

## 学位論文全文に代わる要約 Extended Summary in Lieu of Dissertation

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Name

学位論文題目 : Genetic diversity and structure in two endangered species,  
Title of Dissertation *Shorea albida* (Dipterocarpaceae) and *Quercus hondae* (Fagaceae)  
(絶滅危惧樹木であるフタバガキ科*Shorea albida*とブナ科*Quercus hondae*  
の遺伝的多様性と集団構造)

学位論文要約 :  
Dissertation Summary

“Biological diversity (biodiversity)” means the variability among living organisms, and the conservation is justified by its economic values of bioresources, ecosystem services, aesthetics, and rights of living organisms to exist. However, the current global terrestrial biodiversity is in a critical situation. Ideally, all species and ecosystems should be conserved to keep the current biodiversity levels, but the resources that can be used for conservation are limited. To conserve as many species and ecosystems under restrictions, effective conservation of species and ecosystems utilizing information such as the risk of extinction in each species are necessary. The IUCN red list provides information on classifying taxon at high risk of global extinction, and taxonomies categorized as “critically endangered”, “endangered” or “vulnerable” are evaluated as being at the level of threat. Endangered species have small-isolated populations and are expected to show low genetic diversity due to increases in inbreeding and effects of genetic drift. Lack of genetic diversity will increase the extinction risk of species due to reduced evolutionary or adaptability potentials and compromised reproductive fitness. Therefore, conservation projects with aim at minimizing losses of genetic diversity to prevent the extinction of endangered species are necessary.

Forests have multiple roles as habitats for organisms, modulations of hydrologic flow and soil conservation, and provide benefits for human life. However, natural forests have been decreasing, and numerous tree species in the world are considered as threatened with extinction. Considering negative impacts of forest losses and the extinction of tree species on humanity and global environment, conservation of forests, which are home to a variety of organisms, is an important issue. Population genetic studies of tree species have been concerned with their level of genetic diversity, population structure, hybridization with other related species, population demography, and the presence of past refugia populations. Such information is utilized for conservation for trees and forests; for example, selection of protected forests and its magnitude, introductions of individuals without genetic erosion, and seed resources utilized for forest rehabilitation. However, even though species are in the same threatened categories, the patterns of genetic variation are expected to be different between species because breeding systems, favorite habitats, and responses to climate changes are depending on the species.

Both human activities and climate changes in the past need to be considered as causes forming the current genetic diversity and structure of species. While tropical forests have been rapidly logged in recent years, temperate forests have long been extensively used and altered by human activities. Human activities have caused tree species to decline in population size, resulting in reduced genetic diversity and genetic differentiation among populations. Climate changes in the geological time scales, including transitions in temperature, humidity and sea level have also changed the distribution of tree species. For example, it is considered that suitable habitats for many plants and animals could exist only in small refugia during the last glacial period. The postglacial recolonization patterns of species affect the pattern of genetic variations and genetic structures of the populations. Therefore, both human activities and past climate changes need to be considered as causes forming the current genetic diversity and structure of endangered species. However, the extent to which causes affect the patterns of genetic variation is not yet known for many species. In this study, population genetic analysis was conducted for two endangered tree species of *Shorea albida* and *Quercus hondae* to clarify population genetic variation and the evolution of species, with the aim to suggest conservation ideas for endangered tree species. Finally, concrete conservation strategies for the species are discussed in this study.

Southeast Asia is known as one of the world's major hotspots of species diversity, and Dipterocarpaceae is distributed throughout this region. *Shorea albida*, a member of Dipterocarpaceae is endemic to Borneo Island and dominant or often monodominant in peat swamps of northwestern Borneo. Peat swamps have declined continuously from 1990 to 2015. The population of *S. albida* is also decreasing due to habitat loss, being assessed as vulnerable species on the IUCN red list. The recovery of peat swamp forests from past disturbances and the depletion of scars was not found in the peat swamp forests, composed primarily of *S. albida* in Brunei. Therefore, human interventions are necessary for the conservation and recovery of this species, and genetic information on *S. albida* is expected to be utilized for the conservation and afforestation project of this species. Here the population genetic status was assessed for *S. albida* in Brunei using 18 microsatellite markers. Leaf or cambium samples of *S. albida* were collected from 11 locations in Belait district, Brunei Darussalam (Figure 1). The mean of expected heterozygosity ( $H_e$ ) within populations was 0.402 for *S. albida*. This statistic is not lower than other *Shorea* species investigated in previous studies, even though *S. albida* is specialized to peat swamps and hence restricted to a narrow ecological and geographical area. The density of reproductive individuals of *S. albida* in Brunei is exceptionally higher than common *Shorea* species, and substantial genetic diversity in *S. albida* found in this study may result from a large local tree density. Although genetic divergence among populations was generally low, pairwise  $F_{ST}$  and STRUCTURE analysis indicated that two populations (Labi Road 3 and Ingei) were significantly differentiated from other populations (Figure 1). It is interesting that Labi Road 3 showed a different genetic structure even though it is located near other populations. Genetic differentiation over short geographic distances may arise from restrictions of gene flows caused by short pollen and seed dispersal distances or geographic barriers, or as a legacy of past refugia. If short pollen and seed dispersal distances are creating genetic structure across populations, more genetic differences between populations would be expected. As the geographic barrier which is specific in Labi Road 3 seems to be not apparent, genetic differentiation of the Labi Road 3 and Ingei populations is likely the legacy of past displaced populations rather than the result of limited gene flow. Actually, the research of basal peat ages in the Belait-Baram river basin where most of the *S. albida* populations are sampled in this study, suggested that some sand terraces and sand ridges remained exposing when the sea extended inland in the transgressive period, believed to have started about 7,000 years ago, and peat formation was already initiated at these sites before global sea level stabilization. The analysis using DIYABC in this study with defining three groups: Pop1 (Badas pipeline East, Badas pipeline South, Badas pipeline West, Kuala Balai, Labi Road 1, Labi Road 2, Mendaram, Penjarai, and Penjarai Northeast), Pop2 (Ingei), and Pop3 (Labi Road 3), estimated that the current three genetic groups diverged from the ancestral population 7,500 years ago, corresponding to the high sea-level stand. This suggests that the current genetic structure of *S. albida* was formed in this period of high sea levels. During this period, refugial populations of peat swamp species may have persisted in restricted peatland areas. Refugial populations are small and isolated and therefore tend to diverge from other populations. This interpretation is consistent with the remarkably higher  $F$ -value of Cluster III ( $F = 0.4320$ ), which occupies Labi Road 3, than other populations, suggesting that this population was affected by strong genetic drift. Therefore, it is suggested that the current genetic groups as having been formed in the sea transgression period, and small populations were maintained as refugia.

Fagaceae distributes throughout the north temperate zone and at high altitudes in the tropics. Oak (*Quercus*), a genus of Fagaceae is successful in temperate and tropical forests, and there are over 400 species throughout the Northern Hemisphere. The subgenus *Cyclobalanopsis* is composed of eight evergreen *Quercus* species in Japan: *Q. acuta*, *Q. gilva*, *Q. glauca*, *Q. hondae*, *Q. miyagii*, *Q. myrsinaefolia*, *Q. salicina* and *Q. sessilifolia*. Among these species, *Q. hondae* is the most infrequent species and is found only on lower slopes of lucidophyllous forests in Japan. This species is only found in the parts of Southern Kyushu and Southern Shikoku, and is considered to be a vulnerable species in the IUCN red list due to the very restricted extent and a projection of population decline. Considering that *Q. hondae* is threatened by human-oriented environmental changes and its rarity and restricted distributions, it is expected that *Q. hondae* has lower genetic diversity than other widely distributed *Quercus* species. However, the genetic diversity of this species has not been investigated to date and is necessary for the conservation of this species. Here the population genetic status was assessed for 12 *Q. hondae* populations sampled in Kyushu and Shikoku based on chloroplast DNA sequences (*trnH-psbA* and *trnQ-trnS*) and 11 microsatellite loci (Figure 2). To characterize the patterns seen in *Q. hondae*, sympatric populations of a common species, *Q. glauca* were also analyzed using the same DNA markers. Both chloroplast DNA sequences and microsatellite data indicated that a lower level of genetic diversity and a higher level of genetic differentiation among populations were found in *Q. hondae* compared to *Q. glauca* (Figure 3). These results are reflected in scattered geographical distributions and small sizes of each population in *Q. hondae*. In *Q. hondae*, three populations (Miyazaki University Forest and Tsuruda in Kyushu, and Kamibun in

Shikoku) indicated higher genetic diversity compared to other populations. This is because these three populations are found in seminatural and secondary forest vegetations and they have higher genetic diversity than other populations in the tutelary forests of the Japanese shrine, in which forests are generally smaller and fragmented. Although higher genetic diversity in two Kyushu populations and one Shikoku population suggested the possibility that presence of Pleistocene refugia in both Kyushu and Shikoku, DIYABC analysis for *Q. hondae* suggested that Shikoku populations have diverged from a Kyushu group at 2,640-7,920 years ago suggesting that populations *Q. hondae* in Shikoku have occurred after the last glacial period. The genetic differentiation was not corresponding to geographic distance in *Q. hondae*. Miyazaki University Forest and Tsuruda populations are separated by as much as 70 km, but there is a low level of differentiation between the two populations ( $F_{ST} = 0.0310$ ) and these two populations are occupied by the same genetic cluster (Figure 3). On the other hand, there was a significant degree of differentiation between the Miyazaki University Forest and adjacent populations, such as Tsuma Shrine and Aya-Takeno ( $F_{ST} > 0.10$ ). These results suggest that the effect of genetic drift is greater in smaller populations such as being found in shrines and hence the populations become distinct from each other. This is supported by higher  $F$ -value of clusters in the shrine populations.

This study showed that genetic diversity and the structure are different between two species, suggesting that desirable conservation strategies need to be considered for each species (Figure 4). A suitable solution for *S. albida* is to maintain the current level of genetic diversity within populations because the level of genetic diversity for *S. albida* is not extremely lower compared to other *Shorea* species. The solution for *Q. hondae* is to increase in genetic diversity for populations with low genetic diversity and to prioritize conservation of populations with high genetic diversity because the level of genetic diversity for populations of *Q. hondae* is lower compared to those of *Q. glauca*. On the other hand, although the background forming the current genetic structure is different in each species, the presence of genetic structure in both species indicates that conservation of genetically different populations is recommended. This is because conserving genetically different populations and preventing genetic erosion among them are important for maintaining intraspecific genetic diversity in the species.

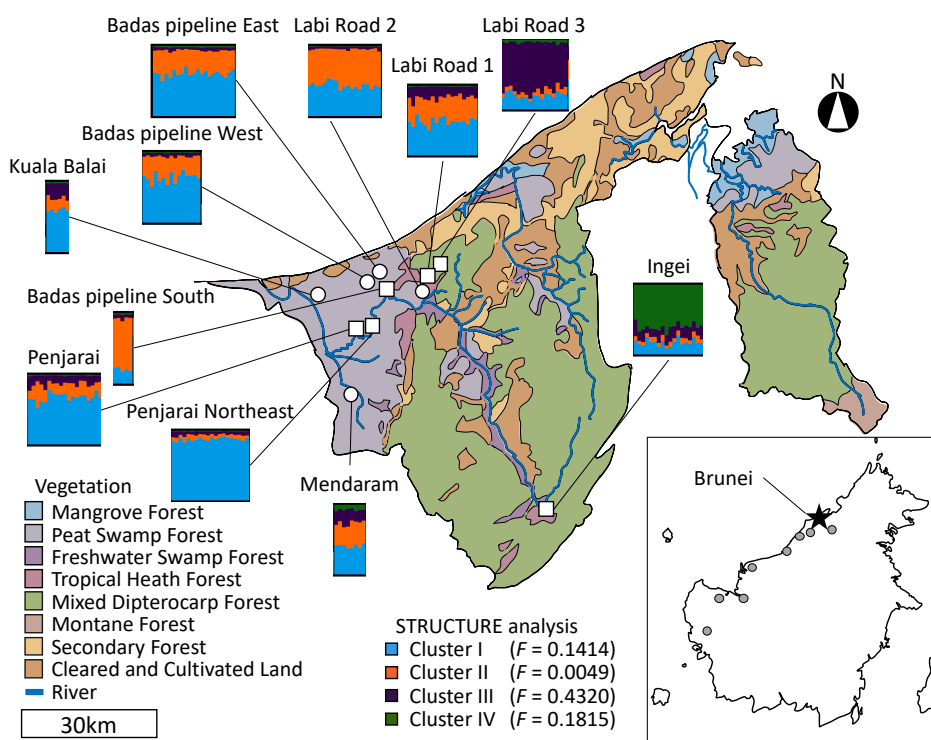


Figure 1. Sampling locations of the 11 *S. albida* populations in this study (open circles and squares) and the result of STRUcTURE analysis at  $K = 4$ . Open squares indicate locations on sand terraces (podzols or shallow peat), and open circles indicate locations on deeper peat. Vegetation map is constructed based on Henrot (1995). The STRUcTURE bar plots represent the assignment probabilities for each genotyped individual. Gray circles on the map at the lower right indicate where *S. albida* is distributed (Ashton 2004).

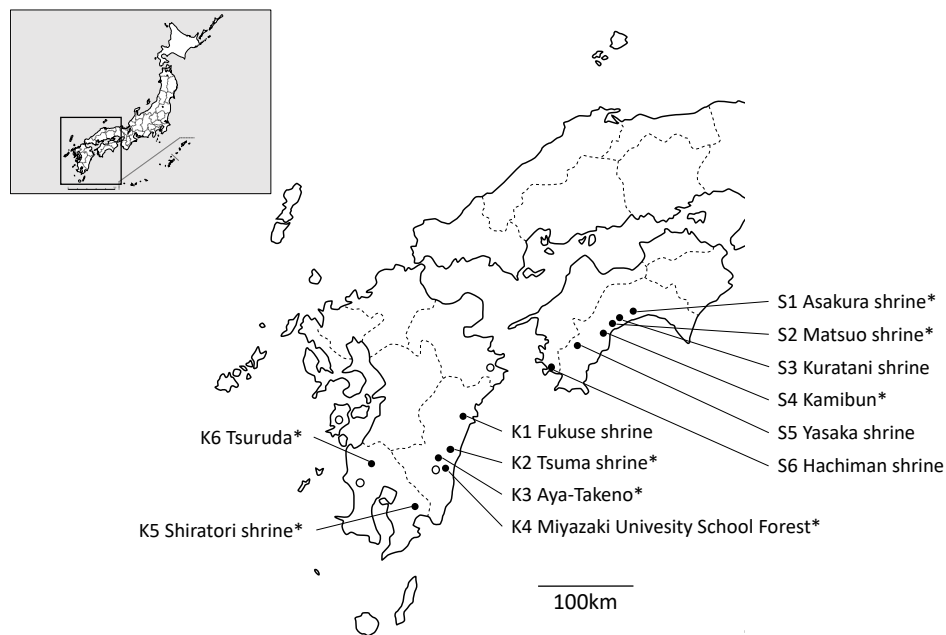


Figure 2. Populations of *Q. hondae* sampled in this study (solid circles). Open circles indicate areas where *Q. hondae* exists, but was not sampled in this study. *Q. glauca* individuals were also collected from populations with asterisks.

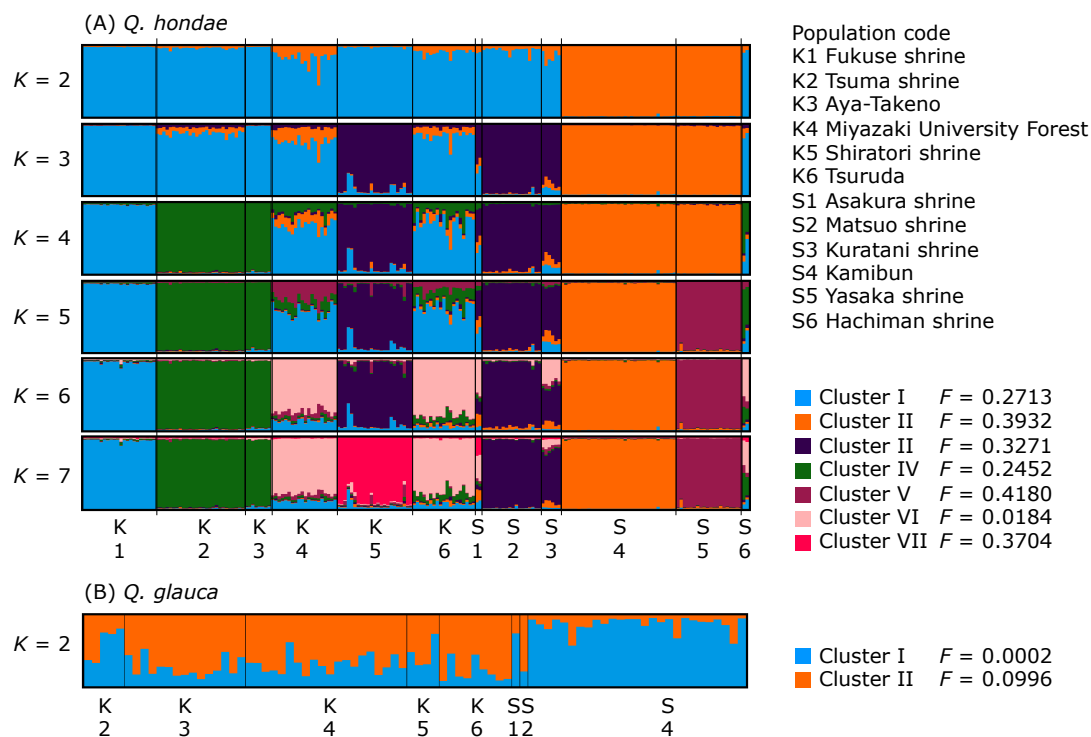


Figure 3. STRUCTURE bar plots of individuals with  $K = 2-7$  for *Q. hondae* (A) and  $K = 2$  for *Q. glauca* (B). Populations were separated by black bars.

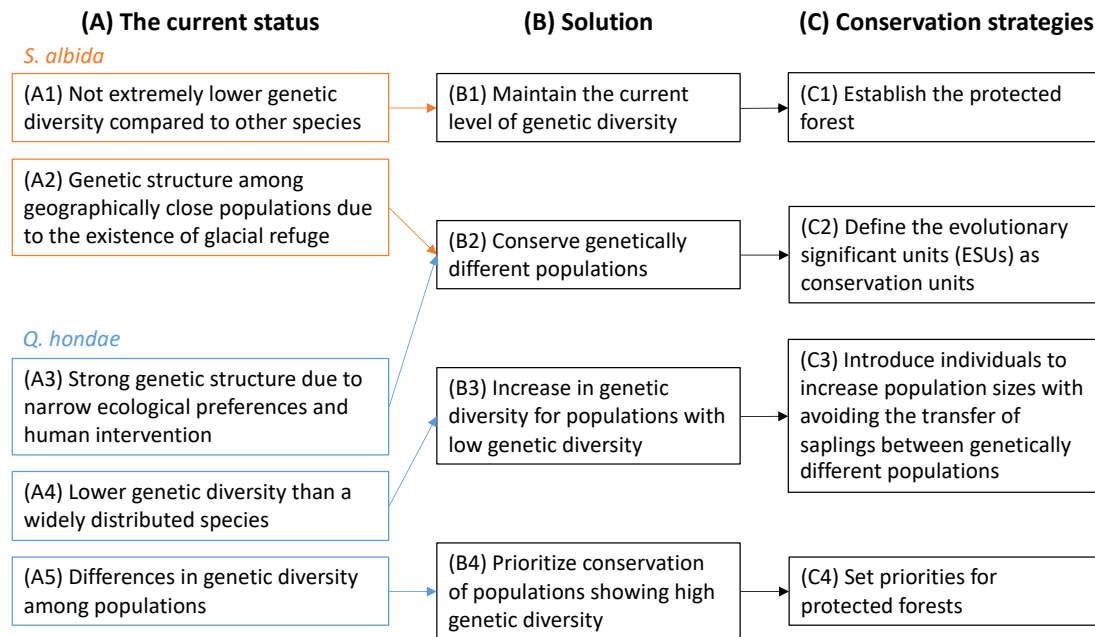


Figure 4. Proposed effective conservation strategies based on the genetic status of *S. albida* and *Q. hondae*.