

Ecological and conservation genomics for the tropical tree species *Metrosideros polymorpha* and *Shorea leprosula*

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Abstract

Tree species, which have developed adaptive traits to survive in diverse environmental conditions, provide excellent opportunities to address the fundamental question of how organisms have adapted and expanded their distributions. The scientific findings regarding adaptive mechanisms of tree species can be applied to predict the responses of forests after climate and/or anthropogenic activities, promote efficiency in the breeding of timber varieties, and determine priorities in forest conservation. Rapid progress in high-throughput sequencing technologies has provided enormous genetic information for various organisms. The genomics is expected to reveal the mechanisms of adaptation and speciation, and thus greatly advance evolutionary and conservation biology of tree species. In this thesis, I aimed to: 1) reveal the genetic bases underlying environmental adaptation of tree species at the genome level and; 2) derive the genetic implications for sustainable conservation of tropical forests.

In Chapters 2 and 3, I investigated the genomic divergence in *Metrosideros polymorpha* (Myrtaceae), which occupies a wide range of ecological habitats and shows adaptive radiation within a species in Hawaiian Islands. The biological functions of genetic variations observed within this species could give us valuable insights into the drastic evolution found in a single lineage.

In Chapter 2, I sequenced and assembled *de novo* genome sequences of one *M. polymorpha* tree to provide the basic genomic parameters about this species and to develop our knowledge about ecological divergences. The assembly yielded 304 Mb genome sequences, half of which were covered by 19 scaffolds with >5 Mb, and contained 30 K protein-coding genes. Demographic history inferred from the genome-wide heterozygosity indicated that this species experienced the dramatic rise and fall in the effective population size possibly according to the past geographic or climatic changes in Hawaiian Islands. The present *M. polymorpha* genome assembly represents a high-quality genome resource useful for future functional analyses of genetic variations or comparative genomics among intra- and inter-species.

In Chapter 3, a *de novo* draft genome sequence provided in Chapter 2 and 2,247 single nucleotide polymorphism (SNP) markers were used to reveal the population genetic structure of nine populations across five elevations and two ages of substrates on Mauna Loa, the island of Hawaii. The nine populations were genetically differentiated according to elevation as well as age of lava but largely admixed, particularly in the lower elevations. A genome scan for the 2,247 SNPs revealed that a small fraction of the genome (35 SNPs on 26 scaffolds; 1.56%) was likely under divergent selection, and alleles on these non-neutral SNPs were fixed in one or more populations. Generalized mixed modeling for pairwise population differentiation according to geographic and environmental variables revealed that population

differentiation in most of the genome followed the isolation-by-distance model, whereas divergence at non-neutral SNPs followed the isolation-by-environment model. Accordingly, the current study reveals the genomic mosaic of *M. polymorpha* comprising contrasting divergence patterns. Although the genome was largely mixed among populations, a small fraction of the genome appears to be subject to environmental selection and responsible for the dramatic divergence in phenotype and adaptation to a wide range of environments.

In Chapters 4 and 5, I focused on the *Shorea leprosula* (Dipterocarpaceae) plantation managed by a private-sector forestry company in Central Kalimantan. Because the family Dipterocarpaceae constitutes the core of biodiversity and is faced with heavy exploitation in the Southeast Asian tropical rainforests, planting of native dipterocarp trees is a valuable policy. However, the genetic concerns of the planted trees and species richness of phyllosphere fungi associated with them have not been evaluated.

In Chapter 4, the genetic diversity of *S. leprosula* and *S. parvifolia* in plantations and those in natural populations were compared using microsatellite markers. Genetic diversity in the planted populations was as high as that in the natural populations. No clear genetic differences between each planted population and the natural forest populations were found. The genetic variation present in planted *S. leprosula* and *S. parvifolia* populations did not appear to deteriorate in the planting system adopted in Indonesia, known as Tebang Pilih Tanam Jalur. These results indicate that the current plantation method practiced in the region is suitable for maintaining the original genetic composition and achieving sustainable use of tropical rainforests.

In Chapter 5, I sought to estimate the species diversity and community structure of phyllosphere fungi in the *S. leprosula* plantation. I conducted a massively parallel amplicon sequencing analysis of fungi collected from the leaves of *S. leprosula*. Phyllosphere fungal compositions and spatial variability were investigated for 31 *S. leprosula* trees across four plots within a plantation stand. In total, 488 fungal operational taxonomic units (OTUs) were recognized in 153,194 ribosomal internal transcribed spacer reads at 95 % OTU identity level. Rare OTUs accounted for the majority of fungal diversity detected in the study site; 200 OTUs (41 %) comprised fewer than 10 reads and 465 OTUs (95 %) were found in fewer than half of the leaf samples. Fungal OTU compositions of *S. leprosula* trees were differentiated within a narrow area of the plantation and even between plots that were separated by 15 m. These findings indicate that highly diverse fungal OTUs form spatially structured communities even within a tropical plantation stand of single tree species.

Overall, this thesis has updated our knowledge about the ecological adaptations of *M. polymorpha* and clearly demonstrated the effectiveness and possibility of genome-wide analyses in the field of ecology and evolution. Further, the appropriateness of the dipterocarp planting in Central Kalimantan was evaluated in terms of genetic and species diversity harbored in the plantation stands. The field of ecological and conservation genetics for wild organisms, including forest trees, is becoming an interesting platform to uncover idiosyncratic ecological phenomena by taking the advantage of massive genomic data. It is expected that we will be able to discuss a more specific evolutionary process regarding fitness-related traits and

predicting the impact of climate change or artificial management on the fitness and longevity of target populations.