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Changes in Plant Species during Succession in a Sago Forest

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Abstract

The variation in solar environments during succession in sago forests is thought to affect the growth of many plant species. To clarify the pattern of plant colonization in sago forests at various successional stages, we constructed eleven 10 m × 10 m quadrats in different solar conditions in sago forests, measured and calculated the relative illumination intensity, collected all plant species in these quadrats, and used two chloroplast gene sequences—the *rbcL* gene of ferns and the *trnL* intron of angiosperms to molecularly identify them. The number of ferns increased while the number of herbaceous species decreased during the process of succession. Moreover, the number of woody species was not significantly correlated with the relative light intensity. Based on these results, it can be concluded that woody species colonized and grew at various successional stages but herbaceous species and ferns did the same in the early and late successional stages, respectively, in the sago forest.

Keywords

Chloroplast DNA (cpDNA), Plant Species, Sago Palm

1. Introduction

Secondary succession tends to happen in various places and it can also start very rapidly at places where the ex-

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isting community is completely destroyed by environmental or artificial factors [1]. Some studies have described secondary succession in tropical forests [2]-[11]. One type of tropical forest, the sago forest, occurs in various areas of Indonesia such as lowlands, coastal regions, and river deltas. Many Indonesian sago forests were destroyed because of governmental development projects; however, rubber and oil palm plantations have been expanded recently through commercial sago extraction, because the sago palm *Metroxylon sagu* Rottb. (Arecaceae) plays an important economic and cultural role in Indonesia [12]. Therefore, it is thought that most sago forests have begun secondary succession in many areas of Indonesia. It is uncertain how the destroyed sago palm forest could be restored in Indonesia.

Old-growth forests can represent an important source of information to understand the processes that drive successional pathways, and plant mortality is a key element of forest dynamics that strongly influence the biological and structural diversity of forest ecosystems [13]. Previous study of the sago palm forest was described the colonization pattern of plant species in sago forests after their development by comparing floras from different successional stages by using DNA barcoding [14], a technique for characterizing the species of organisms, by using a short DNA sequence from a standard and agreed-upon position in the genome, which is very short compared to the entire genome, to identify an unknown sample in terms of a pre-existing classification [15] [16]. This study hypothesized that the numbers of species of ferns, Poaceae, and Cyperaceae colonizing a sago forest were correlated with the amount of solar radiation there, and therefore, their variation would be a clue to determine the forest's successional stage, but because no analyses of solar radiation had been made so far, it remains unclear whether the hypothesis is reasonable. Here, we analysed correlations between the numbers of species of ferns, Poaceae, and Cyperaceae and the measured solar radiation in sago forests in different successional stages in order to verify the previous hypothesis of [14].

2. Materials and Methods

2.1. Plant Materials

The plant materials were from one area of sago palm forests in the Malay Archipelago from March 2014. **Figure 1** shows the location of the collection area used in this study. In this area, we selected four localities, A, B, C, and D, and constructed 11 quadrats (four in A, three in B, one in C and three in D) of 10 m × 10 m and then collected all plant species from them.

2.2. Measurement of Solar Radiation

In order to clarify the illumination intensity in each quadrat, we averaged values obtained from three illuminometers (T-10A: Konica Minolta, Tokyo, Japan) in each quadrat and the control area. These values were of the

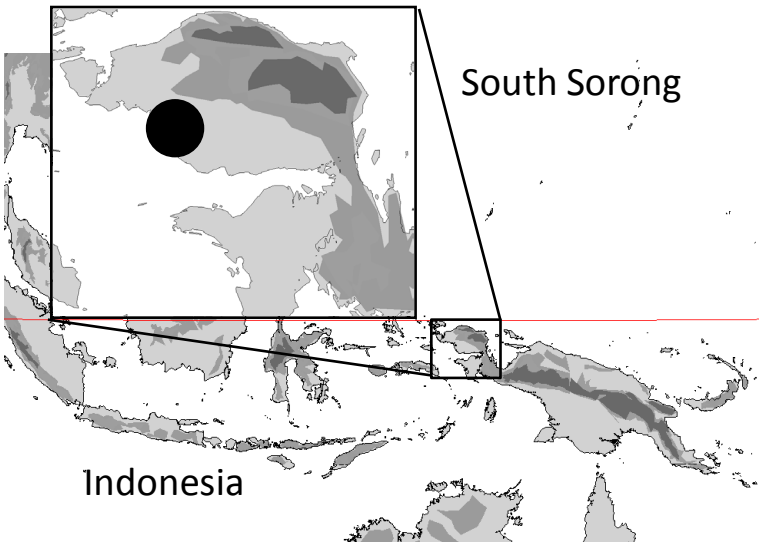

Figure 1. Sampling area of this study.

absolute illumination intensity. We calculated the relative illumination intensity, which was the ratio of the absolute illumination intensity from each quadrat to the value from the control area. These measurements were carried out in just 12 locations, once in each quadrat and in the control area.

2.3. DNA Analyses

Total DNA was isolated from approximately 200 - 300 mg of an air-dried leaf with a Plant Genomic DNA Mini Kit (VIOGENE, Sunnyvale, USA), according to the manufacturers' protocol. Isolated DNA was resuspended in Tris-EDTA (TE) buffer and stored at −20˚C until use. DNA amplification by polymerase chain reaction

(PCR) was carried out in a 50-μL reaction volume containing approximately 50 ng total DNA, 10 mM Tris-HCl buffer (pH 8.3) with 50 mM KCl and 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1.25 U *Taq* DNA polymerase (TOYOBO), and 0.5 μM of each primer. We used the following thermal cycle profile for amplification: 40 cycles of 1.5 min at 94˚C, 2 min at 48˚C, and 3 min at 72˚C, followed by a final extension step of 15 min at 72˚C.

We amplified the *rbcL* and *trnL* introns of cpDNA with primers designed by [17] [18], respectively. After amplification, the reaction products were run on a 1% agarose gel and digitally photographed.

We also confirmed the sequences of these regions. After amplification, the reaction mixtures were subjected to electrophoresis in 1% low-melting-temperature agarose gels for the purification of the amplified products. We sequenced the purified reaction products using a BigDye-terminator Cycle Sequencing Kit (Applied BioSystems) and a Model 3730A automated sequencer (Applied BioSystems) according to the manufacturer's instructions. For sequencing, we used the same primers as those used for amplification. Sequences for each region were pre-aligned with the CLUSTAL X program [19], and ambiguously aligned regions were manually corrected to minimise the number of indels. Alignment for all DNA regions required the inclusion of several indels.

Samples that were identical to a previously published sequence of the DNA Data Bank of Japan (DDBJ) were provided the species name from the previous publication. If samples did not have sequences identical to previously published ones, then a phylogenetic tree was constructed using our sequences and highly similar ones based on a homological search of the DDBJ. These samples were assigned the genus name of the plant species of the sister group of the sample in the phylogenetic tree. For example, when two sequences of our result were the sister group to the species of *Stenochlaena* (Blechnaceae), they were named after *Stenochlaena* sp. 1 and *Stenochlaena* sp. 2 (**Table 1**).

3. Results and Discussion

DNA barcodes are used to identify unknown species of flowering plants [20]. The use of chloroplast DNA (cpDNA) or nuclear DNA (nrDNA) sequences is appropriate for most species of plants because of the relatively fast mutation rate of these sequences [16], and some studies had shown a potential barcode [21]-[24]. Our results of DNA barcoding by using two cpDNA genes—the *rbcL* gene and *trnL* intron of ferns and angiosperms, respectively, added to the morphological identifications and clarified that there were 66 species collected from 42 families in total (**Table 1**). Quadrat B1 had 21 species and was the most species of all quadrats, while only two species grew in quadrat A2, the least species (**Table 1**). Relative illumination intensity was measured to clarify the correlation between the number of species and the solar radiation. The results showed that the quadrat of C was the highest, B1 and D1 were relatively high, D2 and B2 were relatively low, and A1, A2, A3, A4, B3, and D3 were very low (**Table 2**). Although this result suggested that the brightest places did not necessarily have the most species, it suggested that the number of plants decreased in relatively dark places like A1, A2, A3, A4, B3, and D3 (**Table 1** and **Table 2**).

Some studies had been reported the relationship between relative illuminance and plant species [25]-[27]. Previous study of the sago palm forest was hypothesized that the number of Poaceae and Cyperaceae increased in brighter environments [14]. Our results supported that the number of grass and sedge species had a significant correlation with the relative illumination intensity (**Figure 2**). To determine whether this situation was specialized only in Poaceae and Cyperaceae, we compared the numbers of all herbaceous species and the relative illumination intensity. The result was similar to those for Poaceae and Cyperaceae (**Figure 3**). Sago forest succession was considered to reduce solar radiation, and our results suggested that the number of herbaceous species, including Poaceae and Cyperaceae, decreased as sago forest succession progressed. However, why was the number of species not perfectly correlated with the progress of succession in the sago forest? One explanation is the number of ferns and woody species. Previous study of the sago palm forest was suggested that the number of

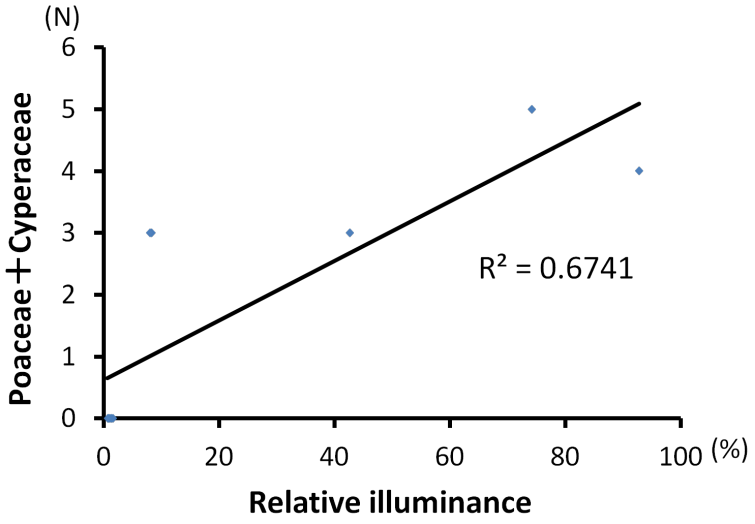

Figure 2. The correlation between the number of grasses and sedges (Poaceae and Cyperaceae) and the relative illumination intensity.

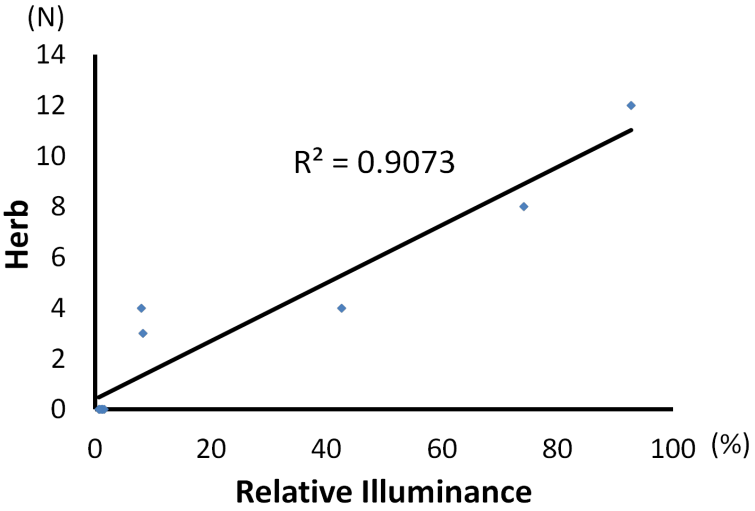

Figure 3. The correlation between the number of herbaceous plant species and the relative illumination intensity.

ferns increased in the dark environment of sago forests [14], and our results could support that suggestion (**Figure 4**). Moreover, our results indicated that the number of woody species had no significant correlation to the relative illumination intensity (**Figure 5**). After adding the numbers of ferns and woody species to those of herbaceous species, there was little correlation between the total number of plant species and the progress of succession in the sago forest. Woody species play a major role in creating terrestrial plant cover when conditions develop to the point where some bare ground and soil is present, and our results suggested that they colonized and grew at various successional stages, while herbaceous species and ferns did so at the early and late successional stages, respectively, of the sago forest.

There has been enormous growth in phylogenetic studies based on molecular data in recent years. In particular, the use of molecular markers has considerably improved our knowledge about past events shaping the genetic diversity within species [28]. The application of phylogenetic analysis is emerging as an important and practical tool for the study of native species and their relatives [29] [30]. Genetic information from native species has provided sources of useful traits for genetic hybridization between native and invasive alien species. Moreover, conscious efforts to search for desirable traits in plants have been underway for the past century, and

Table 1. List of species, accession numbers, and quadrates. No accession number indicates identification by morphological characters. Taxonomic arrangement follows the Engler system. Black circles indicate the collected quadrates.

Taxon	Family	Species	Accession No.	Quadrats										
				A1	A2	A3	A4	B3	D3	B1	B2	D1	D2	C
Pteridophyte														
	Aspleniaceae	*Asplenium nidus*							●					
	Blechnaceae	*Blechnum orientale*	AB981762							●				
		Stenochlaena sp. 1	AB981738	●				●		●	●			
		Stenochlaena sp. 2	AB981745											
	Cyatheaceae	*Cyathea* sp.											●	
		Sphaeropteris glauca	AB981737				●							
	Dennstaedtiaceae	*Histiopteris incisa*	AB981764								●			
		Pteridium aquilinum										●	●	
	Equisetaceae	*Equisetum debile*											●	
	Gleicheniaceae	*Dicranopteris linearis*	AB981739							●	●	●		
	Hymenophyllaceae	*Trichomanes javanica*											●	
	Lycopodiaceae	*Lycopodiella cernua*	AB981757									●		
	Lygodiaceae	*Lygodium microphyllum*	AB981748							●	●			
	Oleandraceae	*Nephrolepis biserata*											●	
		Nephrolepis cordifolia						●					●	
		Nephrolepis sp.											●	
	Pteridaceae	*Acrostichum aureum*								●	●	●	●	
		Ceratopteris thalictroides												●
		Taenitis blechnoides	AB981766			●	●							
		Trichomanes javanica								●	●			
		Pityrogramma calomelanos	AB981780											●
	Polyipodiaceae	*Drynaria sparsisora*	AB981754						●					
	Thelypteridaceae	*Thelypteris* sp.	LC000738							●	●	●		
Angiosperm														
Monocotyledoneae														
	Araceae	*Epipremnum papuanum*	LC000733	●										
	Cyperaceae	*Cyperus bifax*												●
		Cyperus eragrostis	AB981770											●
		Fimbristylis dichotoma	AB981769										●	●
		Fuirena umbellata	AB981755							●	●	●		
		Kyllinga brevifolia	AB981774											●
	Limnocharitaceae	*Limnocharis flava*												●
	Orchidaceae	*Spathoglottis plicata*								●	●			
	Pandanaceae	*Freycinetia* sp.	AB981752						●					
		Pandanus veitchii	AB981746		●									
	Poaceae	*Axonopus* sp.	AB981760										●	
		Imperata cylindrica								●	●	●		
		Paspalum conjugatum										●		
	Pontederiaceae	*Eichornia crassipes*												●

Continued

Angiosperm												
Dicotyledoneae												
Choripetalae												
Anacardiaceae	*Anacardiaceae* sp.	AB981742							●			
Aquifoliaceae	*Ilex* sp. 1	AB981750								●	●	
	Ilex sp. 2	AB981753					●					
	Ilex sp. 3	AB981778					●					
Bombacaceae	*Durio zibethinus*	AB981741						●				
Euphorbiaceae	*Endospermum moluccanum*	LC000737			●			●			●	
	Macaranga pearsonii											●
	Macaranga sp.										●	
	Mallotus sp.	LC000740							●			
Fabaceae	*Fabaceae* sp.	AB981767				●						
Melastomataceae	*Melastoma malabathricum*	AB981768										●
	Mouriri sp.	AB981749						●				
Moraceae	*Artocarpus heterophyllus*	AB981765				●			●			
	Ficus elastica	AB981763						●	●	●	●	
	Ficus sp. 1	LC000739	●									
	Ficus sp. 2	LC000736		●								
Myristicaceae	*Horsfieldia punctatifolia*	AB981751									●	
Myrtaceae	*Syzygium* sp.	LC000734	●									
Onagraceae	*Ludwigia* sp.	AB981743										●
Piperaceae	*Piper* sp.	LC000735		●								
Urticaceae	*Cypholophus* sp.	AB981759									●	
Sympetalae												
Apocynaceae	*Alstonia scholaris*	AB981740						●				
	Trachelospermum sp.	AB981744	●									
Asteraceae	*Asteraceae* sp.	AB981772										●
	Eclipta prostrata	AB981773										●
Convolvulaceae	*Merremia peltata*											●
Gentianaceae	*Fagraea volubilis*	AB981756						●				
Lamiaceae	*Tectona grandis*	AB981761					●					
Plantaginaceae	*Gratiola* sp.	AB981771										●
Rubiaceae	*Alphitonia incana*	AB981758								●		
	Mussaenda scratchleyi							●				
	Mussaenda sp.								●			
	Oldenlandia tenelliflora	AB981775										●
	Uncaria lanosa							●	●	●		
Sapotaceae	*Sapotaceae* sp.	AB981779		●								

Table 2. The relative illumination intensity of each area in south Sorong distinct, West Papua province, Indonesia.

Quadrat Name	A1	A2	A3	A4	B1	B2	B3	C	D1	D2	D3
Relative Illuminance (%)	0.74	1.31	0.99	0.66	74.15	8.00	1.55	92.77	42.66	8.24	1.18

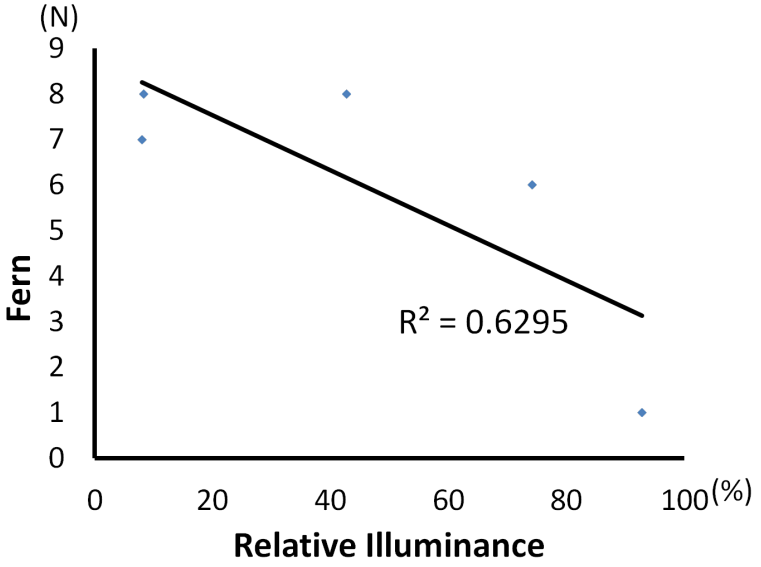

Figure 4. The correlation between the number of ferns and the relative illumination intensity.

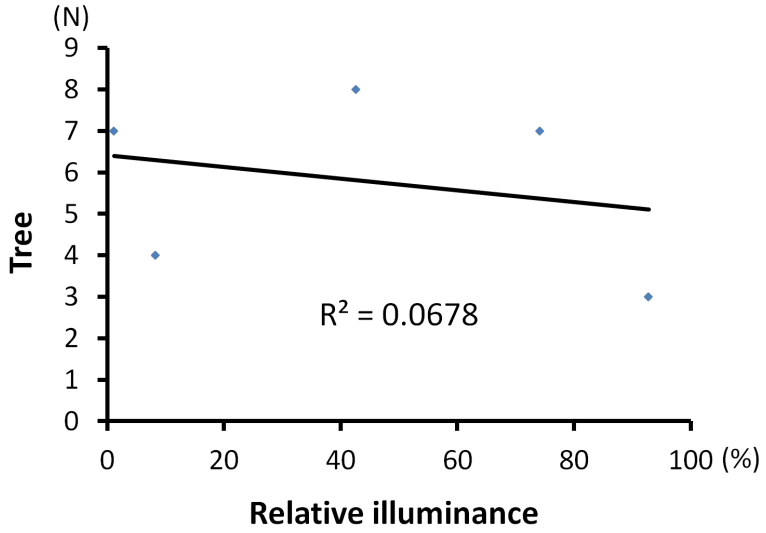

Figure 5. The correlation between the number of woody plant species and the relative illumination intensity.

in recent decades, these species have come to be regarded as important biological resources in need of conservation [31]. Although we consider that our genetic information of a given species in sago palm forests is thus an important research focus to design concerted efforts for their conservation, our research could not distinguish between native and alien plant species. Future analyses should include other areas to protect sago palm forests from genetic disturbance from invasive alien species.

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