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

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学位論文題目：
Title of Dissertation

Phylogeographical study of the genus *Dryobalanops* Gaertn. f. (Dipterocarpaceae) based on nuclear microsatellite markers
(マイクロサテライトマーカーを用いたリュウノウジュ属（フタバガキ科）の系統地理学的研究)

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Dissertation Summary

The Pleistocene glacial events affected the biogeographical distribution patterns of many plant species in both northern and southern hemispheres. During the last decade, phylogeographic studies based on molecular markers have revealed the geographical history, particularly climatic oscillations in the Quaternary period, which have played a major role in shaping the present distribution and genetic structure of many organisms. The geographic area including the Malay Peninsula, Sumatra, Java and Borneo is called Sundaland and known to be a globally important biodiversity hot spot. The lowland forests in this region are now largely dominated by the species of Dipterocarpaceae, but the area has been considered to be limited during the last glacial maximum (LGM). However, recently Cannon *et al.* (2009) proposed controversial view; that is, Sundaland rainforests covered a substantially larger area than at present at the LGM. Population genetic analysis of dipterocarp species will help to elucidate the impact of the Pleistocene glaciations on distribution of the Sundaland tropical rainforests. In this study, extents and patterns of genetic variations on geographical distribution of six species of the genus *Dryobalanops* (*D. aromatica*, *D. beccarii*, two subspecies of *D. oblongifolia*, *D. lanceolata*, *D. rappa* and *D. keithii*) in Dipterocarpaceae, were studied using microsatellite markers. The purposes of this study are (1) to evaluate the abilities of these DNA markers for discriminating among the six *Dryobalanops* species and clarify the relationships among the species, (2) to reveal phylogeographic patterns and demographic histories of two prominent species, *D. aromatica* and *D. beccarii*, and to clarify the late Pleistocene evolutionary history of tropical rainforest in Sundaland, based on a demographic analysis of genetic variation.

Population genetic diversity in the genus *Dryobalanops* Gaertn. f. (Dipterocarpaceae) based on nuclear microsatellite markers

Seven microsatellite markers were used to analyze 46 natural populations of six extant *Dryobalanops* species (N = 700 individuals). All seven microsatellite loci exhibited a large number of alleles per locus,

suggesting that these loci will be of potential use in the studies of genetic diversity, population structure and species relationship of these *Dryobalanops* species. The mean gene diversities at the species level were ranged from 0.392 in *D. rappa* to 0.635 in *D. aromatica*. The genetic differentiation among populations (F_{ST}) of respective species were ranged from 0.156 in *D. keithii* to 0.283 in *D. beccarii*, and all the F_{ST} values were significantly greater than zero (Table 1). These results suggest that gene flow between populations has been limited and intensive genetic drift has occurred in all of the species. The species with narrower distribution, such as *D. keithii* and *D. rappa* tend to have lower levels of genetic diversity compared with widespread species *D. aromatica*.

Table 1 Genetic differentiation among population in six *Dryobalanops* species

Species	No. Population	F_{ST}	*P* value
D. aromatica	8	0.194	<0.0001
D. beccarii	16	0.283	<0.0001
D. lanceolata	10	0.246	<0.0001
D. rappa	4	0.159	<0.0001
D. keithii	2	0.156	<0.0001
D. oblongifolia subsp. *oblongifolia*	5	0.194	<0.0001

Bayesian model-based clustering method (the STRUCTURE analysis) revealed three genetically distinct clusters. *Dryobalanops aromatica* and *D. beccarii* are separated each other (cluster I and II, respectively in Fig. 1a), but the other four species are in one cluster (cluster III in Fig. 1a). The STRUCTURE analysis for the remaining four species revealed three clusters (Fig. 1b). *Dryobalanops keithii* and *D. rappa* (cluster III' in Fig. 1b) were separated from *D. lanceolata* and *D. oblongifolia*, but they were not differentiated each other. Whereas, *D. lanceolata* contained a fraction common in *D. oblongifolia* (cluster I' in Fig. 1b). This pattern suggests that the microsatellite analyses performed in this study were successful in revealing significant genetic heterogeneity between the species and could assist to discriminate most of the *Dryobalanops* species.

This study demonstrates that each *Dryobalanops* species is genetically structured and that areas of high priority of conservation should make to ensure these genetically diverged lineages. The genetic structure data revealed in this study also can be used for silvicultural treatments in these highly potential species.

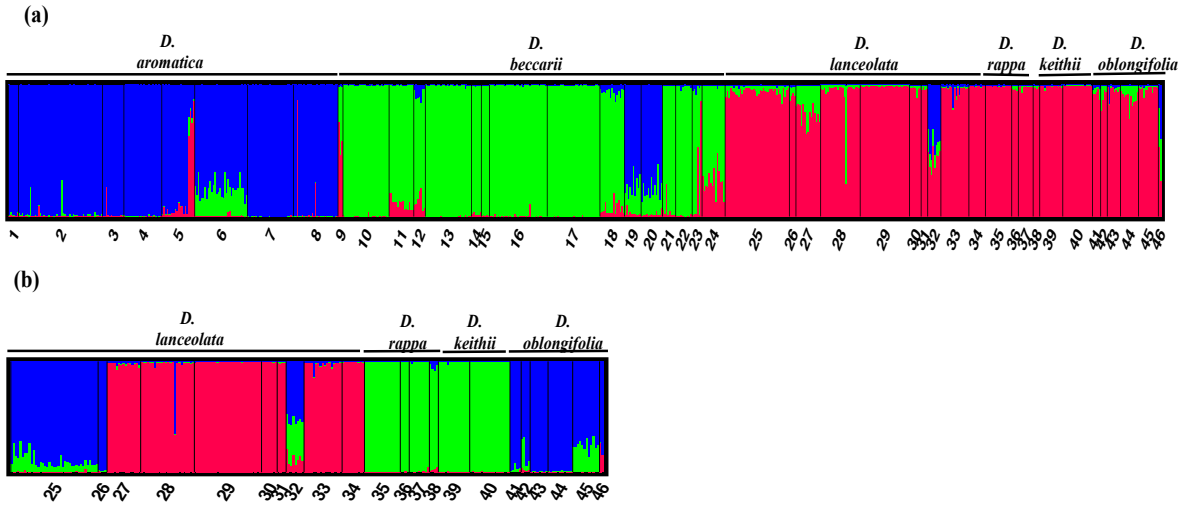

Figure 1 Membership profile for 6 *Dryobalanops* species obtained by STRUCTURE v2.3.4. The result of 8 runs were averaged by CLUMPP v1.1.2 and visualized by DISTRUCT v1.1. The numbers below the boxes are population numbers. (a) Estimated genetic structure for $K = 3$ with 46 populations of 6 *Dryobalanops* species. The cluster in blue is designated cluster I, cluster in green is designated cluster II and the cluster in red is designated cluster III. (b) Estimated genetic structure for $K = 3$ for 4 *Dryobalanops* species with 22 populations. The cluster in blue is designated cluster I', cluster in red is designated cluster II', and cluster in green is designated cluster III'.

Phylogeography and genetic structure of two closely related species, *Dryobalanops aromatica* Gaertn. f. and *Dryobalanops beccarii* Dyer (Dipterocarpaceae) in Sundaland

The phylogeographic patterns were studied on two broadly distributed species, *D. aromatica* and *D. beccarii*. Eight natural populations of *D. aromatica* ($N = 200$) and sixteen natural populations of *D. beccarii* ($N = 235$) were analyzed for this study. The mean unbiased heterozygosity of *D. aromatica* ($H_e = 0.592$) was greater than that of *D. beccarii* ($H_e = 0.400$). This study revealed a higher level of genetic differentiation in *D. beccarii* ($F_{ST} = 0.288$) than in *D. aromatica* ($F_{ST} = 0.198$) (Table 2). The smaller genetic variation but larger differentiation found in *D. beccarii* probably results from its smaller population size and greater extent of isolation.

Table 2 *F*-statistics for *D. aromatica* and *D. beccarii.* The values shown are mean ± standard error, which was estimated using the jackknife method; values in parentheses are 95% confidence intervals, estimated using the bootstrap method. Both are implemented in FSTAT v2.9.3.2.

Species	F_{IT}	F_{ST}	F_{IS}
D. aromatica	0.284 ± 0.052	0.198 ± 0.021	0.107 ± 0.046
	(0.191–0.381)	(0.159–0.233)	(0.030–0.197)
D. beccarii	0.385 ± 0.076	0.288 ± 0.040	0.138 ± 0.114
	(0.270–0.524)	(0.225–0.371)	(-0.022–0.334)

The two species were clearly differentiated by the STRUCTURE analysis although hybridization probably occurred in the area where the two species coexist (Fig. 2a). *Dryobalanops aromatica* could be divided into two genetically distinct groups corresponding to Malay Peninsula-Sumatra and Borneo (Fig. 2b), while *D. beccarii* populations could be divided into four geographically distinct groups including western Sarawak, central inland Sarawak, central coastal Sarawak and Sabah (Fig. 2c). One population in the Malay Peninsula (B7, Gunung Panti) was an admixture of these Bornean clusters, indicating occurrence of long distance migration.

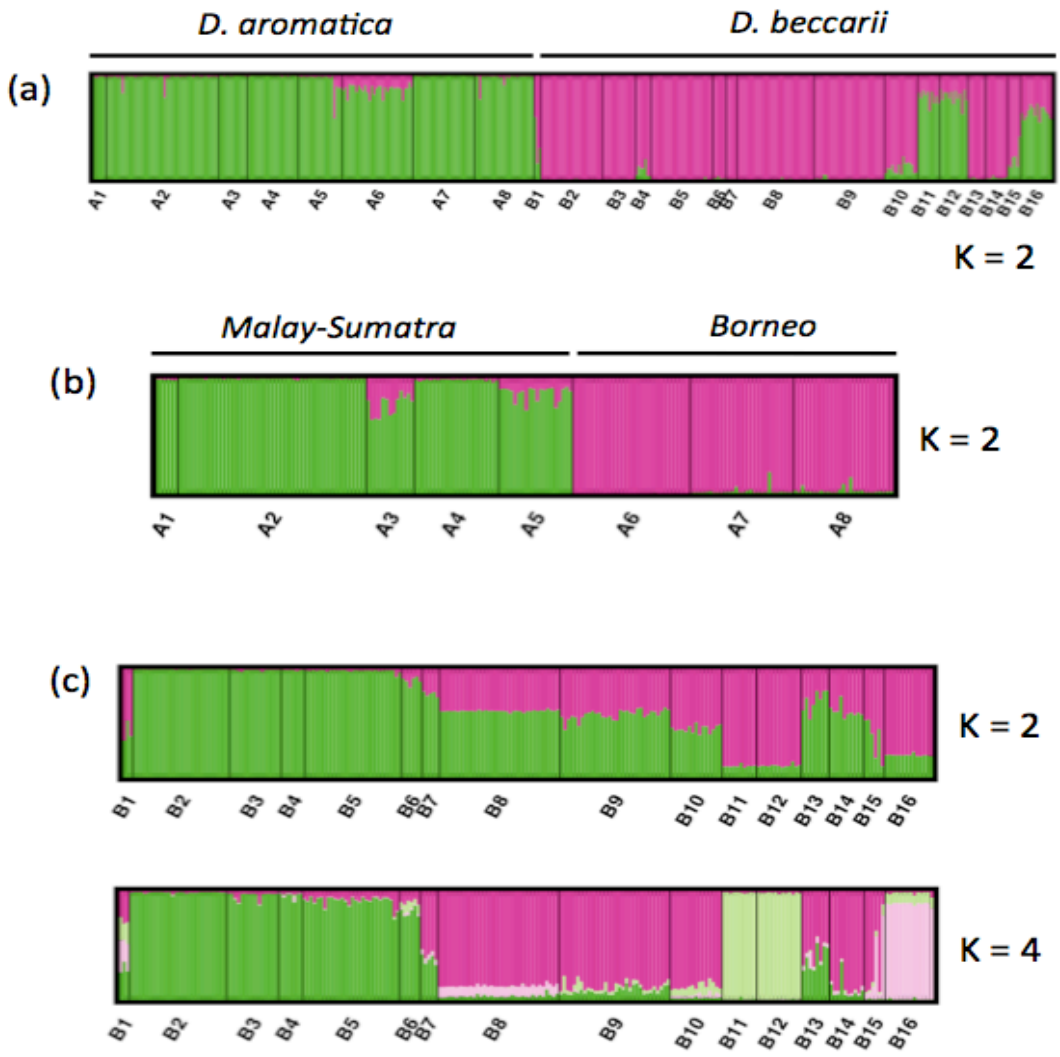

Figure 2 Profile of membership coefficients for each individual of *D. aromatica* and *D. beccarii*, obtained by STRUCTURE v2.3.4. The result of 10 runs were averaged by CLUMPP v1.1.2 and visualized by DISTRUCT v1.1. The numbers below the boxes are population numbers: A1-A8 for *D. aromatica* and B1-B16 for *D. beccarii*.. (a) Estimated genetic structure for $K = 2$ for 24 populations of *D. aromatica* and *D. beccarii*. (b) Estimated genetic structure for $K = 2$ for eight populations of *D. aromatica.* (c) Estimated genetic structure for $K = 2$ and $K = 4$ for 16 populations of *D. beccarii*.

The analysis based on IM (Isolation with Migration) model estimated the time of divergence of *D. aromatica* populations in the Malay Peninsula-Sumatra region and Borneo region to be 7,300–3,600 years ago, i.e. after the last glacial maximum. This analysis also suggested that the ancestral population was twenty times larger than today's populations. This supports the idea that the present tropical rainforest is in a refugial state, and also suggests that the savanna corridor that is hypothesized to have covered the central part of the exposed Sundaland during the last glacial period, if it existed, was not contiguous but rather permeated by rainforests in some places. This study not only clarifies the geographic history of tropical rain forest in Sundaland, but should also be useful for future conservation and efforts to rehabilitate and maintain previously degraded forests.