were analyzed in samples of 269 adult trees collected at altitudes ranging from 68 to 614 m asl. The results show that the population has maintained a high level of genetic diversity at landscape scale and at the three elevations, probably due to its outcrossing breeding system and random mating of reproductive individuals. There was a quantitatively homogenous distribution of genetic diversity at the landscape scale based on F_{ST} and bottleneck tests. SGS at landscape scale was detected within 600 m, but was very weak.

The neighborhood size (N_b) decreased, while the gene dispersal distance increased, as the effective density of adult trees (D_e) decreased (the latter relationship reflecting extensive pollen dispersal). Based on the estimated N_b and D_e values, the estimated genetic neighborhood area ranged from 624.4 to 2879.5 ha at the landscape scale.

Based on the findings, the maximum possible amount of genetic diversity in *P. malaanonan* should be continuously conserved *in situ*. This study indicates that the species should be conserved in an area larger than the minimum conservation area for 624.4 ha (600 to 3000 ha). Thus, the entire Mt. Makiling Forest Reserve should be conserved. Expansion of *in situ* conservation outside the MMFR is also recommended, by establishing a network of protected areas (i.e. national parks, watershed forest reserves, and wilderness areas) containing natural stands of *P. malaanonan* in the country. For *ex situ* germplasm conservation, *P. malaanonan* planting materials in the MMFR can be used in the government's "Enhanced National Greening Program", or similar initiatives. However, collected seedlings and saplings should be spaced more than about 50 m apart to avoid sampling half-sibs. In the future, systematic scientific trials to test the site-specific suitability of species or even provenances for reforestation or land rehabilitation should be established. Thus, for *ex situ* conservation, it is proposed that the genetic diversity and genetic structure among populations of *P. malaanonan* and other dipterocarps distributed throughout their native range in the Philippines should be examined.